



HAL
open science

Continuous production of a biogenic ferric iron lixiviant for the bioleaching of printed circuit boards (PCBs)

Agathe Hubau, Michel Minier, Alexandre Chagnes, Catherine Joulian, Cédric Perez, Anne-Gwenaelle Guezennec

► To cite this version:

Agathe Hubau, Michel Minier, Alexandre Chagnes, Catherine Joulian, Cédric Perez, et al.. Continuous production of a biogenic ferric iron lixiviant for the bioleaching of printed circuit boards (PCBs). Hydrometallurgy, 2018, 180, pp.180-191. 10.1016/j.hydromet.2018.07.001 . hal-02182968

HAL Id: hal-02182968

<https://hal.univ-lorraine.fr/hal-02182968>

Submitted on 22 Aug 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

1 **Continuous production of a biogenic ferric iron lixiviant for the**
2 **bioleaching of printed circuit boards (PCB)**

3 Agathe HUBAU^{1,2,*}, Michel MINIER², Alexandre CHAGNES³, Catherine JOULIAN¹,
4 Cédric PEREZ¹, Anne-Gwenaëlle GUEZENNEC¹

5 ¹BRGM, F-45060 Orléans, France

6 ²Chimie ParisTech, PSL Research University, CNRS, Institut de Recherche de Chimie
7 Paris (IRCP), F-75005 Paris, France

8 ³ GéoRessources - UMR CNRS 7359-CREGU-Université de Lorraine, 2 Rue du Doyen
9 Roubault 54500, BP 10162, Vandœuvre-lès-Nancy Cedex, France

10
11 * Corresponding author E-mail address: a.hubau@brgm.fr

12
13 **Abstract**

14 Ferric iron is a low-cost oxidant frequently used in hydrometallurgy and is particularly
15 suitable to leach various metals from printed circuit boards (PCBs). This paper presents the use
16 of the BRGM-KCC acidophilic consortium to generate ferric iron solution in a bubble column
17 run in continuous mode. The influence of influent ferrous iron concentration, ranging from 1 to
18 9 g L⁻¹ on the bio-oxidation rate was studied in the presence of a solid support. The impacts of
19 the quantity of solid support, the hydraulic residence time (HRT), the culture medium and the
20 type of support were established. Stable performance was achieved over an extended period
21 with a Fe²⁺ oxidation rate of 1400 mg L⁻¹ h⁻¹. Cryogenic scanning electron microscopy was
22 used to observe the attachment of cells on the solid support in different operating conditions
23 and showed that the clogging of the solid support with jarosite precipitates influenced its
24 colonization by the micro-organisms and the stability of the bioprocess. The operating
25 conditions, and especially the influent ferrous iron concentration and nutritive medium
26 composition, also influenced the structure and the abundance of the microbial community.

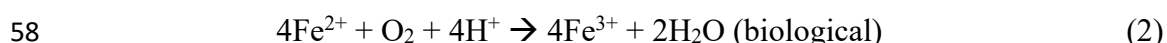
27
28 **Keywords:** Bioleaching / Bio-oxidation / printed circuit boards / acidophilic
29
30

31 1. Introduction

32 The metal content of spent printed circuit boards (PCBs) can reach up to 35% (in weight),
33 including precious and strategic metals even at greater concentrations than in primary resources.
34 For instance, gold and copper are 25 to 250 times and 20 to 40 times more concentrated in spent
35 PCBs than in ores, respectively (Tuncuk et al., 2012). Consequently, spent PCBs are becoming
36 a valuable resource. At the same time, a lack of an appropriate treatment could be a cause of
37 environmental pollution.

38 Today, high-grade PCBs are treated by pyrometallurgy to recover valuable metals but some
39 metals, such as aluminium, iron and precious metals, are lost in the slag during this operation
40 (Cui and Zhang, 2008) and the energy-cost of such processes is high. Moreover, the presence
41 of plastics raises two important issues: first, the emission of toxic dioxins and furans; and
42 second, the presence of plastics can bring about uncontrolled temperature increases leading to
43 smelter damages (Hagelüken, 2006). Currently, the addition of PCBs to smelter feed represents
44 at most 15% of the mass, the rest being metal concentrates. For example, PCBs make up 14%
45 of the total throughput in the Noranda process in Canada and 10% in the Umicore process in
46 Belgium (Cui and Zhang, 2008; Ghosh et al., 2015). Due to this limitation, the quantity of PCBs
47 that are treated annually remains low. Therefore, the design of energy-efficient and cost-
48 effective new processes for efficient metal recovery from PCBs is particularly important.
49 Techniques based on mechanical processes and hydrometallurgy are emerging as alternative
50 solutions.

51 Within this framework, biohydrometallurgy is very promising. In the literature, some studies
52 deal with the use of bioleaching for the treatment of spent PCBs by means of acidophilic micro-
53 organisms (Brandl and al., 2001; Ilyas and al., 2010). The use of such micro-organisms, which
54 are mainly iron and sulfur-oxidising, allows the extraction of various metals such as Cu, Ni and
55 Zn. Although the mechanisms are not completely known, the following main reactions may
56 occur:



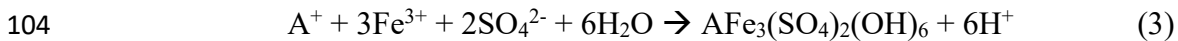
59 The main issue raised by research studies dedicated to the development of bioleaching
60 processes for PCBs treatment is linked to the reproducibility of the results, mainly because of
61 the large heterogeneity of PCBs that leads to large variation of sample composition. Most
62 studies dedicated to acidophilic bioleaching of spent PCBs to date have been performed in
63 simple shake flasks and the quantity of PCBs added for each batch test is often too low to be

64 representative of the whole composition of the PCB sample. Moreover, the results are highly
65 variable as important parameters, such as dissolved oxygen concentration, pH or micro-
66 organisms are diverse. Figure 1 illustrates the discrepancy in copper bioleaching yields reported
67 in the literature for spent PCBs in shake flask and reactor studies.

68 Finally, the addition point of raw material after inoculation influences greatly PCB bioleaching
69 efficiency. This parameter appears particularly important as PCBs may be responsible for the
70 inhibition of microbial growth and activity (Liang et al., 2013). Staggering the production of
71 the lixiviant and the addition of the e-waste in a two-step process can greatly increase leaching
72 rates (Yang et al., 2009; Guezennec et al., 2015). However, this requires the establishment of a
73 stable steady-state operation in the first stage where biomass production and ferrous iron bio-
74 oxidation occur (Reaction 2). The literature shows that a vast number of iron bio-oxidation
75 studies in continuous mode have been done to develop a large range of applications. Some
76 studies are summarised in Table 1. In this Table, the study duration is the time before authors
77 change any parameters once steady state is reached. Most of these works investigated multiple
78 parameters to avoid kinetic limitations and to achieve the highest bio-oxidation rate. The first
79 parameter is the characteristics of the influent, i.e. pH (Kaksonen et al., 2014a; Kinnunen and
80 Puhakka, 2005) and ferrous iron concentration (Gómez and Cantero, 2003) since these
81 parameters influence significantly the kinetics of bio-oxidation. The second parameter is the
82 composition of the gas flow to avoid any limitation in carbon dioxide (required for bacterial
83 growth, as it is the sole carbon source available) and/or oxygen (required for microbial
84 oxidation of ferrous iron, see Reaction 1). Bastías and Gentina (2010) improved their chemostat
85 performance by enriching air with 4% CO₂. Kinnunen and Puhakka (2004) detected an oxygen
86 limitation when using air and used a 99.5%O₂/0.5%CO₂ gas mixture to obtain a maximal
87 oxidation rate of 26.4 g L⁻¹ h⁻¹. This parameter is closely linked to the presence and the type of
88 solid support, which will impact the gas-liquid mass transfer. First studies were performed
89 without using solid support but performance was limited due to limited biomass and cell wash
90 out at low hydraulic residence times (HRT; Halfmeier et al., 1993a). Consequently, many solid
91 supports were developed and investigated (Grishin and Tuovinen, 1988), e.g. polystyrene
92 (Karamanev and Nikolov, 1986), quartz sand (Halfmeier et al., 1993b), Ni-alloy fibres (Gómez
93 and Cantero, 2003) and activated charcoal (Kinnunen and Puhakka, 2004). The goal was to
94 obtain large surface areas and high numbers of attached cells without slowing down mass
95 transfer. Activated charcoal was reported as one of the best solid supports in terms of bio-
96 oxidation performance due to its porosity which enables the retention quantities of large

97 biomass (Jensen and Webb, 1995). Grishin and Tuovinen (1988) reported a maximal oxidation
98 rate of 52.6 g L⁻¹ h⁻¹ in a bioreactor with 50 mL of activated charcoal.

99 However, due to the high concentrations of ferric iron, Ebrahimi et al. (2005) suggested that
100 the presence of jarosite precipitates in the bioreactor modifies the bio-oxidation rates and yields
101 over long periods of time and affects mass transfer near the solid support. The formation of
102 jarosite precipitates was extensively described (Kaksonen et al., 2014c); the following reaction
103 occurs:



105 Where A⁺ is a cation (potassium K⁺, sodium Na⁺, ammonium NH₄⁺, hydronium H₃O⁺).

106 During 6 months, Halfmeier et al. (1993) operated a fixed-bed reactor in continuous mode
107 and revealed transport limitation and long-term stability issues. Kinnunen and Puhakka (2004)
108 highlighted the influence of jarosite accumulation on oxidation performance in a fluidized-bed
109 reactor, with a majority of cells attached on jarosite, creating instabilities when removing these
110 precipitates. They revealed some difficulties to maintain constant jarosite formation rate, and
111 thus, to maintain stable performance (Kinnunen and Puhakka, 2005). Recently, Kaksonen et al.
112 (2014a, 2014c) investigated the use of these precipitates as solid support. Ebrahimi et al. (2005)
113 used this method to develop an airlift reactor that presented stable performance over long
114 periods of time to establish a process of H₂S removal. The use of a settler (Kaksonen et al.,
115 2014a, 2014c) to remove excess sludge maintained stable performance.

116 This paper reports the performance and the limitations obtained for the bio-oxidation of
117 ferrous iron in a bubble column run in continuous mode, which will be used as the first stage
118 of a biolixiviant reactor for leaching spent PCBs. The influence of influent ferrous iron
119 concentration on the bio-oxidation rate was studied over an extended period of time. The
120 influence of the type and quantity of solid support and of the medium composition on bio-
121 oxidation yield were investigated. The colonization of solid support was observed by cryogenic
122 scanning electron microscopy to understand the phenomena that affect the stability of bio-
123 oxidation performance. The main objective of the study was to determine the operating
124 parameters which enabled stable and maximal performance over a period of several weeks.

