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Abstract

This study focused on a pilot scale infiltration of denitrified wastewater through artificially-created soils. The hydraulic performance and sulfide production were evaluated to ensure the system's longevity over the time period needed for autotrophic denitrification. Experiments were carried out over a year in two reactors of 200-L capacity. Sandy and sandy loam soils were tested to represent highly permeable ($K_s = 0.028 \text{ cm/s}$) and permeable ($K_s = 0.0013 \text{ cm/s}$) soils, respectively. The infiltration of denitrified wastewater at a continuous hydraulic rate (130 and $70 \text{ L/m}^2/\text{day}$) through these soils did not lead to the production of large amounts of gaseous hydrogen sulfide ($[\text{H}_2\text{S}] < 2.1 \text{ ppm}$) or aqueous sulfides ($[\text{HS}^- + \text{H}_2\text{S}] < 0.7 \text{ mg/L}$) sulfides in both feeding influent and effluents of the 200-L reactors. Considering the hydraulic performance, no loss in the infiltration capacity was recorded for the sandy soil, whereas a clogging phenomenon was observed after 37 days for the sandy loam soil. Two factors were responsible for this clogging phenomenon. A fine brown layer, known as a biomat, was formed on the infiltrative surface (IS) of the soil, which led to the formation of iron ochre at the bottom of the reactor. As an ascertainment due to the clogging phenomenon faced, sandy soil appeared to be the best choice as it didn't contain organic matter, which could lead to the biomat formation and therefore, to a clogging phenomenon.

Keywords: domestic wastewater; autotrophic denitrification; sulfide generation; clogging phenomenon.

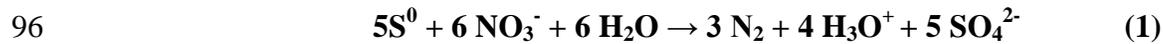
48 **INTRODUCTION**

49 In rural areas, residential wastewater is usually managed using an on-site wastewater
50 system, commonly called septic systems. According to Canada Statistics (2011), 13.3 M
51 households were not connected to a centralized system. Among them, 1 M are in the province of
52 Quebec (MDDEFP, 2013). A septic system typically consists firstly in a physical treatment using
53 a septic tank. The septic tank ensures the settling of heavy solids to form a sludge and the
54 flotation of the light suspended solids and grease to form a scum layer. According to MDDELCC
55 (2015), discharge standard requires that suspended solids in wastewater from septic tank should
56 be lower than 100 mg/L. In addition to the septic tank, the wastewater joins a drain field which
57 ensures the biodegradation of organic matter and the reduction of pathogenic organisms. Studies
58 on the effectiveness of underground disposal shows that wastewater has a high acceptable quality
59 in terms of Biochemical Oxygen Demand after 5 days (BOD₅), suspended solid, phosphorus,
60 nitrogen. According to these studies, fecal coliforms are closed to zero. However, depending on
61 the local regulations, more compact biological processes, such as aerobic treatment units (ATUs)
62 or biofilters, can be installed after the septic tank, replacing drain fields (Buchanan, 2014). In
63 such cases, the treated wastewater flows to an infiltration bed (sometimes referred to as a
64 polishing bed) into which it infiltrates. Thus, the treated wastewater undergoes an additional
65 purification step through the soil before reaching the groundwater. This purification zone allows
66 further filtration of particulates and biomass growth and provides low-cost reduction in the
67 concentrations of some contaminants (Lowe and Siegrist, 2008). However, some deficiencies can
68 remain because of the biological, chemical and/or physical interactions that occur between the
69 intrinsic components of the soils and the remaining residual contaminants in treated wastewater
70 (Baveye et al., 1998).

71 The infiltration capacity is mainly evaluated by the saturated hydraulic conductivity (Ks),
72 which measures the ability of soil to transmit water (Lindbo, 2014). For example, the hydraulic
73 conductivity of highly restrictive soil such as clay is 100 to 1 000 times lower than that of highly
74 permeable sandy soils (Baveye et al., 1998). According to Lowe and Siegrist (2008), sandy and
75 sandy silty soils exhibit good infiltration and follow an unsaturated flow regime. Over time, with
76 a continuous infiltration of effluent, wastewater can reach the saturated hydraulic conductivity
77 zone of a soil. Furthermore, after a long period of constant wastewater infiltration, a biozone or
78 biomat might form on the infiltration surface (IS) (Lowe and Siegrist, 2008). Both of these
79 phenomena are due to the accumulation of microbial biomass and/or organic matter in the soil
80 (Lowe and Siegrist, 2008). These phenomena are usually welcomed, as they result in more
81 uniform infiltration and, to a certain degree, better wastewater purification through
82 biotransformation and/or sorption of the contaminants (Hargett et al., 1981; Lowe and Siegrist,
83 2008; Siegrist and Thresher, 1985). However, over time, the infiltration capacity of treated
84 wastewater through the soil seems to be influenced by the nature of the biomat. According to
85 White and West (2003), the formation and nature of a biomat have a more important effect on
86 the infiltration of treated wastewater than the initial soil permeability. Acting as a barrier, the
87 biomat influences the infiltration capacity of the treated wastewater through the soil, leading to a
88 clogging phenomenon, which enhances hydraulic failure and oxygen depletion, leading to
89 anaerobic conditions in the soil (Siegrist and Thresher, 1985). Many biological and chemical
90 reactions could occur depending on the components present in both treated wastewater and soil.
91 Therefore, it is important to ensure the harmlessness of the by-products.

92 Many studies investigated the performance of an autotrophic denitrification system to
93 reduce nitrates to nitrogen gas from the water using elemental sulfur (Equation 1) (Ben-Khaled,
94 2016; Christianson et al., 2015; Kimura et al., 2002; Soares, 2002; Van Der Hoek et al., 1992).

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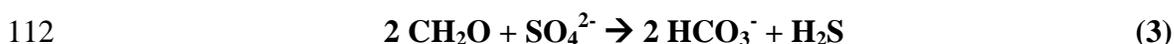
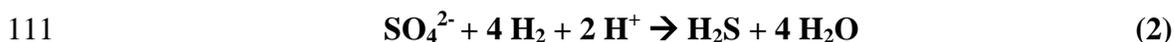


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98 Ben-Khaled (2016) used elemental sulfur and limestone in an on-site treatment system. In
99 another study, elemental sulfur and oyster shells were also shown to be highly efficient in
100 removing nitrates and nitrites (100% nitrate removal), with initial concentrations ranging from
101 25 to 30 mg/L from marine water systems (Simard et al., 2015). In these processes, denitrifying
102 bacteria use nitrate as an electron acceptor, sulfur as an electron donor and an inorganic source of
103 carbon such as CO_2 and HCO_3^- (Zhang and Shan, 1999). The main advantage of these processes
104 is that no external carbon is required. However, one of the main drawbacks is the release of high
105 quantities of sulfates (100-200 mg/L), the degree to which depends on the initial concentration of
106 nitrate ions present in water and the removal capacity of the process used.

107 Despite the fact that many studies pointed out the high performances of nitrate removals
108 from wastewater using an autotrophic denitrification process, few of them were interested in the
109 potential production of sulfides following Equations 2 and 3.

110



113 Table 1 summarizes some recent researches related to the potential release of sulfide
114 products from denitrified groundwater or domestic wastewater. Among these studies, Zhang and
115 Shan (1999) mentioned a sulfide concentration ranging from 1.5 to 10 mg/L during the treatment
116 of an effluent from a septic tank, using sulfur and limestone. Otherwise, the other authors briefly
117 mentioned that the production of sulfide products is very low, without quantifying them (Sierra-
118 Alvarez et al., 2007; Xu et al., 2016). Meanwhile, other workers did not detect sulfide generation
119 while removing nitrates from groundwater (Kimura et al., 2002; Soares, 2002).

120 According to the Eh-pH diagram, sulfide may be present in liquid phase under different forms
121 (H_2S , HS^- or S^{2-}) (Lens and Hulshoff Pol, 2004). However, only the molecular sulfide form (H_2S) can be
122 distributed between the gas and the liquid phases. These concentrations are related to the solubility of
123 $H_{2S(aq)}$ and the partial pressure in air following the Equation 4, involving the Henry's law constant (K_H).
124 For instance, the Henry's law constant is estimated at 0.131 mol/L/bar at 1 bar and 15°C (Reddy
125 and DeLaune, 2008).

126

$$127 \quad [H_2S]_{aq} = K_H P_{H_2Sg} \quad (4)$$

128

129 where $[H_2S]_{aq}$ is the concentration of H_2S in liquid phase (mol/L), K_H is the Henry's law
130 constant (mol/L/bar) and P_{H_2Sg} is the partial pressure of H_2S in the air (bar).

131

132 During the infiltration of denitrified wastewater (DW) through the soil, the sulfate-
133 reducing bacteria (SRB) present in soils can use the organic matter as electron donors and the
134 sulfates as electron acceptors, resulting in the reduction of sulfate to sulfide (Zhou et al., 2011).

135 Sulfide generation could be responsible for many bad effects such as negative impacts on human
136 health, unpleasant odor and corrosion. The effects of sulfide on human health vary from a simple
137 olfactory irritation to severe lung irritation, dizziness and collapse leading to death within 4 – 8 h
138 after exposure (above 500 ppm) (Beauchamp et al., 1984; Chou, 2003; Glass, 1990; Partlo et al.,
139 2001; Reiffenstein et al., 1992). All these drawbacks could highly affect septic system
140 performance and be harmful for users. The SRB activity is dependent upon factors such as the
141 temperature, the pH of the wastewater, anaerobic conditions and the availability of a
142 biodegradable carbon source (Barton and Hamilton, 2007). Liang (2008) emphasizes that a
143 temperature varying between 28 and 32°C is optimum for most SRB. Moreover, the SRB species
144 usually exist in a range of pH varying between 5.5 and 9.0 (Liang, 2008; Nielsen et al., 1998).
145 Thus, the SRB are able to produce sulfide under the usual pH and temperature conditions
146 encountered in soils. Anaerobic environments are highly dependent on the Oxidation Redox
147 Potential (ORP) of the media, which should be lower than -100 mV to support SRB activity
148 (Abhilash et al., 2015; Huan et al., 2013). Some SRB species can use H₂ as an electron donor or,
149 more commonly, simple or complex organic matter such as propionate, butyrate, lactate, ethanol,
150 pyruvate, malate, fumarate and glycerol (Liu et al., 2015).

