The role of polysaccharides and diatom exudates in the redox cycling of Fe and the photoproduction of hydrogen peroxide in coastal seawaters

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Abstract

The effect of artificial acidic polysaccharides (PS) and exudates of *Phaeodactylum tricornutum* on the half-life of Fe(II) in seawater was investigated in laboratory experiments. Strong photochemical hydrogen peroxide (H_2O_2) production of 5.2 to 10.9 nM (mg C)$^{-1}$ h^{-1} was found in the presence of PS and diatom exudates. Furthermore when illuminated with UV light algal exudates kept the concentration of ferrous iron in seawater (initial value 100 nmol L^{-1}) elevated for about 50 min. Since no stabilising effect of PS on Fe(II) in the dark could be detected, enhanced photoreduction seems to be the cause. This was confirmed by a simple model of the photochemical redox cycle of iron. Diatom exudates seem to play an important role for the photochemistry of iron in coastal waters.

22

23 **1 Introduction**

24 Marine phytoplankton contributes significantly to the CO_2 exchange between
25 atmosphere and ocean, thus impacting atmospheric CO_2 concentrations (Falkowski *et*
26 *al.* 1998). Global marine primary productivity shows great spatial and temporal
27 variability, caused primarily by variable light, zooplankton grazing and nutrient
28 distributions. In addition to the macronutrients (P, N), iron is an essential trace
29 element for photo-autotrophic organisms (Geider *et al.* 1994; Falkowski *et al.* 1998;
30 Morel *et al.* 2003). Several large scale iron fertilization experiments have revealed
31 that in 40% of the surface ocean, the so called High Nutrient Low Chlorophyll
32 (HNLC) areas, iron is at least partially responsible for limitation of phytoplankton
33 growth (Boyd *et al.* 2007). However, iron limitation can occur in coastal areas as well
34 (Hutchins *et al.* 1998) and here the supply of Fe through upwelling and resuspension
35 determine its cycling.

36 Free hydrated Fe(III) concentrations in seawater are very low ($<10^{-20}$ mol L^{-1}) (Rue *et*
37 *al.* 1995) and the more soluble Fe(II) is rapidly oxidised (Millero *et al.* 1987; Millero
38 *et al.* 1989; King *et al.* 1995; Gonzalez-Davila *et al.* 2005, 2006). Thus concentrations
39 of dissolved Fe in the ocean should be very low. However, over 99% of the dissolved
40 iron in seawater is reported to be bound by organic compounds (Rue *et al.* 1995; van
41 den Berg 1995; Croot *et al.* 2000; Boye 2001) and these ligands can maintain the
42 concentrations typically seen in the ocean (Johnson *et al.* 1997). Iron binding ligands
43 in seawater mainly consist of bacterial siderophores (Macrellis *et al.* 2001; Butler
44 2005) and possibly planktonic exudates like acidic polysaccharides (PS) (Tanaka *et*
45 *al.* 1971). Transparent exopolymer particles (TEP), which are rich in acidic

46 polysaccharides, are ubiquitous in the surface ocean (Passow 2002). TEP has been
47 shown to bind ^{234}Th (Passow *et al.* 2006) and are therefore a prime candidate to bind
48 iron.

49 The main oxidation pathway of Fe(II) to Fe(III) is the reaction with O_2 and
50 H_2O_2 according to the Haber-Weiss mechanism (Millero *et al.* 1987; Millero *et al.*
51 1989; King *et al.* 1995). This oxidation can be inhibited (Theis *et al.* 1974; Miles *et*
52 *al.* 1981) or accelerated (Sedlak *et al.* 1993; Rose *et al.* 2002, 2003a) in the presence
53 of organic compounds. The decrease in apparent oxidation rate is suggested to be due
54 to stronger photoreduction of Fe(III) (Kuma *et al.* 1995) or stabilisation of Fe(II)
55 (Santana-Casiano *et al.* 2000; Rose *et al.* 2003b; Santana-Casiano *et al.* 2004).