125 **2. Material and methods**

126 **2.1 Growth medium and bacterial culture.** The inoculum used for the cultures was
127 obtained from the BRGM-KCC microbial consortium subcultured on pyrite tailings
128 (Guezennec et al., 2017). The predominant micro-organisms in the culture are affiliated to the
129 acidophilic genera *Leptospirillum*, *Acidithiobacillus* and *Sulfobacillus*. Two culture media were

130 used: 0Km medium consisted of (g L^{-1}): 3.70 $(\text{NH}_4)_2\text{SO}_4$, 0.81 H_3PO_4 85%, 0.48 KOH, 0.52
131 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, while the culture medium 0Cm had the same composition but only 0.4 g L^{-1}
132 $(\text{NH}_4)_2\text{SO}_4$. Ferrous iron was added as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ to obtain ferrous iron concentrations
133 ranging from 1 to 9 g L^{-1} . 1Km and 9Km denote 0Km culture media containing 1 and 9 g L^{-1}
134 of ferrous iron, while 1Cm and 9Cm denote 0Cm culture media containing 1 and 9 g L^{-1} of
135 ferrous iron respectively. Growth media were adjusted to pH 1.2 using concentrated H_2SO_4 .

136 **2.2 Experimental setup.** Continuous culture experiments were carried out in two separate
137 150 mL (working volume) bubble columns (4.5 cm in diameter, 30 cm in height) immersed in
138 a water bath to maintain the temperature at $36 \text{ }^\circ\text{C}$. A schematic diagram of the bubble column
139 used for this study is given in Figure 2. The growth medium was fed continuously to the bottom
140 of the bubble column and the effluent removed at the desired dilution rate by means of variable-
141 speed pumps. The cell suspension was aerated with air flow between 20 and 30 L h^{-1} (2.2 to
142 3.3 vvm). Air was enriched with CO_2 to obtain a partial pressure of 1% CO_2 . Ferrous-iron
143 oxidation kinetics was investigated at various residence times, ranging from 1 to 18 h. To avoid
144 cell wash-out, solid support was introduced in the bubble column, with an apparent volume
145 varying from 33 to 100 mL. Solid supports were either activated charcoal (Organosorb11®
146 from Desotec) or polyvinyl chloride beads (Tefanyl® VR VRGR 366 from Mitsubishi
147 Chemical Performance Polymers). The latter were used as received while the charcoal was
148 washed with 5 mL g^{-1} of water and 1 mL g^{-1} of diluted sulfuric acid (pH 1.3), dried at 40°C for
149 20 hours and sieved to remove particles smaller than 1 mm prior to use. The bioreactor was
150 inoculated with the BRGM-KCC culture at 10% v/v. The bubble column was first operated in
151 batch mode in order to establish the biomass and then switched into continuous mode when the
152 redox potential reached 900 mV vs. SHE. All experiments were performed in non-sterile
153 conditions, to suit more accurately the industrial reality.

154 Finally, biotic and abiotic shake flasks experiments were done to determine the chemical
155 oxidation of ferrous iron by the activated charcoal itself. Flasks (250 mL) contained 100 mL
156 9Cm medium with 10 g of activated charcoal. Biotic tests were inoculated with 10% (v/v)
157 BRGM-KCC. Abiotic shake flasks were autoclaved with activated charcoal, 9Cm medium was
158 filter sterilized ($0.2 \text{ }\mu\text{m}$ syringe filter from ClearLine in cellulose acetate) and manipulations
159 were performed under a laminar air flow hood.

160 **2.3 Analytical techniques.** Oxygen and carbon dioxide concentrations in the off-gas from
161 the bioreactor were analysed by using ADC MGA 3000 Multi-Gas Analyzer (Analytical
162 Development Company Ltd). Total iron concentration was determined by flame atomic
163 absorption spectroscopy (AAS) using a Varian SpectrAA-300. Ferrous iron concentration was

164 determined by using the correlation between redox potential and total iron concentration as
165 reported by Yue et al. (Yue et al., 2014). Estimated values of ferrous iron concentration deduced
166 from this correlation were occasionally confirmed experimentally by performing cerium (IV)
167 titration with a Metrohm titrode (Pt-pH) in sulfuric acid medium (Yue et al., 2016).

168 **2.4 Bacterial community monitoring.**

169 *2.4.1 Cell counting.* The number of micro-organisms in the supernatant was monitored by
170 counting on Thoma cell counting chamber. The same methodology was used to count attached
171 cells on the solid support, after cell detachment performed by slowly shaking with a mixing
172 shaker (Heidolph Reax 2) at minimum speed (24 rpm) 500 mg of solid support in 1.5 mL of
173 fresh acidic medium (OKm with pH <2) with 0.25% v/v Tween 20 (from Sigma Aldrich), for 1
174 hour.

175 *2.4.2 Cryogenic scanning electron microscopy (Cryo-SEM).* This method was used to
176 observe the attachment of cells onto the solid support without prior steps of preparation. Fresh
177 aliquots of solids sampled from the bubble column were introduced on a copper frame with
178 carbon paste. Samples were immersed into liquid nitrogen at -176 °C and a pressure depression
179 enabled to reach -210 °C (slush nitrogen). From this point, samples were set under vacuum and
180 transferred into the SEM (Hitachi S-4500 field-emission operated at 1 kV). They were warmed
181 to -70 °C to remove ice that was formed on the sample due to moisture condensation and cooled
182 to -105 °C for observations. Detection of secondary electrons was performed on the surface or
183 inside samples after cryo-fracturing. Energy dispersive X-ray spectroscopy (EDX) was used to
184 characterize the sample composition with SEM operated at 5 or 15 kV.

185 *2.4.3 DNA extraction and capillary electrophoresis-single strand conformational*
186 *polymorphism (CE-SSCP) monitoring of the bacterial community structure.* The CE-SSCP
187 technique was used on polymerase chain reaction (PCR) product of 16S rRNA gene to obtain
188 a diversity profile and to determine relative abundances of the detected bacterial strains.
189 Samples containing 0.5 mL solid support or 2 mL liquid phase were taken from the bubble
190 column. Solid samples and pellets (from centrifugation of the liquid samples for 10 minutes at
191 14000 g) were washed by re-suspension in Tris buffer (100 mM, pH 8) until pH reached around
192 7. Genomic DNA was extracted with the FastDNA Spin Kit for Soil and using the
193 manufacturer's protocol (MP Biomedicals) with a FastPrep®-24 at a speed of 5 m s⁻¹ for 30 s.
194 Amplification of the 16S rRNA gene from DNA extracts was performed with C-1000
195 thermocycler (Biorad) with the w49 forward primer and the 5'-end FAM-labeled w34 primer,
196 as in Hedrich et al. (2016). For CE-SSCP analysis, one microliter of 5- to 500-fold diluted PCR
197 product, 0.4 µL of Genescan-600 LIZ internal standard (Applied Biosystems) and 18.6 µL of

208 deionized formamide HiDi (Applied Biosystems) were heated for 5 minutes at 95 °C for
209 denaturation and immediately cooled on ice for 10 minutes. Fragment analyses were conducted
210 with the ABI Prism 310 (Applied Biosystems). The software GeneScan (Applied Biosystems)
211 was used to realign the peak profiles based on internal standard migration, assign peak position
212 and calculate relative abundances on the basis of peak areas.

203 3. Results

204 3.1 The influence of ferrous iron concentration on bio-oxidation

205 Activated charcoal produced from black granular coal was chosen as support for this work
206 because high bio-oxidation rates can be achieved with this type of support (Jensen and Webb,
207 1995), especially due to its high specific area of 950 m² g⁻¹. It should be noted that the rate of
208 the chemical oxidation of ferrous iron into ferric iron in the presence of activated charcoal is
209 very low, as demonstrated by the studies of Kuznetsova et al. (1995) and Ahumada et al. (2002)
210 with severe conditions (high temperature and O₂ pressure). In our conditions, measures in sterile
211 shake flasks in 9Cm medium gave a chemical oxidation rate reaching 65 mg L⁻¹ h⁻¹ with clean
212 activated charcoal. This rate was much lower than the one in biotic shake flasks, with bio-
213 oxidation rates easily reaching 240 mg L⁻¹ h⁻¹. It confirms that this material is mainly acting as
214 a solid support for bacteria, and its ability to catalyse ferrous iron oxidation is negligible
215 compared to bio-oxidation.

216 In order to examine the kinetics of bio-oxidation at different influent ferrous iron
217 concentrations, 15 g of activated charcoal were introduced in the bioreactor and the HRT was
218 set to 12 h. This mass of activated charcoal corresponded to an apparent volume of 33 mL.
219 Table 2 shows the bio-oxidation yield and rate obtained in these conditions with ferrous iron
220 concentration in the influent medium ranging from 1 to 9 g L⁻¹.

221 The maximum bio-oxidation rate obtained was 375 mg L⁻¹ h⁻¹ with an influent concentration
222 of 4.5 g L⁻¹ of ferrous iron. This maximal oxidation rate was very low compared to those
223 reported in the literature. At higher influent ferrous iron concentrations, both the bio-oxidation
224 yield and rate were lower. Moreover, substantial variations in these rates were observed with
225 the same operating conditions.

226 Bio-oxidation performance as a function of HRT is presented for bioreactors with 1 and 9 g
227 L⁻¹ influent ferrous iron in Figure 3. For fixed operating conditions, performance were not stable
228 even at 10 HRT. Consequently, each dot of the figure, which represents the bio-oxidation yield
229 or rate for one fixed HRT, depicts the most common value obtained in these fixed operating
230 conditions. Error bars depict the range of all other obtained values in such conditions. Figure 3

231 shows large discrepancies in bio-oxidation yield and rate when 9 g L⁻¹ ferrous iron was used
232 whatever the HRT. This was mainly due to precipitate formation on the solid support (see
233 below). At 1 g L⁻¹ ferrous iron, between 80% and 100% bio-oxidation yield was obtained for
234 high HRT. However, when the HRT decreased below 4 hours, iron bio-oxidation yield
235 decreased sharply. A maximum oxidation rate of 450 mg L⁻¹ h⁻¹ was obtained at 1 g L⁻¹ ferrous
236 iron and HRT of 2 hours.

237

238 **3.2 The influence of the quantity of activated charcoal on bio-oxidation**

239 In order to minimize the instabilities obtained at high influent ferrous iron concentrations,
240 the impact of the quantity of solid support was investigated. The amount of activated charcoal
241 influences the amount of biomass attached onto and into the solid, and therefore, the
242 performance of the bioreactor. Table 3 shows the bio-oxidation rate for both influent ferrous
243 iron concentrations (1 g L⁻¹ and 9 g L⁻¹ Fe²⁺) at 6 h HRT.

244 The quantity of activated charcoal at 1 g L⁻¹ did not seem to influence iron oxidation since
245 100% bio-oxidation was obtained in all cases at 6 h HRT. Higher oxidation rates were observed
246 at influent ferrous concentration of 9 g L⁻¹ when larger quantities of activated charcoal were
247 used; however, the performance was still not stable. It is interesting to highlight that, at fixed
248 HRT, the addition of activated charcoal into the bioreactor was responsible for an instantaneous
249 increase in bio-oxidation yield and rate followed by a gradual decrease (data not shown). Figure
250 4 shows lower instabilities when larger amounts of activated charcoal were used at 9 g L⁻¹. The
251 iron bio-oxidation yield gradually decreased with decreasing HRT and oxidation rate varied
252 between 400 and 700 mg L⁻¹ h⁻¹.