151 Over the last years, many researchers have developed autotrophic denitrification
152 processes to meet the regulations, which are more stringent in terms of contaminant releases
153 from wastewater to the environment and especially nitrates. Despite the fact that these processes
154 allowed the decrease of the concentration of nitrate below 5 mg NO₃⁻-N/L, avoiding
155 eutrophication and cyanobacteria developments, they require a better understanding of the
156 behavior of by-products generated such as sulfate to ensure the safety of these processes. In this
157 context, this research project aims to ensure that there is no generation of by-products during the

158 denitrification of domestic wastewater using elemental sulfur and limestone, which can represent
159 a potential risk for the environment. More specifically, it was necessary to ensure that there is no
160 reduction of sulfates into aqueous and/or gaseous sulfides during the infiltration of the denitrified
161 wastewater through soils having different permeabilities at a pilot scale.

162 **MATERIALS AND METHODS**

163 **Experimental set-up**

164 Pilot-scale experiments were conducted at an experimental wastewater treatment station
165 located in Rivière-du-Loup (Quebec, QC, Canada). Figure 1 presents a schematic diagram of the
166 septic system used at the experimental treatment station. During the sampling period, the average
167 temperature in the city varied between -29.8 and +22.7°C (Canada, 2014-2015). However, for
168 decentralized residential sites, the temperature of the raw wastewater exiting a house rarely drops
169 below 10°C.

170 As shown in Figure 2 (a, b), two 200-L-capacity reactors (internal diameter of 58 cm and
171 height of 95 cm) made of high-density polyethylene (HDPE) were installed at the experimental
172 wastewater station. These experiments were carried out over a year using two different soil
173 samples [sandy (100% sand) and sandy loam (85% sand-15% loam)]. The sand used for these
174 experiments was obtained from Biomix (Quebec, QC, Canada), while the loam fraction used in
175 the soil mixture was collected along the St. Charles River (Quebec, QC, Canada) and passed
176 through a 53- μ m sieve. The loam fraction was then mixed with a beach sand fraction (15%-85%)
177 to obtain the targeted properties of a permeable soil. The gravel, devoid of its fine particles,
178 represents the distribution system over the infiltration area and was of a size greater than or equal
179 to 1.5 cm. Approximately 180 kg of sand was used to fill the first reactor (60 cm in height),
180 while a mixture of 105.5 kg of sand and 18.6 kg of loam was used to fill the second reactor

181 (30 cm in height). Actually, the septic field design was based on the regulation established by the
182 Province of Quebec (MDDELCC, 2015). According to this regulation, soil height depends on its
183 permeability (highly permeable and permeable soils require 60 and 30 cm of natural soil,
184 respectively). Moreover, this regulation indicates that the septic field should be composed of
185 back fill (60 cm), gravel (30 cm) and natural soil (30 or 60 cm), which represents at least 120 cm
186 depth (3.93 ft depth). Bulk densities were measured according to the real diameter of 50 cm
187 instead of 58 cm (presence of tarpaulin and plastic layers). Thus, sand reactor density was
188 estimated at 1.52 g/cm³ (calculated by using the diameter of 50 cm and the height of 60 cm for
189 the volume and 180 000 g for the mass), while, the bulk density of the sandy loam reactor was
190 estimated at 2.1 g/cm³ (calculated by using the diameter of 50 cm and the height of 30 cm for the
191 volume and 124 100 g for the mass).

192 **Denitrified domestic wastewater**

193 The pilot-scale reactors were fed in semi-continuous mode with DW emerging from a
194 decentralized wastewater treatment plant, producing approximately 1 500 L/day (representative
195 of a residence with three bedrooms) (MDDELCC, 2015). Table 2 summarizes the main
196 characteristics of this DW (the influent of the present study). Then, the reactors were daily
197 supplied with fresh denitrified wastewater, which was twice a week collected and characterized
198 with reactors effluents during the 365 days of experiments. The entire process chain consisted of
199 a septic tank, an organic-based biofilter and a sulfur- and limestone-based autotrophic
200 denitrification reactor (Figure 1). This latter allows to reach nitrate concentration, during the
201 sampling period, under 5 NO₃⁻N-mg/L.

202 To simulate a conventional infiltration of the treated wastewater through the soil, two
203 multichannel peristaltic pumps were used to deliver the wastewater continuously into the reactors

204 during the feeding period via a system that allowed for homogeneous distribution over the entire
205 surface of the reactor, thus avoiding the surface effect (Figure 2c.). The feeding period (supply
206 sequences) lasted 3 h each day (from 8 a.m. to 9 a.m., from 10 a.m. to 11 a.m. and from 12 p.m.
207 to 1 p.m.). In the present study, the wastewater loading rates were based on an isolated three-
208 bedroom household (MDDELCC, 2015). Based on the regulation established by the Province of
209 Quebec concerning the infiltration of domestic wastewater (MDDELCC, 2015), the infiltration
210 of denitrified wastewater through different soils using typical hydraulic loading rates (HLR)
211 (65 L/m²/day for the highly permeable soil and at 35 L/m²/day for the permeable soil) has
212 already been studied at laboratory scale (Ghorbel et al., 2016). The results obtained at laboratory
213 scale showed that no important sulfide generation occurs after 104 days of experiments. Thus,
214 the HLR was doubled to get results in a reasonable experimental timeframe but also to afford
215 favorable conditions for sulfide generation (higher sulfate and organic matter concentrations)
216 (Lowe and Siegrist, 2008). Therefore, the daily HLRs were set at 130 and 70 L/m²/day for the
217 highly permeable and permeable soils, respectively. Twice a week, samples of approximately
218 240 mL were collected from the bottom of the reactors. Various parameters were measured on-
219 site at the inlet and outlet of the reactors.

220 **Determination of soil sample permeability**

221 The hydraulic conductivities of both sandy and sandy loam soils were determined using a
222 constant load test according to Darcy's Law based on Longpré-Girard et al. (2016) and Martel
223 and Gélinas (1996). For this purpose, a 2.54-cm-diameter and 45-cm high column was used. The
224 soil was gradually introduced into the column and compacted to obtain a relative soil density of
225 1.8 g/cm³. Two types of saturation were then applied to the system. First, carbon dioxide (CO₂)
226 was circulated at low pressure (3 - 5 psi) for 30 min, and then, upward flowing water saturation

227 was applied using a Mariotte vessel (Musy and Soutter, 1991). The first CO₂ saturation was
228 applied to remove the oxygen, while the second water saturation was conducted to provide a
229 constant pressure head on the tested material. According to Darcy's Law, the permeability (Ks)
230 was determined by measuring the outlet flow from the column as follows:

231

$$232 \quad K_s = Q L / S \Delta h \quad (5)$$

233

234 *where Ks is the permeability coefficient (cm/s), S is the cross-sectional area of the column*
235 *(cm²), L is the column length (cm), Δh is the loading difference between the input and output (cm)*
236 *and Q is the outlet flow rate (cm³/s).*

237

238 **Analytical methods**

239 A laser particle sizer (Partica Laser Scattering LA-950V2, Laser Particle Size Analyzer,
240 Tokyo, Japan) was used to determine the particle size distribution of the different soil samples.

241 The concentration of the organic carbon present in the soils was determined using a
242 CHNS Leco Analyzer (TruSpec Micro, Michigan, MI, USA). During each series of
243 measurement, two certified control powders have been used (Sulfamethazine from USA and
244 OAS from UK) to check the accuracy of the results. The concentration of the dissolved organic
245 carbon present was determined using a TOC-analyzer (TOC-VCPH model, Shimadzu Scientific
246 Instruments, Columbia, MD, USA). A certified organic carbon control solution (100 μg/mL)
247 from SCP Science (Canada) was used to control of the accuracy of the results. Using a H₂S
248 detector (ToxiRAE Pro, San Jose, CA, USA), the gaseous hydrogen sulfide was measured at the

249 outlet of the autotrophic denitrification system, at the inlet and above each reactor. As reactors
250 were buried, H₂S detector was introduced carefully above the soil in air compartment to perform
251 the measure.

252 The pH, ORP and Dissolved Oxygen (DO) were measured using a Thermo Scientific
253 Orion STAR A322 conductivity portable meter. Before each series of pH measurements, a
254 Ag/AgCl reference cell was calibrated using certified solution (pH = 2.00, 4.00, and 7.00). The
255 ORP cell was verified before each series of measurements using a certified solution (ORP =
256 470 mV).

257 All colorimetric methods used for the determination of sulfate, sulfide and Chemical oxygen
258 demand (COD) contents were performed using a UV-Vis spectrophotometer (Varian Cary® 50,
259 Varian Inc., Mississauga, ON, Canada). The sulfate analyses were performed using a modified
260 version of the method described by Bertolacini et al. (1957). A certified control solution (Multi-
261 element Ion Chromatography standard sol II in H₂O, Fluka, Sigma Aldrich, Canada) and an
262 ammonium sulfate solution (10 000 µg S/mL, Plasma Cal SCP Science, Canada) were used to
263 validate the accuracy of the measurements.

264 The dissolved sulfide analyses were also performed using a modified colorimetric method
265 according to Cline (1969). The chemical oxygen demand (COD) was determined according to
266 the method MA. 315 - COD 1.1 developed by the CEAEQ (MDDEFP, 2014). The calibration
267 curve was prepared from a potassium biphthalate stock solution (10 000 mg O₂/L). Additionally,
268 a certified control solution (500 mg/L solution, calibration standard, RTC) was used to ensure the
269 quality of the results for each series of measurements.

270 **RESULTS AND DISCUSSION**

271 **Pilot-scale experiments**

272 In the following study, the pilot-scale experiments were carried out on two different soil
273 samples (highly permeable and permeable). According to the particle size distribution (Ghorbel
274 et al., 2016), the mean particle size was estimated to be 378 and 269 μm , whereas the D_{10} values
275 were approximately 163 and 30 μm for the highly permeable and permeable soils, respectively,
276 indicating that the latter contained higher amounts of small particles than the highly permeable
277 soil. Their hydraulic conductivities (K_s) were estimated at 0.028 cm/s for the highly permeable
278 soil (100% sand) and 0.0013 cm/s for the permeable soil (85% sand-15% silt) (Gouvernement du
279 Québec, 2015). The experiments were conducted on the highly permeable soil (reactor R1) over
280 a single year, but the assays carried out on the permeable soil (reactor R2) were stopped after
281 10 weeks of operation due to a clogging phenomenon. The factors that affected this clogging are
282 discussed in section 3.2.

