



HAL
open science

Simple or complex organic substrates inhibit arsenite oxidation and *aiiA* gene expression in two β -Proteobacteria strains

Tiffanie Lescure, Catherine Joulian, Clément Charles, Taoikal Ben Ali Saanda, Mickael Charron, Dominique Breeze, Pascale Bauda, Fabienne Battaglia-Brunet

► To cite this version:

Tiffanie Lescure, Catherine Joulian, Clément Charles, Taoikal Ben Ali Saanda, Mickael Charron, et al.. Simple or complex organic substrates inhibit arsenite oxidation and *aiiA* gene expression in two β -Proteobacteria strains. *Research in Microbiology*, 2020, 171 (1), pp.13-20. 10.1016/j.resmic.2019.09.006 . insu-02298717

HAL Id: insu-02298717

<https://insu.hal.science/insu-02298717v1>

Submitted on 27 Sep 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Journal Pre-proof



Simple or complex organic substrates inhibit arsenite oxidation and *aioA* gene expression in two β -*Proteobacteria* strains

Tiffanie Lescure, Catherine Joulian, Clément Charles, Taoikal Ben Ali Saanda, Mickael Charron, Dominique Breeze, Pascale Bauda, Fabienne Battaglia-Brunet

PII: S0923-2508(19)30100-7

DOI: <https://doi.org/10.1016/j.resmic.2019.09.006>

Reference: RESMIC 3741

To appear in: *Research in Microbiology*

Received Date: 21 June 2019

Revised Date: 4 September 2019

Accepted Date: 6 September 2019

Please cite this article as: T. Lescure, C. Joulian, C. Charles, T. Ben Ali Saanda, M. Charron, D. Breeze, P. Bauda, F. Battaglia-Brunet, Simple or complex organic substrates inhibit arsenite oxidation and *aioA* gene expression in two β -*Proteobacteria* strains, *Research in Microbiology*, <https://doi.org/10.1016/j.resmic.2019.09.006>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier Masson SAS on behalf of Institut Pasteur.

1 **Simple or complex organic substrates inhibit arsenite oxidation and *aioA* gene**
2 **expression in two β -*Proteobacteria* strains**

3

4 Tiffanie Lescure^{a,b}, Catherine Joulian^a, Clément Charles^a, Taoikal Ben Ali Saanda^a, Mickael
5 Charron^a, Dominique Breeze^a, Pascale Bauda^c, Fabienne Battaglia-Brunet^{a,b*}

6

7 ^aBRGM, 3 avenue Claude Guillemin, 45060 Orléans, France

8 ^bBRGM, ISTO, UMR 7327, BP 36009, 45060 Orléans, France

9 ^cLIEC UMR 7360 CNRS-Université de Lorraine, Campus Bridoux, rue du Général
10 Delestraint, 57070 Metz, France

11 *Corresponding author: f.battaglia@brgm.fr, (+33)2.38.64.39.30

12

13 E-mail addresses: tiffanielescure@gmail.com (T. Lescure), c.joulian@brgm.fr (C. Joulian),
14 clement_cm45@yahoo.fr (Charles C.), taoikalali@hotmail.fr (Ben Ali Saanda T.),
15 m.charron@brgm.fr (Charron M.), d.breeze@brgm.fr (Breeze D.), [pascale.bauda@univ-](mailto:pascale.bauda@univ-lorraine.fr)
16 lorraine.fr (Bauda P.), f.battaglia@brgm.fr (Battaglia-Brunet F., *correspondence and
17 reprints)

18

19

20

21

22

23

24

25

26

27

28 **Abstract**

29 Microbial transformation of arsenic species and their interaction with the carbon cycle play a
30 major role in the mobility of this toxic metalloid in the environment. The influence of simple
31 or complex organic substrates on arsenic bio-oxidation was studied using two bacterial
32 strains: one – the *arsenivorans* strain of *Thiomonas delicata* – is able to use AsIII as sole
33 energy source; the other, *Herminiimonas arsenicoxydans*, is not. Experiments were performed
34 at two AsIII concentrations (75 and 2 mg/L). At 75 mg/L As, for both strains, expression of
35 *aioA* gene decreased when yeast extract concentration was raised from 0.2 to 1 g/L. At 2
36 mg/L As, the presence of either yeast extract or simple (succinate or acetate) organic
37 substrates in the medium during bacterial growth decreased the AsIII-oxidation rate by both
38 strains. When added specifically during oxidation test, yeast extract but not simple organic
39 substrates seems to have a negative effect on AsIII oxidation. Taken together, results confirm
40 the negative influence of simple or complex organic substrates on the kinetics of microbial
41 AsIII oxidation and suggest that this effect results from different mechanisms depending on
42 the type of organic substrate. Further, for the first time, the influence of a complex organic
43 substrate, yeast extract, on *aioA* gene expression has been evidenced.

44

45 **Keywords:** *Betaproteobacteria* strains; organic substrates; arsenite oxidation; *aioA* gene
46 expression

47

48

49 1. Introduction

50 High concentrations of the toxic metalloid arsenic (As) in soils may be the result of pollution
51 from mining, industrial activities or agricultural practices, or they may be due to the
52 geochemical background. In the case of mining and metallurgical activities, As is released as
53 a by-product in the waste (tailings) or from drainage galleries [1].

54 Biological arsenite (AsIII) oxidation processes, coupled to iron oxidation and precipitation,
55 can be used advantageously for effective bioremediation of mine water [1,2] and, where soils
56 are concerned, to attenuate the environment toxicity, since arsenate (AsV) is less toxic than
57 AsIII. This oxidizing reaction is of significant ecological importance as it leads to the
58 stabilization of arsenic in the environment since AsV is more readily adsorbed than AsIII by
59 carrier phases such as iron or manganese oxides [3,4].

60 Bacteria able to oxidize AsIII and reduce AsV have been found in many terrestrial and aquatic
61 environments [5-10]. Some bacteria possess genes allowing oxidation as well as genes
62 conferring the ability to reduce AsV to AsIII via the Ars resistance system. Although this
63 AsV-reduction detoxification intracellular reaction is energy consuming, it is widely found in
64 microbial communities because AsIII thus obtained is excreted out of the cell, or is
65 sequestered as reduced glutathione (GSH) or other thiol conjugates, for example in yeast [11].

