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Sulfur and oxygen stable isotope tracing in Zero valent iron based Insitu remediation system for metal contaminants

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Abstract:

In the present study, controlled laboratory column experiments were conducted to understand the biogeochemical changes during the microbial sulfate reduction. Sulfur and oxygen isotopes of sulfate were followed during sulfate reduction in zero valent iron incubated flow through columns at a constant temperature of 20 ± 1°C for 90 days. Sulfur isotope signatures show considerable variation during biological sulfate reduction in our columns in comparison to abiotic columns where no changes were observed. The magnitude of the enrichment in $\delta^{34}S$ values (‰) ranged from + 9.4 to +10.3‰ compared to initial value of 2.3‰, having total fractionation ΔS between biotic and abiotic columns as much as 6.1‰. Sulfur isotope fractionation was directly proportional to the sulfate reduction rates in the columns. Oxygen isotopes in this experiment seem less sensitive to microbial activities and more likely to be influenced by isotopic exchange with ambient water. A linear relationship is observed between $\delta^{34}S$ and $\delta^{18}O$ in biotic conditions and we also highlight a good relationship between $\delta^{34}S$ and sulfate reduction rate in biotic columns.

Keywords: Sulfur isotopes, oxygen isotopes, zero valent iron, in-situ remediation

41 **1. Introduction**

42 *In-situ* groundwater remediation of metal contaminants has emerged as a sustainable option in
43 recent years for various reasons, e.g. economic feasibility and energy consumption in pump and
44 treats methods, site accessibility etc… (Farhadian et al., 2008). For in-situ remediation, various
45 possibilities have also been explored for instance by enhancing natural attenuation, providing
46 electron donors (Satyawali et al., 2010) or by using reactive barrier materials (Benner et al.,
47 1999; Waybrant et al., 2002). Zero valent iron (Fe°) is getting large attention lately as a reactive
48 material for in-situ applications (Dries et al., 2005; Burghardt and Kassahun, 2005; Wilkin and
49 McNeil, 2003). The highly reducing nature and relatively larger available specific surface area
50 make Fe° a suitable medium for groundwater contaminant removal. There have been already
51 many successful installation of Fe° based remediation system in last decade (Phillips et al., 2010;
52 Liyuan Liang, 2003; Gu et al., 1998; Rowland, 2002). Fe° has been successfully used in lab scale
53 as well as field scale applications, dealing with wide range of groundwater contaminants e.g.
54 chlorinated compounds and metals (Dries et al., 2005; Gandhi et al., 2002; Rangsivek and Jekel,
55 2005), radioactive material (Burghardt and Kassahun, 2005), arsenic (Su and Puls, 2003;
56 Nikolaos et al., 2003) etc... However, the biogeochemical dynamics and contaminants behavior
57 in subsurface environments are still poorly understood, as real field sites often encounter
58 problems due to little or no control over fluxes and changes in subsurface processes with time or
59 seasons. Regular chemical analysis and monitoring may not be a practical or economic option in
60 many isolated sites. These complications often make it difficult to understand the actual
61 processes (biotic/abiotic) contributing in contaminant removal, needless to say that this
62 distinction is rather very important in designing realistic remediation strategy for any particular
63 site.

64 Stable isotopes have emerged as a potential tool in understanding the dynamics of pollutants in
65 water systems (Fritz et al., 1994; Houhou et al., 2010; Krouse, 2000; Mast et al., 2001; Wu et al.,
66 2011). Relatively easy analysis and bulk information makes isotope study a practical and viable
67 option. The characterization and quantification of electron-accepting processes, like nitrate, iron
68 and sulfate reduction are extremely valuable in estimating the sustainability and longevity of
69 degradation process in any subsurface system (Knöller et al., 2006). This characterization is of
70 primary importance in case of in-situ treatment process, where there is little control over
71 changing processes in subsurface environments. Biological sulfate reduction has been observed

72 multiple times in similar kind Fe° based treatments systems (Van Nooten et al., 2007; Gu et al., 73 2002).