283 The evolution of the various parameters studied for both reactors R1 and R2 are presented
284 in Figures 3 and 4. Table 2 shows the main characteristics of the feed solution (sampled from a
285 20-L-capacity storage tank of DW produced by the denitrification system). According to these
286 results, the ORP value was approximately -40 mV, and the DO was estimated at 5.59 mg/L.
287 These values were different from the ORP (-300 mV) and DO (< 2 mg/L) of the DW measured
288 directly in the denitrification system, which were very low (Ben-Khaled, 2016). The storage tank
289 was closed tightly and directly connected by pipes to the denitrified reactor. The DW from the
290 tank then passed through the top of the reactors, simulating the infiltration of DW through the
291 soil.

292 Despite that no open-channel flow was used from the pump to the reactor and that only
293 closed-conduit flows were used, we have noticed that dissolved oxygen values increased
294 between the denitrified wastewater and those directly measured in the denitrification reactor.
295 Thus, the pipes ensure the aeration/oxygenation of the DW, which is very beneficial to avoid
296 sulfide generation. This can also be achieved by installing a drain field, as it is separated from
297 the wastewater distribution box by pipes (Service des eaux municipales, 2007). Sulfide
298 generation is highly dependent on the environmental conditions of the medium such as
299 temperature, pH and ORP. The evolution of temperature, pH, ORP and DO of the feed solution
300 (DW) and of the effluents from reactors R1 and R2 during sampling are presented in Figure 3.

301 According to our results presented in Figure 3a, the temperature of the feed solution
302 (DW) and of the effluents from the reactors R1 and R2 did not reach below 4.6°C during the
303 winter (day 1 to 163), while the outside temperature reached -20°C (Figure 3a). The system was
304 underground and insulated by the soil, which prevented its freezing. During the summer (day 164
305 to 346), the highest temperature recorded for the effluents from reactors R1 and R2 was 19°C.
306 According to Huan et al. (2013), low temperatures (lower than 7°C) could highly affect and
307 delay the generation of gaseous and aqueous sulfides. However, an increase in the temperature
308 from 15 to 38°C lead to a 7% increase in the sulfide production, reaching its maximum at 30°C
309 (Huan et al., 2013). Indeed, in the present study, while the temperature gradually increased
310 during summer, a generation of aqueous and gaseous sulfides was recorded for the feed solution
311 with levels reaching 0.7 mg/L and 2.1 ppm, respectively.

312 Another important parameter has also been emphasized by many authors, who indicated
313 that a wide range of pH (4.5 and 9.2) seemed to be suitable for the generation of sulfide
314 (Benedetto et al., 2005; Cervantes et al., 2006; Gutierrez et al., 2009; Holland and Turekian,

2010; Liu et al., 2015). According to Figure 3b, the pH of the feed solution (DW) and of the effluent emerging from the highly permeable (reactor R1) and permeable (reactor R2) soils were quite stable over the period of 365 days, with average values estimated at 6.68 for the feed solution (DW), 7.00 for the effluent of reactor R1 and 7.30 for the effluent of reactor R2.

As shown in Figure 3, there was an obvious correlation between the pH and the temperature. Actually, the higher temperatures of the summer caused an increase in molecular vibration, which consequently led to a decrease of hydrogen bonds being formed, leading to an increase of H^+ and a decrease in pH (Down and Lehr, 2005). However, the increase of pH values for the effluents emerging from the reactors occurred due to the basic initial soil pH (8.40 and 9.95 for the highly permeable and permeable soils, respectively). In addition, a slight increase of the pH values can be due to the release of CO_2 into the environment during microbial activity as explained by Deepa and Krishnaveni. (2012) and Yuan et al. (2013). The latter has previously been mentioned by Deepa and Krishnaveni (2012) for treated municipal wastewater (pH varying between 7.4 and 8.0) after they passed through columns containing soil with an initial pH of 6.8. Moreover, such results have also been mentioned by Yuan et al. (2013) in a similar study with an increase of pH values from the top (7.0-7.5) to the bottom of the soil column (8.5-9.0). However, pH values were still in the optimum range mentioned by Yeh (2000) for the production of sulfide due to the reduction of sulfates by SRB.

The values of the ORP measured for the feed solution (DW) varied over the sampling period. The ORP values measured downstream and into the denitrification reactor were estimated at -40 mV and -200 mV, respectively (Figure 3c). Indeed, a large variation in the ORP values was observed at the beginning, with a significant decrease from the 64th to the 191st day, and then an average value of -94 ± 45 mV ($n = 25$) was attained. After this period, the ORP

338 values of the feed solution (DW) increased, becoming positive with an average value of
339 $+101 \pm 42$ mV ($n = 40$). Moreover, it appeared that ORP is highly dependent on the pH value
340 (James, 2004). We can hypothesize that the decrease in pH (due to the increase in the
341 temperature), starting from the 200th day, had a large impact on the ORP values, causing them to
342 increase. Despite the fluctuation observed at the beginning of the experiments, the ORP values of
343 both reactor R1 and reactor R2 effluents increased throughout the sampling period, with average
344 values of $+103 \pm 45$ ($n = 77$) and $+75 \pm 15$ ($n = 6$) mV, respectively. Such ORP values indicate
345 an environment that is insufficiently reducing to generate sulfide formation (Annable, 2008).
346 Indeed, many authors have noted that ORP values of at least -100 mV are required for the SRB
347 activity (Abhilash et al., 2015; Huan et al., 2013). The same observation can be made for the
348 evolution of the DO of the feed solution (DW) over the sampling period. Indeed, the results
349 revealed an important fluctuation of the DO at the beginning of the experiments, followed by an
350 increase after the 66th day. The average DO concentration was estimated at 4.9 ± 1.4 mg/L ($n =$
351 71) over the one-year sampling period. The concentration of the DO measured in both the
352 reactors R1 and R2 effluents were always higher at 7.8 ± 1.5 and 5.2 ± 1.4 mg/L, respectively,
353 than that of the feed solution (4.9 mg/L), indicating that the system aerated the water. According
354 to several studies, these conditions of high ORP values and DO concentrations prevent the
355 production of sulfide, as the SRB need very low ORP values and anaerobic conditions to grow
356 (Annable, 2008). In contrast to clay soils, sandy and sandy loam soils, mainly composed of large
357 and coarse grains, are well known for their good water percolation and gas exchange abilities.
358 These qualities reduce the development of anaerobic zones and, consequently, the reduction of
359 sulfates to sulfides by the SRB (Anderson and Halsey, 1980).

360 Figure 4 presents the evolution of the concentration of Dissolved organic carbon (DOC),
361 sulfate and aqueous ($\text{H}_2\text{S}_{(\text{aq})}$) and gaseous sulfide ($\text{H}_2\text{S}_{(\text{g})}$). Despite the high hydraulic
362 conductivity of sandy soils, and the short residence time of the DW (Sandhu, 2013), a slight
363 purification was observed during the infiltration of the DW ($\text{DOC} = 8.5 \pm 1.8 \text{ mg/L}$; $n = 77$)
364 through the highly permeable soil (reactor R1), leading to further degradation of the remaining
365 organic matter ($\text{DOC} = 6.8 \pm 1.7 \text{ mg/L}$; $n = 90$) (Figure 4a). Regarding the sandy loam soil, the
366 average value of the DOC ($9.5 \pm 3.2 \text{ mg/L}$; $n = 15$) was higher than the values measured for the
367 feed solution ($\text{DOC} = 8.5 \pm 1.8 \text{ mg/L}$; $n = 77$). This difference could be explained by the quantity
368 of the organic carbon initially present in this soil (0.33%) in comparison to the sandy soil
369 ($< 0.05\%$) and by the release of organic matter from the silt present in a higher proportion in the
370 sandy loam soil. Moreover, the concentrations of the sulfates measured in the reactors R1 and R2
371 effluents, 146 mg/L and $161 \pm 39 \text{ mg/L}$ ($n = 15$), respectively, were similar to that of the feed
372 solution ($137 \pm 43 \text{ mg/L}$; $n = 81$), indicating that the SRB did not reduce sulfates to sulfide in
373 both types of soils (Figure 4b).

374 Denitrified wastewater was measured at the output of the denitrification process before
375 reaching the storage tank from which we supplied the reactors. Over the one-year sampling
376 period, the highest level of $\text{H}_2\text{S}_{(\text{g})}$ recorded was around 14 ppm for the output directly of the
377 denitrification reactor and at 2.1 ppm for the feeding solution (DW pumped from the storage
378 tank) (Figure 4d). This sulfide concentration (14 ppm) produces an offensive and foul odor
379 (Chou, 2003). According to the Canadian Centre for Occupational Health and Safety (2005), a
380 concentration of 100 ppm causes severe symptoms such as respiratory tract, eye irritation and
381 loss of smell. A range of concentrations varying between 500 and 1 000 ppm leads to collapse
382 and even death. The concentration of gaseous hydrogen sulfide in reactors R1 and R2 did not

383 exceed 0.5 ppm, indicating that no hydrogen sulfide was produced during the infiltration of DW
384 through both the highly permeable and permeable soils. This observation was confirmed by the
385 evolution of the sulfate concentrations over the sampling period, which remained similar to the
386 initial concentration of the feed solution.

387 Although the COD/SO₄²⁻ ratio occasionally reached 2.3 (on the 308th day), which is higher
388 than the minimal ratio of 0.67 indicated in other studies for the production of H₂S (Biswas and
389 Tapas, 2012; Dar et al., 2008; Velasco et al., 2008), no sulfide generation occurred.
390 Theoretically, there was enough organic matter in the DW (up to a ratio of 2.3) to promote the
391 activity of the SRB and therefore generate sulfide. However, the two soils used during these
392 experiments (highly permeable and permeable) enabled increases both of the DO (7.9 and
393 5.3 mg/L) and the ORP values (+103 and +75 mV), which are critical parameters for the activity
394 of the SRB and therefore the production of sulfide.