56 In marine systems H_2O_2 functions as a strong oxidant or a reductant (Millero
57 *et al.* 1989; Croot *et al.* 2005). Thus it is important for the cycling of organic
58 compounds and trace metals like Fe (Millero *et al.* 1989). H_2O_2 is the most stable
59 intermediate in the reduction of O_2 to H_2O and is mainly produced in the water
60 column by photochemical reactions involving dissolved organic matter (DOM) and
61 O_2 (Cooper *et al.* 1988; Scully *et al.* 1996; Yocis *et al.* 2000; Yuan *et al.* 2001). Light
62 absorbed by DOM induces an electron transfer to molecular oxygen, forming the
63 superoxide anion radical, which undergoes disproportionation to form hydrogen
64 peroxide. Hence light, O_2, H_2O_2 and organic compounds are important factors in the
65 very complex chemistry of iron in seawater.

66 Increased photochemical reduction of Fe(III) in the presence of sugar acids has
67 been reported (Kuma *et al.* 1992; Ozturk *et al.* 2004; Rijkenberg *et al.* 2005) but for
68 polysaccharides no such studies have been carried out so far. However, the relative
69 abundance of polysaccharides in marine dissolved organic matter (DOM) is about

50% (Benner *et al.* 1992) and in phytoplankton derived DOM the fraction of polysaccharides can be up to 64% (Hellebust 1965; Hellebust 1974). In the study reported here we investigate the effect of PS and algal exudates on the photochemical redox cycle of iron and production of H_2O_2.

2 Materials and Methods

2.1 General

Three different types of experiments were conducted to investigate the effect of PS and diatom exudates in combination with UV light on the speciation of iron and the production of H_2O_2. All experiments were conducted at a constant temperature (about 20°C) in the laboratory. In experiments 1 and 3 were samples were exposed to UV radiation, UV transparent 3 L Tedlar bags were used as incubation containers. Experiment 2 was conducted in 30 mL polystyrene screw cap tubes, without UV irradiation.

The natural coastal seawater (SW) was collected in July 2006 off Lepe near Southampton (UK), filtered through 0.2 μm membranes and stored at 5°C. Organic matter was removed from a part of this SW via photo-oxidation with strong UV radiation. The so called "organic-free" UVSW (Donat *et al.* 1988) was also stored at 5°C.

We used gum xanthan, laminarin and carrageenan (all from Sigma) as the artificial PSs. The molecular weight of laminarin is 7700 g mol^{-1} (Rice *et al.* 2004) and 43% (w/w) of the molecule is carbon. For gum xanthan and carrageenan no specifications could be found but we assumed a carbon content of ~40% (w/w).

Diatom exudates were collected as the 0.4 μm filtrate of a senescent culture of *Phaeodactylum tricornutum* grown in f/2 medium. Ford and Percival (1965) separated a significant amount of a water-soluble glucan from an aqueous extract of *Phaeodactylum tricornutum*, and their results showed this polysaccharide to be a typical chrysolaminarin with essential similar properties to the p-1,3-linked glucan, laminarin.

Philips 40TL12 and Philips 40T'05 lamps, respectively, were used as a light source for the irradiation of samples with UVB and UVA light during experiments 1 and 3. Irradiance was measured with a UVA (315-400 nm) sensor type 2.5, a UVB (280-315 nm) sensor type 1.5 (INDIUM-SENSOR, Germany) and a spherical quantum sensor SPQA 2651 (LI-COR) for the photosynthetically active radiation (PAR, 400-700 nm). Sensors were coupled to a data logger LI-1400 (LI-COR). The following irradiance values were used for all light incubations during this study: UVB=0.3 W m^{-2}, UVA=17.6 W m^{-2} and PAR=3.8 W m^{-2}. For all experiments samples were held in UV transparent 3 L polyvinyl fluoride (PVF, Tedlar) bags (SKC Inc., USA), fitted with a polypropylene hose for filling and sub-sampling.

