This is the authors' final version of the contribution published as:

The publisher's version is available at:

1 **Abiotic reductive deiodination of iodinated organic compounds and X-ray contrast**

2 **media catalyzed by free corrinoids**

3

4 Fatima El-Athman [a], Lorenz Adrian [b,c], Martin Jekel [a], Anke Putschew [a,*]

5 [a] Technische Universität Berlin, Department of Environmental Science and Technology, Chair

6 of Water Quality Engineering, Straße des 17. Juni 135, 10623 Berlin, Germany

7 [b] Helmholtz-Centre for Environmental Research GmbH – UFZ, Department Isotope

8 Biogeochemistry, Permoserstraße 15, 04318 Leipzig, Germany

9 [c] Technische Universität Berlin, Department of Biotechnology, Chair of Geobiotechnology,

10 Ackerstraße 76, 13355 Berlin, Germany

11 * Corresponding author:

12 Phone: 0049 30 314 25480. Fax: 0049 30 314 79621. E-mail: anke.putschew@tu-berlin.de

13   **Graphical abstract**
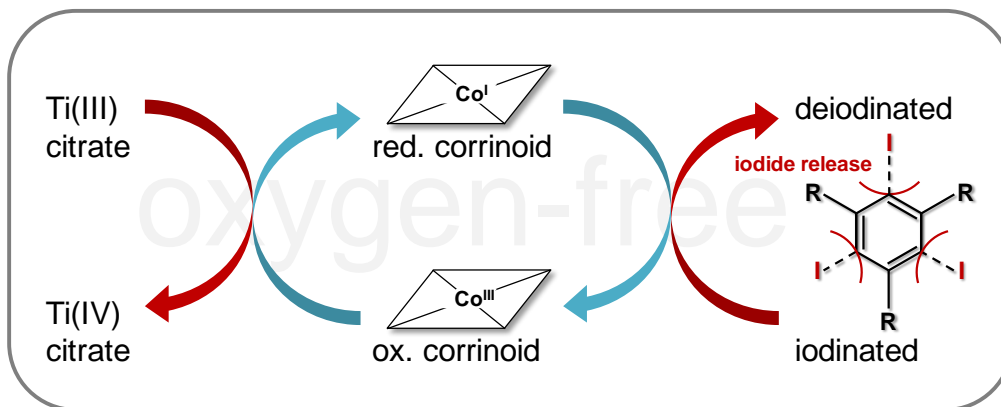
14

15

16   **Highlights**

17   • Deiodination of iodinated contrast media can be catalyzed by B_{12} derivatives

18   • Linear correlation between iodide release rate and corrinoid concentration

19   • Deiodination degree depends on the oxidation state of the cobalt center of B_{12}

20   • Decrease of the iodinated compounds follows first-order kinetics

21   • Deiodination is influenced by the substitution pattern of bound iodine

Abstract

Iodinated X-ray contrast media are known for their stability concerning deiodination in the aquatic environment under aerobic conditions. In this study, we demonstrate the abiotic reductive deiodination of the iodinated contrast media iopromide, iopamidol and diatrizoate in the presence of corrinoids. In addition, triiodinated benzoic acid derivatives with iodine atoms bound at different positions were investigated. Corrinoids like cyanocobalamin (vitamin B_{12}) and dicyanocobinamide served as electron shuttles and as catalysts between the reducing agent (e.g., titanium(III) citrate) and the electron accepting iodinated compound. The concentration decrease of the iodinated compounds followed first-order kinetics with rate constant k_{obs} depending on the iodinated compound. A linear correlation between the rate of iodide release and the corrinoid concentration was observed, with deiodination rates for dicyanocobinamide twice as high as for vitamin B_{12}. Reducing agents with a less negative standard redox potential like dithiothreitol or cysteine caused slower deiodination as the cobalt center was only reduced to its Co^{II} oxidation state. With a temperature increase from 11 to 23 °C, the concentrations of released iodide doubled. A complete deiodination was only observed for the iodinated contrast media but not for structurally similar iodinated benzoic acid derivatives.

Keywords: deiodination, X-ray contrast media, corrinoids, vitamin B_{12}, abiotic, bank filtration

_____

Abbreviations
ICM: iodinated contrast media
TIBA: triiodobenzoic acid
ATIA: 5-amino-2,4,6-triiodoisophthalic acid
B_{12}: cyanocobalamin
$(CN)_2$Cbi: dicyanocobinamide
MV: methyl viologen
BV: benzyl viologen
DTT: dithiothreitol

1 Introduction

Triiodinated benzoic acid derivatives are widely used as contrast media for X-ray examinations of organs and vessels with more than 100 g of contrast media applied for a single examination (Speck and Hübner-Steiner, 1999). The compounds are very polar and stable and are excreted unchanged via urine within hours after application (Speck and Hübner-Steiner, 1999). As a consequence, iodinated X-ray contrast media (ICM) represent the major contribution of halogenated organic compounds in hospital wastewater, municipal waste water and surface waters in Berlin (Germany) shown by the AOI (adsorbable organic bound iodine) determined by differentiated AOX analysis (Oleksy-Frenzel et al., 2000). By conventional wastewater treatment, the AOI is only reduced by 20% (Putschew and Jekel, 2006), indicating a high stability of the triiodinated benzoic acid structure of ICM. In the last decade, the behavior of ICM in different technical processes has been studied extensively. Ozonation has been shown to lead to a fast concentration decrease of iopromide, whereas an AOI decrease has only been observed with uneconomical high ozone dosages (Putschew et al., 2007). In contrast, a complete deiodination could be achieved by reductive processes such as metal-catalyzed hydrodeiodination (Knitt et al., 2008; Stieber et al., 2011), electrochemical reduction (Radjenovic et al., 2013; Zwiener et al., 2009) and disinfection (Duirk et al., 2011).

Studies investigating the biotic transformation of ICM in aerobic water–soil and water–sediment systems revealed the production of various metabolites with transformed side-chains but without deiodination (Batt et al., 2006; Kormos et al., 2010; Kormos et al., 2011; Schulz et al., 2008). In contrast to the results observed in aerobic environment, deiodination has been found to occur under anoxic/anaerobic biotic conditions. Redeker et al. (2014) detected different partially deiodinated transformation products of diatrizoate in anaerobic batch tests with sediment from a polishing pond and detected the same products in groundwater and technical wetland samples. A partial deiodination of ICM was also detected for the anoxic/anaerobic bank filtration with an AOI decrease of around 63% (Grünheid et al., 2005;

78 Schittko et al., 2004; Wiese et al., 2011). Complete deiodination by a microbial consortium has

79 been shown for the ICM precursor 5-amino-2,4,6-triiodoisophthalic acid (Lecouturier et al.,

80 2003) and for 2,4,6-triiodophenol where the involvement of novel anaerobic bacteria has been

81 suggested (Oba et al., 2014). Nevertheless, studies focusing on biological deiodination are

82 scarce compared to those regarding dechlorination and debromination. Various bacteria,

83 including the genera *Dehalobacter*, *Desulfitobacterium*, *Desulfuromonas*, *Sulfurospirillum*,

84 *Geobacter*, *Dehalogenimonas* and *Dehalococcoides*, have been found to remove chlorine or

85 bromine substituents from aromatics (Löffler et al., 2013). They all use corrinoid-containing

86 enzymes to catalyze the reductive dehalogenation (Holliger et al., 1998). Corrinoids are

87 organometallic complexes with a central cobalt ion that can be present in three different

88 oxidation states (+I, +II and +III), enabling the corrinoid to function as electron shuttle between

89 an electron donor and a halogenated compound.