253

254 **3.3 The influence of the growth media composition on bio-oxidation**

255 Silverman and Lundgren (1959) detailed the composition of a growth medium (9K) adapted
256 to the growth of acidophilic microorganisms, e.g. *Acidithiobacillus ferrooxidans*. Collinet-Latil
257 (1989) modified this medium to obtain optimal bioleaching performance with the BRGM-KCC
258 consortium. In the present study, this medium (0Km) was compared with the 0Cm medium,
259 which contains the same quantities of KOH, H₃PO₄ and MgSO₄·7H₂O and ten times less
260 (NH₄)₂SO₄ in the presence of 1 g L⁻¹ or 9 g L⁻¹ ferrous iron and 15 g or 30 g of activated
261 charcoal, respectively (Figures 5 and 6).

262 Results obtained with both media were quite similar at 1 g L⁻¹ ferrous iron but better
263 performance were obtained with the 1Cm medium at low residence time (1.1 hour): the
264 maximal oxidation rate increased from 450 to 900 mg L⁻¹ h⁻¹ when nutritive medium was

265 changed from 1Km to 1Cm. Concerning the bioreactor with influent ferrous iron concentration
266 of 9 g L⁻¹, reducing the quantity of ammonium ions allowed a more stable performance at high
267 HRT, and enabled higher iron oxidation yields. The lower ammonium concentrations may have
268 led to a reduction in jarosite precipitation, as explained below. In 9Cm medium, maximal
269 oxidation rate reached 1400 mg L⁻¹ h⁻¹ at 6.3 hours HRT. In these conditions, bio-oxidation
270 performance was within the range of the results presented in the literature (Table 1).

271

272 **3.4 The influence of the solid support on bio-oxidation**

273 The bio-oxidation rate in the presence of a highly porous support (activated charcoal) and a
274 non-porous support (polyvynil chloride beads) is shown in Figures 7 and 8 with an influent
275 ferrous iron concentration of 1 g L⁻¹ and 33 mL of solid support or 9 g L⁻¹ and 66 mL of solid
276 support, respectively. The same apparent volume was used to compare both supports and both
277 bioreactors were first operated in batch mode during 45 h to favour the attachment of the
278 biomass. At 1 g L⁻¹ ferrous iron, similar performance was obtained with both supports at high
279 HRT (HRT > 2 h). However, the bio-oxidation rate was obviously affected by the type of solid
280 support at low HRT (1h): it sharply decreased from 900 mg L⁻¹ h⁻¹ to 500 mg L⁻¹ h⁻¹ with the
281 use of PVC beads. The performance notably decreased at 9 g L⁻¹ with PVC as less than 20% of
282 influent ferrous iron was oxidized under such conditions whatever the residence time. The lack
283 of porosity of PVC support was probably not favourable to the development of attached biomass
284 in the bioreactor, since available surface area for cells attachment was low.

285

286 **3.5 The characteristics of solid support and bacterial community structure during bio-oxidation**

287
288 *3.5.1 The influence of operating conditions on the solid support.* Cryo-SEM was used to
289 observe the colonization of activated charcoal particles by bacteria. Such a technique is
290 particularly suitable for this study since it allows the observation of fresh biological materials
291 as no dehydration step is required during sample preparation and it is possible to obtain high-
292 resolution images at low voltage. This method also enables the breaking of the charcoal
293 particles into sub-particles without damaging the biofilm in order to observe the interior of
294 porous material: an example of ore breakage was described by Kelly and Burgio (1983).

295 Figures 9-11 show Cryo-SEM pictures inside and outside charcoal particles in biotic
296 conditions in the presence of 1 and 9 g L⁻¹ ferrous iron and in abiotic conditions in the presence
297 of 9 g L⁻¹ ferric iron in 0Km culture medium. Figure 9A shows the presence of many bacteria
298 inside activated charcoal particles in the 1Km media. Cells seemed to be attached on the surface

299 into the pores of the solid support. An organic substance was observed around these cells by
300 Cryo-SEM but its fast degradation by the electron beam prevented the acquisition of any
301 pictures. It was supposed that this substance was composed of extracellular polymeric
302 substances. Only very few cells were observed in the presence of 9 g L⁻¹ ferrous iron and none
303 are visible on Figure 9B. Figures 9B and 9C show the presence of an organic framework but
304 this framework seemed different from the one observed in Figure 9A since its resistance against
305 electron beam was greater. This organic framework is likely composed of bitumen substances
306 given that charcoal is a bituminous compound.

307 Figure 10 shows the presence of precipitates onto the particle surface in all samples. These
308 precipitates formed a layer on the solid support, which seemed to be more spread in samples
309 colonized under biotic conditions in the presence of 9 g L⁻¹ ferrous iron (Figure 10B) and in
310 Erlenmeyer under abiotic conditions in the presence of 9 g L⁻¹ ferric iron (Figure 10C) when
311 compared to 1Km medium (Figure 10A). It is interesting to highlight that the activated charcoal
312 surface seemed to be entirely coated by precipitates in the presence of 9 g L⁻¹ iron (Figures 10B
313 and 10C) while less precipitate covered the charcoal surface in the presence of 1 g L⁻¹ iron
314 (Figure 10A), leading to the formation of small pores.

315 The edge of a representative charcoal particle after colonization in a growth medium
316 containing 9 g L⁻¹ ferrous iron is shown in Figure 11. Pseudocubic structures, which are
317 attributed to ammonium jarosite precipitation (Kaksonen et al., 2014), were observed in the
318 particle pores. It can be inferred that the precipitate was actually a mixture of ammonium
319 jarosite (pseudocubic structure) and potassium jarosite since EDX analyses demonstrated the
320 presence of potassium and sulfate (Figure 12).

321
322 *3.5.2 Bacterial community structure.* Microbial diversity profiles were determined in
323 bioreactors by 16S rRNA gene CE-SSCP. A comparison was performed between solid and
324 liquid phases in bioreactors for two different influent ferrous iron concentrations (1 g L⁻¹ with
325 15 g of activated charcoal and 9 g L⁻¹ with 30 g of activated charcoal) for HRT ranging from 1
326 to 10 hours (Figure 13). Results implied that conditions in the bioreactor were unsuitable for
327 the growth of *Acidithiobacillus caldus*, as it remained undetected despite this bacterium being
328 a member of the BRGM-KCC consortium initially used to inoculate the bioreactor. This can be
329 explained by the absence of reduced sulfur compounds in the bioreactor since *Acidithiobacillus*
330 *caldus* is a sulfur-oxidizing bacterium. Likewise, *Sulfobacillus thermosulfidooxidans* was not
331 detected in any samples. The temperature maintained at 36 °C in the bioreactor was probably
332 too low since the optimal growth temperature is 51 °C for this bacterium (Watling et al., 2008).

333 Conversely, *Leptospirillum ferriphilum* and *Sulfobacillus benefaciens*, which are present in the
334 BRGM-KCC consortium, were present in the solid and the liquid phases in the bioreactor but
335 their relative abundance varied when the operating conditions changed. Especially, influent
336 ferrous iron concentration seemed to have an influence on the bacterial growth since 9 g L⁻¹
337 ferrous iron favoured the growth of *Sulfobacillus benefaciens*, with an average proportion of
338 60%, while 1 g L⁻¹ favoured *Leptospirillum ferriphilum* growth, with an average proportion of
339 80%. This may be explained due to the higher affinity for the ferrous iron substrate and greater
340 tolerance of ferric iron of *L. ferriphilum* over *Sulfobacillus* spp. (Norris, 2007). The proportions
341 of these two organisms were similar in liquid and solid phases. Two unknown micro-organisms
342 were detected in the bioreactor at 9 g L⁻¹ ferrous iron for 3.5 and 5.1 hours. One of these was
343 also detected in the other bioreactor at 1 g L⁻¹ ferrous iron.

344 Finally, cell distribution between solid and liquid phases was determined in the same
345 conditions as the previous characterization of diversity profiles. Figure 14 gives the distribution
346 for both bioreactors. When influent ferrous iron concentration was 1 g L⁻¹, the number of cells
347 remained stable for HRT varying between 1 and 3 hours; the cells were also well distributed
348 between the solid and the liquid phases. For the same iron concentration and HRT of 6.4 hours,
349 the number of cells increased noticeably (at least by 0.5 Log) and a large proportion of cells
350 were attached onto activated charcoal. In the presence of 9 g L⁻¹ ferrous iron in the influent, the
351 increase in cell number at high HRT was less than on 1 g L⁻¹ ferrous iron and the cells were
352 more or less equally distributed between the liquid and the solid phases. For HRT of 6 hours,
353 the total cell number was much lower compared to the results obtained at 1 g L⁻¹ Fe²⁺ whereas
354 the cell number was of the same order of magnitude for lower HRT in both conditions. It must
355 be noted that these data are only an estimate of the number of cells in the bioreactor given the
356 low accuracy for cell counting (inaccuracy arose from the difficulty to collect all cells from
357 activated charcoal and from counts on Thoma cell chamber).

358 **4. Discussion**

359 Observations with Cryo-SEM highlighted the fact that activated charcoal from the bioreactor
360 containing 9 g L⁻¹ ferrous iron was less colonized than in the presence of 1 g L⁻¹ ferrous iron.
361 Furthermore, Cryo-SEM demonstrated that activated charcoal particles from 9Km bioreactor
362 were entirely coated with precipitates, while particles from 1Km bubble column were only
363 partially clogged. Consequently, one hypothesis is that instabilities in 9Km bioreactor
364 performance resulted from the presence of precipitates, which coat the charcoal surface and
365 cause difficulties for the bacterial attachment. For unchanged operating conditions (fixed HRT),

366 this clogging phenomenon seemed to occur according to the following cycle: (i) High bio-
367 oxidation yield and rate were observed since ferrous iron was intensively oxidized to ferric iron
368 by the cells, which initially colonized the pores of activated charcoal; (ii) Activity of the bacteria
369 decreased because of limitation of the diffusion of nutrients, carbon dioxide, oxygen and ferrous
370 iron resulting from the accumulation of potassium jarosite and ammonium jarosite that coated
371 the external charcoal surface (cf Reaction 3); (iii) Attrition between the particles was
372 responsible for coating destruction, which led to an increase in bacterial activity, and therefore,
373 an increase in bio-oxidation yield and rate until new jarosite precipitation phenomena occurred.
374 The age of solid support greatly affected the performance. It was thus difficult to determine
375 accurately the cell distribution between the liquid and the solid phases since the clogging
376 phenomenon and the high porosity of activated charcoal limited the cell collecting procedure.

377 This hypothesis could explain the difference in diversity profiles between both bioreactors:
378 *Sulfobacillus benefaciens* was able to take advantage of different energy and carbon sources, as
379 it is a mixotrophic species (Johnson and al., 2008). If carbon dioxide and ferrous iron were not
380 available due to the coating of the solid support, this species could use cellular waste to survive,
381 at the expense of *Leptospirillum ferriphilum*, which is a strict autotroph (Mi and al., 2011).