395 There are some differences between natural soils and the artificially-created soils used in
396 the present study that can affect the observation made related to the generation of sulfide during
397 the infiltration of denitrified wastewater through the soils. Natural soils have a high spatial
398 heterogeneity with respect to their physical, chemical and biological properties. Natural soils are made
399 of solid matter, water and air. The solid matter could be composed of organic or mineral material. The
400 bulk density ranges from values lower than 0.5 g/cm³ in organic soils to 1.8 g/cm³ in mineral soils
401 (Reddy and DeLaune, 2008). Depending on the season, these soils could be under drained or flooded
402 conditions, which highly affect air-filled pore space of soil and thereby the soil aeration by oxygen
403 diffusion. This latter governs most of the biogeochemical reactions. Thus, from a biological point of view,
404 facultative and obligate anaerobes bacteria predominate when the oxygen is depleted, generally in poorly
405 drained soils with fine-textured soils. The oxidized compounds are reduced through chemical and

406 biological processes, whereas aerobic bacteria predominate in well-drained, coarser-textured soils and
407 oxidize the reduced forms present in soils. Moreover, compared to the artificial soil columns
408 (mineral soil), oxygen governs most of the biogeochemical reactions. It is important to mention
409 that the oxygen in soil is needed for microbial respiration but also to plant root respiration
410 through diffusion and mass flow. Moreover, the application of animal waste, compost, sewage
411 sludge or fertilizer can also result in oxygen depletion in the soil (Reddy and DeLaune, 2008).

412 **Clogging phenomenon**

413 The accumulation of treated wastewater at the surface of the reactor (persistent ponding)
414 was first detected in the sandy loam soil after 37 days of continuous flow rate infiltration. Once
415 the overflow occurred, wastewater feeding was stopped, and the water level was monitored at a
416 reference point. Figure 5 shows the ponding depth (level of the liquid accumulated on the
417 surface) for a period of 35 days. According to the results, over time, the clogging of the system
418 led to a decrease in the infiltration rate. The numerous reasons responsible for this clogging
419 phenomenon will be explored below. During the first 15 days after ponding, the system was fed
420 with 17.2 L of DW per day and then stopped; the infiltration rate of the wastewater was
421 followed. According to the results, the infiltration velocity of the DW into the soil was estimated
422 to be approximately 1.6 cm/day from day 0 to day 15. Thereafter, the pump was switched on,
423 and the permeable soil reactor was fed with the DW for another 7 days. However, the ponding
424 phenomenon persisted, and the infiltration velocity decreased, leading to an average value of
425 0.5 cm/day that tended to reach a steady state. As mentioned by Lowe and Siegrist (2008), this
426 state is called the “end state”. This state occurred in the reactor R2 (sandy loam soil) after 9
427 weeks of infiltration, while the 1st reactor R1 (sandy soil) never reached the end state. When the
428 reactor R2 reached this end state, the feeding of this reactor was completely stopped, and the soil

429 profile was analyzed to understand why the clogging phenomenon occurred. For the same soils,
430 no rapid and severe clogging had occurred during previous laboratory-scale experiments carried
431 out by Ghorbel et al. (2016). These differences between laboratory and pilot scales might be due
432 to the higher loading rate that can lead to a decrease in the infiltrative capacity and a loss of
433 permeability, as discussed by Hargett et al. (1981). Several hypotheses were explored to explain
434 the clogging phenomenon, which occurred in the second reactor with the permeable soil at pilot
435 scale. The reactor was fully dismantled and soil cores were sampled as a function of depth in
436 several separate sections of about 1 cm each. Thus, tests were conducted on thin soil sections
437 collected at 17 different depths. The particle size distribution, moisture and organic carbon
438 contents of these thin sections were measured to identify the chemical or physical changes. The
439 results are presented in Figure 6.

440 First, the potential migration of the fine particles through the sandy particles (thereby
441 limiting the infiltration of the DW) was explored. Figure 6a shows the particle size distribution
442 of the 17 different soil sections of the second reactor as a function of the particle diameter.
443 Regardless of the depth of the sections, the results showed a good superposition of the particle
444 size distribution curves, indicating that no fine particles had migrated to the gravel at the bottom
445 of the reactor. Therefore, it was concluded that the observed clogging phenomenon was not due
446 to the migration of fine particles creating an impermeable layer.

447 Figure 6b presents the moisture content measured for the 17 different thin soil sections
448 collected. Regardless of the depth of the sections, a soil moisture content of $12.5 \pm 1.1\%$ ($n = 17$)
449 was measured for almost all soil samples. Unfortunately, these results did not allow for the
450 detection of variations in soil moisture contents in the soil profile.

451 Next, further investigations were carried out to explain the clogging effect. Lowe and
452 Siegrist (2008) assumed that clogging is a highly time-dependent process during which
453 wastewater pollutants such as humic matter fills the pore of the soil. Other authors found that
454 biological activities, especially under aerobic conditions, lead to the formation of by-products
455 such as an extra polymeric substance (EPS), which cause the clogging phenomenon (McKinley
456 and Siegrist, 2011; Winstanley and Fowler, 2013). Additionally, many studies have shown that
457 the presence of organic carbon in the infiltration surface causes substantial soil clogging (Siegrist
458 and Thresher, 1985; Winstanley and Fowler, 2013). Figure 7a shows a thin brown layer of
459 approximately 5 mm, which formed on the infiltration surface of the reactor R2. This thin layer
460 had a different color than the rest of the soil profile, which was similar to the color of the initial
461 soil. This layer, also referred to as a biomat, is generally due to the deposition of organic matter.
462 It usually influences the infiltration capacity of a soil regardless of its initial permeability (White
463 and West, 2003). The organic carbon analysis, performed on the different soil samples collected,
464 showed that the quantities of organic carbon were similar across all of the 17 soil samples (less
465 than 2.0%). However, the biomat layer showed a high enrichment of organic matter with a
466 quantity of organic carbon reaching 7.5% (Figure 6c). Therefore, the biomat highly influenced
467 the infiltration of treated wastewater through the soil by clogging the surface.

468 During the complete dismantling of the second reactor (permeable soil), a red and
469 gelatinous layer was found at the bottom, which prevented the complete removal of the DW from
470 the reactor (Figure 7b). A complete analysis of this sludge showed that its ferric content of
471 112 g/kg was 14 times higher than the 7.70 g/kg of the initial soil sample. As described by Ford
472 (1985), iron sludge can play an important role in the clogging phenomenon. Moreover, Smart
473 and Herbertson (1992) correctly described this red-brown gelatinous material as iron ochre.

474 Ferrous iron compounds were oxidized deep in the soil to insoluble ferric oxide (Fe_2O_3),
475 blocking tank adapter and causing the clogging phenomenon (Smart and Herbertson, 1992).
476 According to Lindbo et al. (2014), the biological oxidation of iron requires free oxygen, a source
477 of organic matter and iron and a temperature above 5°C . Other key factors for soil saturation and
478 iron redox reactions include retention time and soil depth (Lindbo, 2014). The presence of
479 stagnant water favors anaerobic conditions. Thus, the ferrous iron present in soil migrates to the
480 ponding-water using the iron-reducing bacteria (Ford, 1985), followed by their oxidation in the
481 interface between the anaerobic and aerobic zones, forming a layer. This process also depends on
482 the chemical species present in the submerged soil and on the second law of thermodynamics
483 (change in Gibbs free energy or ΔG°). Following a precise order of preference, bacteria first use
484 oxygen (O_2) to oxidize the organic matter, followed by nitrates (NO_3^-), oxide of manganese
485 (MnO_2), oxide of iron (Fe_2O_3), sulfate (SO_4^{2-}) and, finally, CO_2 for methane fermentation (Reddy
486 and DeLaune, 2008). Because iron ions precede sulfates, the presence of ferrous ions limits the
487 formation of sulfide ions (Baveye et al., 1998).

488 Therefore, unlike for the small scale (Ghorbel et al., 2016), where much lower sulfide
489 occurred (<0.15 ppm and <0.2 mg/L for gaseous and aqueous sulfide, respectively), a slight
490 emission of H_2S was observed in the feed solution, while temperature increased. This emission
491 of H_2S seemed proportionally related to changes in temperature and pH. Although the increase of
492 both temperature and pH is slight and restricted to the feed solution, this was responsible of the
493 increase of the solubility of organic compounds which were more available for the
494 microorganisms, leading to an increase of the speed of both biological reactions and conversion.

495 Moreover, the study of the infiltration in sandy soil proved that no clogging and sulfide
496 generation occurred whereas, we highlighted that, for the permeable soil, a biomat layer

497 appeared on the infiltrative surface followed by the formation of iron ochre at the bottom of the
498 reactor both causing the clogging phenomenon. Additionally, the biomat itself indirectly led to
499 the formation of the iron ochre. Actually, the appearance of the biomat layer first led to flood the
500 soil which became depleted in oxygen. This state allowed the ferric ions present in the soil to
501 migrate in water by means of iron-reducing bacteria (Ford, 1985) followed by the oxidation of
502 the latter in contact with air (bottom of reactor). This resulted in the formation of iron ochre at
503 the bottom of the reactor R2. Thus, these two phenomena are responsible for the phenomenon of
504 clogging observed in the reactor R2. Moreover, the reduction of ferrous ion
505 ($\Delta G^0 = -115.0$ kJ/mol) avoided sulfide generation in the same time as the sulfates reduction
506 ($\Delta G^0 = -104.7$ kJ/mol) for the permeable soil. High hydraulic loading rate could also be one of
507 the reasons that can be responsible to the clogging phenomenon observed for the reactor R2.
508 Actually, the high hydraulic loading rate induces the presence of high organic matter content to
509 the reactor, which may have accelerated the biomat formation in the infiltration surface.

510 Finally, we could assume that no important sulfide was generated downstream the
511 autotrophic-denitrification system during the infiltration of DW through highly permeable soil
512 over one year sampling. As mentioned by Sierra et al. (2007), we can also confirm that a further
513 treatment step (cascade aeration with a sand field) is important for a safe infiltration of the
514 denitrified soil, as long as the organic matter is present at a low amount. Indeed, a wastewater
515 with low BOD₅ will ensure the oxygen flow to the system (Erickson, 2000).

516 CONCLUSION

517 This study focused on the behavior of artificially-created soils infiltrated by treated
518 wastewater containing large quantities of sulfates from a decentralized domestic wastewater
519 treatment process based on autotrophic sulfur denitrification. The results indicated that the
520 wastewater treated by the autotrophic sulfur-limestone process did not lead to the production of
521 problematic amounts of aqueous or gaseous sulfide ($\text{H}_2\text{S}_{(\text{aq})} < 0.1 \text{ mg/L}$ and $\text{H}_2\text{S}_{(\text{g})} < 0.5 \text{ ppm}$) in
522 the outflow. The concentration of sulfide in the feeding solution (denitrified wastewater) did not
523 exceed 0.7 mg/L of aqueous sulfide and 2.1 ppm of gaseous sulfide. This observation might be
524 explained by the fact that the DO and ORP values did not reach target values conducive to the
525 SRB activity. Moreover, because this process was installed downstream of a secondary process,
526 there was less easily biodegradable residual organic carbon available for the SRB.