2.2 Specific Experiments

2.2.1 Experiment 1: Effect of polysaccharides on the photogeneration of H_2O_2

Four pairs of Tedlar bags were filled with MQ water and concentrated solutions of three different PSs were added to three pairs of these bags. For this experiment carrageenan, gum xanthan and laminarin were used. The PSs were dissolved in MQ water by sonicating for 30 min. The final concentration of PS was

10 mg L^{-1} in about 2.3 L. The last pair of bags served as control and contained no PS. One bag of each pair was placed in the dark the other was illuminated with UV light for 270 min. H_2O_2 was measured 1 h before illumination and after 0, 10, 30, 90, 270 min in the light and the dark sample.

2.2.2 Experiment 2: Effect of polysaccharides on the oxidation of Fe(II) in seawater in the dark

Ten clean polystyrene screw cap tubes (30 mL) were filled with the natural Solent seawater (0.2 μm filtered) and another ten tubes were filled with the organic-free Solent Seawater. To 5 tubes of each treatment gum xanthan was added to a final concentration of 1 mg L^{-1} and the samples were sonicated for 30 min. Initially Fe(II) equivalent to 200 nmol L^{-1} was added to all tubes, and Fe(II) and H_2O_2 measured after 0, 2, 6, 18, 54 min. Temperature, salinity, oxygen concentration and pH were measured before the iron addition and at the end of the experiment.

2.2.3 Experiment 3: Effect of diatom exudates and UVA/B radiation on the oxidation of Fe(II) in seawater

Three Tedlar bags were filled with about 1 L of organic-free seawater (0.2 μm filtered). One bag served as a control and no further additions were made. To the second bag 100 nmol L^{-1} Fe(II) were added. To the third bag an addition of diatom exudates and 100 nmol L^{-1} Fe(II) was made. The amount of diatom exudates added to the sample was chosen in order to reach a concentration of PS similar to natural Solent seawater (0.4 mg glucose eq. L^{-1}). Ferrous iron concentration was measured

139 over a 60 min period after the iron addition. The UV light was switched on for the
140 whole experiment right after the addition of iron to the sample bags. Temperature,
141 salinity, oxygen concentration, pH and total iron were measured before the iron
142 addition and at the end of the experiment. H_2O_2 in the organic-free seawater was
143 adjusted to an initial concentration of 5 nmol L^{-1} and was measured again at the end of
144 the experiment.

145

146 *2.3 Analyses*

147 Iron concentrations in the samples were determined using a colorimetric
148 method described by Stookey (1970) and Viollier *et al.* (2000). Briefly Ferrozine (the
149 disodium salt of 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine) forms a
150 magenta coloured tris complex with ferrous iron. The water soluble complex is stable
151 and quantitatively formed in a few minutes at pH = 4-9 after adding an aqueous
152 0.01 mol L^{-1} Ferrozine solution. The absorbance was measured with a Hitachi U-1500
153 at 562 nm in 10 cm cuvettes buffered with an ammonium acetate buffer adjusted to
154 pH = 5.5, and compared to a calibration curve made by standard additions to the
155 sample water. Standards were prepared from a 10 mmol L^{-1} Fe(II) stock solution
156 ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 0.1 mol L^{-1} HCl) diluted in 0.01 mol L^{-1} HCl. Total iron
157 was determined by previous reduction of the iron present in the sample under acid
158 conditions over 2 h at room temperature by adding hydroxylamine hydrochloride
159 (1.4 mol L^{-1} in 5 mol L^{-1} HCl) as the reducing agent. The detection limit of this
160 method is about 8 nmol L^{-1} of Fe(II) and the standard error is about 20%. All
161 Reagents were from Sigma-Aldrich and at least p.a. grade. All solutions were
162 prepared in MQ water (18 MΩ cm^{-1}) purified with a Millipore deionisation system.

Samples were prepared in 30 mL polystyrene screw cap tubes. All equipment has been carefully acid washed prior to use.