66 Another way in which bacteria derive energy from arsenic is via the dissimilatory reduction of
67 AsV into AsIII by anaerobic respiration via the Arr system, using AsV as a final electron
68 acceptor [12]. The AsIII oxidation function in bacteria is encoded by the *aio* operon [13],
69 which comprises up to six genes (*aioB*, *aioA*, *aioS*, *aioR*, *aioX* and *aioE*). Two of them encode
70 the two AsIII oxidase enzyme subunits: *aioB*, which encodes the small subunit containing the
71 Rieske [2Fe-2S] cluster; and *aioA*, which encodes the large subunit containing a catalytic
72 molybdopterin and a cluster [3Fe-4S]. These two genes were originally identified and isolated
73 from two β -proteobacteria: *Alcaligenes faecalis* [14] and *Herminiimonas arsenicoxydans*

[15]. The other four *aio* genes correspond to the following functions: *aioS* encodes a histidine kinase sensor, *aioR* a transcriptional regulator, *aioX* an oxyanions-ligand protein, and *aioE* an AsIII oxidation electron transporter [16]. Santini and van den Hoven [17] studied a chemolithoautotrophic bacterium, strain NT26 that derives energy from AsIII oxidation but is not able to grow on minimal medium in the presence of arsenic when its *aioA* gene is inactivated. This experiment demonstrated the use of arsenic as an energy source by some bacteria. Many other bacteria have been identified as carrying genes encoding AsIII oxidase [18, 19]. This is the case for the “*Thiomonas (T.) arsenivorans*” strain, a chemolithoautotrophic, acid-tolerant and mobile Gram-negative rod [5,20]. It has been proven elsewhere that this bacterium, by oxidizing AsIII into AsV, can both create energy and detoxify the environment [5]. Some publications suggest a very significant effect of organic substrates on bacterial AsIII oxidation in the presence of oxygen. Challan-Belval et al. [21] have shown that addition of yeast extract substantially reduces the rate of AsIII oxidation by bacteria organized in biofilms. Bachate et al. [22] observed a decrease in enzymatic AsIII-oxidase activity of two pure strains as the total concentration of organic substrates (acetate and yeast extract) in the growth medium increased. Lescure et al. [23] observed a negative effect of organic substrates input, with concentrations from 0.08 to 0.4 g of carbon/L, corresponding to 0.2 to 1 g/L yeast extract, on the AsIII-oxidation rate constant of soil microbial communities. It therefore appears that organic substrates slow down the transformation of AsIII into AsV by bacteria in aerated conditions, and may decrease the retention of As in the environment. Nandre et al. [24] showed that the presence of acetate in the growth medium induced a lower *aioA* gene expression in diverse bacterial strains isolated from tannery effluent and soils. Organic substrates may inhibit the *aio* encoded oxidation processes, because bacteria would preferentially metabolize simple or complex organic substrates providing more energy than AsIII. Organic substrates may also induce a decrease

99 in AsIII bioavailability by complexation reactions [25]. Because of these complexation
100 reactions, if AsIII oxidation was mainly or exclusively a detoxification mechanism, the
101 presence of a high concentration of organic substrate may have a protective effect against
102 AsIII, inducing an attenuation of the detoxification process.

103 However, to date, the influence of the concentration and nature of different organic substrates
104 on biological AsIII oxidation has not been studied systematically.

105 The objective of this work was to study the influence of two types of organic substrates,
106 complex or simple organic substrates, on the ability to oxidize AsIII of two β -*proteobacteria*
107 strains presenting different metabolisms. One of these, a strain of *Thiomonas delicata* referred
108 to as “*T. arsenivorans*” – uses AsIII as energetic substrate; the other,
109 *Herminiimonas (H.) arsenicoxydans*, does not. The hypothesis to be tested through this
110 experiment was that bacterial AsIII oxidation could be inhibited differently by organic
111 substrates when AsIII is used or not as energy source. A common complex organic substrate
112 was chosen: yeast extract, a reference complex organic substrate in laboratory studies that can
113 promote the growth of both strains. In parallel, because the strains do not use any common
114 simple organic substrate, two simple organic substrates were selected; acetate for *H.*
115 *arsenicoxydans* and succinate for “*T. arsenivorans*”. Two sets of experiments were carried
116 out. First, AsIII oxidation and *aioA* gene expression were studied in batch growth experiments
117 with a relatively high concentration of arsenic allowing strain growth with AsIII as sole
118 energy source: 75 mg.L⁻¹ (or 1 mM) AsIII. Then, AsIII oxidation kinetics were determined at
119 an initial AsIII concentration more compatible with the concentrations commonly found in the
120 pore water of polluted soils (0.5–2 mg.L⁻¹ AsIII, [26]).

121

122

123

2. Materials and methods

2.1. Bacterial strains

Two AsIII-oxidizing microorganisms carrying an *aioA* gene were selected as models; "*Thiomonas arsenivorans*" (DSM 16361), a mixotrophic and optional autotrophic strain able to grow on AsIII and using only the AsIII oxidation reaction as an energy source; *Herminiimonas arsenicoxydans* (DSM 17148), a heterotrophic strain perfectly characterized [27].

These two strains belong to the subdivision of β -*Proteobacteria*. "*T. arsenivorans*" was isolated by BRGM [5]. *H. arsenicoxydans* [28] was kindly provided for this study by the laboratory of Molecular Genetics, Genomics, Microbiology (GMGM) of Louis Pasteur University (Strasbourg, France), where the strain was isolated.

2.2. Culture media

"*T. arsenivorans*" was maintained in minimal CAsO1 medium [29] with 100 mg.L⁻¹ AsIII, and *H. arsenicoxydans*, which is a heterotrophic bacterium, in the Luria-Bertani (LB) medium, then sub-cultured in CAsO1 medium amended with 1 g.L⁻¹ yeast extract before each experiment.

The CAsO1 liquid medium is a mixture of 500 mL of each of two solutions. Solution A contains 0.5 g KH₂PO₄, 0.5 g K₂HPO₄, 0.5 g NaCl, 0.05 g (NH₄)₂SO₄ and 1 mL of trace elements in solution [29]. The pH of this solution was adjusted to 6.0 before autoclaving for subsequent growth of "*T. arsenivorans*", and to 7.2 for growth of *H. arsenicoxydans*. Solution B (500 mL) contains 0.1 g CaCl₂ and 0.1 g MgSO₄. Both solutions were autoclaved separately and mixed after cooling. 10 mL of a solution of 10 g.L⁻¹ AsIII (As₂O₃), filtered at 0.22 μ m,

148 were added to the culture medium. A concentrated solution of 50 g/L yeast extract was
149 prepared, filtered at 0.22 μm and then stored at 4°C.

150 2.3. *aioA* gene expression with 75 mg.L⁻¹ AsIII

151 The aim of the first experiment was to determine the influence of a complex organic substrate,
152 yeast extract, on the expression of the *aioA* gene during the growth of the bacteria, in the
153 presence of 1 mM AsIII, a concentration that provides enough energy for the growth of
154 “*Thiomonas arsenivorans*” in batch conditions [5].