527 Another observation was the occurrence of a clogging phenomenon in the permeable soil.
528 Many hypotheses were proposed to explain the loss of permeability, which involved the
529 formation of a biomat layer on the infiltration surface, and the formation of iron ochre in the
530 sandy loam soil. To avoid these problems during the operation of a functioning full-scale
531 wastewater infiltration system, a further treatment step (cascade aeration) with a sand field is
532 important for a safe infiltration of the denitrified wastewater.

533 Further experiments should be performed with different hydraulic loading rates including
534 typical hydraulic loading rates (65 L/m²/day for the highly permeable soil and at 35 L/m²/day for
535 the permeable soil) and the same hydraulic loading rates as those used in this study
536 (130 L/m²/day for the highly permeable soil and at 70 L/m²/day for the permeable soil) to further
537 understand the formation of the clogging phenomenon. Moreover, denitrified wastewater
538 infiltration into a real soil (with higher organic matter and the presence of plants) would be very

539 interesting to have a better comprehension of the mechanisms responsible of the potential sulfide
540 generation.

541 **ACKNOWLEDGMENTS**

542 The writers are grateful for the financial and technical support of Premier Tech Aqua, the Natural
543 Sciences and Engineering Research Council of Canada and Research Funds of Quebec Nature
544 and Technology. Mrs. Ginette Bélanger and Mrs. Myriam Chartier are acknowledged for their
545 significant contribution. Sincere thanks are extended to Mrs. Jihen Ben Khaled and Mr. André
546 Ouellet

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708

709 **FIGURE LIST**

710 **FIG. 1** Schematic representation of the septic system used (modified from PTA,
711 2015)

712 **FIG. 2** Experimental design used for pilot scale experiments : a. Schematic
713 representation of the composition of the different reactors, b. Photography of
714 the reactors (by Leila Ghorbel) and c. Photography of the denitrified
715 wastewater distribution system (by Leila Ghorbel)

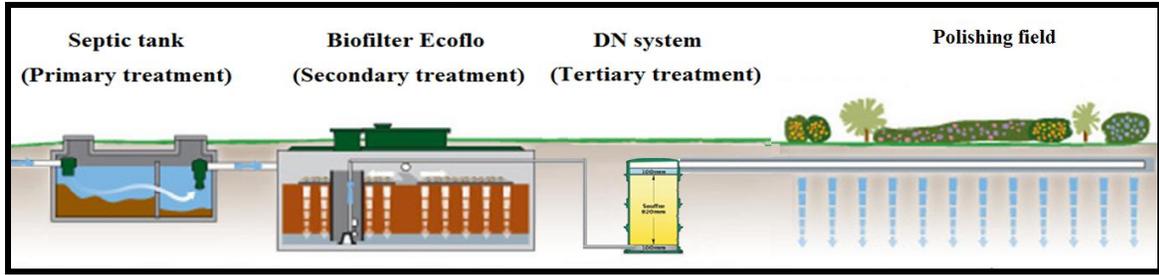
716 **FIG. 3** Evolution of the temperature (a.), pH (b.), ORP (c.) and Dissolved Oxygen
717 (d.) during the infiltration of denitrified wastewater (feeding solution)
718 through highly permeable soil (reactor R1) and permeable soil (reactor R2)

719 **FIG. 4** Evolution of the concentrations of DOC (a.), SO_4^{2-} (b.), $\text{H}_2\text{S}_{(lq)}$ (c.) and $\text{H}_2\text{S}_{(g)}$
720 (d.) during denitrified wastewater (feeding solution) infiltration through
721 highly permeable soil (reactor R1) and permeable soil (reactor R2)

722 **FIG. 5** Infiltration rate of treated wastewater through the sandy loam soil (R2)
723 during the clogging period

724 **FIG. 6** Investigation of soil sections to explain the clogging phenomenon : Particle
725 size distribution (a.), Moisture content (b.) and Organic carbon content (c.)

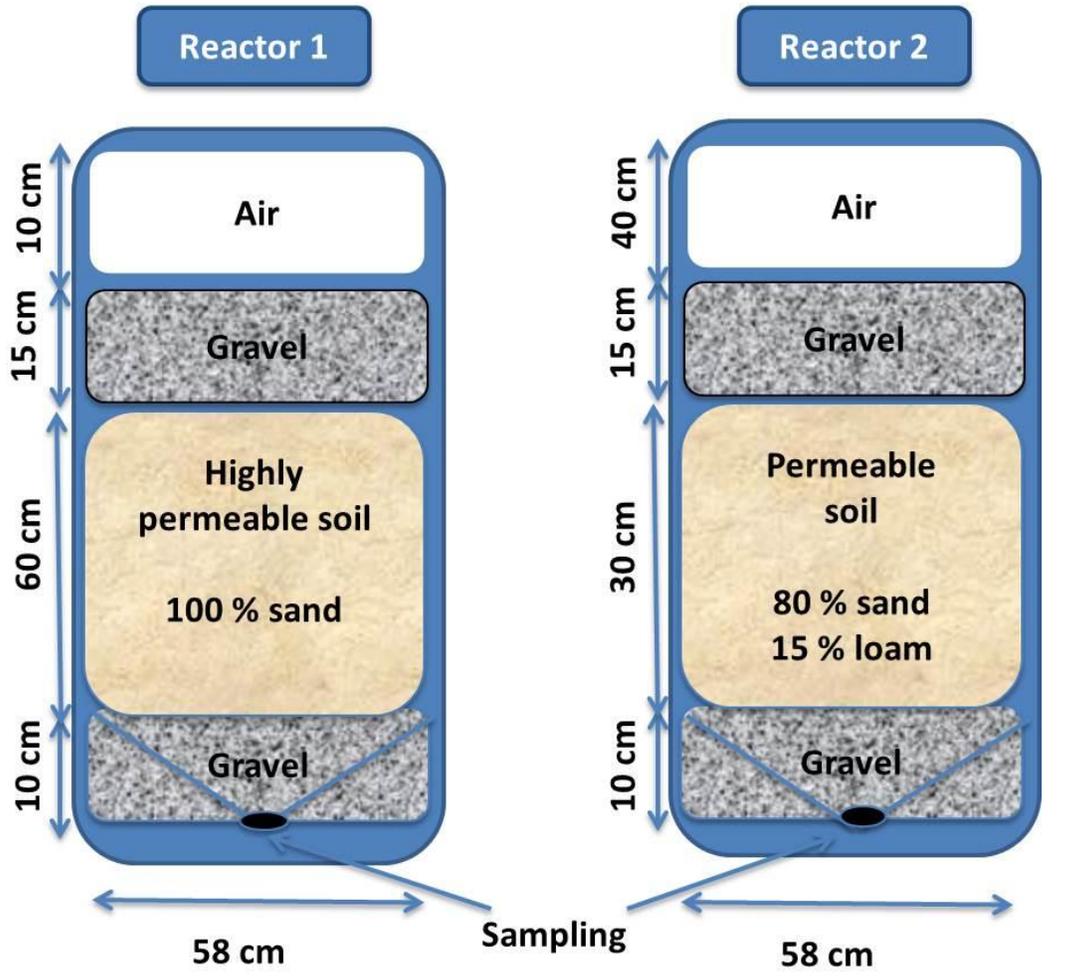
726 **FIG. 7** Formation of biomat layer (a.) and iron ochre (b.), responsible of clogging
727 phenomenon (Photography by Leila Ghorbel)



728

729

730 **Fig. 1**



a.

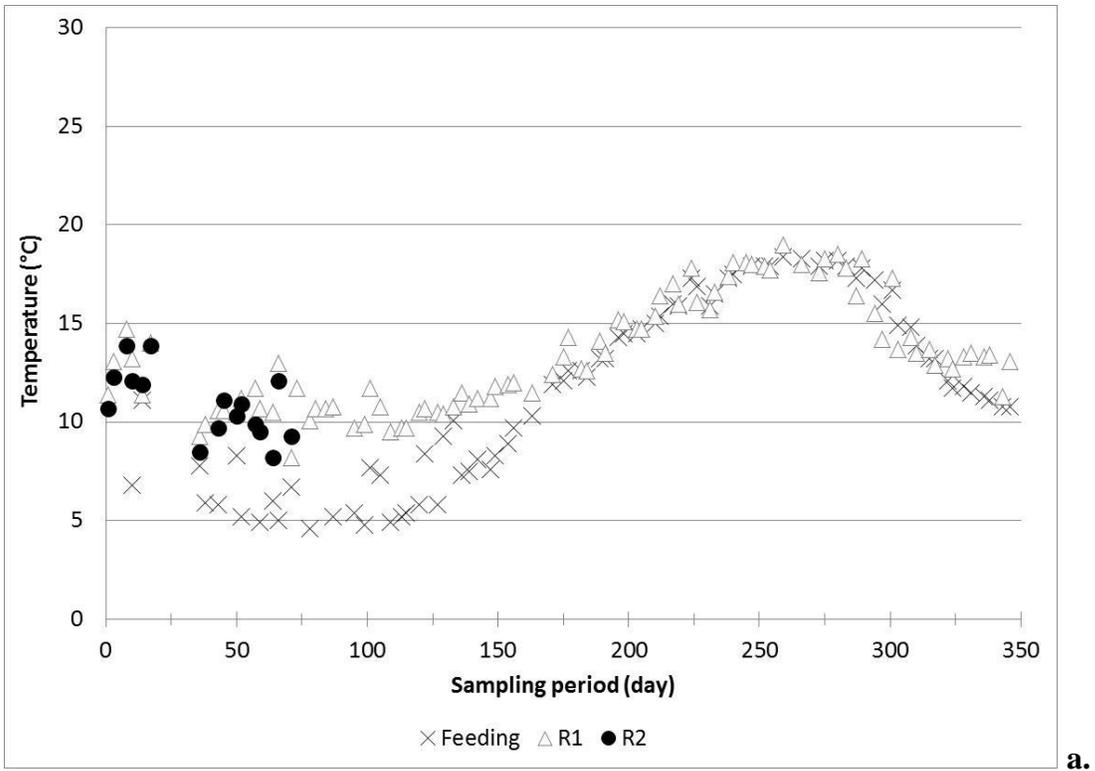


b.