Concentrations of dissolved mono- and polysaccharides were determined semi quantitatively using another colorimetric method described by Myklestad *et al.* (1997). Briefly the absorbance of the strong coloured complex of 2,4,6-tripyridyl-s-triazine (TPTZ) formed with iron reduced by monosaccharides or previously hydrolyzed polysaccharides at alkaline pH is measured at 595 nm in 2.5 cm cuvettes and compared to a calibration curve prepared from D-glucose in MQ water. Total sugar concentration was determined after hydrolysis of the acidified sample in a sealed glass ampoule at 150°C for 90 min. The detection limit was 0.02 mg glucose eq. L^{-1} and the standard error was about 3%. All glassware and reagents were prepared as described by Myklestad *et al.* (1997).

For the determination of hydrogen peroxide (H_2O_2) a chemiluminescence flow injection analysis (FIA-CL) described by Yuan and Shiller (1999) was used. The method is based on oxidation of luminol by hydrogen peroxide in an alkaline solution using Co(II) as a catalyst. Our flow injection system generally resembled that described by Yuan and Shiller (1999) but as a detection unit we used the photosensor module H8443 (Hamamatsu) with a power supply and a signal amplifier. The voltage signal was logged every second using an A/D converter and logging software (PMD-1208LS, Tracer DAQ 1.6.1.0, Measurement Computing Corporation). The chemiluminescence peaks were evaluated by calculating their area. The detection limit was 0.1 nmol L^{-1} and the standard error was 4%. All reagents and solutions were prepared as described by Yuan and Shiller (1999). Since ferrous iron in the sample shows a significant positive interference (Yuan *et al.* 1999) H_2O_2 was measured in

parallel samples without added Fe(II) or after one hour when most of the iron was reoxidised.

A WTW 315i T/S system was used to determine temperature and salinity in the sample. Oxygen was measured using a WPA OX20 oxygen meter. The dissolved organic carbon (DOC) content in the 0.2 µm filtered samples was measured with a Shimadzu TOC-VCSN system via high temperature catalytic oxidation (HTCO) on Pt covered Al_2O_3 beads. The detection limit of this method is ~3 µmol L^{-1} and the precision is ±2 µmol L^{-1}.

The UV photooxidation system consisted of a fan cooled 1 kW medium pressure mercury lamp (Hanovia), with 10 x 200 mL quartz tubes mounted around the axial lamp. After 6 h of UV irradiation the samples were considered "organic-free" (UVSW) (Donat *et al.* 1988). To remove the resulting high concentrations of H_2O_2 the organic-free water was treated with activated charcoal. The charcoal had previously been washed several times with HCl, ethanol and MQ water to remove contaminants. After stirring for 30-40 min the charcoal was removed by filtration through a 0.2 µm polycarbonate membrane. The H_2O_2 concentration in the resulting water was less than 0.5 nmol L^{-1} and no contamination with iron was detectable.

3 Results and discussion

3.1 *Experiment 1: Effect of polysaccharides on the photochemical production of H_2O_2*

The first experiment, examining the effect of polysaccharides on the photochemical production of H_2O_2, showed that within 270 min (4.5 h) of