155 “*T. arsenivorans*” inoculum was cultivated in 20 mL of CAsO1 medium (pH 6.0) containing
156 75 mg.L⁻¹ AsIII without organic substrate, in a cottoned 40 mL glass bottle. The inoculum of
157 *H. arsenicoxydans* was prepared in CAsO1 medium (pH 7.2) containing 75 mg.L⁻¹ AsIII and
158 supplemented with 1 g.L⁻¹ yeast extract. Two CAsO1 culture media containing, respectively,
159 0.2 and 1 g.L⁻¹ yeast extract were inoculated, at pH 7.2 for *H. arsenicoxydans* and pH 6.0 for
160 “*T. arsenivorans*”. All flasks were incubated aerobically at 25°C under stirring. At t=0 and
161 then twice per day, bacterial growth was monitored by enumerating bacterial cells with a
162 Thoma cell under an optical microscope and by absorbance measurements at 620 nm. 1.5 mL
163 of each culture were sampled in a 2 mL sterile microtube and centrifuged for 10 min at
164 5,000 x g. Pellets were frozen in liquid nitrogen and stored at -80°C until RNA/DNA co-
165 extraction. In parallel, 2 mL samples were filtered at 0.22 microns in sterile flasks and kept at
166 4°C until further AsV analysis. Abiotic controls prepared in the same conditions showed that
167 AsIII was not oxidized abiotically [23].

168

169 2.4. AsIII oxidation kinetics

170 The aim of the second experiment was to quantify the influence of simple and complex
171 organic substrates on the AsIII-oxidation rate when concentrations of AsIII are so low that the
172 kinetics must be carried out over a short time, i.e. less than one hour. For this purpose, a

173 standardized cell concentration was used, allowing easier comparison of the results obtained
174 in the different conditions. Acetate was used for *H. arsenicoxydans* as it is the only simple
175 organic substrate used as the sole source of carbon and energy by this strain [27]. For “*T.*
176 *arsenivorans*”, which does not use acetate, succinate was chosen, since it is a simple organic
177 substrate used efficiently by this strain [5]. Yeast extract was chosen as a complex organic
178 substrate used by both strains, allowing comparison of results with a common substrate. Each
179 strain was sub-cultured (1% volume) into 100 mL of CAsO1 medium containing 2 mg.L⁻¹ of
180 AsIII at the appropriate pH (pH 6.0 for “*T. arsenivorans*”, pH 7.2 for *H. arsenicoxydans*) and
181 different concentrations of organic substrate: 0.05, 0.2 or 1 g.L⁻¹ yeast extract for both strains;
182 0.05, 0.2 or 1 g.L⁻¹ of succinate for “*T. arsenivorans*” or acetate for *H. arsenicoxydans*.
183 Incubations were carried out aerobically for 3 days at 25°C under static conditions, to avoid
184 the formation of cell aggregates (oxygen was not limiting). For all of the sub-culture
185 conditions (0.05, 0.2 and 1 g.L⁻¹ of organic substrates), two conditions were compared in
186 triplicate to determine AsIII oxidation kinetics: the liquid phase of the bacterial suspension
187 that oxidized 2 mg/L AsIII was either deprived of organic substrate, or contained 0.2 g.L⁻¹ of
188 organic substrate.

189 Appropriate sub-culture volumes were sampled at the end of the growth phase in order to
190 adjust the final cell densities in the suspensions to values close to 2 x 10⁷ cells.mL⁻¹. After
191 centrifugation (30 minutes at 8,500 x g), pellets were washed with 20 mL of arsenic-free
192 CAsO1 medium. After a second centrifugation (30 minutes at 8,500 x g), pellets were re-
193 suspended in 130 mL of arsenic-free CAsO1 medium, with or without 0.2 g.L⁻¹ of organic
194 substrate. 20 mL of the resulting bacterial suspensions were distributed in 60 mL sterile
195 cottoned flasks.

196 At t=0, 400 µL of 100 mg.L⁻¹ AsIII solution were added to the flasks to obtain a concentration
197 of 2 mg.L⁻¹ AsIII, mixed immediately and sampled for bacterial cells counting and for arsenic

198 speciation. The flasks were incubated at 25°C, under static conditions, and AsIII oxidation
199 was monitored over 45 minutes, with 2.5 mL-sampling every 15 minutes. The samples were
200 filtered at 0.2 µm in sterile microtubes immediately after sampling and were promptly diluted
201 tenfold in sterile ultrapure water and stored at 4°C for subsequent As speciation. Blank
202 experiments were prepared in the same conditions without cells. The specific AsIII-oxidation
203 rate was calculated as the ratio of linear AsIII oxidation over 45 minutes and the cell
204 concentration measured at the start of the experiment (this concentration did not significantly
205 vary during the 45 minutes of the experiment).

206

207 *2.5 Speciation and quantification of arsenic*

208 AsIII and AsV were separated using resins (AG® 1-X8 Resin, Cat. # 140-1431, Biorad) as
209 described in [2], and a fraction of the sample remaining in the tube was used for total As
210 quantification. Quantification of As in tubes containing AsV, AsIII and total As was carried
211 out by electrothermal atomic absorption spectrophotometry (Varian SpectrAA 220Z).

212

213 *2.6. RNA and DNA co-extraction and cDNA synthesis*

214 RNA and DNA co-extractions were performed on cell pellets stored at -80°C, using the
215 NucleoSpin® RNA II kit (Macherey-Nagel) in accordance with the manufacturer's
216 recommendations, including a separate DNA elution step with the NucleoSpin® DNA/RNA
217 Buffer Set (Macherey-Nagel). The column was then placed on a new 1.5 mL collection tube
218 and the manufacturer's protocol was continued with the digestion of DNA followed by RNA
219 elution. An additional digestion step was needed to remove residual DNA from the RNA
220 extract. To that end, extracted RNA was mixed with 7 µL of buffer and 1 µL of Turbo DNase
221 (2 U; Ambion, ThermoScientific) and then incubated at 37°C for 10 min. Finally, a new RNA
222 purification step was performed with the NucleoSpin® RNA II kit (Macherey-Nagel). The

223 RNA was finally eluted in 60 μ L and assayed by BioPhotometer spectrophotometer
224 (Eppendorf). The quality of extracted RNA was checked on 1% agarose gel (25 min at 100 V)
225 and by calculation of the A_{260}/A_{230} and A_{260}/A_{280} ratios. They were stored in LoBind
226 microtubes (Eppendorf), at -80°C until the reverse-transcription PCR (RT-PCR) step. The
227 reaction mixture used for the RT-PCR step consisted of 1 μ L iScriptTM reverse transcriptase
228 (BioRad), a variable volume of RNA extract (so as to obtain 50 ng per tube), 4 μ L 5XTM
229 iScript reaction mix (BioRad), and ultrapure nuclease-free water (qsp 20 μ L). RT-PCR
230 consisted of 5 min at 25°C , 30 min at 46°C , 5 min at 85°C and hold at 15°C .