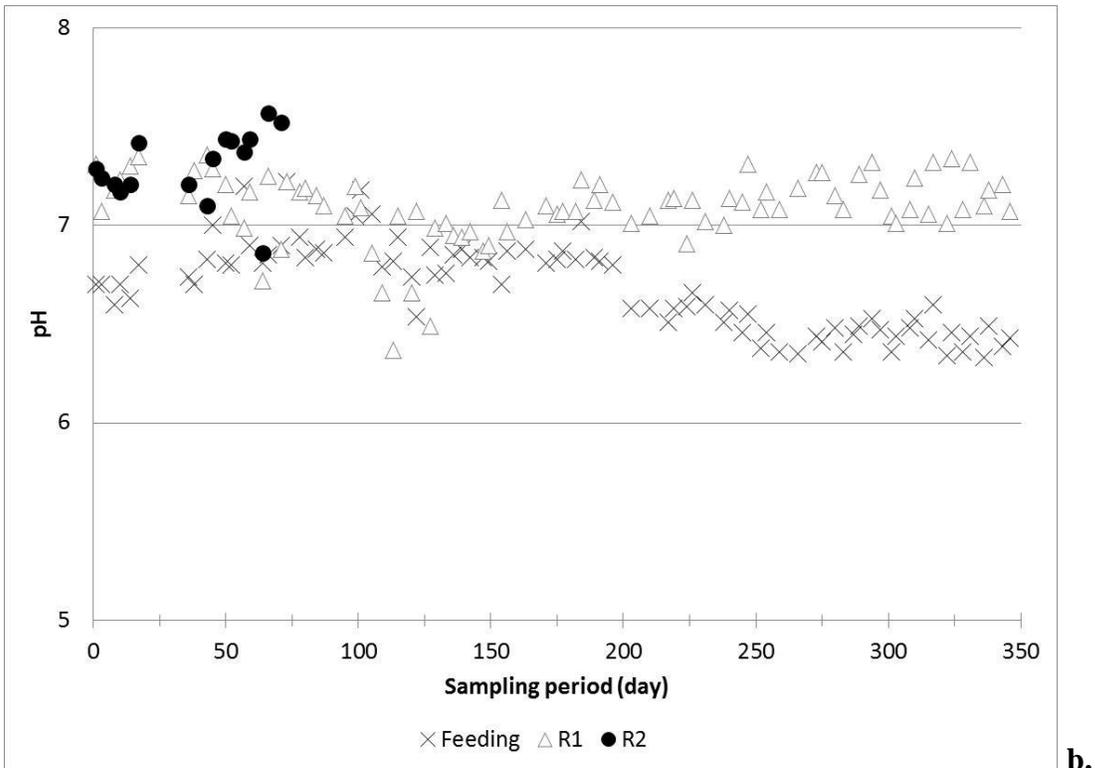


c.

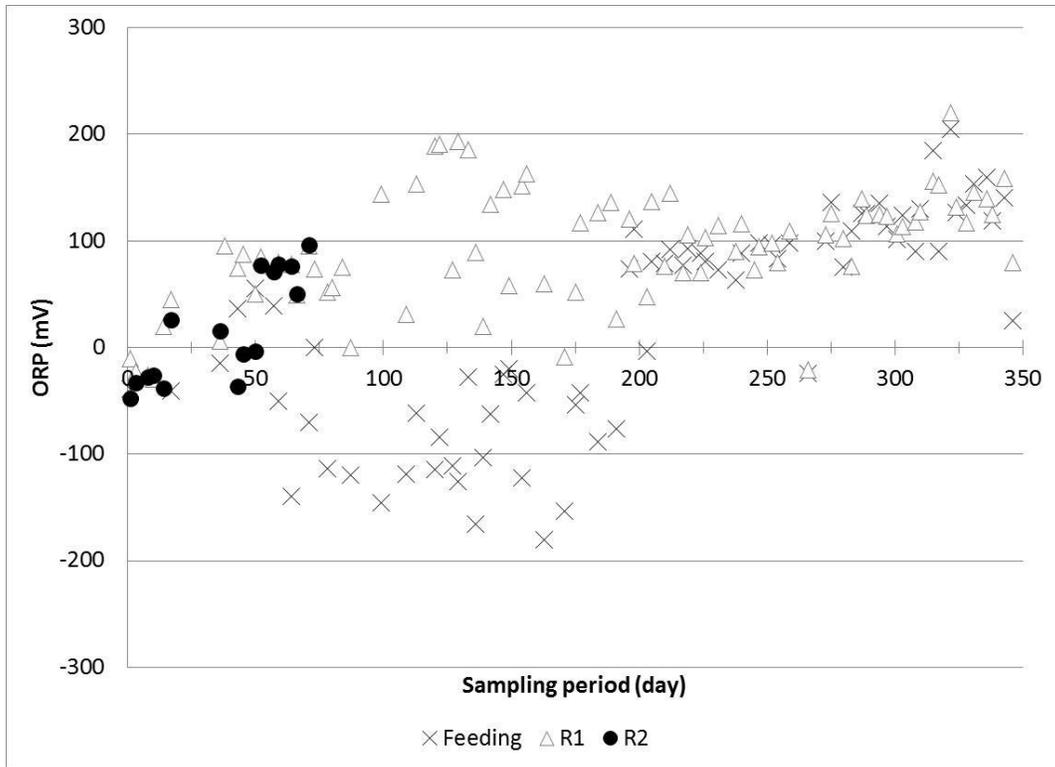
Fig. 2.



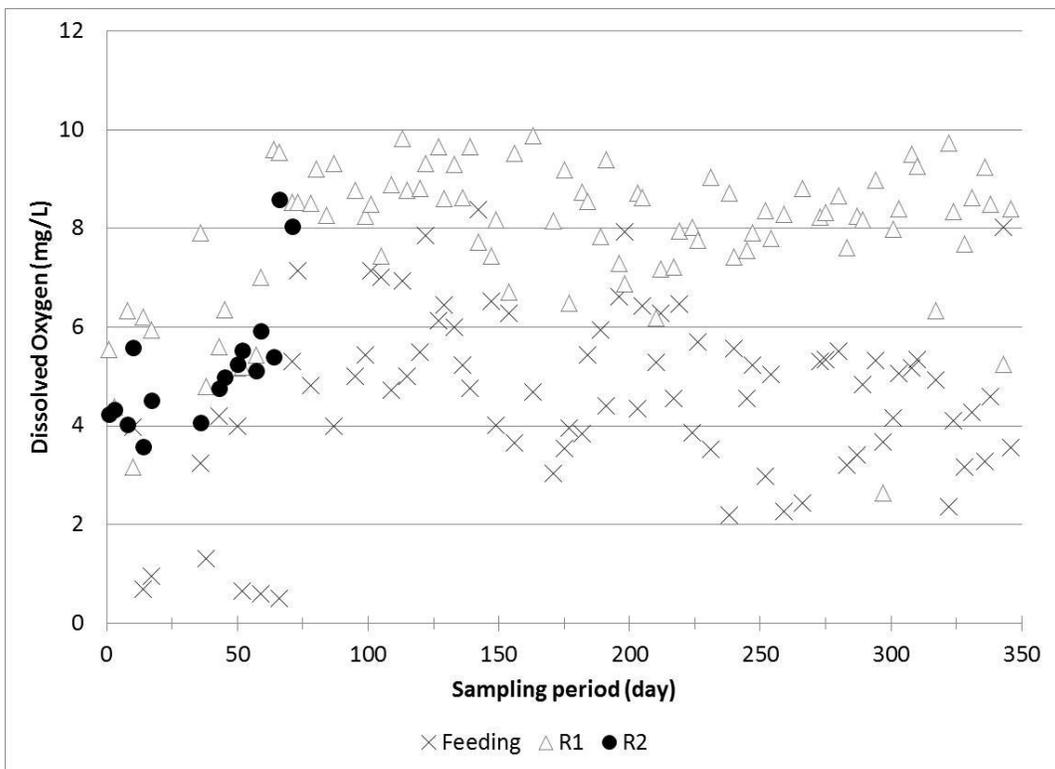
a.



b.

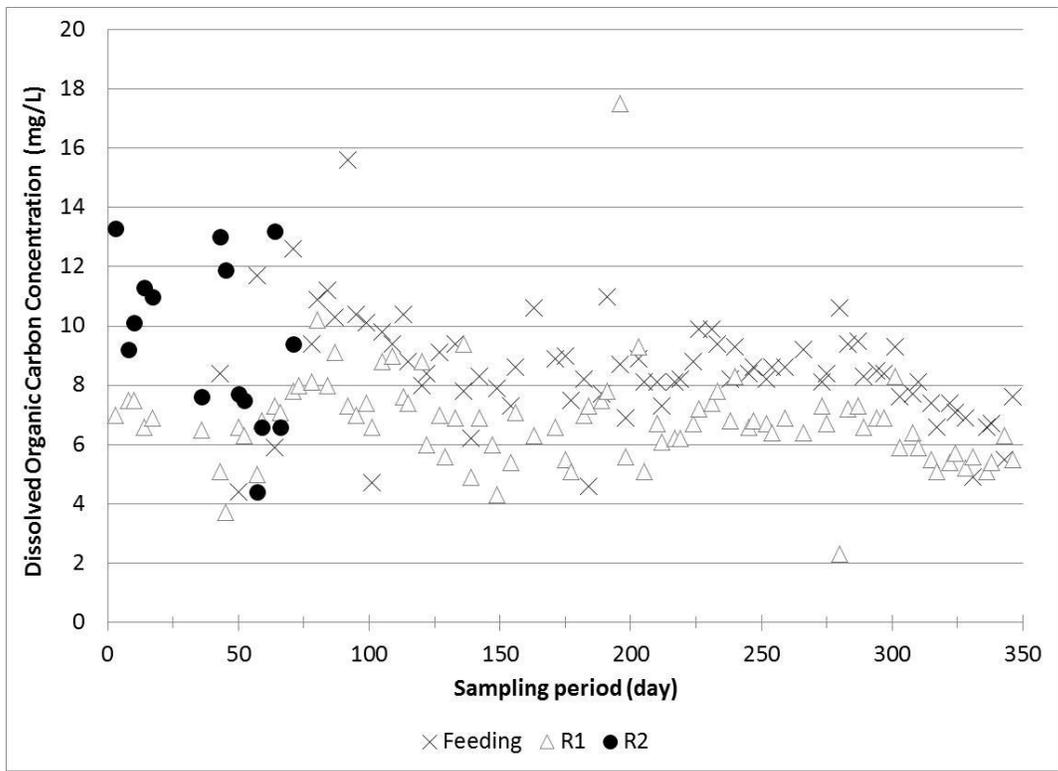


c.