90 Apart from microbiological reduction, an abiotic dehalogenation catalyzed by heat-inactivated

91 enzymes or commercially available free corrinoids has been shown for several chlorinated

92 compounds (Gantzer and Wackett, 1991; Krone et al., 1989a; Krone et al., 1989b; Neumann et

93 al., 2002). Therefore, it is conceivable that apart from microbiological dehalogenation, abiotic

94 dehalogenation catalyzed by free corrinoids, released to the environment from lysed

95 microorganisms, plays a role in natural degradation processes.

96 To our knowledge, abiotic deiodination catalyzed by cobalt-containing corrinoids has not yet

97 been shown. We hypothesized that free corrinoids could contribute to the deiodination of ICM

98 observed in nature. In this study, we have chosen three ICM and three other triiodinated benzoic

99 acid derivatives as model compounds to test whether abiotic deiodination by free corrinoids is

100 possible. The dependence of the abiotic deiodination on different parameters, e.g., type and

101 concentration of the corrinoid, structure of the iodinated compound and temperature was

102 studied. Our results provide new insight into the degradation of ICM and other iodinated

103   benzoic acid derivatives and improve the prediction of contaminant fate during bank filtration

104   and (managed) artificial groundwater recharge.

105

2   Material and Methods

2.1   Chemicals

Iopromide and diatrizoate were kindly provided by Bayer-Schering AG (Berlin, Germany). Iopamidol, 2,4,6-triiodobenzoic acid (TIBA), 2,3,5-TIBA, 5-amino-2,4,6-triiodoisophthalic acid (ATIA), cyanocobalamin (B_{12}), dicyanocobinamide (($CN)_2$Cbi) (structures are given in Appendix, Figures A.1–A.3), methyl viologen (MV), benzyl viologen (BV), dithiothreitol (DTT), cysteine and titanium(III) chloride were purchased from Sigma Aldrich (Steinheim, Germany). Titanium(III) citrate was prepared as previously described (Zehnder and Wuhrmann, 1976). All chemicals used were of analytical grade. Ultrapure water was produced from deionized water with a water purification system (maxima ultrapure water; ELGA, Ubstadt-Weiher, Germany).

2.2   Experiments

All experiments were carried out under oxygen-free conditions within an anaerobic chamber (95% N_2, 5% H_2, Coy Laboratories, Grass Lake, USA) or a glovebox (100% N_2, GS E-Line, Glovebox Systemtechnik, Maisch, Germany).

Experiments were conducted with ultrapure water buffered with either potassium acetate (pH 5.8) or phosphate buffer (pH 7) on microtiter plates commonly used for the determination of enzyme activity. The reaction solution was equilibrated to room temperature or to 30 °C and contained 0.33 mM titanium(III) citrate, 1 mM MV and different concentrations of either B_{12} or ($CN)_2$Cbi. Reactions were started by the addition of the electron acceptor iopromide (30 or 100 µM). The total sample volume in each microtiter well was 210 µL. The MV solution reduced by titanium(III) citrate is deep-blue and an absorption decrease indicates its oxidation and a redox reaction. The absorbance at 578 nm ($\varepsilon_{578} = 9.78$ mM^{-1} cm^{-1}) was measured every 2 min with a plate reader (PowerWave HT, BioTek Instruments GmbH, Bad Friedrichshall, Germany and Ao Microplate Reader, Azure Biosystems Inc., Dublin, USA) for high-resolution

7

132 that is necessary for the determination of the initial iodide release and turnover numbers. The

133 iodide release rates (a_{iodide}) were calculated from the average slope of the linear part (over

134 10 min) of the absorption curves ($\Delta E/\Delta t$) using the sample volume (V), the extinction

135 coefficient of reduced MV and the path length (equation (1)).

136 (1) $\quad a_{iodide} = \frac{\Delta E}{\Delta t} \cdot \frac{V}{\varepsilon_{MV} \cdot d \cdot 2}$

137 The plates contained triplicate samples as well as triplicate buffer controls, controls without

138 iodinated electron acceptor and controls without corrinoids.

139 Tests on microtiter plates were repeated in serum bottles with a reaction volume of 5 mL to

140 allow chemical analysis of reaction products. In addition, tests with different iodinated

141 compounds and different reducing agents were conducted in serum bottles. Unless otherwise

142 stated, the tests in serum bottle were carried out at room temperature and pH 7. The test mixtures

143 contained buffered ultrapure water, 0.22 mM reducing agent (titanium(III) citrate, MV, BV,

144 DTT or cysteine), 1 µM corrinoid and 30 µM of the iodinated compounds. MV and BV were

145 reduced by titanium(III) citrate: They were the only reducing agents present at the start of the

146 reaction since titanium(III) citrate was completely oxidized. After the start of the reaction by

147 the addition of the iodinated compound, the bottles were sealed with butyl stoppers and

148 aluminum crimp caps and stored in the dark without agitation under oxygen-free conditions.

149 Samples were taken at different time intervals and the reaction was stopped by introducing air

150 into the samples. All experiments were conducted in duplicate or triplicate.

151

152 **2.3 Analyses**

153 Iodinated compounds were quantified without any pre-treatment by liquid chromatography

154 with UV detection (LC-UV, Agilent 1200, Waldbronn, Germany). For separation, a C18

155 column (Phenomenex, Luna 3 µm C18(2); 150×2 mm) was used and the column oven was set

156 to 30 ℃. The injection volume was 10 µL. Ultrapure water and methanol, both acidified with

157   0.1% trifluoroacetic acid, were used as eluent with a flow rate of 0.25 mL min^{-1}. Iodide was

158   quantified by ion chromatography with UV detection (IC-UV, HP 1100, Waldbronn, Germany)

159   using an anion exchange column (Dionex, IonPac AS9-SC; 4×250 mm) and an eluent

160   containing 2.2 mM Na_2CO_3 and 0.75 mM $NaHCO_3$ with a flow rate of 1 ml min^{-1} and an

161   injection volume of 10 µL. For more details on calibration and chromatographic conditions see

162   Appendix (Table A.1–A.4). Photometric determinations of B_{12} were made using a Perkin Elmer

163   Lambda 12 UV-VIS spectral photometer (Rodgau, Germany) and quartz cuvettes (path length

164   1 cm) with a lid to keep the solution oxygen-free.