210 illumination large amounts (140-240 nmol L^{-1}) of H_2O_2 were formed due to the
211 addition of 10 mg L^{-1} of polysaccharides to MQ water (Figure 1). The H_2O_2
212 concentrations in all samples increased linearly during the experiment, after the light
213 was switched on. Gum xanthan showed the highest photochemical production of H_2O_2
214 followed by carrageenan and laminarin, which can be explained by their different
215 absorptivity at <400 nm (Figure 2). The addition of laminarin led to a net
216 accumulation rate of H_2O_2 of 22.5 nmol L^{-1} h^{-1}, which was twice as high as that for
217 pure MQ water (12.3 nmol L^{-1} h^{-1}). The H_2O_2 accumulation during illumination of the
218 MQ water was probably due to organic matter leaching from the resin of the filter
219 cartridge of the MQ system. However, the DOC concentration in MQ water was
220 <<10 µmol L^{-1}. H_2O_2 accumulation rates of 36.2 nmol L^{-1} h^{-1} and 43.4 nmol L^{-1} h^{-1}
221 were determined in samples with added carrageenan and gum xanthan, respectively.
222 The photochemical production of H_2O_2 was thus 3-4 times higher in the presence of
223 carrageenan and gum xanthan compared to pure MQ water. Linear H_2O_2
224 accumulation rates of similar magnitude have been reported by Cooper *et al.* (1988)
225 and Miller *et al.* (1995) in natural seawater samples. The main structural differences
226 between the molecules of these three PSs are that laminarin has a linear structure of
227 linked glucose monosaccharide units, carrageenan has sulphur containing groups and
228 gum xanthan has a branched structure incorporating uronic acid groups. The PS
229 concentration used in our experiment is equivalent to about 4 mg L^{-1} organic carbon
230 leading to normalised H_2O_2 generation rates of 5.2 nmol L^{-1} (mg C)$^{-1}$ h^{-1} (laminarin),
231 9.1 nmol L^{-1} (mg C)$^{-1}$ h^{-1} (carrageenan) and 10.9 nmol L^{-1} (mg C)$^{-1}$ h^{-1} (gum xanthan).
232 These values are up to 29 times higher than the rate of 0.38 nmol L^{-1} (mg C)$^{-1}$ h^{-1}
233 reported by Price et al. (1998) for the >8000 Da fraction of natural DOM in the
234 Western Mediterranean even though the light bulbs used in our study typically

produced only 25% of the UVB radiation 39% of UVA and 1% of PAR of the calculated natural irradiance found in midday summer sun in the Mediterranean (Zepp *et al.* 1977). The polysaccharides in our study caused strong photogeneration of H_2O_2 even under low light exposure probably due to the absence of removal processes such as enzymatic decomposition of H_2O_2 (Moffett *et al.* 1990). Photochemical production rates of H_2O_2 in the Atlantic Ocean and Antarctic waters are much lower ranging from 2.1 to 9.6 nmol L^{-1} h^{-1} (Obernosterer 2000; Yocis *et al.* 2000; Yuan *et al.* 2001; Gerringa *et al.* 2004). Gerringa et al. (2004) calculated a net production rate of 7 nmol L^{-1} h^{-1} at irradiance levels of 2.8 (UVB), 43 (UVA) and 346 W m^{-2} (VIS/PAR) in 0.2 μm filtered water from the eastern Atlantic close to the Equator. These low rates are presumably due to lower DOC concentrations and higher decay rates due to colloids or enzymatic activity in natural waters (Moffett *et al.* 1990; Petasne *et al.* 1997). Our experiments suggest that PSs may have had a significant indirect effect on Fe oxidation due to the enhanced photochemical production of H_2O_2.

3.2 Experiment 2: Effect of gum xanthan on the oxidation of Fe(II) in the dark

Differences in the rate of Fe(II) oxidation due to added gum Xanthan were small, both in the natural SW and the UVSW samples (Figure 3 and 4). However, the oxidation of Fe(II) in the natural SW samples (with or without gum xanthan) (Figure 3) was much slower than that in the respective DOM-free UVSW samples (Figure 4). Half-life values and oxidation rates of organic-free seawater can be calculated according to Millero and Sotolongo (1989) and Millero *et al.* (1987). Under our experimental conditions the calculated half-life was 25 s for the ambient H_2O_2 concentrations and 82 s under O_2 saturation. These theoretical values can be compared

259 to measured Fe(II) half-life values of 42 s (UVSW) and 35 s (UVSW+PS). The
260 measured values resemble the theoretical values under the ambient H_2O_2 conditions.
261 This indicates that the high H_2O_2 concentration had a stronger oxidising effect on
262 Fe(II) than the dissolved O_2 in the samples.

263 For the natural SW sample the theoretical half-life of 43 s under O_2 saturation
264 does not fit the measured data well. The half-life of Fe(II) in the natural SW sample
265 (Figure 3) was ~17 times (11.9 min) and with PS added ~19 times (13.3 min) longer
266 than theoretical value. The measured data followed the exponential oxidation curve
267 calculated for the low H_2O_2 concentration of these samples whereas the high O_2
268 content seemed to not accelerate the measured oxidation of Fe(II).

