

Dehalogenation of α -hexachlorocyclohexane by iron sulfide nanoparticles: Study of reaction mechanism with stable carbon isotopes and pH variations

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21 Abstract

The biodegradation of hexachlorocyclohexanes (HCHs) is known to be accompanied by isotope 22 fractionation of carbon $({}^{13}C/{}^{12}C)$, but no systematic studies were performed on abiotic degradation 23 of HCH isomers by iron (II) minerals. In this study, we explored the carbon isotope fractionation 24 25 of α -HCH during dechlorination by FeS nanoparticles at different pH values. The results of three 26 different experiments showed that the apparent rate constants during dehalogenation of α -HCH by 27 FeS increased with pH. The lowest apparent rate constant value α-HCH during dehalogenation by FeS was 0.009 d⁻¹ at pH value of 2.4, while the highest was 1.098 d⁻¹ at pH 11.8. α -HCH was 28 completely dechlorinated by FeS only at pH values 9.9 and 11.8, while the corresponding apparent 29 rate constants were 0.253 d⁻¹ and 1.098 d⁻¹, respectively. Regardless of the pH used, the 1.2.4-30 trichlorobenzene (1,2,4-TCB), 1,2-dichlorobenzene (1,2-DCB), and benzene were the dominant 31 degradation products of α -HCH. An enrichment factor (ϵ_C) of -4.7 ± 1.3 ‰ was obtained for α -32 HCH using Rayleigh model, which is equivalent to an apparent kinetic isotope effect (AKIEC) 33 value of 1.029 ± 0.008 for dehydrohalogenation, and of 1.014 ± 0.004 for dihaloelimination, 34 35 respectively. The magnitude of isotope fractionation from this study suggests that abiotic isotope fractionation by FeS must be taken into account in anoxic sediments and aquifers contaminated 36 with HCH isomers, when high concentrations of FeS are present in the above-mentioned anoxic 37 38 environments.

39

40 Keywords:

41 dehalogenation, HCH, CSIA, FeS, dehydrohalogenation, reductive dechlorination

43 **1. Introduction**

The abiotic transformation of emerging and priority pollutants has been less studied compared to 44 45 biodegradation. This is the case of abiotic reductive dehalogenation, a process that can mimic the anoxic transformation of halogenated contaminants (i.e. chlorinated ethenes, chlorinated ethanes, 46 hexabromocyclododecane (HBCD), etc) in the environment (Wacławek et al., 2019). The 47 contaminants can be abiotically transformed by iron (II) minerals (He et al., 2015) formed either 48 49 by microbial activity or occurring in nature as part of the site geochemistry. The minerals capable of abiotic contaminant transformation include iron sulfides (mackinawite (FeS), pyrite (FeS₂), 50 51 greigite (Fe₃S4)) and additional iron (II) minerals such as magnetite (Lee and Batchelor, 2002), green rust (Ayala-Luis et al., 2012), and phyllosilicate clays (biotite (Kriegman-King and Reinhard, 52 1992) and vermiculite (Bae and Lee, 2012)). Among the most important of these iron (II) minerals 53 is iron sulfide (FeS), a mineral with reductive properties that appears naturally in various anoxic 54 environments (in concentrations often exceeding 10 µmol/g dry weight (Li et al., 2016)), being 55 associated with sulfate-reducing bacteria that grow in anoxic aquifers and sediments. The natural 56 formation of FeS involves two steps. The first step is biotic and consists in HS⁻ ion generation by 57 sulfate-reducing microorganisms from sulfate, as well in parallel Fe(II) generation from naturally 58 occurring Fe(III) oxyhydroxides by iron-reducing microorganisms. The second step results in the 59 60 rapid precipitation of poorly crystalline and reactive iron sulfide species. Considering that the accidental pollutions or the unsound disposal practices may lead to the spreading of organohalogen 61 62 contaminants into environment, those compounds can be transformed, in anoxic sediment and aquifers, by biotic or abiotic processes. Beside biodegradation by various bacteria, the 63 organohalogen contaminants can also be abiotically transformed, in the presence of the reactive 64 iron sulfide species, as electrons are donated from the iron sulfide to the halogenated contaminants 65 (i.e. HCHs, HBCDs, etc). 66

FeS nanoparticles have also been synthesized under laboratory conditions by various methods and 67 they were used in the elimination of different organohalogen contaminants. Previous studies have 68 69 reported the capacity of FeS to reductively dehalogenate various halogenated organic pollutants, such as hexachloroethane (Butler and Hayes, 1998), trichloroethylene (Butler et al., 2013; He et 70 al., 2010), y-hexachlorocyclohexane (y-HCH) (Liu et al., 2003) and hexabromocyclododecane 71 (HBCD) (Li et al., 2016). (Nie et al., 2020) recently showed that the degradation of 72 73 trichloroethylene by biotic FeS was six time faster than its degradation by abiotic FeS. The higher reductive activity of the biogenic FeS detected by (Nie et al., 2020) was attributed to a higher 74 monosulfide (HS⁻ ion) content and to more structural Fe^{2+} on the surface. 75