3   Results and Discussion

3.1   Catalyzing role of corrinoids

To investigate whether corrinoids have catalyzing effects on the reductive deiodination of iodinated X-ray contrast media, iopromide was treated with reduced MV, a radical cation, as electron donor and B_{12} or $(CN)_2Cbi$ as a potential catalyst. A reduction reaction is indicated by a decolorization of the reaction solution which is monitored photometrically. Only a very slight absorption decrease over time was recorded for the control tests without corrinoids, whereas in the presence of B_{12} and $(CN)_2Cbi$, a strong decrease of the absorption was observed (Appendix, Figure A.4). The decolorization was consistently higher with $(CN)_2Cbi$ than with B_{12}. The increase of the iopromide concentration from 30 to 100 µM increased the decolorization as well.

We investigated whether the absorption decrease of MV is caused by deiodination or by other reactions of the iopromide molecule. The removal of one iodine atom requires two electrons (equation (2) and Appendix, Figure A.5).

(2) $R–X + 2MV^{+\bullet} + H^+ \rightarrow R–H + 2MV^{2+} + X^-$
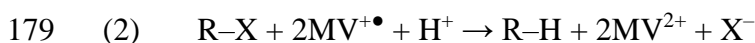
Using the specific absorption coefficient of MV ($\varepsilon_{578} = 9{,}78$ mM^{-1} cm^{-1}), we calculated the expected iodide concentration from MV decolorization and compared it with measured iodide values. The consistency of measured and calculated iodide concentrations (**Figure 1**) confirms that the absorption decrease is caused by deiodination consuming two electrons and that no other reactions occur.

185
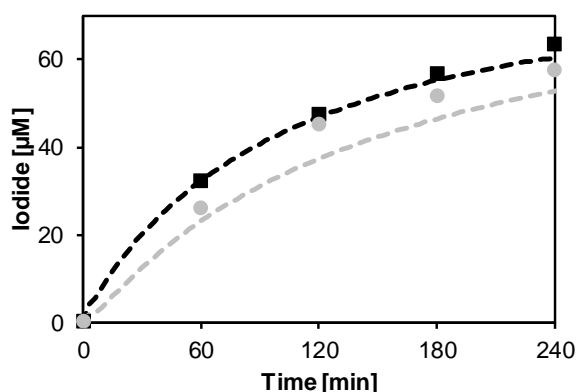
Figure 1. Iodide release calculated based on MV absorption measurements (dashed lines) and measured iodide concentrations (symbols) in reaction bottles over time. 0.33 mM reduced MV, 5 µM B_{12} (grey, ●) or 5 µM $(CN)_2Cbi$ (black, ■) and 100 µM iopromide; pH 5.8, 30 °C. n = 3, MV absorption SD = ± 0.04, SD error bars of measured iodide concentrations are smaller than the symbol size.

In the next step, we investigated the effect of the corrinoid concentration on the initial

deiodination by photometrical measurements on microtiter plates which produce high-

resolution data without any errors caused by sampling or other steps necessary before analysis.

194
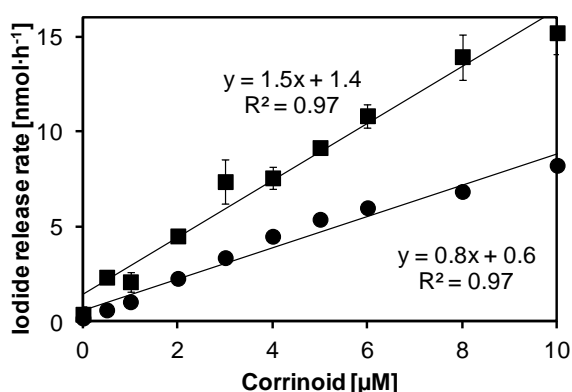
Figure 2. Iodide release rate over corrinoid concentration with 0.33 mM reduced MV, B_{12} (●) and $(CN)_2Cbi$ (■) and 30 µM iopromide; pH 7, room temperature. n = 3, most SD error bars are smaller than the symbol size.

The iodide release rate was found to be linearly dependent on the B_{12} and $(CN)_2Cbi$

concentration over the investigated range of up to 10 µM corrinoid (**Figure 2**). Using $(CN)_2Cbi$

as catalyst resulted in higher iodide release rates than using B_{12}. For a corrinoid concentration

of 10 µM, $(CN)_2Cbi$ showed an iodide release with a turnover number of $2.2 \cdot 10^{-3}$ s^{-1} (mol

iodide released per mol corrinoid per second) which is about twice as high as the turnover

number determined for B_{12} ($1.2 \cdot 10^{-3}$ s^{-1}). Both turnover numbers were calculated using the

11

204 regression lines from **Figure 2** with $R^2 = 0.97$ for both lines. These results are in line with a

205 previous study that also showed a linear correlation between the dehalogenation rate of 0.8 mM

206 trichloroacetate and B_{12} concentrations of up to 0.1 µM (Neumann et al., 2002). However, we

207 were able to document the linearity for a broader B_{12} range and much lower concentrations of

208 the halogenated compound.

209 Differences in the catalyzing properties of B_{12} and $(CN)_2Cbi$ in our experiments can be

210 explained by their different ligands at the α- and the β-side of the central cobalt ion (Appendix,

211 Figure A.3). Depending on the form of vitamin B_{12}, the ligand on the β-side is a cyano-, methyl-

212 or deoxyadenosyl-group coordinated by a cobalt-carbon bond and removed in the super-

213 reduced state (Co^I) (Banerjee and Ragsdale, 2003; Schneider and Stroiński, 1987). The ligand

214 at the α-side consists of nitrogen in a dimethylbenzimidazole group which is covalently bound

215 to a ribose and a phosphate group (ó Proinsias et al., 2013). The α-side ligand is either bound

216 (base-on) or not bound (base-off) to the cobalt ion, depending on the pH (ó Proinsias et al.,

217 2013). In contrast to B_{12}, $(CN)_2Cbi$ has a cyanide ligand at the α- and at the β-side and is missing

218 the dimethylbenzimidazole, the ribose and the phosphate group of B_{12} (ó Proinsias et al., 2013).

219 The observed different turnover numbers for iopromide could be explained by a possible

220 difference in the electron transfer which is mediated by $(CN)_2Cbi$ from the α- and the β-side of

221 the corrin ring whereas B_{12} mediates it only from the β-side.

222

223 **3.2   Influence of the reducing agent**

224 Along the infiltration zone and aquifer passage, different redox zones are present which

225 facilitate the transformation and degradation of different organic compounds. For reductive

226 transformation processes during bank filtration such as deiodination, a low redox potential is

227 one of the key factors (Wiese et al., 2011).