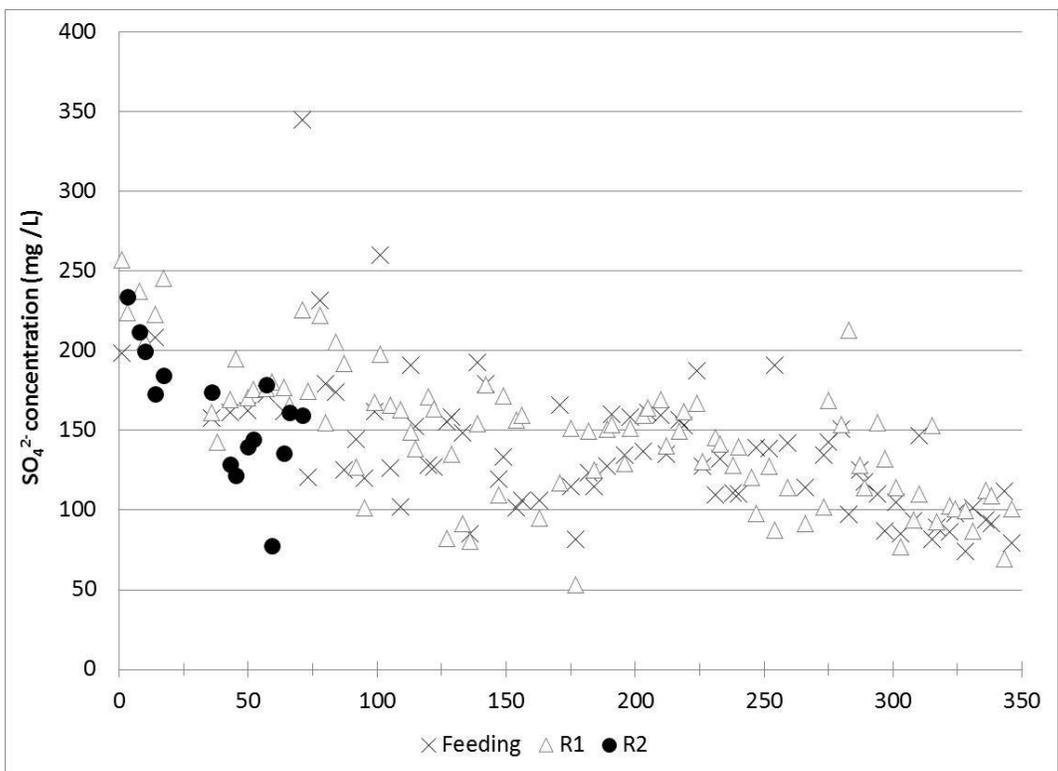


d.

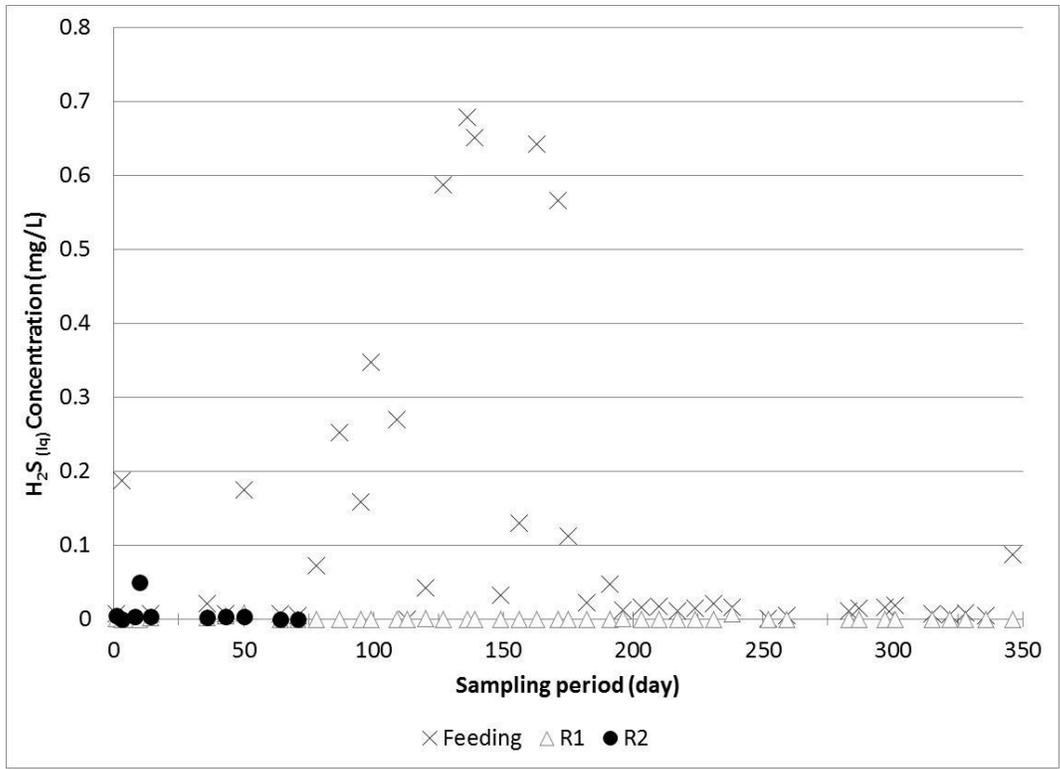
Fig. 3.



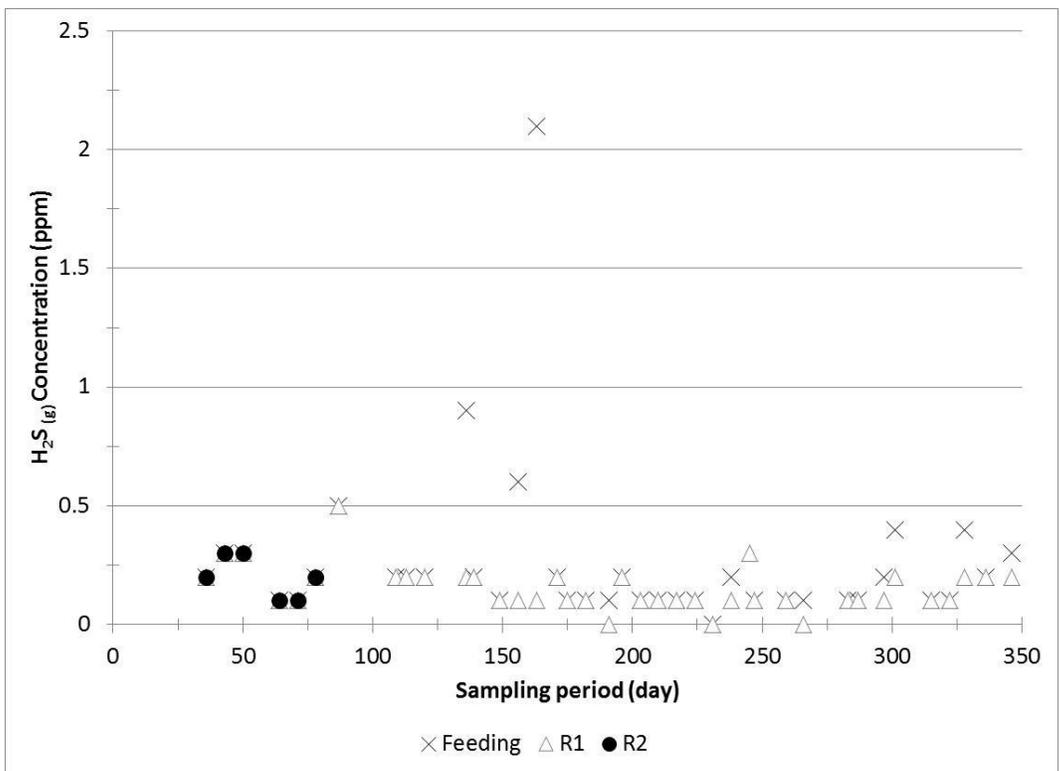
a.



b.



c.



d.

Fig. 4.