Compound specific isotope analysis (CSIA) of carbon isotopes is using the preferential 76 transformation of lighter isotopomers during a biotic or abiotic reaction, therefore leading to an 77 enrichment of heavier isotopes in the residual phase of substrate (Elsner and Imfeld, 2016). In order 78 to relate the change of bulk isotope ratios of chemicals to the extent of the target compound 79 80 degradation, the Rayleigh model has been extensively used in environmental studies (Julien et al., 2018). The carbon, hydrogen and chlorine based CSIA is currently available for more simple 81 organic contaminants, such as BTEX (Elsner and Imfeld, 2016; Lesser et al., 2008; Vogt et al., 82 2016) and chlorinated ethenes (Kuder et al., 2013; Marco-Urrea et al., 2011), and these concepts 83 are already applied in the monitoring of contaminated sites (Hunkeler, 2008). Conversely, the 84 concepts and applications of CSIA for larger molecules including chemical classes such as 85 pesticides, flame retardants, and other emerging products are still nascent areas of research and 86 urgently need further development (Elsner and Imfeld, 2016). Hexachlorocyclohexane (HCH) 87 belongs to the organochlorine compounds and its γ -isomer (Lindane) is a globally applied broad-88 spectrum insecticide (Muller and Kohler, 2004). HCH has been classified as persistent organic 89 pollutant by the Stockholm Convention due to his adverse effects on humans and environment 90 (Wacławek et al., 2019). HCH consists of eight isomers (α -, β -, γ -, δ -, ϵ -, η -, υ -, and ι -HCH), of 91

which only the α-HCH is a chiral compound. CSIA has been applied in the past to characterize the
biodegradation of HCH isomers using sulfate-reducing bacteria (Badea et al., 2009), fermentative
microorganisms (Badea et al., 2011), a Dehalococcoides strain (Liu et al., 2020a), aerobic cultures
(Sphingobium spp (Bashir et al., 2013), and Sphingobium indicum Strain B90A (Liu et al., 2019)),
some studies involving also multi-element (C-Cl) CSIA (Liu et al., 2020a).

97 This study aims to investigate the mechanism and pathway of α -HCH dehalogenation by FeS 98 nanoparticles using the carbon isotope fractionation under anoxic conditions, in order to be able to 99 differentiate this process from biotic degradation in the field. To this end, the influence of pH on 100 the degradation rate were included, degradation products were identified and a mass balance of 101 substrate and products was established.

102 2. Materials and Methods

103 *2.1. Chemicals*

FeSO₄ · 7 H₂O (99% analytical purity) was purchased from Honeywell (Seelze, Germany), while
sodium sulfide hydrate (with 65.6 % Na₂S content) was purchased from Sigma–Aldrich (Munich,
Germany). α-HCH (99%) and hexachlorobenzene (HCB) (99%), 1,2,4-trichlorbenzene (1,2,4TCB) (99%), 1,2-dichlorobenzene (1,2-DCB) (99%) and benzene (99%) were obtained from
Sigma–Aldrich (Munich, Germany). Anhydrous Na₂SO₄ and hydrochloric acid (HCl) was
purchased from Sigma–Aldrich (Munich, Germany). Dichloromethane (DCM) (99.8 %) was
purchased from Carl Roth (Karlsruhe, Germany).

111 2.2. Synthesis of FeS nanoparticles

FeS nanoparticles were synthesized by adding 250 mL solution of 0.2 M Na₂S over 250 mL solution 0.2 M FeSO₄, under gaseous N₂ flow. The experiment was performed up to 6 h on a water bath heated by a combined hot-plate magnetic-stirrer device (Biosan, Riga, Latvia) at 30 °C and mixed at 1000 rpm to homogenize the FeS nanoparticles. FeS precipitate was divided in 11 parts (in 50 mL Eppendorf conical tubes) and FeS nanoparticles were centrifuged at 6000 rpm for 10

minutes and afterwards washed with N2-flushed water. The FeS precipitate was frozen with liquid 117 nitrogen and then freeze dried for about 48 h at about -80 °C. The dried powder of FeS was 118 collected and stored in small Eppendorf plastic tubes of 2 mL sealed with teflon band until use. 119 The FeS nanoparticles were characterized by determination of specific surface, X-Ray diffraction 120 (XRD), Raman spectroscopy. Scanning electron microscopy (SEM), as well by Fourier-transform 121 infrared spectroscopy (FTIR). The Raman and XRD peaks showed that mackinawite is the main 122 crystalline phase of FeS nanoparticles, while the specific surface of the FeS was $32.58 \text{ m}^2/\text{g}$, 123 which is similarly with the specific surface reported by (Liu et al., 2003) (see Supplementary 124 125 Material).

126 2.3. Degradation experiments

The dehalogenation experiments were performed in 300 mL anaerobic bottles crimped by 127 polytetrafluoroethylene (PTFE)-coated butyl septa. In the first experiment, the dehalogenation 128 reaction was performed in duplicates by adding 165 mL buffer mixture solution K₂HPO₄0.1 129 M/KH₂PO₄ 0.1 M (in a ratio of about 94 to 6) over 1.5 g FeS nanoparticles (final pH of 8.10 \pm 130 0.04). The bottles were firstly flushed with nitrogen and then α -HCH was added from an acetone 131 solution (3.43 mM α-HCH), to a theoretical concentration of approximatively 20.7 μM (see Tab.2). 132 Also, a control bottle was prepared in duplicates by spiking 165 mL deionized water with α-HCH 133 of the same concentration of 20.7 µM. In the first experiment, the dechlorination reaction was 134 performed for 22 days in an incubator at 30 °C and 125 rpm. In the second experiment, the 135 dehalogenation reaction was performed in three bottles by adding 165 mL deionized water over 136 1.5 g FeS nanoparticles (FeS concentration of 9.0 g/L). The pH of the three aquatic solutions was 137 adjusted with 2-3 mL of 1M NaOH / 1M HCl to three final pH values: 2.4, 5.3 and 11.8. In this 138 case also, the bottles were firstly purged with nitrogen and then α -HCH was added from the same 139 acetone solution (3.43 mM α -HCH), to a concentration of approximatively 20 μ M. In the 2nd 140 experiment, the dechlorination reaction was performed for 32 days in an incubator at 30 °C and 141

125 rpm. The third experiment was carried out in 120 mL bottles screwed gas-tight by a butyl 142 septum by mixing 1g of FeS nanoparticles with 110 mL N₂ flushed-water (FeS concentration of 143 9.0 g/L) and adjusting the pH to 9.9. In the 3rd experiment, the bottle was firstly purged with 144 nitrogen and then spiked with α -HCH in acetone to a concentration in water of about 30 μ M. In all 145 three experiments, control bottles without FeS nanoparticles were used. At regular intervals, 14 mL 146 aliquots of α-HCH solution were taken with syringes for extraction with 1 mL DCM that contains 147 148 hexachlorobenzene (HCB) as internal standard, as previously described (Badea et al., 2009). The extraction and analysis of slurry samples of FeS nanoparticles was performed by ultrasonication 149 150 and was similarly with the one of aliquots (see Supplementary Material).