228 We investigated the influence of the redox potential on the deiodination by using reducing

229 agents with less negative redox potentials than titanium(III) citrate to simulate different redox

12

230　environments. The tests were performed with different reducing agents and with B_{12} as catalyst

231　and iopromide as electron acceptor over a period of several days by measuring the iodide release

232　in serum bottles with 20 mL total sample volume.
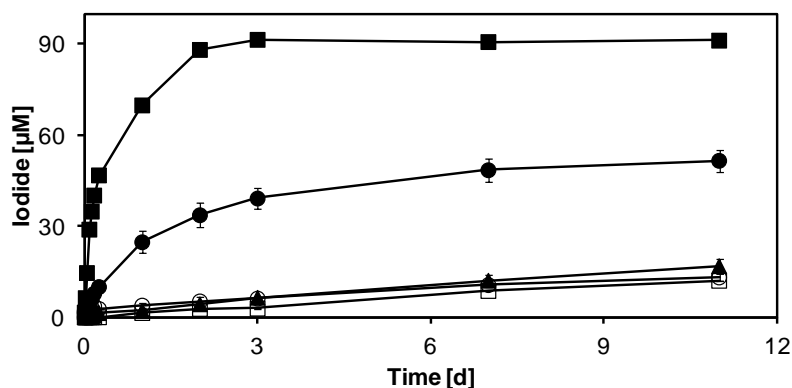
233

234　**Figure 3.** Iodide release over time from 30 µM iopromide in the presence of 1 µM B_{12} and
235　0.22 mM of a reducing agent: titanium(III) citrate (■), reduced MV (●), cysteine (▲), DTT (○)
236　and reduced BV (□); pH 7, room temperature. n = 2, most SD error bars are smaller than the
237　symbol size.

238　In the presence of titanium(III) citrate, the complete deiodination of 30 µM iopromide, resulting

239　in an iodide concentration of 90 µM, was reached within three days (**Figure 3**). With MV, an

240　iodide concentration of about 50 µM (56% deiodination) was released after 11 days. For DTT,

241　cysteine and BV, the iodide concentration was significantly lower, varying between 12 and

242　17 µM (13–19% deiodination) after 11 days. The five reducing agents differ in their standard

243　redox potentials, increasing in the following order: titanium(III) citrate (–480 mV (Zehnder and

244　Wuhrmann, 1976)), MV (–450 mV (Bird and Kuhn, 1981)), BV (–360 mV (Bird and Kuhn,

245　1981)), DTT (–330 mV (Cleland, 1964)), cysteine (–220 mV (Jocelyn, 1967)). The

246　concentration of released iodide correlated with the standard redox potential and the three

247　reducing agents with the less negative redox potentials effected only a low deiodination. When

248　comparing the redox potentials of the two oxidation states of B_{12}, it appears that the reduction

249　from Co^{III} to Co^{II} with a standard redox potential of +514 mV (Lexa and Saveant, 1983) is far

250　easier than the reduction from Co^{II} to Co^{I} with a potential of –496 mV (Lexa and Saveant, 1983)

251　(for base-off). To investigate to which oxidation state B_{12} has been reduced by the different

reducing agents, the absorption spectra of B_{12} reduced with an excess of titanium(III) citrate, DTT or cysteine were recorded during deiodination. To ensure complete reduction of B_{12}, the solution of B_{12} with titanium(III) citrate was stored for four days before the test was started by the addition of iopromide. Before adding iopromide, B_{12} was present in the super-reduced Co^I state with a peak at 386 nm (**Figure 4**). The addition of iopromide led to a decrease of the Co^I-peak with an absorbance increase at 470 nm which corresponds to the Co^{II} state according to published data (Beaven and Johnson, 1955; Smith and Woods, 1994). One hour after the addition of iopromide, a reverse effect was noticeable: The Co^I-peak increased again and nearly reached its initial maximum while the Co^{II} peak almost disappeared indicating re-reduction of Co^{II} to Co^I with the remaining titanium(III) citrate. The iodide concentration at different times revealed that after one hour, the deiodination had been already completed (data not shown). This indicated that the oxidation of Co^I to Co^{II} by iopromide was faster than the re-reduction of Co^{II} to Co^I by titanium(III) citrate. These results correspond to similar findings for the dechlorination of tetrachloroethene (Gantzer and Wackett, 1991). The Co^{III} state, represented by an absorption peak at 360 nm was obtained by introducing oxygen (**Figure 4**).
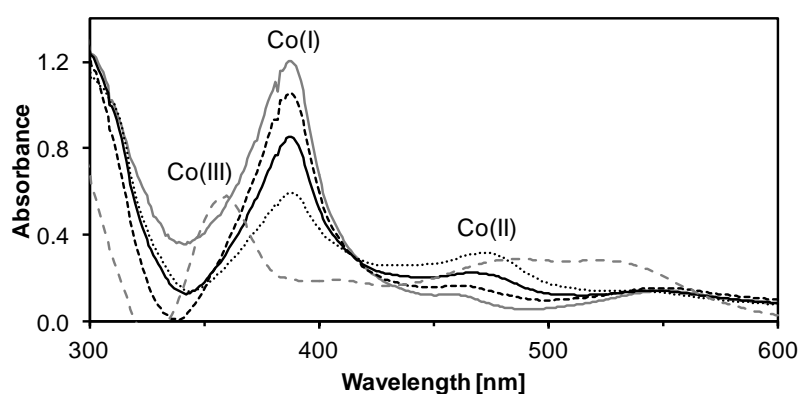
Figure 4. Absorption spectra of 50 µM B_{12} reduced by 0.22 mM titanium(III) citrate: before adding 30 µM iopromide (grey solid line), 5 min after (black solid line), 10 min after (dotted line), 60 min after (black dashed line) and after introducing oxygen (grey dashed line).

In the presence of 300 mM DTT or 300 mM cysteine, the observed absorption spectra indicate that DTT and cysteine were only able to reduce B_{12} to the Co^{II} state within four days (Appendix, Figure A.6), which is in agreement with data from a previous study (Smith and Woods, 1994).

14

274 Within the first hour after the addition of 30 µM iopromide, the spectral profiles remained

275 unchanged despite the deiodination occurring (iodide release not shown). It can be assumed

276 that due to the high excess of the reducing agent, B_{12} was immediately re-reduced to its Co^{II}

277 state after donating the electron to iopromide. Therefore, the absorption spectra of B_{12} suggest

278 that the low deiodination rates obtained with DTT and cysteine (**Figure 3**) were caused by the

279 higher standard redox potentials compared to titanium(III) citrate and the resulting oxidation

280 states of the B_{12}. Only the highly reactive super-reduced Co^{I} state of B_{12} seems to be an effective

281 catalyst for a complete deiodination of iopromide.

282

283 **3.3 Influence of temperature**

284 The influence of temperature on the deiodination was examined using iopromide, titanium(III)

285 citrate and B_{12} at 11, 23 or 33 °C. With increasing temperature, the deiodination increased

286 (**Table 1**). Samples taken after 5 h showed the highest differences in iodide concentrations in

287 dependence on the temperature. After 5 h, the iodide concentration at 23 °C was already more

288 than twice as high as the concentration at 11 °C and four times higher than the concentration at

289 33 °C. For the tests at 23 and 33 °C, the influence of temperature decreased with reaction time

290 because a near complete deiodination resulting in about 90 µM iodide was already reached after

291 24 h.