382 It is consistent with results obtained when the growth medium composition was changed:
383 the decrease in ammonium concentration limited ammonium jarosite precipitation, thus limiting
384 the coating of solid support. It enabled more stable performance. In these conditions, maximal
385 oxidation rates were obtained at $1400 \text{ mg L}^{-1} \text{ h}^{-1}$ in the presence of 9 g L^{-1} ferrous iron in the
386 influent. In the literature, it is reported that the stability of jarosite depends on the standard
387 enthalpy of formation. Thus, potassium jarosite is more stable than sodium and hydronium
388 jarosite, which are more stable than ammonium jarosite (Gaborreau and Vieillard, 2004; Gramp
389 et al., 2008; Kaksonen et al., 2014b). Modifying the potassium concentration in the medium
390 could improve bacteria attachment onto the pores surface of the charcoal by avoiding potassium
391 jarosite precipitation.

392 The nature of the support seemed to have a high impact on bio-oxidation performance since
393 the use of non-porous polyvinyl chloride beads reduced the iron bio-oxidation yield to less than
394 20% while it reached 99% in the same conditions with activated charcoal. This was probably
395 due to a difference in porosity, which influenced the number of accessible sites for cell
396 attachment: with 33 mL, the accessible surface with activated charcoal is estimated to $14\,520$
397 m^2 while it is estimated to 0.048 m^2 with PVC beads. The zeta potential of solid supports may
398 also play a role in the attachment of acidophilic cultures, as reported by Halfmeier et al. (1993b)
399 : it is assumed that the adsorption of cells onto solid supports due to electrostatic and Van der

400 Walls forces is the first step of the creation of biofilms. Acidophilic cultures, positively charged
401 with protonation, will better adsorb to negatively charged surfaces.

402 Concerning these results, one way to improve the kinetics could be to change the solid
403 support. A study with woven nylon performed by Svirko et al. (2009) give good results in terms
404 of ferrous iron bio-oxidation rates, with $4 \text{ g L}^{-1} \text{ h}^{-1}$. This might be explained by the creation of
405 a framework that enables the biomass to get attached without affecting mass transfer.

406 The highest oxidation rate obtained in 9Cm was $1400 \text{ mg L}^{-1} \text{ h}^{-1}$ at HRT of 6.3 hours.
407 Concerning the 1Cm medium, the highest oxidation rate obtained was $900 \text{ mg L}^{-1} \text{ h}^{-1}$ at a
408 minimum HRT of 1.1 h. To go further in the design of the bubble column, it is therefore
409 mandatory to take into account the second stage of the bioreactor. If the bubble column must
410 regenerate high concentrations of ferric iron due to the inhibition of bacterial activity in the
411 second stage, it is necessary to test higher influent ferrous iron concentrations and minimize
412 ferric iron precipitation. If the bubble column aims at the development of a fresh culture with
413 planktonic cells, the use of activated charcoal is not recommended as cells are strongly attached
414 onto the pores surface of solid support particles.

415 **5. Conclusion**

416 To recover metals from different resources, particularly spent printed circuit boards, the
417 production of a biogenic lixiviant solution is of great interest. However, the monitoring of the
418 process should be controlled and the stability of this production over long periods of time is
419 necessary. In this work, a bubble column inoculated with BRGM-KCC microbial consortium
420 in the presence of activated charcoal was used for ferric lixiviant generation. Results were not
421 reproducible at 9 g L^{-1} in 0Km medium and were much lower than literature oxidation rates.
422 The use of higher quantities of activated charcoal did not improve the rates but the stability
423 improved. The use of polyvinyl chloride beads as solid support decreased the oxidation rates.
424 However, with a modified medium containing a ten-fold lower ammonia concentration,
425 maximal bio-oxidation rates were obtained with activated charcoal with long-term stability:
426 $1400 \text{ mg L}^{-1} \text{ h}^{-1}$ with 9 g L^{-1} ferrous iron at HRT of 6.3 h and $900 \text{ mg L}^{-1} \text{ h}^{-1}$ with 1 g L^{-1} ferrous
427 iron at the minimal HRT experimented, 1.1 h. These results were satisfactory since they were
428 in the range of the kinetics found in the literature and they were maintained during extended
429 periods of time. Cryo-SEM observations of activated charcoal particles demonstrated the
430 presence of precipitates on the external surface of activated charcoal (ammoniojarosite and
431 potassiojarosite). The clogging of charcoal pores was likely to explain the decrease of the
432 bubble column performance, at 9 g L^{-1} particularly, by trapping cells into solid support.

433 Consequently, according to the age of the support, mass transfer (Fe^{2+} , nutrients, O_2 and CO_2)
434 from the liquid phase to the bacteria was reduced and oxidation kinetics were lower than
435 expected. The microbial diversity profiles in the bioreactor were different in presence of 1 or 9
436 g L^{-1} of ferrous iron : mixotrophic cells were in higher proportions in 9 g L^{-1} bubble column, as
437 mass transfer were limited due to the clogging whereas autotrophic cells (particularly
438 *Leptospirillum ferriphilum*) were in higher proportions in 1 g L^{-1} bubble column. With this
439 knowledge, operating conditions of such a bioreactor can be adapted to be adequate to the
440 subsequent step of the process, i.e. the leaching of PCBs.

441 6. Acknowledgements

442 This work was supported by the Chair “Mines Urbaines” from ParisTech foundation supported
443 by Eco-systèmes. Authors would like to thank Christopher G. Bryan for his careful reading of
444 the paper.

445 7. References

446 Ahumada, E., Lizama, H., Orellana, F., Suárez, C., Huidobro, A., Sepúlveda-Escribano, A.,
447 Rodríguez-Reinoso, F., 2002. Catalytic oxidation of Fe(II) by activated carbon in the presence
448 of oxygen. Effect of the surface oxidation degree on the catalytic activity, Carbon, 40, 2827-
449 2834.

450 Arshadi, M., Mousavi, S.M., 2014. Simultaneous recovery of Ni and Cu from computer
451 printed circuit boards using bioleaching: Statistical evaluation and optimization, Bioresource
452 Technology, 174, 233–242.

453 Arshadi, M., Mousavi, S.M., 2015. Multi-objective optimization of heavy metals
454 bioleaching from discarded mobile phone PCBs: Simultaneous Cu and Ni recovery using
455 *Acidithiobacillus ferrooxidans*, Separation and Purification Technology, 147, 210–219.

456 Bai, J., Gu, W., Dai, J., Zhang, C., Yuan, W., Deng, M., Luo, X., Wang, J., 2016. The
457 catalytic role of nitrogen-doped carbon nanotubes in bioleaching copper from waste printed
458 circuit boards, Pol. J. Environ. Stud., 25 (3), 951-957. Bas, A.D., Deveci, H., Yazici, E.Y., 2013.
459 Bioleaching of copper from low grade scrap TV circuit boards using mesophilic bacteria,
460 Hydrometallurgy, 138, 65–70.

461 Bastías, M., Gentina, J. C. , 2010. Variables affecting the growth and ferrous oxidation
462 capacity of *L. Ferrooxidans* in continuous culture. Hydrometallurgy 104, 351–355.

463 Brandl, H., Bosshard, R., Wegmann, M., 2001. Computer-munching microbes: metal
464 leaching from electronic scrap by bacteria and fungi. *Hydrometallurgy* 59, 319–326.

465 Bryan, C.G., Watkin, E.L., McCredden, T.J., Wong, Z.R., Harrison, S.T.L., Kaksonen, A.H.,
466 2015. The use of pyrite as a source of lixiviant in the bioleaching of electronic waste.
467 *Hydrometallurgy*, 152, 33–43.

468 Chen, S., Yang, Y., Liu, C., Dong, F., Liu, B., 2015. Column bioleaching copper and its
469 kinetics of waste printed circuit boards (WPCBs) by *Acidithiobacillus ferrooxidans*.
470 *Chemosphere*, 141, 162-168.

471 Collinet-Latil, M.N., 1989. PhD thesis, Lixiviation bactérienne par *Thiobacillus*
472 *ferrooxidans* et *Thiobacillus thiooxidans* d'un concentré de flottation arsénopyriteux aurifère
473 (réfractaire à la cyanuration directe), University of cellular biology and microbiology Aix-
474 Marseille.

475 Cui, J., Zhang, L., 2008. Metallurgical recovery of metals from electronic waste: A review.
476 *Journal of Hazardous Materials*, 158, 228-256.

477 Ebrahimi, S., Fernandez Morales, F.J., Kleerebezem, R., Heijnen, J.J., Van Loosdrecht,
478 M.C.M., 2005. High-Rate Acidophilic Ferrous Iron Oxidation in a Biofilm Airlift Reactor and
479 the Role of the Carrier Material, *Biotechnology and Bioengineering*, 90(4), 462-472.

480 Gaboreau, S., Vieillard, P., 2004. Prediction of Gibbs free energies of formation of minerals
481 of the alunite supergroup, *Geochimica et Cosmochimica Acta*, 68(16), 3307–3316.

482 Ghosh, B., Ghosh, M.K., Parhi, P., Mukherjee, P.S., Mishra, B.K., 2015. Waste Printed
483 Circuit Boards recycling: an extensive assessment of current status, *Journal of Cleaner*
484 *Production*, 94, 5-19.

485 Gómez J.M., Cantero D., 2003. Kinetic study of biological ferrous sulphate oxidation by
486 iron-oxidising bacteria in continuous stirred tank and packed bed bioreactors, *Process*
487 *Biochemistry*, 38, 867-87.

488 Gramp, J.P., Jones, F.S., Bigham, J.M. & Tuovinen, O.H., 2008. Monovalent cation
489 concentrations determine the types of Fe(III) hydroxysulfate precipitates formed in bioleach
490 solutions, *Hydrometallurgy*, 94, 29–33.

491 Grishin, S.I., Tuovinen, O.H., 1988. Fast kinetics of Fe²⁺ oxidation in packed-bed reactors,
492 *Appl. Microbiol. Biotechnol.*, 54, 12, 3092-100.

493 Gu, W., Bai, J., Dai, J., Zhang, C., Yuan, W., Wang, J., Wang, P., Zhao, X., 2014.
494 Characterization of extreme acidophile bacteria (*Acidithiobacillus ferrooxidans*) bioleaching
495 copper from flexible PCB by ICP-AES, *Journal of Spectroscopy*, 2014, 269351.

496 Gu, W., Bai, J., Dong, B., Zhuang, X., Zhao, J., Zhang, C., Wang, J., Shih, K., 2017a.
497 Catalytic effect of graphene in bioleaching copper from waste printed circuit boards by
498 *Acidithiobacillus ferrooxidans*, *Hydrometallurgy*, 171, 172-178.

499 Gu, W., Bai, J., Dong, B., Zhuang, X., Zhao, J., Zhang, C., Wang, J., Shih, K., 2017b.
500 Enhanced bioleaching efficiency of copper from waste printed circuit board driven by nitrogen-
501 doped carbon nanotubes modified electrode, *Chemical Engineering Journal*, 324, 122-129.

502 Guay, R., Silver, M., Torma, A.E., 1977. Ferrous iron oxidation and uranium extraction by
503 *Thiobacillus ferrooxidans*, *Biotech. Bioeng.* 19, 727-740.

504 Guezenec, A.G., Bru, K., Jacob, J., d'Hugues, P., 2015. Co-processing of sulfidic mining
505 wastes and metal-rich post-consumer wastes by biohydrometallurgy, *Minerals Engineering*, 75,
506 45–53.