231

232 2.7. *aioA* gene and transcripts quantification by qPCR

233 The *aioA* gene involved in AsIII oxidation and its transcripts were quantified by quantitative
234 real-time PCR (qPCR), respectively from DNAs and cDNAs obtained from cultures of “*T.*
235 *arsenivorans*” and *H. arsenicoxydans*. qPCR was carried out using m4-1F forward
236 (GCCGGCGGGGGNTWYGARRAYA) and m2-1R reverse
237 (GGAGTTGTAGGCGGGCCKRTRTGDAT) primers, as designed in [30] and applied in
238 [31]. The expected product size was 110 bp. Each primer was used at a concentration of
239 0.3 μ M, with 100 ng of T4GP32 (MP Biomedicals), in 1X IQ SYBR Green Supermix
240 (BioRad) and a final volume of 20 μ L. The program was run on a CFX Connect (BioRad) and
241 consisted of an initial denaturation at 95°C for 3 min, followed by 50 cycles of 95°C for 10 s,
242 54°C for 20 s, 72°C for 10 s, and a data acquisition step at 80°C for 30 s. At the end, a
243 melting curve analysis was performed by measurement of the SYBR Green I signal intensities
244 during a 0.5°C temperature increment every 10 s, from 65°C to 95°C . Negative template
245 controls confirmed the absence of contaminant DNA. Six-point serial decimal dilution of a
246 linearized plasmid carrying the *H. arsenicoxydans aioA* gene was used to generate a linear
247 calibration curve of threshold cycle versus a number of gene copies ranging from 10^1 to 10^6 .

248 All measurements were run in duplicates. Results were expressed as ratios of *aioA* transcripts
249 relative to the *aioA* gene (*aioA* mRNA/DNA ratio).

250

251 2.8. Statistical analysis

252 Statistical analysis were performed with XLSTAT 2019: normality of the data was first
253 verified using the tests of Shapiro-Wilk, Anderson-Darling, Lilliefors and Jarque-Bera. Then,
254 significance of differences between results obtained at different substrate concentrations was
255 evaluated using an Anova parametric test. Differences between modalities were analysed with
256 a confidence interval of 95% using the methods of Tukey and Dunnett.

257

258 3. Results

259 3.1. Expression of *aioA* gene during bacterial growth in the presence of 75 mg.L⁻¹ AsIII

260 Arsenite oxidation activity in batch conditions was monitored together with the detection and
261 quantification of *aioA* gene expression and growth of the pure strains. Fig. 1A. shows that
262 expression of the *aioA* gene by “*T. arsenivorans*” was mainly detectable during early growth
263 (Fig. 1C). Maximum *aioA* gene expression was measured at 24 h, corresponding to about
264 60 mg.L⁻¹, i.e. 80% of oxidized AsIII (Fig. 1E), and tended to be higher in the presence of
265 0.2 g.L⁻¹ than 1 g.L⁻¹ of yeast extract (average *aioA* mRNA/DNA ratios were, respectively,
266 0.032 and 0.0015 in the presence of 0.2 g.L⁻¹ and 1 g.L⁻¹ yeast extract). However, due to a
267 large variability between replicates, the difference observed between ratios obtained at 0.2
268 and 1 g/L yeast extract was not statistically significant. Expression of the *aioA* gene for
269 *H. arsenicoxydans* was detectable throughout the duration of the experiment (Fig. 1B). As for
270 “*T. arsenivorans*”, maximum expression was detected in the early growth (Fig. 1D) and AsIII
271 oxidation phase (t=5 h), and corresponds to 20–30 mg.L⁻¹ (26-40%) of oxidized AsIII (Fig.

272 1F). Here, in the maximum *aioA* gene expression phase, expression was significantly greater
273 in the presence of 0.2 g.L⁻¹ than 1 g.L⁻¹ of yeast extract (at t=5h, average *aioA* mRNA/DNA
274 ratios were 1.6 x 10⁻³ and 3.3 x 10⁻⁴ in the presence of 0.2 g.L⁻¹ and 1 g.L⁻¹ of yeast extract,
275 respectively).

276

277 For *T. arsenivorans*, AsV concentrations were always similar whatever the concentrations of
278 yeast extract (Fig 1E), whereas for *H. arsenicoxydans*, AsV concentration was slightly higher
279 with 1 g.L⁻¹ yeast extract at t=5h (Fig. 1F). No significant AsV oxidation was observed in
280 sterile media (Fig. 1G). For both strains, bacterial growth was higher at 1 g.L⁻¹ than at 0.2 g.L⁻¹
281 of yeast extract (Fig. 1C and 1D). Consequently, the lower the yeast extract concentration,
282 the higher the specific AsIII oxidation.

283

284 3.2. Oxidation kinetics with 2 mg.L⁻¹ AsIII

285 Cells at the end of the growth phase, that grew at different concentrations of a simple organic
286 substrate or yeast extract oxidized AsIII in a minimum medium containing 2 mg.L⁻¹ of AsIII
287 and no organic substrate (Fig. 2) (cell growth was negligible throughout the 45 min duration
288 of the AsIII-oxidation kinetics). In the blank experiments without cells, AsV concentration
289 remained lower than the quantification limit. Examination of the kinetics shows that oxidation
290 of AsIII was more efficient with cells that grew on yeast extract (Fig. 2B and 2D) than on
291 simple substrates (Fig. 2A and 2C), and more efficient with "*T. arsenivorans*" (Fig. 2A and
292 2B) than with *H. arsenicoxydans* (Fig. 2C and 2D). *H. arsenicoxydans* did not grow well on
293 acetate, thus the rates obtained with this strain and a simple substrate were very low (Fig. 2C).
294 The results expressed as specific oxidation rates (Fig. 3) confirm these observations: for both
295 strains, a significant decrease of specific AsIII oxidation rates was observed when yeast
296 extract concentration in the growth medium increased (Fig. 3A). The same trend was

297 observed for “*T. arsenivorans*” grown on succinate as a simple organic substrate (Fig. 3B). *H.*
298 *arsenicoxydans* did not grow well in the presence of acetate, although the trend is less clear.
299 However, for this strain, the specific AsIII oxidation rate was lower at 1 g.L⁻¹ ($5.6 \cdot 10^{-11}$
300 $\mu\text{g}/\text{cell}/\text{min}$) than at 0.05 g.L⁻¹ of acetate ($2 \cdot 10^{-10}$ $\mu\text{g}/\text{cell}/\text{min}$).