292 **Table 1.** Deiodination of iopromide with B_{12} at different temperatures

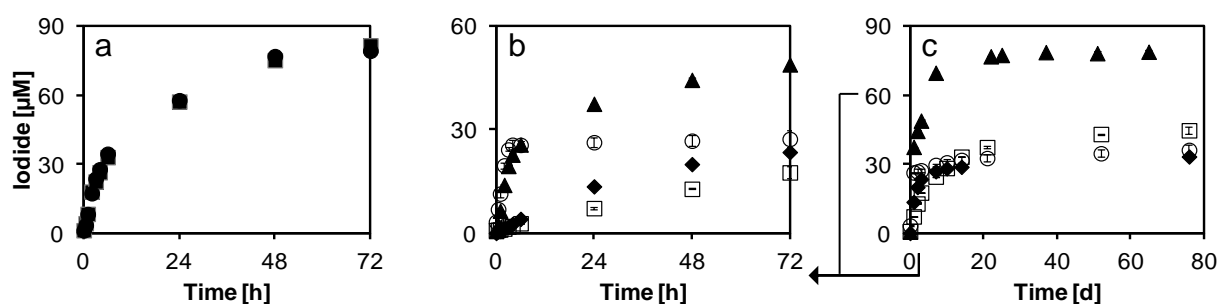| Temperature [°C]	Iodide [µM][a]		
	5 h	24 h	48 h
11	15.5	45.1	60.2
23	42.3	80.0	89.4
33	61.9	84.3	87.8

293 [a] Iodide release of 30 µM iopromide with 5 µM B_{12}; pH 5.8.

294

15

3.4 Different iodinated compounds

In addition to iopromide, the reductive deiodination was also examined for the ICM iopamidol and diatrizoate as well as for other iodinated benzoic acid derivatives (2,4,6- and 2,3,5-TIBA and ATIA) in the presence of titanium(III) citrate and B_{12}. For a better understanding of the deiodination of the selected compounds, samples were taken over a longer period of time and the iodide concentrations (**Figure 5**) as well as the concentrations of the initial compounds (**Figure 6**) were quantified.

Figure 5. Formation of iodide over time from 30 µM of different iodinated compounds in the presence of 1 µM B_{12} and 0.22 mM titanium(III) citrate; pH 7, room temperature. (a): Iopromide (■) and iopamidol (●) (symbols are strongly overlapping). (b) and (c): Diatrizoate (▲), 2,4,6-TIBA (□), 2,3,5-TIBA (○) and ATIA (♦). n = 2, SD error bars are smaller than the symbol size.

Based on the measured iodide concentrations, both iopromide and iopamidol were nearly completely deiodinated within three days and show similar iodide concentration curves over time (**Figure 5a**). For diatrizoate and the other tested iodinated benzoic acid derivatives, the concentration of released iodide was significantly lower (**Figure 5b** and **c**). **Figure 5b** illustrates the iodide release within the first 72 hours, whereas **Figure 5c** shows a period of 76 days. While diatrizoate was almost completely deiodinated after 20 days, 2,4,6- and 2,3,5-TIBA and ATIA showed only a deiodination of around 33% after 50 days with only a very slight further increase of the iodide concentration until day 76. These data suggest that for these compounds, one iodine atom was released relatively fast whereas the second and third iodine atoms were not removed under the tested conditions. **Figure 6** shows the decreasing concentrations of the tested ICM over time. Taking the logarithm of the concentration shows that the deiodination can be

16

319 described by a first-order rate law with the rate constant k_{obs} (Appendix, equation (A.1) and

320 (A.2)).

321
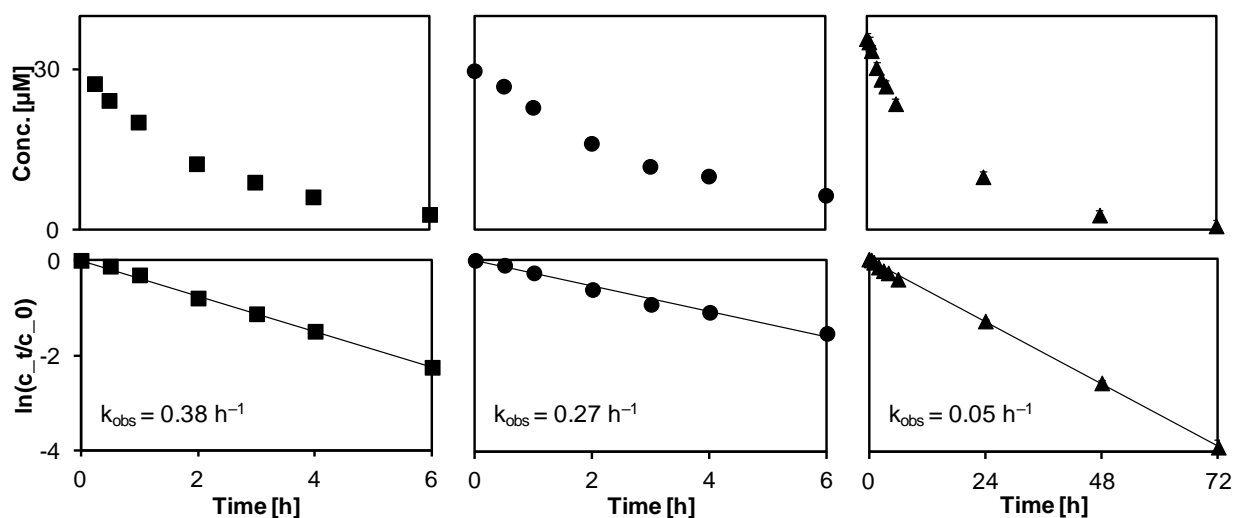
Figure 6. Reduction of 30 µM iopromide (■), iopamidol (●) and diatrizoate (▲) in the presence of 1 µM B_{12} and 0.22 mM titanium(III) citrate; pH 7, room temperature. Upper graphs: concentration decrease over time. Lower graphs: plot of ln (c_t/c_0) and first-order rate constants k_{obs}. n = 2, SD error bars are smaller than the symbol size.

326 The concentration decrease of 2,4,6-TIBA, 2,3,5-TIBA and ATIA also followed first-order

327 kinetics (Appendix, Figure A.7). For 2,3,5-TIBA, the highest k_{obs} value of 1.0 h^{-1} was observed

328 which is more than a hundred times higher than that for 2,4,6-TIBA (Appendix, Table A.5).

329 Since both compounds only differ in the position of their iodine atoms, it seems that an iodine

330 atom is released much faster in the presence of an iodine atom in ortho position. This is

331 consistent with data from electron density modeling of chlorinated aromatics showing that a

332 halogen substituent has a less negative partial charge on the apical site of the carbon-halogen

333 bond ("sigma hole") when the neighboring carbon atoms are also substituted by halogens

334 (Cooper et al., 2015).