507 Guezenec, A.G., Joulain, C., Jacob, J., Archane, A., Ibarra, D., de Buyer, R., Bodénan, F.,
508 d'Hugues, P., 2017. Influence of dissolved oxygen on the bioleaching efficiency under oxygen
509 enriched atmosphere, *Miner. Eng.* 106, 64–70.

510 Halfmeier, H., Schafer-Treffenfeldt, W., Ressus, M., 1993a. Potential of *Thiobacillus*
511 *ferrooxidans* for waste gas purification: Part 1. Kinetics of continuous ferrous iron oxidation,
512 *Appl. Microbial. Biotechnol.* 40, 416-420.

513 Halfmeier, H., Schafer-Treffenfeldt, W. & Ressus, M., 1993b. Potential of *Thiobacillus*
514 *ferrooxidans* for waste gas purification: Part 2. Increase in continuous ferrous iron oxidation
515 kinetics using immobilized cells, *Appl. Microbial. Biotechnol.* 40, 582-587.

516 Hedrich, S., Guezenec, A.-G., Charron, M., Schippers, A., Joulain, C., 2016. Quantitative
517 Monitoring of Microbial Species during Bioleaching of a Copper Concentrate. *Frontiers in*
518 *Microbiology* 07, 2044.

519 Hong, Y., Valix, M., 2014. Bioleaching of electronic waste using acidophilic sulfur oxidising
520 bacteria, *Journal of Cleaner Production*, 65, 465–472.

521 Ilyas, S., Anwar, M.A., Niazi, S.B., Ghauri M.A., 2007. Bioleaching of metals from
522 electronic scrap by moderately thermophilic acidophilic bacteria. *Hydrometallurgy*, 88, 180–
523 188.

524 Ilyas, S., Lee, J.C., 2014. Bioleaching of metals from electronic scrap in a stirred tank
525 reactor, *Hydrometallurgy*, 149, 50–62.

526 Ilyas, S., Lee, J.C., Chi, R.A., 2013. Bioleaching of metals from electronic scrap and its
527 potential for commercial exploitation. *Hydrometallurgy*, 131-132, 138–143.

528 Ilyas, S., Ruan, C., Bhatti, H. N., Ghauri, M. A., Anwar, M. A., 2010. Column bioleaching
529 of metals from electronic scrap. *Hydrometallurgy* 101, 135–140.

530 Işildar, A., van de Vossenberg, J., Rene, E.R., van Hullebusch, E.D., Lens, P. N. I., 2015.
531 Two-step bioleaching of copper and gold from discarded printed circuit boards (PCB). *Waste*
532 *Management*, 57, 149-157.

533 Jensen, A.B., Webb, C., 1995. Ferrous sulphate oxidation using *Thiobacillus ferrooxidans*:
534 a review, *Process Biochemistry*, 30, 3, 225-236.

535 Johnson, D. B., Joulain, C., d'Hugues, P., Hallberg, K. B., 2008. *Sulfobacillus benefaciens*
536 sp. nov., an acidophilic facultative anaerobic Firmicute isolated from mineral bioleaching
537 operations. *Extremophiles* 12, 789–798.

538 Kaksonen, A.H., Morris, C., Rea, S., Li, J., Wylie, J., Usher, K.M., Ginige, M.P., Cheng,
539 K.Y., Hilario, F. & du Plessis, C.A., 2014a. Biohydrometallurgical iron oxidation and
540 precipitation: Part I — Effect of pH on process performance, *Hydrometallurgy*, 147-148, 255–
541 263.

542 Kaksonen, A.H., Morris, C., Rea, S., Li, J., Usher, K.M., McDonald, R.G., Hilario, F.,
543 Hosken, T., Jackson, M. & du Plessis, C.A., 2014b. Biohydrometallurgical iron oxidation and
544 precipitation: Part II — Jarosite precipitate characterization and acid recovery by conversion to
545 hematite, *Hydrometallurgy*, 147-148, 264–272.

546 Kaksonen, A.H., Morris, C., Hilario, F., Rea, S. M., Li, J., Usher, K.M., Wylie, J., Ginige,
547 M.P., Cheng, K.Y. & du Plessis, C., 2014c. Iron oxidation and jarosite precipitation in a two-
548 stage airlift bioreactor, *Hydrometallurgy*, 150, 227–235.

549 Karamanev, D.G., Nikolov, L.N., 1988. Influence of Some Physicochemical Parameters on
550 Bacterial Activity of Biofilm: Ferrous Iron Oxidation by *Thiobacillus ferrooxidans*,
551 *Biotechnology and Bioengineering*, 31, 295-299.

552 Karwowska, E., Andrzejewska-Morzuch, D., Łebkowska, M., Tabernacka, A., Wojtkowska,
553 M., Telepko, A., Konarzewska, A., 2014. Bioleaching of metals from printed circuit boards
554 supported with surfactant-producing bacteria, *Journal of Hazardous Materials*, 264, 203-210.

555 Kelly, W.C., Burgio, P.A., 1983. Cryogenic scanning electron microscopy of fluid inclusions
556 in ore and gangue minerals, *Economic Geology*, 78, 1262-1267.

557 Kinnunen, P.H.M., Puhakka, J.A., 2004. High-Rate Ferric Sulfate Generation by a
558 *Leptospirillum ferriphilum*-Dominated Biofilm and the Role of Jarosite in Biomass Retainment
559 in a Fluidized-Bed Reactor, *Biotechnology and Bioengineering*, 85(7), 697-705.

560 Kinnunen, P.H.M. & Puhakka, J.A., 2005. High-rate iron oxidation at below pH 1 and at
561 elevated iron and copper concentrations by a *Leptospirillum ferriphilum* dominated biofilm,
562 *Process Biochemistry*, 40, 3536–3541.

563 Kuznetsova, N.I., Likholobov, V.A., Gurrath, M., Boehm, H.P., 1995. Promotion effect of
564 carbon on the oxidation of ferrous ions by oxygen in the presence of sodium nitrite, *Applied*
565 *Catalysis A/General*, 128, 41-52.

566 Liang, G., Mo, Y., Zhou, Q., 2010. Novel strategies of bioleaching metals from printed
567 circuit boards (PCBs) in mixed cultivation of two acidophiles, *Enzyme and Microbial*
568 *Technology*, 47, 322–326.

569 Liang, G., Tang, J., Liu, W., Zhou, Q., 2013. Optimizing mixed culture of two acidophiles
570 to improve copper recovery from printed circuit boards (PCBs). *Journal of Hazardous*
571 *Materials*, 250-251, 238–245.

572 Liang, G., Ti, P., Liu, W., Wang, B., 2016. Enhanced bioleaching efficiency of copper from
573 waste printed circuit boards (PCBs) by dissolved oxygen-shifted strategy in *Acidithiobacillus*
574 *ferrooxidans*. *J Mater Cycles Waste Manag*, 18, 742-751.

575 MacDonald, D.G., Clark, R.H., 1970. The oxidation of aqueous ferrous sulphate by
576 *Thiobacillus ferrooxidans*, *Can. J. Chem. Eng.* 48, 669-676.

577 Mäkinen, J., Bachér, J., Kaartinen, T., Wahlström, M., Salminen, J., 2015. The effect of
578 flotation and parameters for bioleaching of printed circuit boards. *Minerals Engineering*, 75,
579 26–31.

580 Mi, S., Song, J., Lin, J., Che, Y., Zheng, H., Lin, J., (2011). Complete genome of
581 *Leptospirillum ferriphilum* ML-04 provides insight into its physiology and environmental
582 adaptation. *The Journal of Microbiology* 49, 890–901.

583 Mrážíková, A., Kaduková, J., Marcinčáková, R., Velgosová, O., Willner, J., Fornalczyk, A.,
584 Saternus, M., 2016. The effect of specific conditions on Cu, Ni, Zn and Al recovery from PCBs
585 waste using acidophilic bacterial strains, *Arch. Metall. Mater.*, 61 (1), 261–264.

586 Nie, H., Yang, C., Zhu, N., Wu, P., Zhang, T., Zhang, Y., Xing, Y., 2014. Isolation of
587 *Acidithiobacillus ferrooxidans* strain Z1 and its mechanism of bioleaching copper from waste
588 printed circuit boards. *J Chem Technol Biotechnol*, 90, 714–721.

589 Norris, P.R., 2007. Acidophile Diversity in Mineral Sulfide Oxidation. In: Rawlings D.E.,
590 Johnson D.B. (eds) *Biomining*. Springer, Berlin, Heidelberg.

591 Ojumu, T. V., Hansford, G. S., Petersen, J., 2009. The kinetics of ferrous-iron oxidation by
592 *Leptospirillum ferriphilum* in continuous culture: The effect of temperature. *Biochemical*
593 *Engineering Journal* 46, 161–168.

594 Ojumu, T. V., Petersen, J., 2011. The kinetics of ferrous ion oxidation by *Leptospirillum*
595 *ferriphilum* in continuous culture: The effect of pH. *Hydrometallurgy* 106, 5–11.

596 Ojumu, T. V., Petersen, J., Hansford, G. S., 2008. The effect of dissolved cations on
597 microbial ferrous-iron oxidation by *Leptospirillum ferriphilum* in continuous culture,
598 *Hydrometallurgy*, 94, 69–76.

599 Priya, A., Hait, S., 2018. Extraction of metals from high grade waste printed circuit board
600 by conventional and hybrid bioleaching using *Acidithiobacillus ferrooxidans*,
601 *Hydrometallurgy*, In Press.

602 Rodrigues, M.L.M., Leão, V.A., Gomes, O., Lambert, F., Bastin, D., Gaydardzhiev, S.,
603 2015. Copper extraction from coarsely ground printed circuit boards using moderate
604 thermophilic bacteria in a rotating-drum reactor. *Waste Management*, 41, 148-158.

605 Silverman, M.P., Lundgren, D.G., 1959. Studies on the chemoautotrophic iron bacterium
606 *Ferrobacillus ferrooxidans* I. An improved medium and a harvesting procedure for securing
607 high cell yields *J.Bacteriol*, 77, 642-7.

608 Sinha, R., Chauhan, G., Singh, A., Kumar, A., Acharya, S., 2018. A novel eco-friendly
609 hybrid approach for recovery and reuse of copper from electronic waste, *Journal of*
610 *Environmental Chemical Engineering*, 6 (1), 1053-1061.

611 Svirko, L., Bashtan-Kandybovich, I., Karamanev, D., 2009. Experimental Study of Ferrous
612 Iron Biooxidation by *Leptospirillum Ferriphilum* in Different Biofilm Reactors. *Advanced*
613 *Materials Research* 71–73, 263–266.

614 Tuncuk, A., Stazi, V., Akcil, A., Yazici, E. Y., Deveci, H., 2012. Aqueous metal recovery
615 techniques from e-scrap: *Hydrometallurgy in recycling*, *Miner. Eng.*, 25, 28–37.

616 Wang, J., Bai, J., Xu, J., Liang, B., 2009. Bioleaching of metals from printed wire boards by
617 *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* and their mixture. Journal of
618 Hazardous Materials, 172, 2–3, 1100-1105.

619 Wang, L., Li, Q., Li, Y., Sun, X., Li, J., Shen, J., Han, W., Wang, L. 2017. A novel approach
620 for recovery of metals from waste printed circuit boards and simultaneous removal of iron from
621 steel pickling waste liquor by two-step hydrometallurgical method, Waste Management, In
622 Press.