151 2.4. Control experiment

152 In order to demonstrate that the decrease in concentration of α -HCH and the formation of the degradation products in the three experiments performed with FeS nanoparticles are attributable to 153 the dehydrohalogenation processes that are taking place on the surface of FeS, an additional control 154 155 experiment (experiment no. 4) was performed at three different pH values. The control experiments were performed using two 300 mL anaerobic bottles crimped by polytetrafluoroethylene (PTFE)-156 silicon septa. The first control bottle was preparing by dissolving HgCl₂ in 250 mL deionized water 157 to a final concentration of 82 mg/L HgCl₂ in order to prevent any biodegradation of α -HCH, while 158 the pH was adjusted to 7.0 by adding 0.4 mL 1M NaOH. In the second control bottle, the pH of 159 160 250 mL deionized water was adjusted to 9.99 using 2-3 mL of 1M NaOH / 1M HCl. In the third control bottle, the pH of 250 mL deionized water was adjusted to 11.7 with 0.9 mL 1M NaOH. In 161 all three control bottles, 1 mL of solution 3.92 mM of α -HCH was added in both control bottles to 162 final concentration of about 15.6 μM. Here too, 14 mL aliquots of α-HCH solution were taken with 163 syringes for extraction with 1 mL DCM that contains hexachlorobenzene (HCB) as internal 164 standard, the last sample for first and third control bottles (pH 7.0 and 11.7 respectively) being 165

taken after 17 days and the last one for the second control bottle (pH of 9.9) after 10 days. The control experiment was performed up to 17 days in an incubator at 30 °C and 125 rpm. 167

2.5. GC-MS analysis 168

For the three degradation experiments with FeS, the GC-MS measurements were carried out on a 169 Varian 450 GC-240 MS (Varian, Inc, USA), equipped with an ion trap mass analyzer configurated 170 in positive electron ionization mode (70 eV). To identify and characterize the structure of the 171 degradation products, the ion trap mass analyzer was configured in scan mode. The samples (1 μ L 172 DCM in volume) were injected at 260 °C into a split injector, with the split ratio adjusted to 20, 173 while the flow rate of carrier gas (helium) was 1 mL/min. The α-HCH and its degradation products 174 were separated on a CP-Sil 8 CB (8% phenyl polysilphenylene-siloxane) capillary column (30 m 175 $\times 0.25 \text{ m} \times 0.25 \text{ \mu}\text{m}$) using the following temperature program: 40 °C initial temperature (held for 176 3 min), 4 °C min⁻¹ to 120 °C (0 min), 20 °C min⁻¹ to 200 °C (0 min) and finally 10 °C min⁻¹ to 280 177 °C (and held for 5 min). 178

In the experiment 4 (control experiment), the GC-MS measurements were carried out on a Perkin 179 Elmer Clarus 580 GC (Waltham, Massachusetts, USA), equipped with an Clarus SQ-8 quadrupole 180 181 mass analyzer configurated in positive electron ionization mode (70 eV). The samples (1 µL DCM in volume) were injected into a split injector, with the split ratio adjusted to 10, while the flow rate 182 of carrier gas (helium) was 1 mL/min. The α-HCH and its degradation products were separated on 183 184 a DB5 MS (5% phenyl polysilphenylene-siloxane) capillary column (30 m \times 0.25 m \times 0.25 μ m) using the same temperature program as described in the paragraph above. 185

2.6. GC-C-IRMS analysis 186

The carbon isotope composition of α -HCH was analyzed using a gas chromatograph (Thermo 187 Trace 1310 GC, Thermo Scientific) coupled via a CONFLOW IV (Thermo Scientific, Germany) 188 to a Delta V Advantage mass spectrometer (Thermo Scientific, Germany), as described previously 189

190 (Zamane et al., 2020). The oxidation furnace of the interface (Thermo Scientific, Germany) 191 containing Pt, Ni, and CuO was set to 1000 C° in order to completely combust α -HCH. The furnace 192 was conditioned for 120 minutes with O₂ before each batch run of samples. The flow rate of carrier 193 gas (helium) was 2.0 mL/min, while the injector was operated in split mode at 280°C. A DB-5MS 194 GC column (60 m × 0.25 mm, 0.25 µm film thickness) was used for chromatographic separation 195 of α -HCH from HCB. The temperature program of the GC oven was described previously (Badea 196 et al., 2011) (see Supplementary Material).

197 2.7. Carbon stable isotope calculations

198 The carbon isotope signatures were reported in δ -notation (‰) relative to the Vienna Pee Dee 199 Belemnite (V-PDB) (Coplen, 2011), as follows:

200
$$\delta^{13} C [\%] = \left(\frac{R_{sample} - R_{stan \, dard}}{R_{stan \, dard}}\right) \times 1000$$
(1)

where the ${}^{13}C/{}^{12}C$ ratio of the sample (R_{sample}) is reported relative to the ${}^{13}C/{}^{12}C$ ratio of the VPDBstandard ($R_{standard}$).