335 Within the first two hours, the degradation curve of 2,3,5-TIBA showed an almost linear

336 progression (Appendix, Figure A.7) and the coefficient of determination of the first-order

337 linearization yields high inaccuracy ($R^2 = 0.87$). These findings suggest that the concentration

338 decrease of 2,3,5-TIBA could theoretically be even faster (resulting in a higher k_{obs} value) and

339 that the deiodination was limited by the previous reaction step, the electron transfer between

17

340   titanium(III) citrate and the corrinoid. Except for k_{obs} of 2,3,5-TIBA, the k_{obs} values confirm the

341   observations made from the iodide concentrations (**Figure 5**): The concentration decrease of

342   the three ICM is faster than that of 2,4,6-TIBA and ATIA while iopromide and iopamidol show

343   higher k_{obs} values than diatrizoate (Appendix, Table A.5). It can be assumed that the side chains

344   of the highly polar ICM induce a more positive partial charge on the iodine atoms than the

345   carboxy groups of the TIBA, whereby the withdrawing effect increases and higher k_{obs} values

346   can be reached. However, k_{obs} only gives information about the release rate of the first iodine

347   atom because the compounds can no longer be quantified after the structure of the molecule has

348   changed. The analysis of the transformation products and the complete reaction kinetics will be

349   the subject of future work.

350

351   **3.5   Environmental significance**

352   Halogenated aromatics, such as ICM, are one of the largest groups of environmental

353   contaminants with dehalogenation being one of the most important processes for their

354   degradation. An improved biodegradability has been observed for deiodinated transformation

355   products where the large iodine atoms are no longer hindering further degradation processes

356   (Redeker et al., 2014; Stieber et al., 2011). Therefore, the transformation of ICM under natural

357   conditions like during bank filtration and the understanding of the transformation pathways are

358   of great interest. Along the infiltration zone and aquifer passage, different redox zones are

359   formed by the consumption of oxygen and of further electron acceptors such as nitrate. Previous

360   studies have shown that side-chain transformation of ICM is occurring under aerobic conditions

361   while the deiodination is only possible under anoxic/anaerobic conditions (Grünheid et al.,

362   2005; Kormos et al., 2011; Schittko et al., 2004). Natural organic matter (NOM) present in the

363   soil increases the oxygen demand and thus supports the formation of anoxic zones.

364   Furthermore, NOM such as humic acids may function as electron shuttle for reductive processes

365   (Uchimiya and Stone, 2009; Van der Zee, Frank P. and Cervantes, 2009).

366 Our results show that an abiotic deiodination of ICM catalyzed by corrinoids is possible and

367 that it can lead to complete deiodination. Corrinoids are synthesized by several sediment

368 bacteria, e.g., denitrifying bacteria, methanogens and acetogens (Löffler et al., 2013; Martens

369 et al., 2002; Yan et al., 2012), and are required as co-factors for reductive dehalogenase

370 enzymes (Adrian et al., 2000; Kube et al., 2005; Yan et al., 2012). From the perspective of

371 remediation, a dehalogenation by free corrinoids provides several advantages compared to a

372 dehalogenation by enzyme-bound corrinoids. For instance, the loss of substrate specificity in

373 the absence of a protein structure has been reported for iron porphyrins (Wade and Castro,

374 1973). Furthermore, many dehalogenase enzymes of the strictly anaerobic organohalide-

375 respiring bacteria were found to be O_2-sensitive (Maillard et al., 2003; Wohlfarth and Diekert,

376 1997), whereas changing redox conditions do not pose irreversible damage to free corrinoids.

377 Our findings show that cobinamide, the precursor of cobalamin, can already function as a

378 catalyst for abiotic deiodination, proving that the biosynthetic pathway to cobalamin has not to

379 be completed for abiotic deiodination. It has also been shown that some dehalogenating strains

380 require specific corrinoid co-factors for their enzyme activity and cell growth (Adrian and

381 Löffler, 2016). In contrast, we assume that for the abiotic dehalogenation of ICM, all corrinoids

382 as well as their precursors and degradation products, containing only the core corrinoid

383 structure, might function as catalysts. High concentrations of sediment-bound corrinoids, e.g.,

384 B_{12} concentrations up to $0.1 \, \mu mol \, kg^{-1}$ (Nishijima and Hata, 1978; Schneider and Stroiński,

385 1987), have been reported.

386 Cooper et al. (2015) investigated the biotic dehalogenation of different chlorinated and

387 brominated compounds by organohalide-respiring bacteria and found that compounds showing

388 abiotic dehalogenation caused lower microbiological growth yields. The dehalogenation of

389 these molecules might be partially uncoupled from the energy metabolism and cell growth of

390 the microorganisms (Cooper et al., 2015). If biological dehalogenation is indeed occurring, it

391 is not easy to distinguish between abiotic corrinoid- and biotic enzyme-catalyzed reactions.

392 Different isotope effects observed by compound-specific stable isotope analysis might be used

393 for a differentiation of both dehalogenation pathways (Renpenning et al., 2014).

4 Conclusion

In this study, the abiotic deiodination of iodinated contrast media and other iodinated benzoic acid derivatives in the presence of free corrinoids were investigated. The corrinoids cyanocobalamin (vitamin B_{12}) and dicyanocobinamide were found to function as strong catalysts between the electron accepting iodinated compound and a reducing agent. The deiodination rate linearly depended on the corrinoid concentration and dicyanocobinamide, a precursor of vitamin B_{12}, was found to be a stronger catalyst than B_{12} itself. The deiodination degree and rate further depended on the structure of the iodinated compound as well as on the standard redox potential of the reducing agent. While strong reducing agents such as titanium(III) citrate could reduce the corrinoid to its super-reduced Co^I state, reducing agents with a less negative standard redox potential like dithiothreitol or cysteine reduced it only to the Co^{II} state, causing a slower and incomplete deiodination.

Since the investigated ICM showed very high abiotic deiodination rates, we conclude that abiotic deiodination catalyzed by free corrinoids present in sediments could play a role in the deiodination of ICM during bank filtration and might thus be used for managed ground water recharge. Based on our results, reasons for slower reaction rates and incomplete deiodination of ICM in nature could be attributed to lower ICM concentrations, competing electron acceptors in the water–soil matrix, lower temperatures and higher redox conditions, enabling the corrinoid reduction only to its Co^{II} state, as well as insufficient retention times. An adjustment of the mentioned parameters to near-natural conditions will be covered in future research. In particular, the deiodination will be investigated with lower ICM and corrinoid concentrations in a natural water matrix. Apart from organic compounds competing with the ICM as electron acceptors, further reducing agents and electron shuttles are expected in a natural water matrix. For instance, quinones as model compounds of humic acids are known for their catalyzing capability (Uchimiya and Stone, 2009; Van der Zee, Frank P. and Cervantes, 2009).