301
302 These specific rates were compared with those obtained when AsIII-oxidation kinetics were
303 determined in the presence of organic substrates (Fig. 4 and Table 1). A negative effect of the
304 addition of yeast extract was observed systematically for both strains, whatever the pre-
305 culture condition: rates decreased by 13.6% to 39.1% for “*T. arsenivorans*” and by 10% to
306 25.9% for *H. arsenicoxydans*. In contrast, no effect on AsIII-oxidation rates was detected with
307 simple organic substrates (acetate or succinate).

308

309

310 4. Discussion

311 A negative effect of organic substrates on bacterial AsIII-oxidation activity was observed with
312 two pure strains presenting contrasting metabolisms, one able to use AsIII as an energy source
313 (“*T. arsenivorans*”), the other not (*H. arsenicoxydans*). This study has shown, for the first
314 time, the negative effect of a complex organic substrate, yeast extract, on the expression of the
315 *aioA* gene encoding the catalytic subunit of the AsIII-oxidase in the Aio system. So far, the
316 main factor studied influencing the expression of the *aioA* gene was the presence and
317 concentration of arsenic III or V. Expression of the *aioA* gene is induced by the presence of
318 As in most AsIII-oxidizing bacteria [32] including several strains belonging to the *Thiomonas*
319 genus [33]. However, in some strains, *aioA* gene expression is also observed in the absence of
320 arsenic: *Thiomonas* sp. 3AS [7], *Ralstonia* sp. 22 [34] and *Agrobacterium tumefaciens* 5A
321 strain, for which AsIII oxidation seems regulated by quorum-sensing [35], and for some

322 strains of *Pseudomonas* [36, 37]. In *H. arsenicoxydans*, the expression of genes in the *aio*
323 operon is related to the presence of AsIII and to the expression of genes involved in motility
324 [38]. Our results are in accordance with those of Nandre et al. [24], who observed a higher
325 expression of *aioA* genes under acetate starvation condition in seven strains belonging to
326 different genera, for one single time of incubation (8 h), in presence of 1.33 mM of As. Here,
327 the phenomenon was observed with a complex organic substrate (yeast extract) and correlated
328 with early growth phase.

329 The specific AsIII-oxidation rates obtained as part of this study are of the same order of
330 magnitude (Table 2) as the V_{\max} of AsIII oxidation determined for *Variovorax* sp. 24 [39] and
331 *Agrobacterium albertimagni* AOL15 [40].

332 The specific oxidation rate was lower when cells had been grown with a high substrate
333 concentration. Several authors have observed that simple or complex organic substrates
334 influence the activity of AsIII-oxidizing bacteria. Santini et al. [41] reported that the AsIII-
335 oxidizing strain NT-26, able to use AsIII for energy, showed strong AsIII-oxidase activity
336 when it was grown without any organic substrate, the addition of yeast extract resulting in a
337 decrease in AsIII-oxidizing activity. Challan-Belval et al. [21] observed that AsIII oxidation
338 by a bacterial consortium containing "*T. arsenivorans*", grown as biofilm in microplate
339 experiments with pozzolana, was drastically decreased in the presence of yeast extract,
340 dropping from 100% to 34%. This result was explained by the supply of nutrients present in
341 yeast extract, including electron donors other than AsIII. Bachate et al. [22] studied the
342 influence of various parameters (temperature, pH and growth medium) on AsIII-oxidation
343 rate by bacterial cells washed and re-suspended in 75 mg.L⁻¹ AsIII-containing medium. They
344 followed AsIII oxidation by these cells for 1 hour. The strains were heterotrophic bacteria,
345 phylogenetically related to the genera *Bordetella* and *Achromobacter*. These authors observed
346 that the growth conditions have an effect on AsIII-oxidation rate: the latter was maximal

347 when the strains were cultured with only 0.4 g.L⁻¹ yeast extract, decreased by 25 to 50% with
348 1 g.L⁻¹ yeast extract, and by 85% with 0.4 g.L⁻¹ of yeast extract and 20 mM (1.18 g.L⁻¹) of
349 acetate. They also observed a decrease in the enzymatic AsIII-oxidase activity of both strains
350 with the increase of total organic substrate concentration in the growth medium. The present
351 results are consistent with those of Bachate et al. [22], although the studied strains belong to
352 different genera and come from very different environments: “*T. arsenivorans*” and *H.*
353 *arsenicoxydans* were isolated from a mining site and a wastewater treatment plant
354 respectively, whereas the origin of strains of Bachate and al. [22] was a garden soil.

355 Here, bacterial specific AsIII-oxidation rate was adversely affected for both strains by the
356 presence of simple or complex organic substrates in growth medium. This phenomenon was
357 observed both at relatively high AsIII concentrations (75 mg.L⁻¹) and at the lower
358 concentrations of arsenic (2 mg.L⁻¹) commonly detected in groundwater or soil pore water on
359 polluted sites. This negative effect on AsIII oxidation was observed with a complex organic
360 substrate (yeast extract), as well as with at less one simple organic substrate that do not
361 provide nitrogen, i.e. succinate, suggesting that the observed phenomenon is not linked to
362 nitrogen metabolism but rather to energy or carbon source. Thus for both “*T. arsenivorans*”, a
363 mixotrophic strain using AsIII as energy source, and *H. arsenicoxydans*, a heterotrophic
364 strain, arsenic oxidation and metabolism of organic substrates seem to be linked.

365 Several hypotheses may explain the negative effect of organic substrates on *aioA* gene
366 expression and the bacterial specific AsIII-oxidation rate. Nandre et al. [24] hypothesized
367 cross protection in the carbon starvation stress wherein nutrient deprived cells show higher
368 resistance against other stresses. However, this phenomenon could hardly explain results
369 obtained with bacteria such as “*T. arsenivorans*” usually growing in mineral or oligotrophic
370 media. In bacteria using AsIII as an energy source, such as NT-26 or “*T. arsenivorans*”, the
371 phenomenon could be related to competition between AsIII and organic substrates as an

372 energy source: in the presence of high energy-providing organic molecules, cells do not have
373 to acquire energy from the AsIII oxidation reaction, and therefore express the *aioA* gene more
374 weakly. In bacteria unable to gain energy from AsIII oxidation (like *H. arsenicoxydans*) and
375 in which the ability to oxidize AsIII can be a detoxification mechanism [41], the presence of a
376 high concentration of organic substrate may have a protective effect against AsIII.