203 The Rayleigh equation was used to quantify isotope fractionation upon degradation (equation 2):

204
$$\ln\left(\frac{R_{t}}{R_{0}}\right) = (\alpha - 1)\ln\left(\frac{C_{t}}{C_{0}}\right)$$
(2)

The C_0 and C_t and are the concentrations of the substrate at times 0 and t, while the R_0 and R_t are the ¹³C/¹²C ratios of the substrate at times 0 and t, respectively. The α is the isotope fractionation factor, and it can further used to calculate the isotope enrichment factor ε (equation 3):

$$\epsilon = (\alpha - 1) \times 1000 \tag{3}$$

The error of the isotope enrichment factor was given as 95% confidence interval (CI) and determined using a regression analysis as described elsewhere (Elsner et al., 2007). The apparent kinetic isotope effect (AKIE) was calculated using equation 4 according to (Elsner etal., 2005)

213
$$AKIE \approx \frac{1}{1 + \left(\frac{n}{x} \cdot z \cdot \frac{\varepsilon}{1000}\right)}$$
(4)

where x is the number of reactive positions, z is the number of positions in intramolecular competition and n is the number of atoms of the molecule of a target element. The error propagation method was used to estimate uncertainty of the AKIE, as described elsewhere (Fischer et al., 2010).

217 **3. Results and discussions**

218 3.1. Dehalogenation of α -HCH by iron sulfide nanoparticles

 α -HCH was dechlorinated in all degradation experiments at reaction times of up to 32 days. The 219 220 main degradation products formed at all pH during dehalogenation were (Fig. 1, pathway (A)): 221 1,2,4-trichlorbenzene (1,2,4-TCB), 1,2-dichlorobenzene (1,2-DCB), benzene and, as intermediate, pentachlorocyclohexene (PCCH), tentatively identified by GC-MS by comparing its mass 222 spectrum with those of the NIST library and with the previous degradation studies of α -HCH 223 (Suar et al., 2005). (see Fig. S9, Supplementary Material). In the control bottles, no degradation 224 products were detected. For example, in the experiment 1, no degradation products were detected 225 226 and the isotope composition of α -HCH remained constant during the whole experiment indicating that the α-HCH from the control bottle is not biotically or abiotically transformed. Nevertheless, in 227 the experiment 1, the concentration of α -HCH varied from 16.7 μ M at the beginning of the 228 experiment to 7.5 μ M at the end of the experiment. This loss of α -HCH is probably attributable to 229 the volatilization of α-HCH due to the low final volume of aquatic phase (about 54 mL) comparing 230 with the initial one of 166 mL (with results in a large head-space volume), as well to the sorption 231 232 processes. Small amounts of α -HCH might have been adsorbed onto the plastic syringes during sampling procedures. Also in the experiment no.4 without FeS nanoparticles, the evolution of α -233

HCH concentration in the control bottles varied according to the initial pH values. In the control 234 bottle with the pH of 7.0, no degradation products were detected and the concentration of α -HCH 235 evolved from 15.6 µM at the beginning of the experiment to 16.6 µM after 17 days (at the end of 236 the experiment), showing no clear trend (see Fig.2). In control bottle with pH value of 9.9, the 237 concentration of α -HCH decreased from 15.4 μ M at the beginning of the experiment to 11.9 μ M 238 after 10 days (see Fig S7, Supplementary Material). In the control bottle with pH value of 9.9, the 239 pseudo-first-order rate constant was calculated to 0.090 d⁻¹ which is slightly higher than the pseudo-240 first-order rate constant of 0.069 d⁻¹ recorded by (Ren et al., 2006) during alkaline hydrolysis of 241 242 α -HCH performed at pH 9.28 and 25°C, but was lower than the rate constant of 0.384 d⁻¹ (0.0064 h^{-1}) recorded by (Zhang et al., 2014) during alkaline hydrolysis of α -HCH performed at pH 9.78. 243 In the control bottle with pH value of 11.7, the degradation rate was the fastest from all control 244 bottles, since the concentration of α -HCH decreased from 15.5 μ M at the beginning of the 245 experiment to 0.3 µM after just one day (24 h), afterwards its concentration being bellow the 246 detection limit of the GC-MS method. 247

The above-mentioned degradation products (PCCH, 1,2,4-TCB, 1,2-DCB, benzene) showed that 248 the dehydrochlorination was the main degradation pathway of α-HCH by FeS. The proposed 249 250 degradation pathway from this study assumed a simultaneous elimination of two chlorine and two hydrogen atoms from PCCH to produce 1,2,4-TCB, followed by a further reductive dechlorination 251 252 of 1,2,4-TCB to 1,2-DCB and finally to benzene ((Fig. 1, pathway (A)), in contrast with the hydrolysis pathway recently proposed by (Kannath et al., 2019) (Fig. 1, pathway (B)) which 253 suggest an attack of the hydroxyl ion on the molecules of different HCHs isomers. The degradation 254 pathway from this study is also similar to a dehydrochlorination biotic pathways of α -HCH by 255 Sphingomonas paucimobilis B90A proposed by (Suar et al., 2005) and (Lal et al., 2010) (Fig. 1, 256 pathway (C)), but the abiotic dehalogenation reaction is going beyond 1,2,4-TCB with the 257 formation of 1,2-dichlorobenzene and benzene as new degradation products. 258