21

429 **References**

430 Adrian, L., Löffler, F.E., 2016. Organohalide-respiring bacteria. Springer, Berlin.

431 Adrian, L., Szewzyk, U., Wecke, J., Görisch, H., 2000. Bacterial dehalorespiration with

432   chlorinated benzenes. Nature 408 (6812), 580–583. doi:10.1038/35046063.

433 Banerjee, R., Ragsdale, S.W., 2003. The many faces of vitamin B_{12}: Catalysis by cobalamin-

434   dependent   enzymes.   Annu.   Rev.   Biochem.   72,   209–247.

435   doi:10.1146/annurev.biochem.72.121801.161828.

436 Batt, A.L., Kim, S., Aga, D.S., 2006. Enhanced biodegradation of iopromide and trimethoprim

437   in   nitrifying   activated   sludge.   Environ.   Sci.   Technol.   40   (23),   7367–7373.

438   doi:10.1021/es060835v.

439 Beaven, G.H., Johnson, E.A., 1955. The reduction of vitamin B_{12}. Nature 176 (4496), 1264–

440   1265. doi:10.1038/1761264b0.

441 Bird, C.L., Kuhn, A.T., 1981. Electrochemistry of the viologens. Chem. Soc. Rev. 10 (1), 49–

442   82. doi:10.1039/cs9811000049.

443 Cleland, W.W., 1964. Dithiothreitol, a new protective reagent for SH groups. Biochemistry 3

444   (4), 480–482. doi:10.1021/bi00892a002.

445   Cooper, M., Wagner, A., Wondrousch, D., Sonntag, F., Sonnabend, A., Brehm, M.,

446   Schüürmann, G., Adrian, L., 2015. Anaerobic microbial transformation of halogenated

447   aromatics and fate prediction using electron density modeling. Environ. Sci. Technol. 49

448   (10), 6018–6028. doi:10.1021/acs.est.5b00303.

449   Duirk, S.E., Lindell, C., Cornelison, C.C., Kormos, J., Ternes, T.A., Attene-Ramos, M., Osiol,

450   J., Wagner, E.D., Plewa, M.J., Richardson, S.D., 2011. Formation of toxic iodinated

451   disinfection by-products from compounds used in medical imaging. Environ. Sci. Technol.

452   45 (16), 6845–6854. doi:10.1021/es200983f.

453   Gantzer, C.J., Wackett, L.P., 1991. Reductive dechlorination catalyzed by bacterial transition-

454   metal coenzymes. Environ. Sci. Technol. 25 (4), 715–722. doi:10.1021/es00016a017.

455   Grünheid, S., Amy, G., Jekel, M., 2005. Removal of bulk dissolved organic carbon (DOC) and

456   trace organic compounds by bank filtration and artificial recharge. Water Res. 39 (14),

457   3219–3228. doi:10.1016/j.watres.2005.05.030.

458   Holliger, C., Wohlfarth, G., Diekert, G., 1998. Reductive dechlorination in the energy

459   metabolism of anaerobic bacteria. FEMS Microbiol. Rev. 22 (5), 383–398.

460   doi:10.1111/j.1574-6976.1998.tb00377.x.

461   Jocelyn, P.C., 1967. The standard redox potential of cysteine-cystine from the thiol-disulphide

462   exchange reaction with glutathione and lipoic acid. Eur. J. Biochem. 2 (3), 327–331.

463   doi:10.1111/j.1432-1033.1967.tb00142.x.

464   Knitt, L.E., Shapley, J.R., Strathmann, T.J., 2008. Rapid metal-catalyzed hydrodehalogenation

465   of iodinated X-ray contrast media. Environ. Sci. Technol. 42 (2), 577–583.

466   doi:10.1021/es071837y.

467   Kormos, J.L., Schulz, M., Kohler, H.-P.E., Ternes, T.A., 2010. Biotransformation of selected

468   iodinated X-ray contrast media and characterization of microbial transformation pathways.

469   Environ. Sci. Technol. 44 (13), 4998–5007. doi:10.1021/es1007214.

470 Kormos, J.L., Schulz, M., Ternes, T.A., 2011. Occurrence of iodinated X-ray contrast media

471 and their biotransformation products in the urban water cycle. Environ. Sci. Technol. 45

472 (20), 8723–8732. doi:10.1021/es2018187.

473 Krone, U.E., Laufer, K., Thauer, R.K., Hogenkamp, H.P.C., 1989a. Coenzyme F_{430} as a possible

474 catalyst for the reductive dehalogenation of chlorinated C1 hydrocarbons in methanogenic

475 bacteria. Biochemistry 28 (26), 10061–10065. doi:10.1021/bi00452a027.

476 Krone, U.E., Thauer, R.K., Hogenkamp, H.P.C., 1989b. Reductive dehalogenation of

477 chlorinated C1-hydrocarbons mediated by corrinoids. Biochemistry 28 (11), 4908–4914.

478 doi:10.1021/bi00437a057.

479 Kube, M., Beck, A., Zinder, S.H., Kuhl, H., Reinhardt, R., Adrian, L., 2005. Genome sequence

480 of the chlorinated compound-respiring bacterium *Dehalococcoides* species strain CBDB1.

481 Nat. Biotechnol. 23 (10), 1269–1273. doi:10.1038/nbt1131.

482 Lecouturier, D., Rochex, A., Lebeault, J.-M., 2003. Enrichment and properties of an anaerobic

483 mixed culture that reductively deiodinates 5-amino-2, 4, 6-triiodoisophthalic acid, an X-ray

484 contrast agent precursor. Appl. Microbiol. Biotechnol. 62 (5-6), 550–556.

485 doi:10.1007/s00253-003-1296-5.

486 Lexa, D., Saveant, J.M., 1983. The electrochemistry of vitamin B_{12}. Acc. Chem. Res. 16 (7),

487 235–243.

488 Löffler, F.E., Ritalahti, K.M., Zinder, S.H., 2013. *Dehalococcoides* and reductive

489 dechlorination of chlorinated solvents, in: Stroo, H.F., Leeson, A., Ward, C.H. (Eds.),

490 Bioaugmentation for groundwater remediation. Springer, New York, pp. 39–88.

491 Maillard, J., Schumacher, W., Vazquez, F., Regeard, C., Hagen, W.R., Holliger, C., 2003.

492 Characterization of the corrinoid iron-sulfur protein tetrachloroethene reductive

493 dehalogenase of *Dehalobacter restrictus*. Appl. Environ. Microbiol. 69 (8), 4628–4638.

494 doi:10.1128/AEM.69.8.4628-4638.2003.