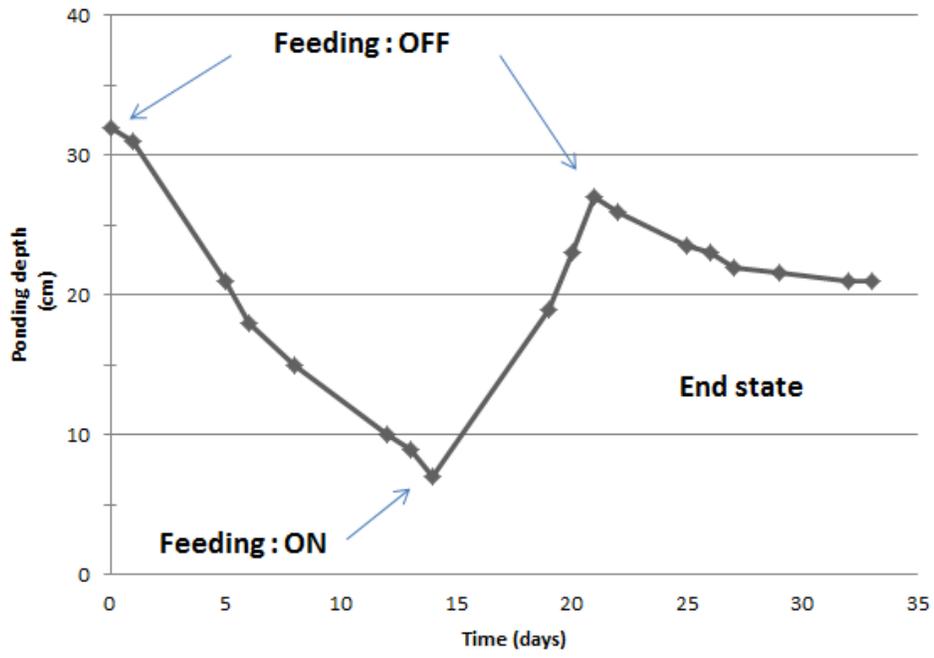
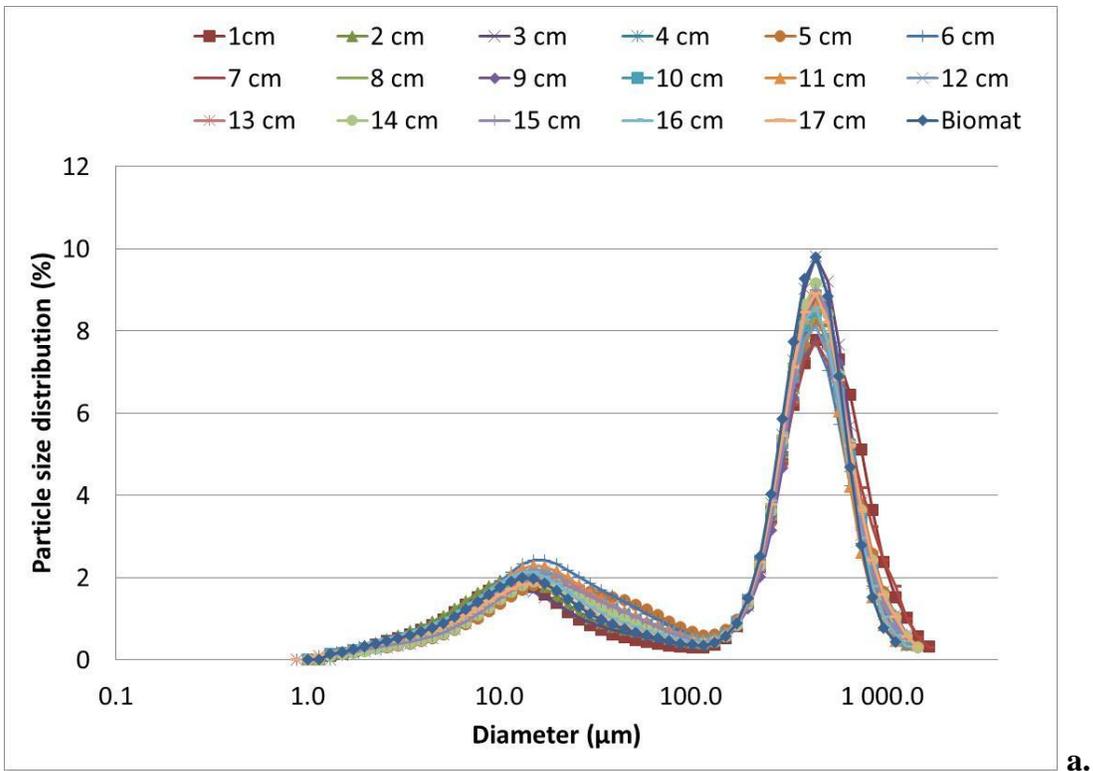
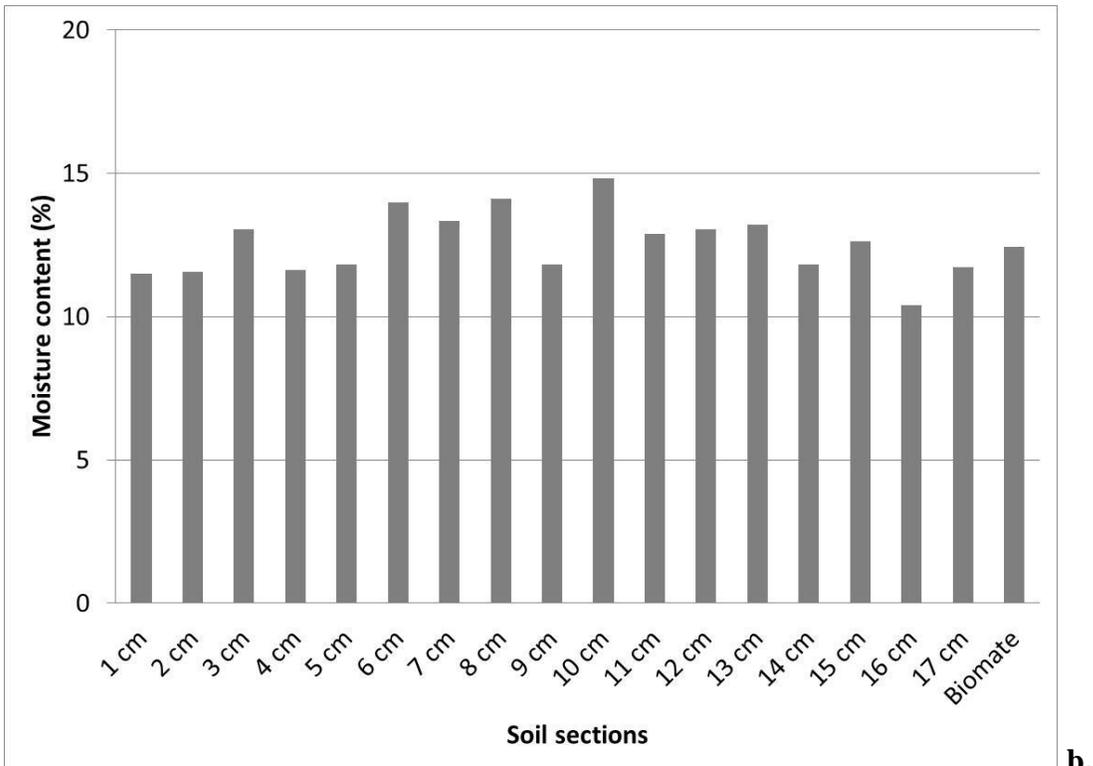


Fig. 5



a.



b.

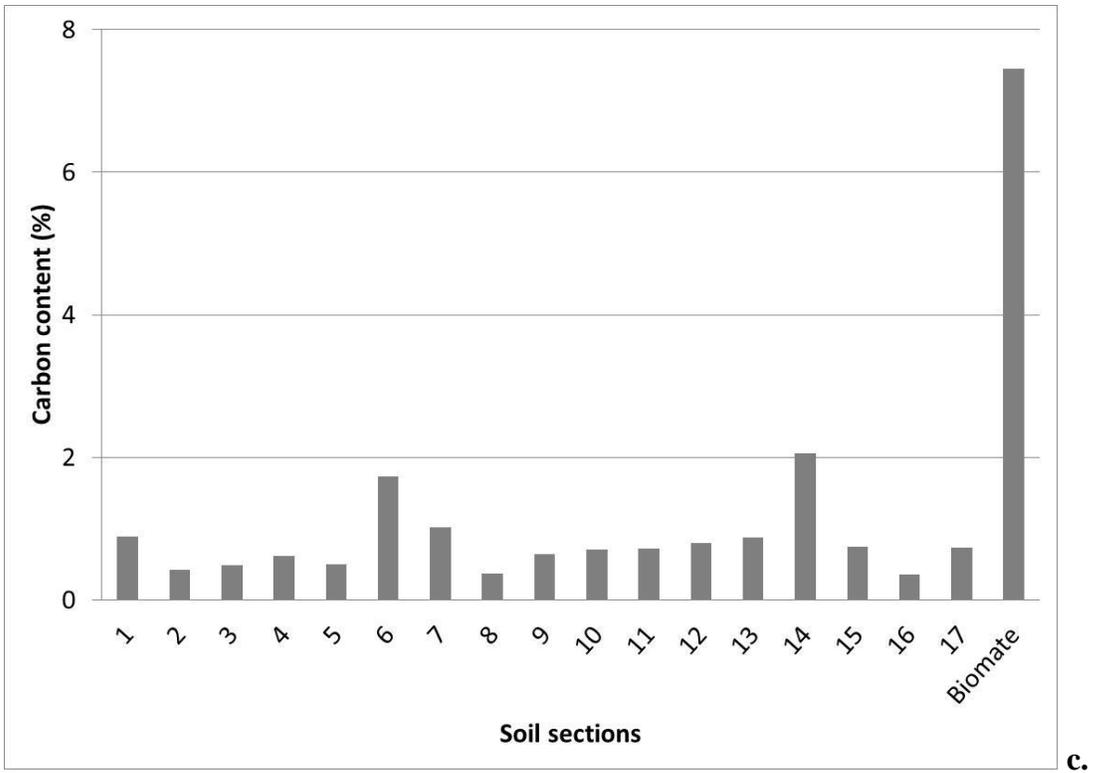


Fig. 6.



a.



b.

Fig. 7

Table 1 Summary of by-products from autotrophic denitrification processes on elemental sulfur

Field	Material	Nitrate removal (%)	SO ₄ ²⁻ (mg/L)	S ²⁻ (mg/L)	Reference
Groundwater	Egg shell	97	350 - 450	Low	Xu et al. (2016)
Domestic wastewater	Limestone	81 - 99	100 - 280	NM*	Ben-Khaled (2016)
Aquaculture wastewater	None	50	136	0.069	Christianson et al. (2015)
Groundwater	Limestone	96	610	<0.048	Sierra-Alvarez et al. (2007)
Groundwater	None	100	640	ND**	Kimura et al. (2002)
Groundwater	None	80	320	ND	Soares (2002)
Septic tank effluent	Limestone	90	300 - 400	1,5 - 10	Zhang et Shan (1998)
Groundwater	Limestone	90	130 - 170	10	Van Der Hoek et al. (1992)

NM : Not mentionned ;ND : Not detected

Table 2 Denitrified wastewater characterization and chemical composition (element concentrations expressed in mg/L) at the beginning of the experiments

Parameters (n = 92)	pH	ORP (mV)	T (°C)	DO	SO ₄ ²⁻ (mg/L)	COD (mg/L)	H ₂ S _(g) (ppm)	H ₂ S _(aq) (mg/L)	DOC (mg/L)	BOD ₅ (mg/L)	NO ₃ ⁻	Na	Ca	S	K	Mg	Si	P	Fe	Al	
Values	6.7 ± 0.2	- 40 ± 5	11.4	5.6 ± 1.0	138 ± 43	63 ± 15	0.2 ± 0.1	0.18 ± 0.00	9.8 ± 1.8	4 ± 1	5 ± 2	144	50.4	50.6	16.9	7.56	3.77	2.83	0.13	0.10	
Typical waste-water values*	6,4 - 7,6	-			300 - 400	< 30		1.5 - 10													

* Source: Zhang and Shan (1999)