The proposed degradation pathway was supported by the absence of monochlorobenzene (MCB) 259 as degradation product. The vicinal dehaloelimination (vicinal reductive dechlorination) of α -HCH 260 by FeS nanoparticles might be just a minor degradation pathway. In contrast with the previous 261 studies (Badea et al., 2011), as the key intermediate of such pathway, the 3,4,5,6-tetrachloro-1-262 cyclohexene (TCCH, Fig. 1 pathway (D)) was detected just in the FeS slurry samples (see Fig. S13, 263 Supplementary Material), but not in the aquatic ones. Since no reference standards of PCCH and 264 265 TCCH were available for calibration, the concentrations (in µM) were calculated only for the 1,2,4-TCB, 1,2-DCB and benzene. The trend of degradation products in aquatic samples was influenced 266 267 by the rate of α-HCH dechlorination which was influenced by the pH on the solutions. The ratio between final concentration of 1,2,4-TCB to 1,2-DCB varied from about 7.3 at pH 8.1 to about 268 32.9 at pH 11.8, indicating that the formation of 1,2-DCB by reductive dechlorination of 1,2,4-269 TCB is favored in heterogenous systems at more neutral pH, as previously found (Mackenzie et 270 al., 2005). α-HCH was degraded completely in aquatic solutions only at pH 11.8 after 11 days, his 271 concentration decreasing from 5.0 μM at the beginning of the 2^{nd} experiment to 0.003 μM after 3 272 days and to further 0.002 μ M after 7 days, afterwards the α -HCH being below the detection limit 273 of the GC-MS method. In comparison, in the same 2^{nd} experiment, the α -HCH concentrations 274 decreased from 8.7 µM at the beginning of the experiment to 5.0 µM (pH 5.3) and respectively 275 from 8.4 µM to 5.6 µM (pH 2.4), after 32 days of degradation, showing a high influence of pH on 276 277 the degradation rate of α -HCH (see Fig. 2). In the 1st experiment, performed at pH 8.1, the α -HCH 278 concentration decreased from 9.0 \pm 0.4 μ M at the beginning of the experiment to 5.6 \pm 0.1 μ M, after 22 days, at the end of the experiment. (Fig. 2). In the 3rd experiment performed at pH 9.9, the 279 apparent degradation rate of α-HCH was slightly slower comparing with the one at pH 11.8 (see 280 281 the apparent rate constants from Tab. 1), the α -HCH concentration decreasing from 5.9 μ M at the beginning of the experiment to 0.5 μ M after 4 days and to 0.041 μ M after 19 days, at the end of the 282 experiment (Fig. 2). This influence of pH on the degradation rate was also observed by (Liu et al., 283

2003) in the degradation study of γ -HCH isomer with FeS nanoparticles. In order to explain the 284 influence of pH on degradation rate, (Butler and Hayes, 1998) suggested that the dehalogenation 285 rate of chlorinated chemicals (i.e. hexachloroethane) at alkaline pH is higher comparing with the 286 reaction rate at acidic pH, possible due to deprotonation of species from the surface of FeS 287 (deprotonated species have a higher reactivity). Nevertheless, in our study, the "apparent" 288 degradation rate of α -HCH might be overestimated and might differ from the "true" degradation 289 290 rate due to the sorption of α -HCH on the surface of FeS nanoparticles. This assumption is supported by the above-mentioned values of measured aquatic concentrations at the beginning of the 291 292 experiments 1, 2 and 3 which were all lower than the initial spiked aquatic concentrations of about $20 \,\mu\text{M}$ (respectively around $30 \,\mu\text{M}$ for 3^{rd} experiment). For the experiment 1, only about 43.7 % 293 $(1.49 \,\mu\text{moles})$ of the total quantity of spiked α -HCH was detected in first aquatic 294 samples taken, the rest being adsorbed on surface of FeS nanoparticles. To quantitively determine 295 the influence of pH on the degradation rate, the kinetics of α-HCH degradation by FeS was assessed 296 by considering the dehalogenation reaction to follow a pseudo first order kinetic. Therefore, the 297 apparent rate constants (k_a) for the dehalogenation of α -HCH were calculated at different pH values 298 (Tab.1), in the three different experiments, having all of them the same level of FeS to liquid ratio 299 (the same FeS concentration of about 9 g/L) in the respective bottles. In the acidic pH domain, the 300 apparent rate constant value showed only a small increase from 0.009 d⁻¹ at pH 2.4 to 0.014 d⁻¹ at 301 pH 5.3 (both in 2nd experiment). In the alkaline pH domain, the apparent rate constants increased 302 from $0.018 \pm 0.002 \text{ d}^{-1}$ at pH 8.1 (1st experiment) to 0.25 d⁻¹ at pH 9.9 (3rd experiment), while the 303 highest apparent rate constant of 1.1 d⁻¹ was recorded at pH 11.8 (2nd experiment). The value of 304 apparent rate constant recorded at pH 11.8 (1.1 d⁻¹) was higher than the rate constant of 0.384 d⁻¹ 305 (0.0064 h^{-1}) recorded by (Zhang et al., 2014) during alkaline hydrolysis of α -HCH performed at 306 pH 9.78. These increases in k_a values clearly showed that the apparent rate constants during 307 308 dehalogenation of α -HCH by FeS increased with pH, which was also shown by (Liu et al., 2003)

for γ -HCH isomer. By comparing the apparent rate constant of 0.25 d⁻¹ recorded during dehalogenation of α -HCH by FeS at pH 9.9 (3rd experiment), with the pseudo-first-order rate constant of 0.090 d⁻¹ recorded during hydrolysis of α -HCH in the control bottle with same pH value of 9.9 (4th experiment), it can be concluded that the kinetics of α -HCH dehalogenation of by FeS at moderate alkaline pH values (up to 10) is different from the one of hydrolysis. Nevertheless, at very high pH values (higher than 10) the contribution of hydrolysis can became significant.