74 Quantification of sulfate reduction by following dissolved concentrations of sulfate and co-75 existing sulfide in groundwater is often a challenge due to possible dilution, mixing or mineral 76 precipitation processes. Precipitation of dissolved sulfide is particularly important in case of Fe° 77 based systems where abundant Fe^{2+} is available in groundwater for possible FeS precipitation. It 78 has been shown that sulfate reducing bacteria preferentially removes lighter isotopes of sulfur 79 and oxygen from sulfate molecule resulting into isotopic enrichments of both heavier isotopes 80 i.e. ^{18}O and ^{34}S in residual sulfate (Fritz et al., 1989).

81 In this study, we followed the evolution of $\delta^{34}S$ and $\delta^{18}O$ from dissolved sulfate in groundwater 82 within lab scale Fe° based flow through columns, which were primarily designed for heavy metal 83 remediation from groundwater. Aim of this study was to characterize the isotope fractionation 84 and changes during the long-term remediation processes and also to determine the impact of 85 microbiology on isotopic signature in subsurface redox environment. We summarize here the 86 results of sulfur and oxygen isotope fractionation in Fe° based lab scale in-situ remediation 87 systems over 90 days of experiment.

88

89 **2. Material and Methods**

90 **2.1. Column design**

91 Four double jacketed flow through glass columns (30 cm length x 4.5 cm I.D, total liquid volume 92 ≈ 480 mL) were setup in laboratory for ~ 30 weeks at controlled temperature (20 ± 1°C), with the 93 primary aim of groundwater contaminant (metals) removal using Fe° as a reactive material. 94 Columns were filled with sediment obtained from a heavy metal contaminated site in Belgium 95 from a depth of ≈ 32 m, more description about this site is given elsewhere (Vanbroekhoven et 96 al., 2008). Efforts were made to design a lab scale concept of *in-situ* reactive barrier using two 97 types of Fe°differing in particles size and source, , i.e. granular zero valent iron (gFe°, Gotthard 98 Maier, Germany) and micro zero valent iron (mFe°, Högenäs, Sweden) with an average particle 99 size of 0.25-2 mm and 20-40 µm respectively. For each column, the first bottom half (~ 240 mL) 100 was filled with an aquifer/ Fe° mixture with ratios of 80:20 and 98:2 v/v for gFe° and mFe°

101 respectively. The second (upper) half of all columns was filled only with aquifer (Figure 1).
102 Filling of columns was performed under nitrogen atmosphere in a glove box. Simulated
103 groundwater, which was prepared in lab corresponding to the site characteristics (Table 1), was
104 injected in parallel through the columns using a peristaltic pump at a constant flow rate of 1 ± 0.2
105 mL/h. A slight over pressure (0.01 Bar) of N_2 was maintained in the feeding bottles to avoid air
106 contact during column feeding. All tubes and fittings used in the experiment were acid washed
107 and flushed with nitrogen before use.
108 For each Fe^0 type, two columns were set-up, of which one was fed with a small dose of glycerol
109 (0.1% v/v of inlet water) to enhance indigenous microbial activity, and the column was exposed
110 to gamma radiations (IONISOS, Dagneux, France), with minimum absorbed radiation dose of 25
111 kGy, before injecting groundwater to restrict all microbial activities.

112 **2.2. Analytical methods**

113 For observation and counting of bacterial cells, defined volumes of samples (from 50 to 1000
114 µL) were extracted from the columns using a nitrogen filled plastic syringe, by injecting the
115 nitrogen and extracting equal amount of liquid from column. Samples were immediately diluted
116 in deionized water and filtered onto a black polycarbonate filter, 0.22 µm (Nuclepore,
117 Whatman). The filter was incubated 15 min in the dark with 1 mL filtered deionized water mixed
118 with 1 µL DAPI (4',6-diamidino-2-phenylindole) solution (1 mg/mL, SIGMA). This mixture was
119 removed by filtration, and the filter was rinsed 2 times with 1 mL filtered deionized water. The
120 filter was then mounted on a glass slide with Citifluor (Biovailey), and observed with an optical
121 microscope (Zeiss Axio Imager Z1) equipped with Filter Set 49 for DAPI, UV HBO lamp and a
122 digital camera. Bacteria were enumerated on 10 independent fields (each of 5800 μm^2). Cell
123 counts were calculated considering the volume of the sample used and filter surface area
124 calculations on an average basis. Sulfate concentration was analyzed with a spectrophotometer
125 operating at λ 540° using specific analysis kits (Merck Spectroquant® kit 1.14548.001,
126 Germany).