269 The DOC content of the natural SW (97 μmol L^{-1}) was almost 10 times higher
270 than of the UVSW. The difference in Fe(II) oxidation between the water types might
271 therefore be due to the stabilisation of Fe(II) against oxidation by natural occurring
272 compounds of the coastal SW (Theis *et al.* 1974; Miles *et al.* 1981; Santana-Casiano
273 *et al.* 2000; Rose *et al.* 2003a; Santana-Casiano *et al.* 2004). These results show that
274 the added gum xanthan was not a good model for natural occurring substances
275 stabilising Fe(II) against oxidation. Initial H_2O_2 concentrations also differed
276 appreciably, with 5 nmol L^{-1} H_2O_2 in the natural SW sample and 270 nmol L^{-1} H_2O_2 in
277 the UVSW sample. UV oxidation in UVSW water during removal of natural DOM
278 must have caused the differences in H_2O_2. We calculated Fe(II) oxidation rates due to
279 O_2 and H_2O_2 in our experiment to investigate if the differing rates could have been
280 caused by differing initial H_2O_2 concentrations. From the comparison between our
281 measured and theoretically calculated values we conclude that a strong effect of H_2O_2
282 on the lifetime of Fe(II) was observed but no effect of gum xanthan was found in this

283 experiment conducted without irradiation. The lower initial H_2O_2 concentrations in
284 the natural SW sample (5 nmol L^{-1} H_2O_2; Figure 3) compared to the UVSW sample
285 (270 nmol L^{-1} H_2O_2; Figure 4) appears to be the major cause for slower Fe(II)
286 oxidation, suggesting that H_2O_2 mainly control the oxidation of Fe(II).

287

288 *3.3 Experiment 3: Effect of diatom exudates and UVA/B radiation on the oxidation*
289 *of Fe(II) in seawater*

290 Initially, the half-lives of Fe(II) in both treatments, those with and without
291 addition of diatom exudates, was quite similar (Figure 5). For the initial 5 min (300 s)
292 a half life of 4.5±0.7 min and 4.0±0.3 min, respectively was determined for Fe(II) in
293 the UVSW without and with added diatom exudates. These values are in the same
294 range as published values (Millero *et al.* 1987; Kuma *et al.* 1995; Croot *et al.* 2002).
295 A remarkable difference between both treatments is clearly visible after about 7 min
296 (420 s) (Figure 5). In the UVSW without exudates the Fe(II) concentration continued
297 decreasing exponentially reaching the detection limit after 20 min, whereas in UVSW
298 with added diatom exudates the Fe(II) concentration remained at about 30 nmol L^{-1}
299 decreasing only very slightly with time. The photochemical effect of the exudates was
300 strong enough to result in a net stabilising effect on Fe(II) after 7 minutes.

301 Differences in H_2O_2 production during the first hour of irradiation were
302 significant between UVSW with and without exudates. In the UVSW sample with
303 added diatom exudates a linear production rate of 33 nmol L^{-1} h^{-1} H_2O_2 was
304 determined whereas in pure UVSW the respective rate was only 5 nmol L^{-1} h^{-1}. The
305 higher production rate of H_2O_2 in the presence of exudates, suggests increased
306 photochemical production of H_2O_2. UVSW without exudates contained 11 µmol L^{-1}

307 DOC and no measurable total MS and PS, whereas UVSW mixed with exudates of
308 *Phaeodactylum tricornutum* contained ~450 µmol L^{-1} DOC, including
309 0.4 mg glucose eq. L^{-1} (i.e. 13 µmol C L^{-1}) total MS and PS. The DOC- normalised
310 H_2O_2 generation rate of 6.1 nmol L^{-1} (mg C)$^{-1}$ h^{-1} calculated from UVSW with
311 exudates indicates that laminarin-like diatom exudates (Ford *et al.* 1965)
312 photochemically produce H_2O_2. However, the high DOC content suggests that there
313 was also other organic matter contributing to the photo-production of H_2O_2.