At the end the of experiment 1, the concentrations of α -HCH and of its main degradation products 315 in the FeS slurry were: $0.840 \pm 0.008 \ \mu mol/g$ for α -HCH, $0.008 \pm 0.003 \ \mu mol/g$ for 1,2,4-TCB and 316 $0.0009 \pm 0.0004 \ \mu mol/g$ for 1,2-DCB, respectively (see Tab. 2). These values can be used to 317 calculate a mass balance. In this respect, the number of moles from the FeS slurry for α -HCH (1.26 318 µmol), 1,2,4-TCB (0.012 µmol) and 1,2-DCB (0.0013 µmol) must be considered, taking into 319 320 account the quantity of FeS nanoparticles (1.5 g). As in many degradation studies, the mass balance is taking into account firstly the final aquatic concentrations of the compounds which were ranged 321 between 5.6 \pm 0.1 μ M for α -HCH (the number of moles of α -HCH was 0.3024 μ mol), 0.844 \pm 322 0.045 μ M for 1,2,4-TCB (0.0455 μ mol) and 0.115 \pm 0.098 μ M for DCB (0.0062 μ mol), 323 respectively (see Tab. 2). The number of moles for each compound were calculated taking into 324 325 account the final volume of aquatic phase (about 54 mL) at the end of the experiment. Nevertheless, due to complex nature of the degradation experiments with FeS nanoparticles (Li et al., 2016), the 326 327 mass balance must include also the amounts of α -HCH, 1,2,4-TCB and 1,2-DCB removed from the degradation bottles by sampling. The number of moles removed from the bottles by sampling 328 were: 0.7508 µmol for α-HCH, 0.0360 µmol for 1,2,4-TCB and 0.0050 µmol for 1,2-DCB, and 329 these values were calculated individually for every compound as sum of numbers of moles of 330 removed in every sampling point (with the volume of 14 mL), taking into account that 1,2,4-TCB 331 and 1,2-DCB were measured only in the last seven, respectively five sampling point (out of eight). 332 The total sum of these values at the end of experiment 1 was 2.42 µmol which means a recovery 333

of 70.4 % in the mass balance, comparing with the initially spiked amount of 3.44 μ mol of α -HCH (the spiked concentration of α -HCH was 20.7 μ M at an initial volume of 166 mL). This slightly difference between the numbers of moles at the beginning and at the end of experiment 1 is probably due to transformation of α -HCH in the intermediates PCCH and TCCH, as well to the more volatile nature of 1,2,4-TCB and 1,2-DCB.

339 3.2. Carbon isotope fractionation in the course of α -HCH dehalogenation by FeS

In the first degradation experiment performed at pHs 8.1 and 11.8, the carbon isotope composition 340 341 of α -HCH changed during dehalogenation. Since a pH value of 8.1 is of more relevance for real environmental conditions (compared to pH of 11.8 where hydrolysis is fast), the isotope 342 fractionation of α-HCH was assessed only at pH value of 8.1. Note that Ren et al (2005) obtained 343 a half-life time of 74 days for hydrolysis of α -HCH at pH 8.3. The δ^{13} C values of δ^{13} C of the 344 345 internal standard HCB stayed constant. The carbon isotope composition of α -HCH increased from -25.8 ± 0.6 % to -23.1 ± 0.4 %, whereas the α -HCH concentrations decreased from $9.0 \pm 0.4 \mu$ M 346 347 to $5.6 \pm 0.1 \,\mu\text{M}$ (Fig. 3A). 1,2,4-trichlorbenzene (1,2,4-TCB) and 1,2-dichlorobenzene (1,2-DCB) were detected after one and respectively 6 days of degradation, in concentrations of 0.014 ± 0.001 348 µM for 1,2,4-TCB and 0.019 µM for 1,2-DCB, respectively. At the end of the experiment (after 22 349 days), the concentrations increased to 0.844 \pm 0.045 μ M (1,2,4-TCB) and to 0.115 \pm 0.098 (1,2-350 DCB) (Fig. 3B), while β -PCCH was detected only as traces. For this experiment, the concentrations 351 352 of 1,2,4-TCB, 1,2-DCB and β -PCCH were smaller than the linearity range of the GC-C-IRMS instrument and thus their isotope compositions could not be determined. Nevertheless, in the 2nd 353 experiment, the evolution of concentration of 1,2,4-TCB in the bottle with the pH of 11.8, allowed 354 355 a determination of isotope signature of 1,2,4-TCB during its formation, as well the elucidation of trends of 1,2,4-TCB, 1,2-DCB and benzene concentrations during dehalogenation of α-HCH by 356 357 FeS performed at more alkaline pHs. In the bottle with the pH of 11.8, the first changes in the concentrations of 1,2,4-TCB, and 1,2-DCB and benzene appeared after about 40 minutes, when 358

the 1st sample was taken (Fig. 4A and 4B), due to the fast dehalogenation reaction. After about 40 minutes, the recorded concentration of 1,2,4-TCB was 8.5 μ M (the first day of the experiment) and increased to a maximum of 15.7 μ M after 3 days of degradation. Afterwards its concentration decreased to 7.9 μ M at the end of the experiment, whereas the isotope composition of 1,2,4-TCB decreased from -31.2 \pm 0.3 ‰ after 3 days of degradation (lighter than the initial isotope composition of α -HCH) to -25.2 \pm 0.1 ‰ after 32 days of degradation (Fig. 4A).