127 Samples for sulfur and oxygen isotope analysis were collected at the outlet of columns using 250
128 mL pre acid washed plastic perplex bottles. Cd-Acetate was already added in the bottles (5% v/v)
129 prior to sample collection, to fix sulfur as CdS, and then an aliquot was filtered through a 0.2
130 µm-nitrocellulose filter before chemical determination of residual sulfate. The amount of sample

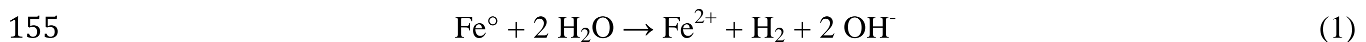
collected varied at different time points during the experiment as the sulfate concentration in the outlet solution changed over the time. However, in any case, a minimum of 5 mg of SO_4 was collected for every sampling point. The analysis was performed as described by (Fritz et al., 1989).

Dissolved sulfate was precipitated as $BaSO_4$ at pH< 4 (in order to remove HCO_3^- and CO_3^{2-} species) by adding a $BaCl_2$ solution. The isotopic analyses on $BaSO_4$ were carried out using a Delta+XP mass spectrometer coupled in continuous-flow mode to a Thermo Elemental Analyzer. Sulfate-isotope compositions are reported in the usual δ-scale in ‰ with reference to V-CDT (Vienna Canyon Diablo Troilite) and V-SMOW (Vienna Standard Mean Ocean Water) according to δsample (‰) = {(Rsample/Rstandard) - 1} x 1000, where R is the $^{34}S/^{32}S$ and $^{18}O/^{16}O$ the atomic ratios. Sulfate-isotope compositions ($\delta^{34}S$ (SO_4) and $\delta^{18}O$ (SO_4)) were measured with a precision of ± 0.3‰ vs. CDT for $\delta^{34}S$ (SO_4) and ±0.8 ‰ vs. VSMOW for $\delta^{18}O$ (SO_4) respectively.

3. Results and Discussion

3.1. Sulfate

Sulfate reduction is a common phenomenon observed in Fe° based PRB's due to favorable growth environment i.e. close to neutral pH and a very low ORP (Oxidation Reduction Potential) conditions (Gu et al., 2002). The efficiency of Fe° in increasing pH and decreasing ORP by releasing OH^- ions and scavenging O_2 (see reaction 1) and production of water born H_2, is well known (Johnson et al., 2008). This water born H_2 can also be a potential electron donor for sulfate reducing bacteria (Karri et al., 2005). In the present study, dissolved sulfate concentration in the column outlet solution was analyzed regularly to follow behavior and activity of sulfate reducing bacteria.



$$Fe^{\circ} + 2\,H_2O \rightarrow Fe^{2+} + H_2 + 2\,OH^- \quad (1)$$

Dissolved sulfate concentrations in the column outlet solution decreased from initial inlet concentrations of 3.95 mmol/L to 0.03 and 0.09 mmol/L in gFe° and mFe° biotic columns respectively (Table 2, Figure 2). Appearance of black patches (believed to be FeS precipitation)

159 in both biotic columns were in agreement with microbial sulfate reduction. Providing glycerol
160 probably also stimulated bacterial growth by acting as carbon source along with availability of
161 H_2. In this experiment no extra microbial culture was added, so only natural population of sulfate
162 reducers were expected to grow.

163 However, in the abiotic columns, sulfate concentrations were unaffected throughout the
164 experiment (Figure 2), which was expected due to absence of viable microbial cells after gamma
165 radiation exposure to sediment and the small dose of formaldehyde (100µL/L) that was added
166 with inlet water to avoid any possibility of microbial growth.

167 Potential sulfate reduction rates (SRR, $nmol.cm^{-3}.h^{-1}$) were calculated using equation (2)
168 described by Stam et al., 2011.