314 Figure 6 shows a schematic of that part of the iron cycle relevant for our
315 experiment. In pure UVSW the added Fe(II) was oxidised rapidly, but in the presence
316 of ligands contained in the diatom exudates Fe(II) formed FeL, which in the light was
317 released as Fe(II) and then oxidised. The Fe(II) concentration could thus remain stable
318 as Fe(II) production from FeL balanced Fe(II) oxidation. We used a simple numerical
319 model based on these processes to model the Fe(II) concentration in our experimental
320 system.

321 The model uses a constant photoproduction term $\boldsymbol{k}_{h\nu}$[FeL] of ferrous iron, and
322 constant oxidation rates with oxygen ($\boldsymbol{k}_{O_2}$). The oxidation rates with hydrogen
323 peroxide ($\boldsymbol{k}_{H_2O_2}$) are assumed to increase linearly with a photoformation rate of
324 33 nmol L^{-1} h^{-1} as measured in this experiment and initial H_2O_2 concentration are set
325 at 4.6 nmol L^{-1}. The initial Fe(II) concentration [$Fe(II)_0$] is set at 100 nmol L^{-1} Fe(II),
326 the amount added in the experiment, and increases in the model by the constant
327 photoreduction of the FeL complex (where L is either EDTA or diatom exudates or a
328 combination of both). The direct photoreduction of inorganic iron colloids and
329 dissolved ferric iron is also possible (Waite *et al.* 1984; Wells *et al.* 1991a; Wells *et*
330 *al.* 1991b; Johnson *et al.* 1994), but rates for these processes are negligibly low. For

both processes together we calculated about 0.004 nmol L^{-1} s^{-1} of Fe(II) for 100 nmol L^{-1} Fe(II) added using the rates reported by Johnson et al. (1994). The model assumes that the concentration of FeL changes only negligibly during the experiment. As loss processes of Fe(II) we included the oxidation of Fe(II) with O_2 and the oxidation with H_2O_2. The latter depends on the increasing H_2O_2 concentrations during the experiment. Since dissociation and formation of FeL are relatively slow (Hudson *et al.* 1992) compared to the photoreduction of FeL and the oxidation of Fe(II) we ignored these processes in the model. The model calculates the change in Fe(II) concentration over time (equation 1).

$$\frac{d[Fe(II)]}{dt} = k_{h\nu}[FeL] - k_{O_2}[Fe(II)_0] - k_{H_2O_2}[H_2O_2][Fe(II)_0] \qquad \text{eq. 1}$$

$$[H_2O_2] = 33/3600 * t + 4.6 \qquad \text{eq. 2}$$

t given in [s], $\boldsymbol{k}_{h\nu}$ and $\boldsymbol{k}_{O_2}$ in [s^{-1}], $\boldsymbol{k}_{H_2O_2}$ in [L $nmol^{-1}$ s^{-1}] and all concentrations given in [nmol L^{-1}].

The parameters $\boldsymbol{k}_{O_2}$, $\boldsymbol{k}_{h\nu}$ [FeL] and $\boldsymbol{k}_{H_2O_2}$ were estimated by fitting the model to the observed data, minimizing the root mean squared model-data misfit, scaled by the assumed variance of the measurements. If the deviations between model and data are independent and normally distributed, the misfit

$$\chi^2 = \sum_i \frac{(d_i - m_i)^2}{\sigma_i^2} \qquad \text{eq. 3}$$

is a χ^2 variable. In this case we can estimate the posterior probability density function (pdf) of the model parameters (assuming a uniform prior) by

$$pdf\left(k_{O_2}, k_{h\nu}[FeL], k_{H_2O_2}\right) \sim \exp\left(\frac{-\chi^2}{2}\right) \qquad \text{eq. 4}$$