The first recorded concentration of 1,2-DCB was 0.31 µM at 40 minutes (in the 1st day of the 365 experiment), then it increased to a maximum of 0.45 µM after 5 days of degradation, and afterwards 366 decreased to 0.24 µM, after 32 days of degradation (Fig. 4B). The concentration of benzene 367 increased from 0.08 µM after 3 days of degradation to 0.12 µM, after 32 days of degradation (data 368 not shown). These changes in concentration of α -HCH degradation products showed that reaction 369 rates of 1,2-DCB and respectively benzene are slower comparing with the initial reaction of α -370 HCH to 1,2,4-TCB. Here too, the concentrations of 1,2-DCB and PCCH and benzene were smaller 371 than the linearity range of the GC-C-IRMS instrument and thus their isotope compositions could 372 not be determined. 373

374 3.3. Quantitative assessment of isotope fractionation during dehalogenation of α -HCH by FeS

The enrichment factor (ϵ_C) of α -HCH dechlorination in the 2nd experiment was calculated using 375 according to the Rayleigh equation (equation 2) and gave a value of $\varepsilon_{\rm C} = -4.7 \pm 1.3$ %, while the 376 correlation coefficients (\mathbb{R}^2) was 0.94 (Fig. 5). This ε_C is higher (in absolute value) than the values 377 of -1.6 ± 0.3 ‰ and -1.0 ± 0.2 ‰ recorded for *S. indicum strain B90A* and *S. japonicum strain* 378 UT26, respectively, for the dehydrochlorination pathways recorded by (Bashir et al., 2013) during 379 aerobic biodegradation of bulk α -HCH, but lower than the $\epsilon_{\rm C}$ of -7.6 \pm 0.4% recorded by (Zhang 380 et al., 2014) during dehydrochlorination of bulk α-HCH induced by alkaline hydrolysis (pH 9.78). 381 382 As demonstrated by (Zhang et al., 2014), the carbon isotope enrichment factors of individual enantiomers ($\varepsilon_{C(+)}, \varepsilon_{C(-)}$) of α -HCH upon chemical transformation were statistically identical with 383

each other and they fall within the respective $\varepsilon_{\rm C}$ of bulk α -HCH within a 95% confidence interval, therefore an enantioselective stable isotope analysis (ESIA) of α -HCH was not investigated in this paper.

Considering dehydrohalogenation as the dominant degradation pathway of α -HCH by FeS, then 387 the recorded values of $\varepsilon C = -4.7 \pm 1.3$ ‰ from this study is also lower than the enrichment factors 388 (εC) of γ -HCH recorded recently (Schilling et al., 2019) during dehydrohalogenation of γ -HCH by 389 390 lindane dehydrochlorinases enzymes LinA1 ($-8.1 \pm 0.3 \%$) and LinA2 ($-8.3 \pm 0.2 \%$). Nevertheless, if the vicinal dihaloelimination is taken into consideration as possible degradation 391 392 pathway of α -HCH by FeS (albeit a minor one), then the recorded ϵ C is similar with the ϵ C of -4.9 \pm 0.1% previously reported by (Zhang et al., 2014) during dihaloelimination of α -HCH by Fe 393 nanoparticles and with the εC of -3.7 \pm 0.8 % reported by (Badea et al., 2011) during 394 dihaloelimination pathway during anaerobic biodegradation of α -HCH by C. pasteurianum. The 395 recorded $\varepsilon C = -4.7 \pm 1.3$ % from this study is also similar to the εC values of -4.2 ± 0.4 % 396 and $-3.6 \pm 0.4\%$ recorded by (Liu et al., 2020b) for α -HCH and γ -HCH, respectively, during 397 anaerobic biodegradation by an enrichment culture. Comparing with the ε C from this study, 398 using the same enrichment culture, (Liu et al., 2020b) recorded a lower εC of $-1.9 \pm 0.3\%$ for 399 β -HCH, but a higher one of 6.4 \pm 0.7‰ for δ -HCH. Regarding the isotope fractionation appeared 400 during the abiotic degradation of organohalides by FeS, (Koster van Groos et al., 2018) recorded 401 an isotope enrichment factor of -19.3 ± 2.4 % during reductive debromination of the 1,2-402 403 dibromoethane by FeS.

For a more comprehensive elucidation of the reaction pathway, the apparent kinetic isotope effect (AKIE_C) (Elsner et al., 2005) was assessed assuming an elimination reaction with concerted bond cleavage at one axial H/Cl pair of α -HCH to form pentachlorocyclohexene (PCCH). In this case, two carbons with axial chlorine hydrogen pairs (x = 2) with two indistinguishable positions (z = 2) were considered in the calculation of AKIE_C, while α -HCH has 6 carbon atoms (n=6). The resulting

AKIE_C, 1.029 ± 0.008 , is slightly lower compared to the AKIE_C value of 1.035 ± 0.004 calculated 409 by (Liu et al., 2019) for LinA1 but higher to the AKIE_C value of 1.011 ± 0.001 calculated for 410 411 LinA2, during a study involving dehydrochlorination of α -HCH by Sphingobium indicum Strain B90A and by corresponding enzymes. The resulting AKIE_C, 1.029 ± 0.008 , is also lower than the 412 value of 1.048 \pm 0.003 recorded by (Zhang et al., 2014) during alkaline hydrolysis of α -HCH. 413 However, if we assume that two C-Cl bonds are stepwise and reductively cleaved during the 414 415 reaction of α -HCH to TCCH, we have to consider that a two-electron transfers to the α -HCH molecule for dichloroelimination can take place stepwise or in a concerted mode (Tobiszewski and 416 417 Namieśnik, 2012). The calculated AKIE_C values of 1.029 ± 0.008 (for a stepwise electron transfer) and of 1.014 ± 0.004 (for a two-electron transfer) are similar to the values of 1.026 ± 0.003 (for a 418 stepwise electron transfer) and of 1.013 ± 0.001 recorded by (Liu et al., 2020b) during anaerobic 419 biodegradation of α-HCH by an enrichment culture. The recorded AKIE_C values from this study 420 are also similar to the values of 1.030 ± 0.0006 (for a stepwise electron transfer) and of 421 1.015 ± 0.0003 (for a two-electron transfer) recorded by (Zhang et al., 2014), during 422 dihaloelimination of α-HCH by Fe nanoparticles. As suggested by (Butler and Hayes, 1998), the 423 predominance of dehydrochlorination in the degradation of α -HCH by FeS from this study, instead 424 of vicinal dihaloelimination, might be attributable to the FeS surface hydrolysis and this creates 425 426 heterogeneously vicinal species (that can be either protonated or deprotonated depending on pH). The heterogeneously vicinal species might favor the dehydrochlorination (elimination of HCl) 427 428 instead of vicinal dihaloelimination (elimination of two Cl atoms).