169 $$SRR = \Delta C \; X \; Q / V \qquad (2)$$

170 Where Q represents the volumetric flow rate of solution through the reactor, ΔC is the difference
171 between inflow (C_0) and outflow (C) sulfate concentration and V is the volume of the sediment
172 contained in the reactor.

173 Sulfate reduction rate (SRR) values in this experiment are consistent with the sulfate reduction as
174 the maximum rate was achieved after 30 days of incubation, after which similar values of SRR
175 were obtained (value of 4 - 4.1 $nmol.cm^{-3}.h^{-1}$, Figure 3a) for both granular and micro biotic
176 columns. Equal and steady values of SRR throughout the experiment are consistent with the
177 equal and limited supply of sulfate in the system. In general the rate of sulfate reduction is also
178 believed to influence the isotope fractionation, and in this study sulfate reduction rate was
179 directly related to the sulfur isotope fractionation (Figure 3b). It is also argued in literature that
180 substrate type may also influence the fractionation, considering H_2 gas producing lower
181 fractionation than organic substrate (Kemp and Thode, 1968; Rittenberg., 1964). As in the
182 present study H_2 as well as glycerol were available as electron donor, individual contribution
183 cannot be established in this experimental setup.

184 **3.2. Cell counting and microscopic observations**

185 Bacterial cell counting was performed using DAPI imaging. Changes in cell numbers during the
186 experiment can give general trends of microbial activity; increases or decreases in bacterial cell

187 count can be related to the overall sulfate reduction rate. Normally, if the sulfate is not limiting
188 and energy source is available, the cell count could increase with time which would further
189 enhance the sulfate reduction.

190 Photographs of DAPI-stained samples (Figure 4) show the presence of dominant rod shaped
191 bacterial cells with an average length of 5 μm. All cells were visually similar in shape and sizes,
192 suggesting the growth of a dominant species. No efforts were made at this step of the experiment
193 to identify the bacterial species.

194 Bacterial cells were counted at day 30 of column test, when dissolved sulfate was completely
195 reduced in biotic columns. An average cell count of 1.9×10^7 and 1.2×10^7 cells/mL were obtained
196 in gFe° biotic and mFe° biotic column, respectively. At the end of the experiment (day 90), the
197 cell concentrations were 1.7×10^7 and 6.5×10^7 cell/mL in gFe° and mFe° biotic column,
198 respectively. These observations suggest that the cell concentration during the experimental period
199 did not change significantly, which is consistent with limited and uniform supply of sulfate and
200 almost constant SRR in the columns. These observations also suggest that due to the limited
201 sulfate doses, the sulfate-reducing bacterial population would continue to consume sulfate in the
202 columns, so the cell concentrations are more likely to be related to the dissolved sulfate supply in
203 this case.

204 **3.3. δ^{34}S variations and origin of sulfur isotope fractionation**

205 Although isotopes of the same element behave the same physically and chemically, reaction rates
206 differ due to the mass difference between the isotopes. This mass difference causes a preferential
207 partitioning, namely isotope fractionation, that results in varying isotopic compositions during
208 reaction. On the one hand, in the biotic columns δ^{34}S enrichment was evident (Figure 5),
209 explicable by a preferred use of lighter sulfur element of SO_4 (^{32}S)by sulfate reducers, which
210 results into abundance of heavier element ^{34}S in the remaining sulfate molecules. On the other
211 hand, no sulfate reducers were active in the abiotic columns, so δ^{34}S values remain almost the
212 same as the initial value (Figure 5). There is no mechanism reported till date for abiotic sulfate
213 reduction.

214 A large range of variation in δ^{34}S (from 4 up to 46‰) has been observed in pure bacterial
215 cultures where sulfate was available abundantly (Kaplan and Rittenberg, 1964; Detmers

216 et.al.,2001). In the present study, the maximum values of $\delta^{34}S$ observed equal +10.3‰ in the
217 mFe° biotic columns, with final enrichment values of +9.4‰. A total ΔS was as much as +6.1‰,
218 while compared between biotic and abiotic processes, where ΔS is the difference of $\delta^{34}S$ (‰)
219 between biotic and abiotic columns. The isotope measurement were followed until 90 days of
220 column operation, after this period SRR was constant so no further fractionation was expected;
221 also sample collection was not possible as the dissolved sulfate concentration was nearly zero.