623 Watling, H. R., Perrot, F. A., Shiers, D. W., 2008. Comparison of selected characteristics of
624 *Sulfobacillus* species and review of their occurrence in acidic and bioleaching environments.
625 Hydrometallurgy, 93, 57–65.

626 Xia, M.C., Wang, Y.P., Peng, T.J., Shen, L., Yu, R.L., Liu, Y.D., Chen, M., Li, J.K., Wu,
627 X.L., Zeng, W.M., 2017. Recycling of metals from pretreated waste printed circuit boards
628 effectively in stirred tank reactor by a moderately thermophilic culture, Journal of Bioscience
629 and Bioengineering, In Press.

630 Xiang, Y., Wu, P., Zhu, N., Zhang, T., Liu, W., Wu, J., Li, P., 2010. Bioleaching of copper
631 from waste printed circuit boards by bacterial consortium enriched from acid mine drainage,
632 Journal of Hazardous Materials, 184, 812–818.

633 Yang, T., Xu, Z., Wen, J., Yang, L., 2009. Factors influencing bioleaching copper from
634 waste printed circuit boards by *Acidithiobacillus ferrooxidans*. Hydrometallurgy, 97, 29–32.

635 Yang, Y., Chen, S., Li, S., Chen, M., Chen, H., Liu, B., 2014. Bioleaching waste printed
636 circuit boards by *Acidithiobacillus ferrooxidans* and its kinetics aspect. Journal of
637 Biotechnology, 173, 24–30.

638 Yue, G., Guezennec, A.G., Asselin, E., 2016. Extended validation of an expression to predict
639 ORP and iron chemistry: Application to complex solutions generated during the acidic leaching
640 or bioleaching of printed circuit boards, Hydrometallurgy, 164, 334-342.

641 Yue, G., Zhao, L., Olvera, O.G., Asselin, E., 2014. Speciation of the $\text{H}_2\text{SO}_4\text{-Fe}_2(\text{SO}_4)_3\text{-}$
642 $\text{FeSO}_4\text{-H}_2\text{O}$ system and development of an expression to predict the redox potential of the
643 $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple up to 150 °C, Hydrometallurgy, 147-148, 196–209.

644 Zhu, N., Xiang, Y., Zhang, T., Wu, P., Dang, Z., Li, P., Wu, J., 2011. Bioleaching of metal
645 concentrates of waste printed circuit boards by mixed culture of acidophilic bacteria. Journal of
646 Hazardous Materials, 192, 2, 614-619.

Table 1: Continuous bio-oxidation studies - experimental conditions and performance.

Strain	Fe ²⁺ (g L ⁻¹)	Reactor	pH	Temp (°C)	Hydraulic residence time	Max oxidation rate (mg L ⁻¹ h ⁻¹)	Study duration (HRT = hydraulic residence time)	References
<i>A. ferrooxidans</i>	0.2 to 3	Stirred tank reactor 4 L	2.2	28	6 to 12.5 h	420	N.D.	MacDonald and Clark, 1970
<i>A. ferrooxidans</i>	5 to 9	Chemostat 10 L	2.3	32	7 to 91 h	830	6 hours	Guay and Silver, 1977
<i>A. ferrooxidans</i>	3.5 to 4	Airlift 1,3 L with polystyrene	1.8 to 2	27	55 min to 12.5 h	1680	3 days	Karamanev and Nikolov, 1986
<i>A. ferrooxidans</i>	9	Airlift 3 L	1.3	30	8 to 50 h	770	3 HRT	Halfmeier et al., 1993a
<i>A. ferrooxidans</i>	9	Airlift 3 L quartz sand – sintered glass rings	1.3	30	8 to 50 h	3600	3 HRT – 6 months	Halfmeier et al., 1993b
<i>A. ferrooxidans</i>	1 to 8.5	Stirred tank reactor 5 L	1.8	30	16 to 100 h	450	1 HRT	Gómez and Cantero, 2003
		Column 1.35 L with Ni alloy fibers			4 to 12.5 h	1100		
mixed culture with <i>L. ferrooxidans</i>	6.9	Fluidized bed reactor 0.9 L with activated charcoal 320 mL or 365 mL	1.1	37	0.2 to 0.6 h	26400	N.D.	Kinnunen and Puhakka, 2004
mixed culture with <i>L. ferrooxidans</i>	7 to 21	Fluidized bed reactor 0.9 L with activated charcoal 285 mL	1	35	3.4 to 4.8 h	10000	N.D.	Kinnunen and Puhakka, 2005
		Fluidized bed reactor 0.4 L with activated charcoal 120 mL			1.9 to 3.4 h			
mixed culture with <i>L. ferrooxidans</i>	5.6	Airlift 3 L with solid supports	0 to 1.8	30	0.25 to 4 h	8100	3 months	Ebrahimi et al., 2005
<i>L. ferriphilum</i>	5	Stirred tank reactor 1 L	1.3	42	7.8 to 67 h	670	3+1 HRT	Ojumu et al., 2008
<i>L. ferriphilum</i>	12	Stirred tank reactor 1 L	1.3	18 - 45	7.8 to 67 h	900	3+1 HRT	Ojumu et al., 2009
<i>L. ferriphilum</i>	12	Stirred tank reactor 1 L	0.8 to 2	42	10 to 66 h	950	3+1 HRT	Ojumu and Petersen, 2011
<i>L. ferrooxidans</i>	9 to 18	Chemostat 1.25 L	1.8	33.5	10 to 33 h	1000	1 HRT	Bastias and Gentina, 2010
mixed culture with <i>L. ferrooxidans</i>	15	Stirred tank reactor 2 stages with settling	1.0 to 2.2	23	6 to 45 h	1100	6 days	Kaksonen et al., 2014a
mixed culture with <i>L. ferrooxidans</i>	15	Airlift 2 stages with settling	1.5	23	8 to 45 h	680 - 810	5 HRT	Kaksonen et al., 2014c

Table 2: Bio-oxidation performances obtained with different ferrous iron concentrations
(HRT = 12 h; Mass of activated charcoal = 15 g).

$[\text{Fe}^{2+}]_{\text{influent}}$ (g L ⁻¹)	Bio-oxidation yield (%)	Bio-oxidation rate (mg L ⁻¹ h ⁻¹)	Experiment duration
1	100%	83	12 HRT
4.5	100%	375	18 HRT
6	21%-70%	105 – 350	27 HRT
9	7%-44%	60 – 330	44 HRT

Table 3: Bio-oxidation rate (r_b ; $\text{mg L}^{-1} \text{h}^{-1}$) and yield (y_c ; %) vs. activated charcoal mass at 6 h HRT for two different Fe^{2+} concentrations.

Activated charcoal (g)	[Fe^{2+}] at 1 g L ⁻¹		[Fe^{2+}] at 9 g L ⁻¹	
	r_b (mg L h^{-1})	y_c (%)	r_b (mg L h^{-1})	y_c (%)
15	165	100%	80-780	6%-55%
30	165	100%	650-1000	40%-70%
45	-	-	500-1500	35%-100%

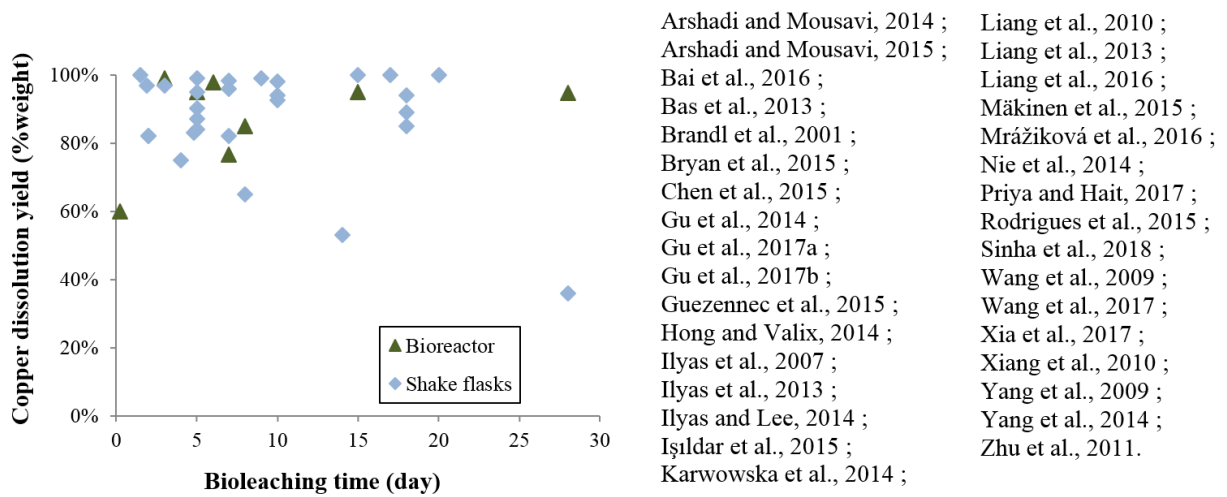


Figure 1: Copper leaching yields versus bioleaching time in bioreactors and shake flasks in printed circuit boards bioleaching studies reported in the literature.

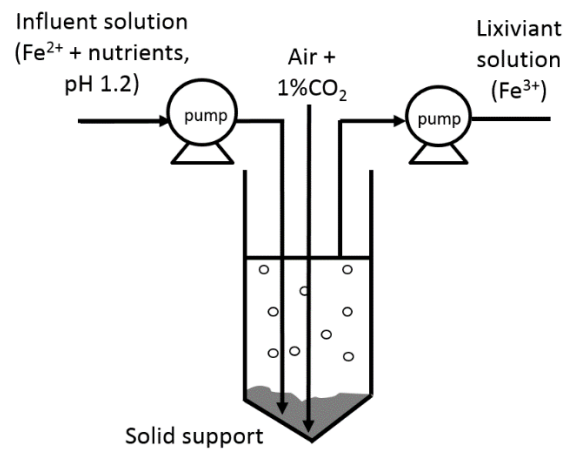


Figure 2: Schematic diagram of the bio-oxidation bubble column

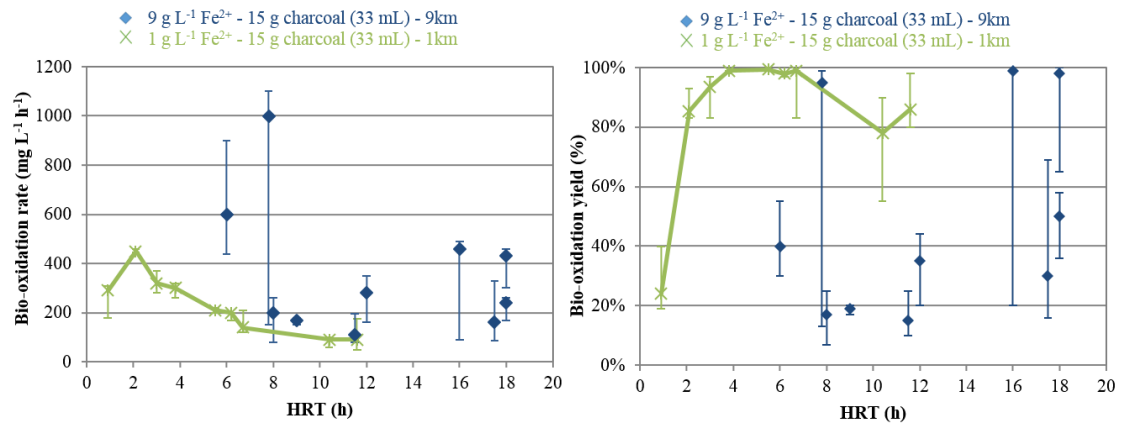


Figure 3: Ferrous iron bio-oxidation at various HRT comprised between 1 and 18 hours in 1Km and 9Km medium in the presence of 15 g of activated charcoal. Error bars depict the range of all obtained values at fixed operating conditions.