377 Another hypothesis would be linked to complexation reactions that may occur between As
378 and some compounds present in complex organic substrates, and thus reduce AsIII toxicity
379 and/or bioavailability. Here, the results of experiments at 2 mg.L⁻¹ As suggest that the
380 complex organic substrate (i.e. yeast extract) has a negative effect on AsIII oxidation,
381 considering the comparison of kinetics with cells in suspension in solution with or without
382 yeast extract. This phenomenon may be related to the complexation of AsIII with certain
383 components of yeast extract such as thiol groups of amino acids that would make As less
384 bioavailable. These complexation reactions would not occur with simple organic substrates.
385 Direct binding of AsIII to cysteine residues of proteins has been observed [43]. Another
386 possible explanation of the negative effect of simple or complex organic substrates on AsIII
387 oxidation may be that the availability of energy sources would favor the AsV-reducing Ars
388 arsenic resistance system [12] because this resistance process consumes energy. Regarding
389 the Ars resistance system, most studies have focused on *arsB* and *ACR3* genes carriers
390 [35,44,45] whose expression was shown to be controlled by the concentration of arsenic and
391 phosphate. Further studies should focus on determination of the effect of organic substrates on
392 AsIII complexation, and on the expression of the *arsC* gene encoding an AsV reductase in the
393 Ars resistance system, which, in parallel with the Aio system, could influence the balance of
394 speciation of inorganic arsenic in aerated or microaerophilic environments.

395

396 **Conflict of interest**

397 The authors declare that there is no conflict of interest regarding the publication of this article.

398

399

400 **Aknowledgements**

401 This work was supported by the French Environment and Energy Management Agency
402 (ADEME), BRGM (convention TEZ 11-16), and the Labex Voltaire (ANR-10-LABX-100-
403 01). We are grateful to the laboratory of Molecular Genetics, Genomics, Microbiology
404 (GMGM) of Louis Pasteur University (Strasbourg, France) for providing the *H.*
405 *arsenicoxydans* strain.

406

407 **References**

- 408 [1] Casiot C, Morin G, Juillot F, Bruneel O, Personné J-C, Leblanc M, Duquesne K,
409 Bonnefoy V, Elbaz-Poulichet F. Bacterial Immobilization and Oxidation of Arsenic in Acid
410 Mine Drainage (Carnoulès Creek, France). *Wat Res* 2003;37: 2929–36.
- 411 [2] Battaglia-Brunet F, Itard Y, Garrido F, Delorme F, Crouzet C, Greffié C, Jouliau C. A
412 simple biogeochemical process removing arsenic from a mine drainage water. *Geomicrobiol*
413 2006;23: 201-211.
- 414 [3] Fuller CC, Davis JA, Waychunas GA. Surface chemistry of ferrihydrite: Part 2. Kinetics
415 of arsenate adsorption and coprecipitation. *Geochim Cosmochim Acta* 1993;199357: 227 1-
416 2282.
- 417 [4] Smedley PL, Kinniburgh DG. A review of the source, behaviour and distribution of
418 arsenic in natural waters. *Appl Geochem* 2002;17: 517-568.

- 419 [5] Battaglia-Brunet F, Joulian C, Garrido F, Dictor MC, Morin D, Coupland K, Johnson BD,
420 Hallberg KB, Baranger P. Oxidation of arsenite by *Thiomonas* strains and characterization of
421 *Thiomonas arsenivorans* sp. nov.. *Antonie Van Leeuwenhoek* 2006;89:99-108.
- 422 [6] Campos VL, Valenzuela C, Yarza P, Kampfer P, Vidal R, Zaror C, Mondaca MA, Lopez-
423 Lopez A, Rossello-Mora R. *Pseudomonas arsenicoxydans* sp nov., an arsenite-oxidizing
424 strain isolated from the Atacama desert. *Syst Appl Microbiol* 2010;33:193-197.
- 425 [7] Duquesne K, Lieutaud A, Ratouchniak J, Muller D, Lett MC, Bonnefoy V. Arsenite
426 oxidation by a chemoautotrophic moderately acidophilic *Thiomonas* sp. : from the strain
427 isolation to the gene study. *Env Microbiol* 2008;10: 228-237.
- 428 [8] Hoefft SE, Kulp TR, Stolz JF, Hollibaugh JT, Oremland RS. Dissimilatory Arsenate
429 Reduction with Sulfide as Electron Donor: Experiments with Mono Lake Water and Isolation
430 of Strain MLMS-1, a Chemoautotrophic Arsenate Respirer. *Appl Environ Microbiol* 2004;70:
431 2741-2747.
- 432 [9] Quéméneur M, Cébron A, Billard P, Battaglia-Brunet F, Garrido F, Leyval C, Joulian C.
433 Population structure and abundance of arsenite-oxidizing bacteria along an arsenic pollution
434 gradient in waters of the upper isle River Basin, France. *Appl Environ Microbiol* 2010;76:
435 4566-4570.
- 436 [10] Cordi A, Pagnout C, Devin S, Poirel J, Billard P, Dollard MA, Bauda P. Determination
437 of physiological, taxonomic, and molecular characteristics of a cultivable arsenic-resistant
438 bacterial community. *Environ Sci Poll Res* 2015;22:13753–13763.
- 439 [11] Rosen BP. Biochemistry of As detoxification. *FEBS Lett* 2002;529:86-92.
- 440 [12] Silver S, Phung LT. Genes and enzymes involved in bacterial oxidation and reduction of
441 inorganic arsenic. *Appl Environ Microbiol* 2005;71:599-608.
- 442 [13] Lett MC, Muller D, Lievremont D, Silver S, Santini J. Unified nomenclature for genes
443 involved in prokaryotic aerobic arsenite oxidation. *J Bacteriol* 2012;194:207-208.

- 444 [14] Anderson LC, Bruland KW. Biogeochemistry of Arsenic in Natural Waters: The
445 Importance of Methylated Species. *Environ Sci Technol* 1991;25:420-427.
- 446 [15] Muller D, Lievremont D, Simeonova DD, Hubert JC, Lett MC. Arsenite Oxidase *aox*
447 Genes from a Metal-Resistant-Proteobacterium. *J Bacteriol* 2003;185:135-141.
- 448 [16] Wang Q, Han Y, Shi K, et al. An Oxidoreductase AioE is Responsible for Bacterial
449 Arsenite Oxidation and Resistance. *Scientific Reports* 2017;7:41536.
- 450 [17] Santini JM, van den Hoven RN Molybdenum-Containing Arsenite Oxidase of the
451 Chemolithoautotrophic Arsenite Oxidizer NT-26. *J Bacteriol* 2004;186:1614-1619.
452
- 453 [18] Oremland RS, Hoelt SE, Santini JM, Bano N, Hollibaugh RA, Hollibaugh JT. Anaerobic
454 Oxidation of Arsenite in Mono Lake Water and by a Facultative, Arsenite-Oxidizing
455 Chemoautotroph, Strain MLHE-1. *Appl Environ Microbiol* 2002;68:4795-4802.
- 456 [19] Santini JM, Sly LI, Wen A, Comrie D, Wulf-Durand PD, Macy JM. New Arsenite-
457 Oxidizing Bacteria Isolated from Australian Gold Mining Environments-Phylogenetic
458 Relationships. *Geomicrobiol J* 2002;19:67-76.
- 459 [20] Battaglia-Brunet F, El Achbouni H, Quemeneur M, Hallberg KB, Kelly DP, Joulian C.
460 Proposal that the arsenite-oxidizing organisms, *Thiomonas cuprina* and '*Thiomonas*
461 *arsenivorans*' be reclassified as strains of *Thiomonas delicata*, and emended description of
462 *Thiomonas delicata*. *Intl J Syst Environ Microbiol* 2011;61:2816-2821.
- 463 [21] Challan-Belval S, Garnier F, Michel C, Chautard S, Breeze D, Garrido F. Enhancing
464 pozzolana colonization by As(III)-oxidizing bacteria for bioremediation purposes. *Appl*
465 *Microbiol Biotechnol* 2009;84: 565-573.
- 466 [22] Bachate SP, Khapare RM, Kodam KM (2012) Oxidation of arsenite by two beta-
467 proteobacteria isolated from soil. *Applied Microbiology and Biotechnology* 93: 2135-2145