222 Sulfide produced by sulfate reducing bacteria was not considered to be associated with isotope
223 fractionation in this study as sulfide was very likely to be precipitated with Fe(II) available in the
224 plume to form insoluble iron monosulfide and this phenomenon is known to not be associated
225 with sulfur isotope fractionation effect (Canfield et al., 1992).
226 Figure 6 shows that the dissolved sulfate concentration and $\delta^{34}S$ (‰) follow an inverse trend in
227 both biotic columns, where dissolved sulfate concentration decreases as microbial activity
228 increases, on the other hand $\delta^{34}S$ (‰) value increases through time. This observation confirms
229 that the sulfur isotope fractionation only originates from microbial sulfate reduction avoiding any
230 contribution of sulfate from sediment.

231 **3.4. $\delta^{18}O_{(SO4)}$ variations and origin of the oxygen isotope fractionation**

232 Although significant enrichment of ^{34}S was evident in residual sulfate, a corresponding
233 enrichment of ^{18}O was not observed in this study. Similar observations were previously reported
234 by other researchers (Spence et al., 2005). Sulfur and oxygen isotope followed a different
235 isotopic pattern during sulfate reduction probably because of fundamental differences in the
236 enrichment mechanism. Sulfur isotope fractionation is a purely kinetic effect, whereas oxygen
237 isotope fractionation is influenced by catalysis of isotopic exchange between water and sulfate
238 during sulfate reduction (Fritz et al., 1989., Freney and Ivanov,1983).
239 AN identical pattern shift was observed in $\delta^{18}O$ behavior in both biotic and abiotic processes
240 (Figure 7), so it is very likely that the relatively small variation observed in $\delta^{18}O$ could be due to
241 shift in isotopic equilibrium with ambient water. This leads to the widely believed assumption
242 that the oxygen isotope exchange dominated over kinetic isotope fractionation. $\delta^{18}O$ of ambient
243 water was not analyzed in this experiment, so we were not able to confirm this hypothesis. Direct
244 chemical or microbial oxidation of H_2S to sulfur is accompanied by much smaller oxygen
245 isotope effect. The $\delta^{18}O$ fractionation is normally controlled by ambient surface water. This

246 hypothesis is considered in many studies recently, however no established explanation of this
247 process has been reported yet in literature (Knöller et al., 2006).
248

249 **3.5. Isotope variation: $\delta^{18}O$ vs. $\delta^{34}S$**

250 In Figure 8, linear relationships were observed between $\delta^{34}S$ and $\delta^{18}O$ in the biotic experiments
251 irrespective of the type of Fe° (either granular or micro Fe), although the slopes are slightly
252 different for each in Figure 8. These results suggest that the particle size may affect the overall
253 sulfate reduction process in some cases.

254 It is also argued in literature that there is no simple relationship between SRR and isotope
255 fractionation (Detmers et al., 2001), however in the present study we observed a linear
256 relationship (Fig8b). Brunner et al. (2005) proposed a combined investigation of the influence of
257 sulfate reducing bacteria on the sulfur and oxygen isotopic composition of residual sulfate could
258 be the key to a better understanding of sulfate reduction rate. Böttcher et al. (1998) hypothesized
259 that $\delta^{34}S_{SO4}$ vs $\delta^{18}O_{SO4}$ relationships reflect sulfate reduction rates in marine sediments; the
260 steeper the slope the slower the sulfate reduction rates. In the present study, we observed not so
261 steep slope for $\delta^{34}S_{SO4}$ vs $\delta^{18}O_{SO4}$ relationship (Fig 8), which is consistent with the experimental
262 condition where SRR was stable after initial increase.

263 It is also noteworthy that the $\delta^{34}S_{SO4}$ vs $\delta^{18}O_{SO4}$ relationship does not reflect the bulk SRR but
264 rather cell-specific SRR. So basically, a large number of bacteria with slow cell specific SRR or
265 a small number of bacteria with high cell specific SRR, both can achieve a high bulk SRR
266 (Brunner et al., 2005). But in present study, the number of bacterial cell in both the biotic
267 columns did not increase significantly during the experimental period. .