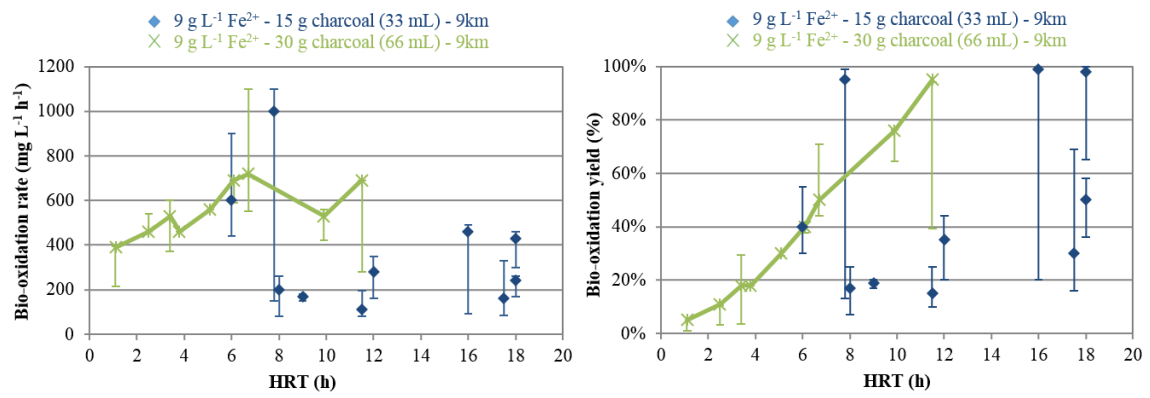


Figure 4: Ferrous iron bio-oxidation at various HRT in 9Km medium in the presence of 15 g and 30 g of activated charcoal. Error bars depict the range of all obtained values at fixed operating conditions.

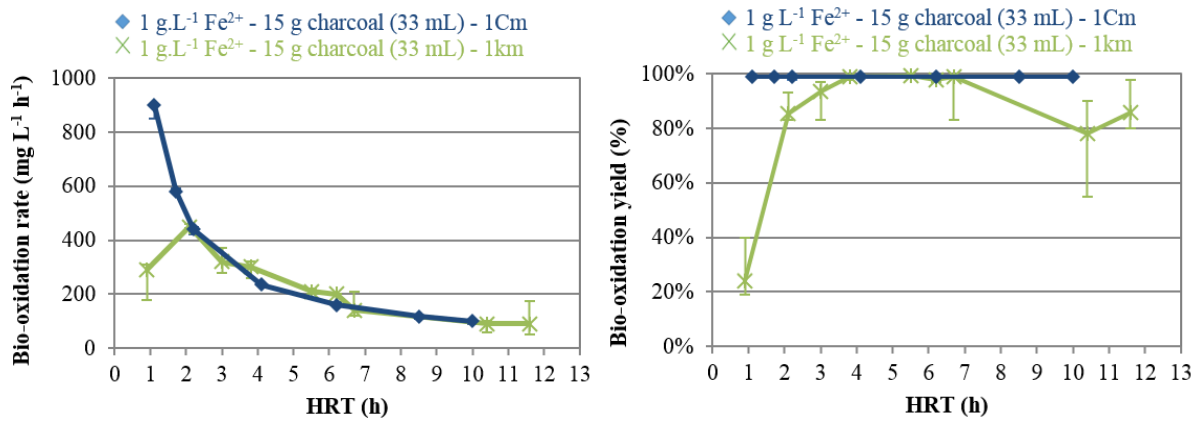


Figure 5: Ferrous iron bio-oxidation at various HRT in 1Km and 1Cm media in the presence of 15 g of activated charcoal. Error bars depict the range of all obtained values at fixed operating conditions.

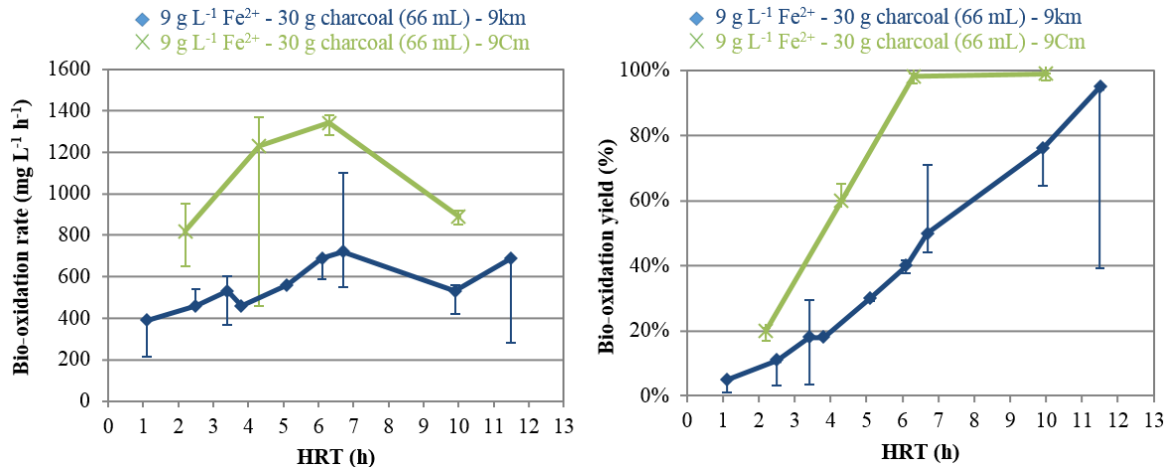


Figure 6: Ferrous iron bio-oxidation at various HRT in 9Km and 9Cm media in the presence of 30 g of activated charcoal. Error bars depict the range of all obtained values at fixed operating conditions.

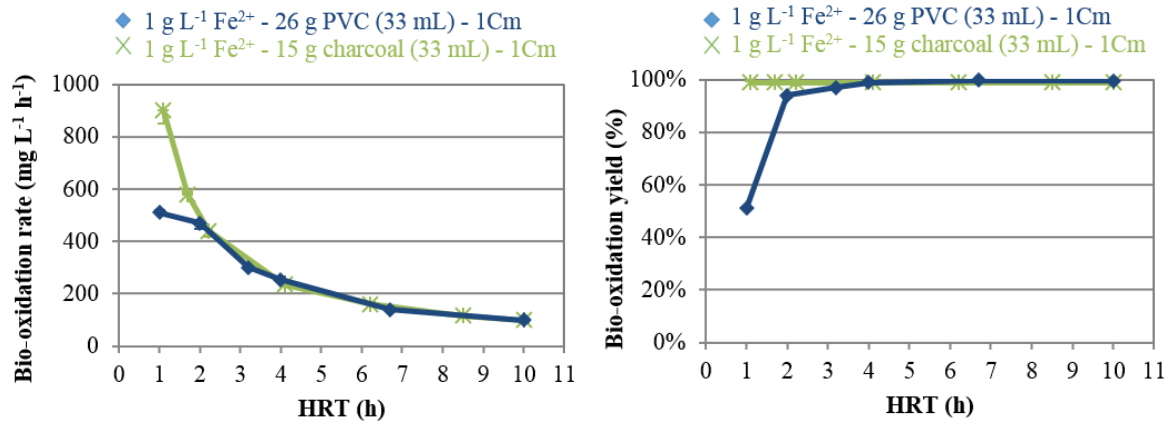


Figure 7: Ferrous iron bio-oxidation at various HRT in 1Cm medium in the presence of 26 g PVC or 15 g activated charcoal (apparent volumes = 33 mL). Error bars depict the range of all obtained values at fixed operating conditions.

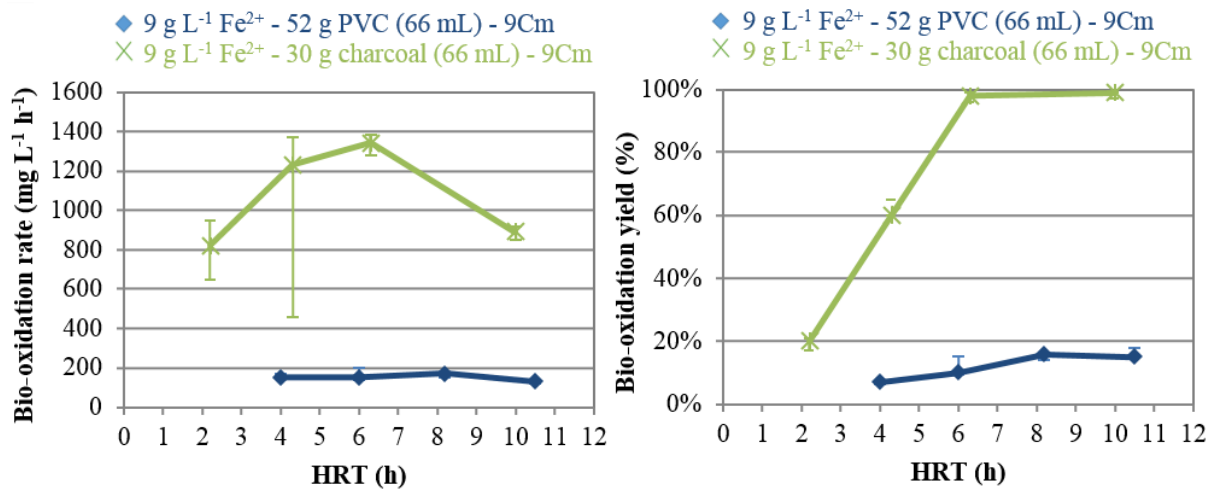


Figure 8: Ferrous iron bio-oxidation at various HRT in 9Cm medium in the presence of 52 g of PVC or 30 g of activated charcoal (apparent volumes = 66 mL). Error bars depict the range of all obtained values at fixed operating conditions.