429 4. Conclusions

430 The results from this study clearly showed that carbon isotope fractionation occurs during 431 dechlorination of α -HCH by iron sulfide nanoparticles. Therefore, beside the biotic isotope 432 fractionation of HCH isomers induced by sulfate-reducing bacteria, the abiotic isotope 433 fractionation by biotic-formed FeS must be considered in anoxic sediments and aquifers

contaminated with HCH isomers, especially if the FeS is present in significant concentrations in 434 those areas. Nevertheless, the degradation of HCH isomers has been studied only with abiotic FeS 435 and therefore the extent of HCHs degradation in the field by biotic FeS is yet to be elucidated. 436 These degradation products identified by GC-MS demonstrated that the dehydrochlorination is the 437 main degradation pathway of the α -HCH by FeS, followed by formation of 1,2,4-TCB and by a 438 novel degradation pathway which involves a to further reductive dechlorination of 1,2,4-TCB to 439 440 1,2-DCB and finally to benzene, without accumulation of monochlorobenzene. The results of the three degradation experiments clearly showed that the apparent first-order rate constants during 441 442 dehalogenation of α -HCH by FeS increased with pH, which confirms the previous studies performed on the degradation of γ -HCH by FeS nanoparticles. Taking into account the novel 443 reaction mechanism proposed in this study, the isotope enrichment factor obtained can be used as 444 reference to assess and quantify the chemical and biological α-HCH transformation in the anoxic 445 environments. The relevance of various dehalogenation reaction mechanisms of α-HCH by FeS 446 are yet to be investigated using multi-element CSIA (i.e. hydrogen vs. carbon vs. chlorine) This 447 approach can provide new perspectives to assess also the environmental fate of all HCH isomers, 448 as well of other contaminants of emerging concern (CECs). 449

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Tab. 1. The values of the apparent rate constants for the dehalogenation of α-HCH calculated at
different pH values. The concentration of FeS was 9 g/L in all experiments.

Crt.	pH value	Apparent rate constant	Experiment			
No.		(d -1)				
1	2.4	0.009	2 nd			
2	5.3	0.014	2 nd			
3	8.1	0.018 ± 0.002	1 st			
4	9.9	0.253	3 rd			
5	11.8	1.098	2 nd			

Tab. 2. The concentration of α-HCH and of its major degradation products (1,2,4-TCB and 1,2-DCB) in aquatic samples and FeS slurry at the end of

the 1^{st} experiment (after 22 days) (pH=11.8).

Theoretical spiked α-HCH concentration (μM)	Final α-HCH aquatic concentration (μM)	Final α-HCH concentration in FeS slurry (μmol/g)	Final 1,2,4-TCB aquatic concentration (µM)	Final 1,2-DCB aquatic concentration (µM)	Aquatic ratio C1,2,4- TCB/C1,2- DCB	Final 1,2,4-TCB concentration in FeS slurry (µmol/g)	Final 1,2-DCB concentration in FeS slurry (µmol/g)	Final sum of α- HCH (μmol)	Final sum of 1,2,4- TCB (µmol)	Final sum of 1,2- DCB (µmol)
20.7	5.6 ± 0.1	0.840 ± 0.008	0.844 ± 0.045	0.115 ± 0.098	7.3	0.008 ± 0.003	0.0009 ± 0.0004	2.3132	0.0935	0.0125

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Fig. 1. The proposed pathway for the reductive dehalogenation of α -HCH with FeS nanoparticles from this study (A); Hydrolysis pathway proposed by (Kannath et al., 2019) (B); Biotic dehydrochlorination degradation pathway proposed by (Suar et al., 2005) (C); Biotic vicinal dihaloelimination pathway proposed by (Badea et al., 2011) (D).



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Fig. 2. The degradation curves of α -HCH by FeS at pH values: 8.1 (**•**) (experiment 1, black line); 2.4 (**•**) and 5.3 (**•**) (experiment 2, cyan line); and 9.9 (experiment 3, green line) (**v**), in three different degradation experiments, as well the evolution of α -HCH concentration in the control experiment (**•**) (experiment 4 without FeS, blue lines) at pH value of 7.0.



Fig. 3. Isotope signatures (\square) vs. concentrations (\blacksquare) of α -HCH during the 1st degradation experiment by FeS at pH of 8.1 (A). Formation of 1,2,4-TCB (\blacktriangle) and 1,2-DCB (\bullet) in the same experiment with FeS (B). The error bars shown the standard deviation of the concentration values between the two replicates, and the standard deviation of the isotope analysis, respectively.



Fig. 4. Isotope signatures (○) vs. concentrations (●) of 1,2,4-TCB during the 2nd degradation
experiment by FeS at pH of 11.8 (A). Formation of 1,2-DCB (◄) in the same experiment (B).



Fig. 5. Calculation of carbon isotope enrichment factor of α -HCH during dehalogenation by FeS according to the Rayleigh model in experiment 1 (pH = 8.1).