268

269 **4. Conclusions**

270 In the present study, we report results from a long-term experiment, designed for groundwater
271 treatment using real site sediment. Sulfur isotope analysis is a good and practically viable option
272 to characterize sulfate reduction activities and sulfate reduction rate in any subsurface system
273 without going for microbial analysis and characterization. Oxygen isotope analysis is also

274 important but needs to be considered in light of ambient water oxygen isotope exchange, as it is
275 more likely to be controlled by ambient water. Sulfur and oxygen isotopes both provide important
276 information and supports the actual practical data obtained during the experiment. However, it is
277 important to see the long term effect of isotope behavior as very less knowledge is available in
278 literature for these kinds of system for long time operations, which is very typical for in-situ
279 remediation treatment systems.

280 Isotope geochemistry can also be used for precise identification of pollution sources, effectiveness
281 of remediation process, and can provide crucial insight into contaminants fate and transport
282 (examples for CAH removal are). Considering the simple methods for isotopic analysis and less
283 analytical cost, these standard analytical tools can easily be incorporated into typical field
284 sampling. As the standard geochemical analysis always retain certain level of uncertainty at times
285 important question might left unanswered. In combination with other chemical and
286 biogeochemical techniques, isotopic analysis can be used for better understanding the processes.

287

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301 **References**

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303 Benner, S.G., Blowes, D.W., Gould, W.D., Herbert, R.B. and Ptacek, C.J., 1999. Geochemistry
304 of a Permeable Reactive Barrier for Metals and Acid Mine Drainage. Environmental
305 Science & Technology 33, 2793-2799.

306 Böttcher, M.E., Brumsack, H.J., De Lange, G.J.,1998. Sulfate reduction and related stable
307 isotope (^{34}S, ^{18}O) variations in interstitial waters from the eastern Mediterranean. In
308 proceedings of the Ocean Drilling program, Scientific results 160, 365-373.

309 Brunner,B., Bernasconi,S.M., Kleikemper, J., Schroth, M.H., 2005. A model for oxygen and
310 sulfur isotope frcationation in sulfate during bacterial sulfate reduction processes.
311 Geochimica et Cosmochimica Acta 69, 4773-4785.

312 Burghardt, D., Kassahun, A., 2005. Development of a reactive zone technology for simultaneous
313 in situ immobilisation of radium and uranium. Environmental Geology 49, 314-320.

314 Canfield, D.E., Raiswell.R., Bottrell, S.H., 1992. The reactivity of sedimentary iron minerals
315 toward sulfide. American journal of Science 292, 659-683.

316 Detmers, J., Brüchert,V., Habicht,K.S., Kuever,J., 2001. Diversity of sulfur isotope
317 fractionations by sulfate reducing prokaryotes. Applied and Environmental Microbiology
318 67, 888-894.

319 Dries, J., Bastiaens, L., Springael, D., Agathos, S.N., Diels, L., 2005. Combined Removal of
320 Chlorinated Ethenes and Heavy Metals by Zerovalent Iron in Batch and Continuous Flow
321 Column Systems. Environmental Science & Technology 39, 8460-8465.

322 Farhadian, M., Vachelard, C.d., Duchez, D., Larroche, C., 2008. In situ bioremediation of
323 monoaromatic pollutants in groundwater: A review. Bioresource Technology 99, 5296-
324 5308.

325 Fritz, P., Basharmal, G.M., Drimmie, R.J., Ibsen, J., Qureshi, R.M., 1989. Oxygen isotope
326 exchange between sulphate and water during bacterial reduction of sulphate. Chemical
327 Geology 79, 99-105.

328 Gandhi, S., Oh, B.T., Schnoor, J.L., Alvarez, P.J.J., 2002. Degradation of TCE, Cr (VI), sulfate,
329 and nitrate mixtures by granular iron in flow-through columns under different microbial
330 conditions. Water Research 36, 1973-1982.