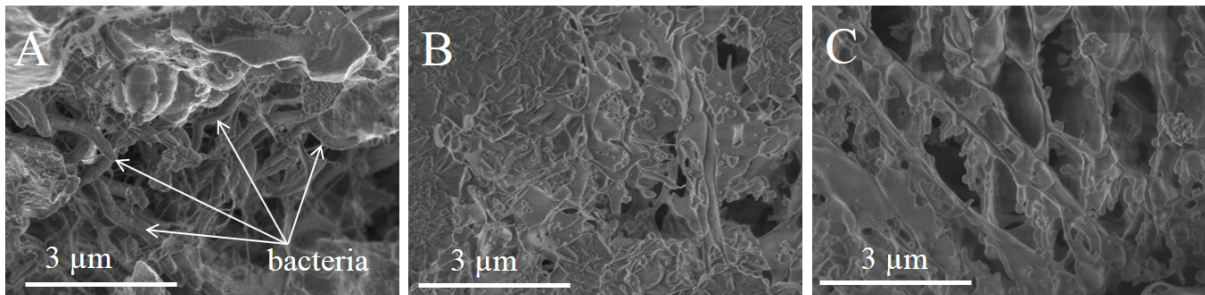


Figure 9: Cryo-SEM pictures inside of activated charcoal particles after colonization by acidophilic bacteria. Experimental conditions in 0Km in the presence of ferrous iron concentrations at 1 g L^{-1} (A) and 9 g L^{-1} (B). For (C), experiments were performed in an Erlenmeyer under abiotic conditions in the presence of 9 g L^{-1} ferric iron.

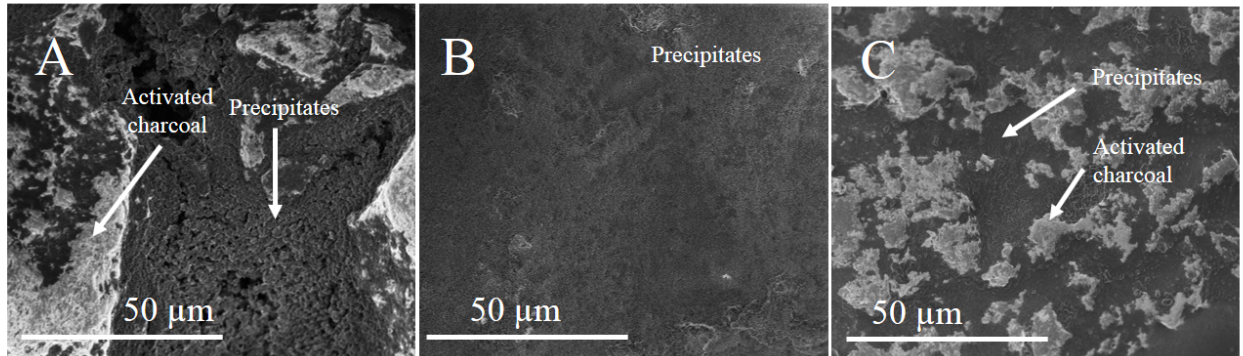


Figure 10: Cryo-SEM pictures of the surface of activated charcoal particles after colonization by acidophilic bacteria. Experimental conditions in 0Km in the presence of ferrous iron concentrations at 1 g L^{-1} (A) and 9 g L^{-1} (B). For (C), experiments were performed in an Erlenmeyer under abiotic conditions in the presence of 9 g L^{-1} ferric iron.

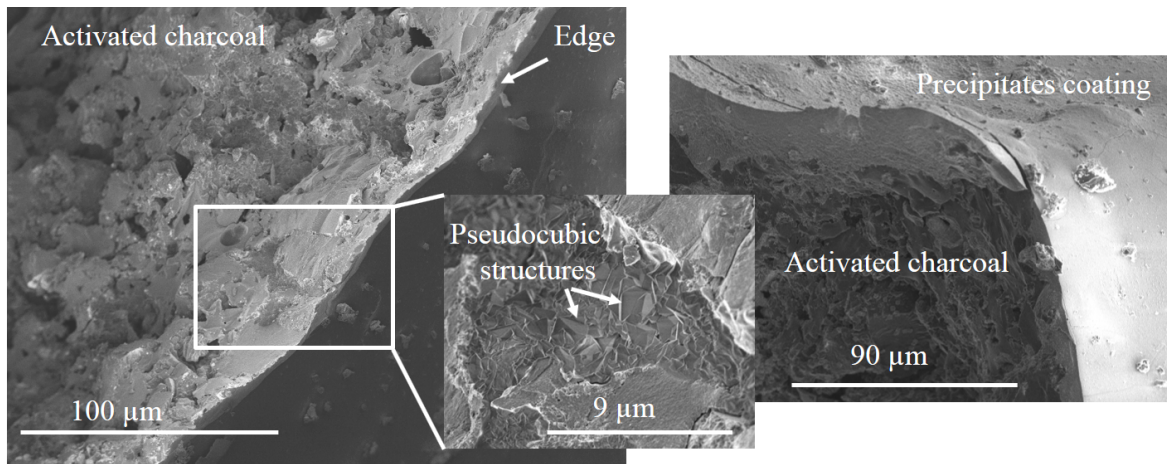


Figure 11: Cryo-SEM observations of the edge of a cryo-fractured particle of activated charcoal after colonization by acidophilic bacteria in 9Km in the presence of ferrous iron concentration of 9 g L⁻¹.

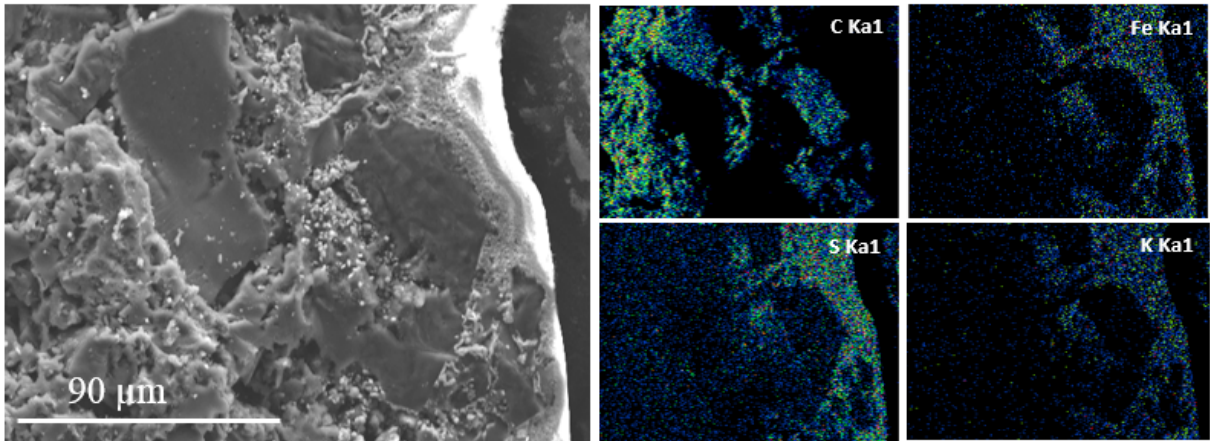


Figure 12: Cryo-SEM observation at 15 kV and EDX cartography of the edge of a cryo-fractured particle of activated charcoal after colonization by acidophilic bacteria in 0Km in the presence of 9 g L⁻¹ ferrous iron.

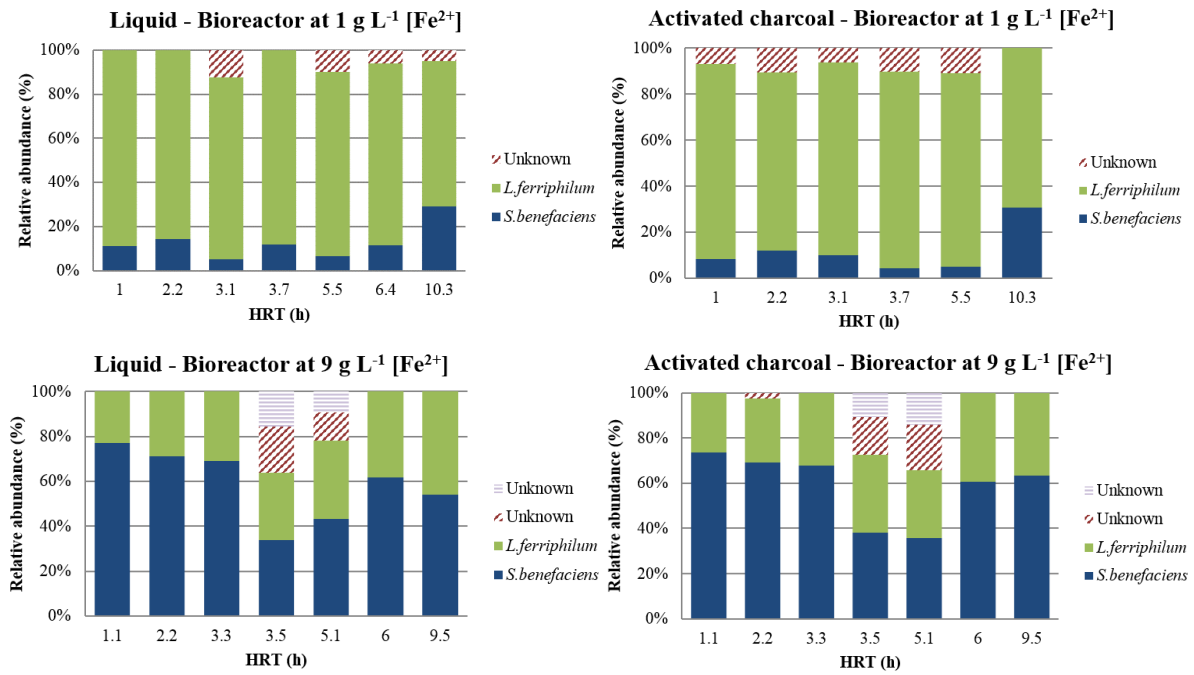


Figure 13: Comparison of bacterial diversity profiles for different HRT in liquid and solid phases (incoming ferrous iron concentrations=1 and 9 g L⁻¹ in 0Km medium).

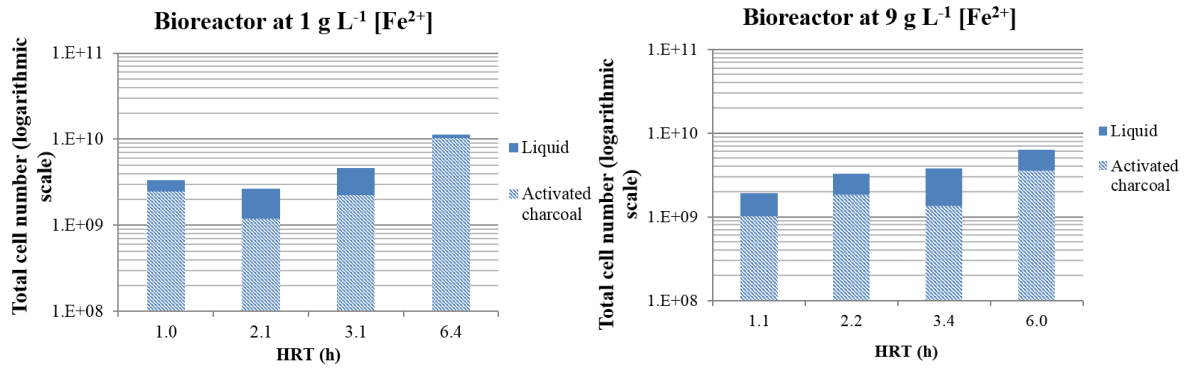


Figure 14: Total cell number in liquid and solid phases in bubble columns with influent with 1 g L⁻¹ Fe²⁺ and 15 g of activated charcoal as well as 9 g L⁻¹ Fe²⁺ and 30 g of activated charcoal in 0Km medium (logarithmic scale).