- 468 [23] Lescure T, Moreau J, Charles C, Ben Ali Saanda T, Thouin H, Pillas N, Bauda P, Lamy
469 I, Battaglia-Brunet F. Influence of organic matters on AsIII oxidation by the microflora of
470 polluted soils. *Env Geochem Health*. 2016;38:911–925.
- 471 [24] Nandre VS, Bachate SP, Salunkhe RC et al. Enhanced Detoxification of Arsenic Under
472 Carbon Starvation: A New Insight into Microbial Arsenic Physiology. *Curr Microbiol*
473 2017;74:614.
- 474 [25] Spuches AM, Kruszyna HG, Rich AM, Wilcox DE. Thermodynamics of the AsIII-thiol
475 interaction: arsenite and monomethylarsenite complexes with glutathione, dihydrogluic acid
476 and other thiol ligands. *Inorg Chem* 2005;44:2964-2972.
- 477 [26] Battaglia-Brunet F, Joulian C, Breeze D, Poirier L, Coulon S, Guérin V (2010)
478 Monitoring of arsenic bio-oxidation and bio-reduction activities in highly As-polluted soils
479 during a phytostabilization field experiment (Phytoperf project). *Consoil* 2010, 11th
480 International UFZ- Deltares/TNO Conference on Management of Soil, Groundwater and
481 Sediment, Salzburg.
- 482 [27] Muller D, Simeonova DD, Riegel P, Mangenot S, Koechler S, Lièvreumont D, Bertin PN,
483 Lett MC. *Herminiimonas arsenicoxydans* sp. nov., a metalloresistant bacterium. *Int J Syst*
484 *Evol Microbiol* 2003; 56:1765–1769.
- 485 [28] Weeger W, Lièvreumont D, Perret M, Lagarde F, Hubert JC, Leroy M, Lett MC.
486 Oxidation of arsenite to arsenate by a bacterium isolated from an aquatic environment.
487 *Biometals* 1999; 12: 141-149.
- 488 [29] Battaglia-Brunet F, Dictor MC, Garrido F, Crouzet C, Morin D, Dekeyser K, Clarens M,
489 Baranger P. An arsenic(III)-oxidizing bacterial population: selection, characterisation, and
490 performance in reactors. *J Appl Microbiol* 2002;93:656-667.
- 491 [30] Quéméneur M. Les processus biogéochimiques impliqués dans la mobilité de l'arsenic :
492 recherche de bioindicateurs. Thèse de doctorat d'université. Thesis, Laboratoire des

- 493 Interactions Microorganismes-Minéraux-Matière Organique dans les Sols (LIMOS), UMR
494 7137 CNRS, Université Henri Poincaré de Nancy. 2008.
- 495 [31] Fernandez-Rojo L, Hery M, Le Pape P, Braungardt C, Desoeuvre A, Torres E, Tardy V,
496 Resongles E, Laroche E, Delpoux S, Joulian C, Battaglia-Brunet F, Boisson J, Grapin G,
497 Morin G, Casiot C. Biological attenuation of arsenic and iron in a continuous flow
498 bioreactor treating acid mine drainage (AMD). *Water Research* 2017;123:594-606.
- 499 [32] Slyemi D, Bonnefoy V. How prokaryotes deal with arsenic. *Env Microbiol Reports*
500 2012; 4: 571-586.
- 501 [33] Hovasse A, Bruneel O, Casiot C, Desoeuvre A, Farasin J, Hery M, Van Dorsselaer A,
502 Carapito C, Arsène-Ploetze F. Spatio-Temporal Detection of the *Thiomonas* Population and
503 the *Thiomonas* Arsenite Oxidase Involved in Natural Arsenite Attenuation Processes in the
504 Carnoulès Acid Mine Drainage. *Front Cell Dev Biol* 2016;4:1-14.
- 505 [34] Lieutaud A, van Lis R, Duval S, Capowiez L, Muller D, Lebrun R, Lignon S, Fardeau
506 ML, Lett MC, Nitschke W, Shoep-Cothenet B. Arsenite oxidase from *Ralstonia* sp. 22:
507 characterization of the enzyme and its interaction with soluble cytochromes. *Journal of*
508 *Biological Chemistry* 2010;285:2433-2441.
- 509 [35] Kashyap DR, Botero LM, Franck WL, Hassett DJ, McDermott TR. Complex regulation
510 of arsenite oxidation in *Agrobacterium tumefaciens*. *J Bacteriol* 2006;188:1081-1088.
- 511 [36] Poirel J, Joulian C, Leyval C, Billard P. Arsenite-induced changes in abundance and
512 expression of arsenite transporter and arsenite oxidase genes of a soil microbial community.
513 *Res Microbiol* 2013;164:457-465.
- 514 [37] Koechler S, Arsène-Ploetze F, Brochier-Armanet C, Goulhen-Chollet F, Heinrich-
515 Salmeron A, Jost B, Lièvreumont D, Philipps M, Plewniak F, Bertin PN, Lett M-C.
516 Constitutive arsenite oxidase expression detected in arsenic-hypertolerant *Pseudomonas*
517 *xanthomarina* S11. *Res Microbiol* 2015;166:205-214.