331 Gu, B., Liang, L., Dickey, M.J., Yin, X., Dai, S., 1998. Reductive Precipitation of Uranium(VI)
332 by Zero-Valent Iron. Environmental Science & Technology 32, 3366-3373.
333 Gu, B., Watson, D.B., Wu, L., Phillips, D.H., White, D.C., Zhou, J., 2002. Microbiological
334 Characteristics in a Zero-Valent Iron Reactive Barrier. Environmental Monitoring and
335 Assessment 77, 293-309.
336 Houhou, J., Lartiges,B.S., France-Lanord, C., Guilmette, C., Poix,S., Mustin,C., 2010. Isotopic
337 tracing of clear water sources in an urban sewer: A combined water and dissolved sulfate
338 stable isotope approach. Water Research 44, 256-266.
339 Ivanov, M.V., Freney, J.R., (ed), 1983. The Biogeochemical Sulphur cycle. John Wiley & Sons.
340 495p.p.
341 Johnson, R.L., Thoms, R.B., O'Brien Johnson, R., Nurmi, J.T., Tratnyek, P.G., 2008. Mineral
342 Precipitation Upgradient from a Zero-Valent Iron Permeable Reactive Barrier. Ground
343 Water Monitoring & Remediation 28, 56-64.
344 Kaplan,I.R., Rittenberg, S.C., 1964. Microbiological fractionation of sulphur isotopes. Journal of
345 General Microbiology 34, 195-212.
346 Karri, S., Sierra-Alvarez, R., Field, J.A., 2005. Zero valent iron as an electron-donor for
347 methanogenesis and sulfate reduction in anaerobic sludge. Biotechnology and
348 Bioengineering 92, 810-819.
349 Kemp, A.L.W., Thode, H.G., 1968. The mechanism of the bacterial reduction of sulphate and of
350 sulphite from isotope fractionation studies. Geochimica et Cosmochimica Acta 32, 71-91.
351 Knöller, K., Vogt, C., Richnow, H.H., Weise, S.M., 2006. Sulfur and Oxygen Isotope
352 Fractionation during Benzene, Toluene, Ethyl Benzene, and Xylene Degradation by
353 Sulfate-Reducing Bacteria. Environmental Science & Technology 40, 3879-3885.
354 Liyuan Liang, A.B.S., Olivia R. W., Gerilynn R. M., Wiwat K., 2003. Predicting the
355 Precipitation of Mineral Phases in Permeable Reactive Barriers. Environmental
356 Engineering Science 20, 635-653.
357 Nikolaidis, N.P., Dobbs, G.M., Lackovic,J.A., 2003. Arsenic removal by zero-valent iron: field,
358 laboratory and modelling studies. Water Research 37, 1417-1425.
359 Phillips, D.H., Nooten, T.V., Bastiaens, L., Russell, M.I., Dickson, K., Plant, S., Ahad, J.M.E.,
360 Newton, T., Elliot, T., Kalin, R.M., 2010. Ten Year Performance Evaluation of a Field-
361 Scale Zero-Valent Iron Permeable Reactive Barrier Installed to Remediate