495   Martens, J.H., Barg, H., Warren, M.J., Jahn, D., 2002. Microbial production of vitamin B_{12}.

496   Appl. Microbiol. Biotechnol. 58 (3), 275–285. doi:10.1007/s00253-001-0902-7.

497   Neumann, A., Siebert, A., Trescher, T., Reinhardt, S., Wohlfarth, G., Diekert, G., 2002.

498   Tetrachloroethene reductive dehalogenase of *Dehalospirillum multivorans*: substrate

499   specificity of the native enzyme and its corrinoid cofactor. Arch. Microbiol. 177 (5), 420–

500   426. doi:10.1007/s00203-002-0409-3.

501   Nishijima, T., Hata, Y., 1978. Distribution of thiamine, biotin, and vitamin B_{12} in lake Kojima.

502   II. Distribution in the bottom sediments. Nippon Suisan Gakk. 44 (8), 815–818.

503   doi:10.2331/suisan.44.815.

504   ó Proinsias, K., Giedyk, M., Gryko, D., 2013. Vitamin B_{12}: Chemical modifications. Chem.

505   Soc. Rev. 42 (16), 6605–6619. doi:10.1039/c3cs60062a.

506   Oba, Y., Futagami, T., Amachi, S., 2014. Enrichment of a microbial consortium capable of

507   reductive deiodination of 2,4,6-triiodophenol. J. Biosci. Bioeng. 117 (3), 310–317.

508   doi:10.1016/j.jbiosc.2013.08.011.

509   Oleksy-Frenzel, J., Wischnack, S., Jekel, M., 2000. Application of ion-chromatography for the

510   determination of the organic-group parameters AOCl, AOBr, and AOI in water. Fresenius

511   J. Anal. Chem. 366, 89–94. doi:10.1007/s002160050016.

512   Putschew, A., Jekel, M., 2006. Iodinated X-ray contrast media, in: Jekel, M., Reemtsma, T.

513   (Eds.), Organic pollutants in the water cycle. Properties, occurrence, analysis and

514   environmental relevance of polar compounds. John Wiley distributor, Weinheim,

515   Chichester, pp. 87–98.

516   Putschew, A., Miehe, U., Tellez, A.S., Jekel, M., 2007. Ozonation and reductive deiodination

517   of iopromide to reduce the environmental burden of iodinated X-ray contrast media. Wat.

518   Sci. Tech. 56 (11), 159–165. doi:10.2166/wst.2007.827.

519   Radjenovic, J., Flexer, V., Donose, B.C., Sedlak, D.L., Keller, J., 2013. Removal of the X-ray

520   contrast media diatrizoate by electrochemical reduction and oxidation. Environ. Sci.

521   Technol. 47 (23), 13686–13694. doi:10.1021/es403410p.

522   Redeker, M., Wick, A., Meermann, B., Ternes, T.A., 2014. Removal of the iodinated X-ray

523   contrast medium diatrizoate by anaerobic transformation. Environ. Sci. Technol. 48 (17),

524   10145–10154. doi:10.1021/es5014714.

525   Renpenning, J., Keller, S., Cretnik, S., Shouakar-Stash, O., Elsner, M., Schubert, T., Nijenhuis,

526   I., 2014. Combined C and Cl isotope effects indicate differences between corrinoids and

527   enzyme (*Sulfurospirillum multivorans* PceA) in reductive dehalogenation of

528   tetrachloroethene, but not trichloroethene. Environ. Sci. Technol. 48 (20), 11837–11845.

529   doi:10.1021/es503306g.

530   Schittko, S., Putschew, A., Jekel, M., 2004. Bank filtration: a suitable process for the removal

531   of iodinated X-ray contrast media? Wat. Sci. Tech. 50 (5), 261–268.

532   Schneider, Z., Stroiński, A., 1987. Comprehensive B_{12}: Chemistry, biochemistry, nutrition,

533   ecology, medicine. de Gruyter, Berlin, 409 pp.

534   Schulz, M., Löffler, D., Wagner, M., Ternes, T.A., 2008. Transformation of the X-ray contrast

535   medium iopromide in soil and biological wastewater treatment. Environ. Sci. Technol. 42

536   (19), 7207–7217. doi:10.1021/es800789r.

537   Smith, M.H., Woods, S.L., 1994. Regiospecificity of chlorophenol reductive dechlorination by

538   vitamin B_{12s}. Appl. Environ. Microbiol. 60 (11), 4111–4115.

539   Speck, U., Hübner-Steiner, H., 1999. Pharmakologie und Toxikologie. Springer, Berlin,

540   Heidelberg, New York.

541   Stieber, M., Putschew, A., Jekel, M., 2011. Treatment of pharmaceuticals and diagnostic agents

542   using zero-valent iron–kinetic studies and assessment of transformation products assay.

543   Environ. Sci. Technol. 45 (11), 4944–4950. doi:10.1021/es200034j.

544 Uchimiya, M., Stone, A.T., 2009. Reversible redox chemistry of quinones: impact on

545   biogeochemical   cycles.   Chemosphere   77   (4),   451–458.

546   doi:10.1016/j.chemosphere.2009.07.025.

547 Van der Zee, Frank P., Cervantes, F.J., 2009. Impact and application of electron shuttles on the

548   redox (bio)transformation of contaminants: a review. Biotechnol. Adv. 27 (3), 256–277.

549   doi:10.1016/j.biotechadv.2009.01.004.

550 Wade, R.S., Castro, C.E., 1973. Oxidation of iron (II) porphyrins by alkyl halides. J. Am. Chem.

551   Soc. 95 (1), 226–230. doi:10.1021/ja00782a040.

552 Wiese, B., Massmann, G., Jekel, M., Heberer, T., Dünnbier, U., Orlikowski, D., Grützmacher,

553   G., 2011. Removal kinetics of organic compounds and sum parameters under field

554   conditions for managed aquifer recharge. Water Res. 45 (16), 4939–4950.

555   doi:10.1016/j.watres.2011.06.040.

556 Wohlfarth, G., Diekert, G., 1997. Anaerobic dehalogenases. Curr. Opin. Chem. Biol. 8 (3),

557   290–295. doi:10.1016/S0958-1669(97)80006-7.

558 Yan, J., Ritalahti, K.M., Wagner, D.D., Löffler, F.E., 2012. Unexpected specificity of

559   interspecies cobamide transfer from *Geobacter spp.* to organohalide-respiring

560   *Dehalococcoides mccartyi* strains. Appl. Environ. Microbiol. 78 (18), 6630–6636.

561   doi:10.1128/AEM.01535-12.

562 Zehnder, A., Wuhrmann, K., 1976. Titanium (III) citrate as a nontoxic oxidation-reduction

563   buffering system for the culture of obligate anaerobes. Science 194 (4270), 1165–1166.

564   doi:10.1126/science.793008.

565 Zwiener, C., Glauner, T., Sturm, J., Wörner, M., Frimmel, F.H., 2009. Electrochemical

566   reduction of the iodinated contrast medium iomeprol: Iodine mass balance and identification

567   of transformation products. Anal. Bioanal. Chem. 395 (6), 1885–1892. doi:10.1007/s00216-

568   009-3098-9.