- 518 [38] Koechler S, Cleiss-Arnold J, Proux C, Sismeiro O, Dillies MA, Goulhen-Chollet F,
519 Hommais F, Lievremont D, Arsene-Ploetze F, Coppee JY, Bertin PN. Multiple controls affect
520 arsenite oxidase gene expression in *Herminiimonas arsenicoxydans*. BMC Microbiol
521 2010;10: 53.
- 522 [39] Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the
523 diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol 2007;45: 2761-2764.
- 524 [40] Salmassi TM, Venkateswaran K, Satomi M, Neilson K, Newman DK, Hering JG.
525 Oxidation of arsenite by *Agrobacterium albertimagni* AOL15, sp. Nov., isolated from hot
526 creek California. Geomicrobiol J 2002;19:53-66.
- 527 [41] Santini JM, Sly LI, Schnagl RD, Macy JM. A new chemolithoautotrophic arsenite-
528 oxidizing bacterium isolated from a gold mine: phylogenetic, physiological, preliminary
529 biochemical studies. Appl Environ Microbiol 2000;66:92-97.
- 530 [42] Ghosh P, Rathinasabapathi B, Teplitski M, Ma LQ. Bacterial ability in AsIII oxidation
531 and AsV reduction: Relation to arsenic tolerance, P uptake, and siderophore production.
532 Chemosphere 2015: 138;995-1000.
- 533 [43] Kitchin KT, Wallace K. The role of protein binding of trivalent arsenicals in arsenic
534 carcinogenesis and toxicity. J Inorg Biochem 2008;102:532-539.
- 535 [44] Achour AR, Bauda P, Billard P Diversity of arsenite transporter genes from arsenic-
536 resistant soil bacteria. Res Microbiol 2007;158: 128-137.
- 537 [45] Achour-Rokbani A, Cordi A, Poupin P, Bauda P, Billar P. Characterization of the *ars*
538 gene cluster from extremely arsenic-resistant *Microbacterium* sp. strain A33. Appl Environ
539 Microbiol 2010;76:948-955.

540 [46] Das S, Jean JS, Kar S, Chou ML, Chen CY. Screening of plant growth-promoting traits
541 in arsenic-resistant bacteria isolated from agricultural soil and their potential implication for
542 arsenic bioremediation. J Haz Mat 2014;272:112-120.

543 [47] Bahar MM, Megharaj M, Naidu R. Kinetics of arsenite oxidation by *Variovorax* sp. MM-
544 1 isolated from a soil and identification of arsenite oxidase gene. Journal of Hazardous
545 Materials 2013;262:997-1003.

546

547

548 **Legends to figures**

549

550 Figure 1. Expression of *aioA* gene represented as the *aioA* mRNA/DNA ratio during growth
551 of *T. arsenivorans* (A) and *H. arsenicoxydans* (B) in the presence of 75 mg.L^{-1} AsIII. ;
552 Evolution of cells concentrations of *T. arsenivorans* (C) and *H. arsenicoxydans* (D) during
553 this experiment. Evolution of AsV concentration with *T. arsenivorans* (E) and *H.*
554 *arsenicoxydans* (F) during this experiment, and in sterile culture media incubated in the same
555 condition (G). Significance of differences between modalities 0.2 and 1 g.L^{-1} yeast extract
556 was evaluated using an Anova parametric test (details in material and methods section).

557

558

559 Figure 2. Kinetics of AsIII oxidation in minimum medium containing 2 mg/L AsIII and no
560 organic substrate. Cells were previously grown in presence of 0.05 , 0.2 or 1 g/L of organic
561 substrate. A: “*T. arsenivorans*” on succinate; B: “*T. arsenivorans*” on yeast extract; C: *H.*
562 *arsenicoxydans* on acetate; D: *H. arsenicoxydans* on yeast extract. Error bars represent the
563 standard error of the mean (triplicates).

564

565
566 Figure 3. Specific AsIII-oxidation rates of kinetics of AsIII oxidation in minimum medium
567 containing 2 mg/L AsIII and no organic substrate; A: *T. arsenivorans* and *H. arsenicoxydans*
568 grown in presence of yeast extract; B: *T. arsenivorans* grown in presence of succinate.
569 Significance of differences between modalities was evaluated using an Anova parametric test
570 (details in material and methods section).

571
572 Figure 4. Kinetics of AsIII oxidation in minimum medium containing 2 mg/L AsIII and 0.2
573 g/L of organic substrate. Cells were previously grown in presence of 0.05, 0.2 or 1 g/L of
574 organic substrate. A: “*T. arsenivorans*” on succinate; B: “*T. arsenivorans*” on yeast extract;
575 C: *H. arsenicoxydans* on acetate; D: *H. arsenicoxydans* on yeast extract. Error bars represent
576 the standard error of the mean (triplicates).

577

578

Table 1. Influence of the presence of 0.2 g.L⁻¹ organic substrate on specific AsIII oxidation rate during the kinetics: variation of AsIII oxidation rates between 0 and 0.2 g.L⁻¹ organic substrates conditions, with pre-cultures grown under different conditions. Significance of variation was tested by ANOVA parametric test.

Strain	Pre-culture conditions	Variation (%) of AsIII oxidation rates between 0 and 0.2 g/L organic substrates during the kinetics
<i>T. arsenivorans</i>	0.05 g/L yeast extract	-13.6
<i>T. arsenivorans</i>	0.2 g/L yeast extract	-39.1
<i>T. arsenivorans</i>	1 g/L yeast extract	-17.7
<i>T. arsenivorans</i>	0.05 g/L succinate	+7.3
<i>T. arsenivorans</i>	0.2 g/L succinate	+3.0 (NS)
<i>T. arsenivorans</i>	1 g/L succinate	+26.3 (NS)
<i>H. arsenicoxydans</i>	0.05 g/L yeast extract	-25.9
<i>H. arsenicoxydans</i>	0.2 g/L yeast extract	-17.9
<i>H. arsenicoxydans</i>	1 g/L yeast extract	-10
<i>H. arsenicoxydans</i>	0.05 g/L acetate	-11.5 (NS)
<i>H. arsenicoxydans</i>	0.2 g/L acetate	NI
<i>H. arsenicoxydans</i>	1 g/L acetate	+106.1

NI: not identified (not observable AsIII oxidation)

NS: not significant

Negative effect	Organic substrate (0.2 g/L) within the kinetics medium has a negative effect on AsIII oxidation rate
Positive effect	Organic substrate (0.2 g/L) within the kinetics medium has a positive effect on AsIII oxidation rate

Table 2. Comparison of the specific oxidation rates obtained in the present study with rates reported in the literature

Bacterial strains	Specific rate ($\mu\text{g/L/min per cell}$)	References
<i>Comamonas</i> sp. ASR11	3.6×10^{-11}	[46]
<i>Pseudomonas</i> sp. ASR1	7.35×10^{-11}	[46]
<i>Variovorax</i> sp. 34	V max 8.85×10^{-11}	[38]
<i>H. arsenicoxydans</i>	0.5 to 9.5×10^{-10}	Present study
<i>Agrobacterium albertimagni</i> AOL15	V max 1.36×10^{-10}	[39]
<i>T. arsenivorans</i>	1 to 3×10^{-9}	Present study
<i>Bordetella</i> sp. SPB-24	V max 1.46×10^{-6}	[22]
<i>Variovorax</i> sp. MM-1	V max 9.23×10^{-6}	[47]