362 Trichloroethene Contaminated Groundwater. Environmental Science & Technology 44,
363 3861-3869.
364 Rangsivek, R., Jekel,M.R., 2005. Removal of dissolved metals by zero-valent iron (ZVI):
365 Kinetics, equilibria, processes and implication for stormwater ruoff reatment. Water
366 Research 39, 4153-4163.
367 Rowland, R.C., 2002. Sulfate-Reducing Bacteria in the Zero-Valent Iron Permeable Reactive
368 Barrier at Fry Canyon, Utah. Handbook of groundwater remediation using permeable
369 reactive barriers. Chapter 10, 281-304.
370 Satyawali, Y., Schols, E., Van Roy, S., Dejonghe, W., Diels, L., Vanbroekhoven, K., 2010.
371 Stability investigations of zinc and cobalt precipitates immobilized by in situ
372 bioprecipitation (ISBP) process. Journal of Hazardous Materials 181, 217-225.
373 Spence, M.J., Bottrell, S.H., Thornton, S.F., Lerner, D.N., 2001. Isotopic modelling of the
374 significance of bacterial sulphate reduction for phenol attenuation in a contaminated
375 aquifer. Journal of Contaminant Hydrology 53, 285-304.
376 Spence, M.J., Bottrell, S.H., Thornton, S.F., Richnow, H.H., Spence, K.H., 2005. Hydrochemical
377 and isotopic effects associated with petroleum fuel biodegradation pathways in a chalk
378 aquifer. Journal of Contaminant Hydrology 79, 67-88.
379 Stam, M.C., Mason, P.R.D., Laverman, A.M., Pallud, C.l., Cappellen, P.V., 2011. $^{34}S/^{32}S$
380 fractionation by sulfate-reducing microbial communities in estuarine sediments.
381 Geochimica et Cosmochimica Acta 75, 3903-3914.
382 Su, C., Puls, R.W., 2003. In Situ Remediation of Arsenic in Simulated Groundwater Using
383 Zerovalent Iron: Laboratory Column Tests on Combined Effects of Phosphate and
384 Silicate. Environmental Science & Technology 37, 2582-2587.
385 Van Nooten, T., Lieben, F.o., Dries, J., Pirard, E., Springael, D., Bastiaens, L., 2007. Impact of
386 Microbial Activities on the Mineralogy and Performance of Column-Scale Permeable
387 Reactive Iron Barriers Operated under Two Different Redox Conditions. Environmental
388 Science & Technology 41, 5724-5730.
389 Vanbroekhoven, K., Van Roy, S., Diels, L., Gemoets, J., Verkaeren, P., Zeuwts, L., Feyaerts, K.,
390 van den Broeck, F., 2008. Sustainable approach for the immobilization of metals in the
391 saturated zone: In situ bioprecipitation. Hydrometallurgy 94, 110-115.

392 Waybrant, K.R., Ptacek, C.J., Blowes, D.W., 2002. Treatment of Mine Drainage Using
393 Permeable Reactive Barriers: Column Experiments. Environmental Science &
394 Technology 36, 1349-1356.
395 Wilkin, R.T., McNeil, M.S., 2003. Laboratory evaluation of zero-valent iron to treat water
396 impacted by acid mine drainage. Chemosphere 53, 715-725.
397 Wu, S., Jeschke, C., Dong, R., Paschke, H., Kuschk, P., Knöller, K., 2011. Sulfur transformation
398 in pilot-scale constructed wetland treating high sulfate-containing contaminated
399 groundwater: A stable isotope assessment. Water Research 45, 6688-6698.
400

401 **Table and Figure Caption**

402

403 **Table 1**

404 Groundwater characteristics.

405

406 **Table 2**

407 Dissolved sulfate (noml/L), sulfur ($\delta^{34}S$, ‰) and oxygen isotope ($\delta^{18}O$, ‰) values in the
408 columns with time (days).

409

410 **Figure 1**

411 Column setup (left schematic diagram and right a picture of column setup in the laboratory).

412

413 **Figure 2**

414 Sulfate concentration (mmol/L) as a function of time (days) in column outlets.

415

416 **Figure 3**

417 (a) Sulfate Reduction Rate (SRR, $nmol.cm^{-3}.h^{-1}$) in biotic columns as a function of time (days).
418 (b) sulfur isotope values ($\delta^{34}S$, ‰) as a function of Sulfate Reduction Rate (SRR, $nmol.cm^{-3}.h^{-1}$)
419 in biotic columns.

420

421 **Figure 4**

422 Photographs of DAPI staining (a) gFe° biotic column and (b) mFe° biotic column.

423

424 **Figure 5**

425 Sulfur isotope variation ($\delta^{34}S$, ‰) as a function of time (days) in biotic (red) and abiotic columns
426 (blue).

427

428 **Figure 6**

429 Comparison of dissolved sulfate (mmol/L) and δ^{34} S(‰) as a function of time for both biotic
430 columns (micro mFe° and granular gFe°).

431

432 **Figure 7**

433 Oxygen isotope ($\delta^{18}O$, ‰) values in columns as a function of time (days).

434

435 **Figure 8**

436 $\delta^{34}S$ (‰) vs. $\delta^{18}O$ (‰) in sulfate for biotic columns.

437
438
439
440