352 (see e.g. D.S. Sivia (2006)). The probability function is well approximated by a
353 multidimensional Gaussian distribution with a maximum value for the best estimated
354 set of parameter values. To obtain an estimate of the variance for this maximum
355 likelihood estimate of the parameters, we also need an estimate of the covariance
356 matrix of the parameters at the minimum of χ^2. This covariance matrix can be
357 estimated as the inverse of the Hessian matrix of χ^2 at the minimum. We can then
358 assume a confidence interval (± one standard deviation) for the best estimates of the
359 parameters, which are $\boldsymbol{k}_{O_2}$ = 6.04e-03±1.20e-03 s^{-1}, $\boldsymbol{k}_{H_2O_2}$ = 1.97e-04±8.59e-05
360 L $nmol^{-1}$ $s^{-1}$and $\boldsymbol{k}_{h\nu}$ [FeL] = 0.22±0.06 nmol L^{-1} s^{-1}. With this high photoreduction rate
361 the model fits the measured data very well (Figure 7) but the oxidation rates for
362 oxygen and H_2O_2 are 30% lower and 105% higher, respectively, than rates reported
363 by Millero *et al.* (1987; 1989). Holding the oxidation rates $\boldsymbol{k}_{O_2}$ and $\boldsymbol{k}_{H_2O_2}$ fixed at
364 values calculated for the given experimental conditions (22 °C, S = 34.2, O_2 saturated,
365 pH = 8.1) according to Millero *et al.* (1987; 1989) the model-data misfit becomes
366 somewhat larger and the model requires a slightly higher Fe(II) photoproduction term
367 $\boldsymbol{k}_{h\nu}$ [FeL] of about 0.24±0.01 nmol L^{-1} s^{-1} to fit the measured data (Figure 7). The
368 larger error margins when fitting all three parameters, compared to fitting only the
369 photoreduction rate, is explained by the strong correlation between the estimates of
370 $\boldsymbol{k}_{H_2O_2}$ and of $\boldsymbol{k}_{h\nu}$ [FeL], meaning that the data can be represented almost equally well
371 with different combinations of these two parameters.

372 The estimated photoproduction rates of Fe(II) are about 50 times higher than the
373 photoreduction rate of inorganic colloidal and dissolved iron calculated before,
374 independent of whether we assume the oxidation rates by Millero et al. (1987, 1989).
375 This indicates high photoreduction of Fe(III) mediated by the added organic material.
376 This high reduction of Fe(III) could have resulted either from direct photoreduction of

377 the FeL or indirectly via light induced (see absorbance spectra Figure 2) formation of
378 superoxide (DOM + hν → DOM*; DOM* + O_2 → $DOM^+ + O_2^-$; and Fe(III) + O_2^-
379 → Fe(II) + O_2) and the subsequent reduction of ferric iron (King *et al.* 1995; Voelker
380 *et al.* 1995; Rose *et al.* 2005; Fujii *et al.* 2006; Rose *et al.* 2006; Waite *et al.* 2006;
381 Garg *et al.* 2007b, 2007a).

382 Since the estimated laminarin concentration of ~1 mg L^{-1} only accounts for
383 ~8% of the DOC content of this sample it is not clear to what extend PS were
384 responsible for the photoreduction during this experiment. Some EDTA
385 (concentration of ~1 µmol L^{-1}) had inadvertently also been added with the diatom
386 exudates, as it was part of the culture media. However, photoreduction of iron from
387 complexes with EDTA seemed to have had only a minor effect. Reported steady state
388 Fe(II) concentrations present under stronger irradiation due to photoreduction of Fe-
389 EDTA complexes are much lower (Sunda *et al.* 2003) than observed in this study.
390 Photo-redox cycling of Fe–EDTA complexes has a larger influence on Fe(III)
391 concentrations than on those of Fe(II) (Sunda *et al.* 2003).

392 Steady state concentrations of photochemical Fe(II) are linearly related to the
393 irradiation energy especially in the UV range (Kuma *et al.* 1995; Rijkenberg *et al.*
394 2005; Rijkenberg *et al.* 2006; Laglera *et al.* 2007). In our study the light intensity was
395 only 25% of the UVB radiation 39% of UVA and 1% of PAR of the calculated natural
396 irradiance in midday summer sun at 40°N (Zepp *et al.* 1977). Therefore under natural
397 coastal conditions, with 4-5 times lower DOC concentrations but a 2.6 to 100 times
398 higher irradiance levels, a photoreductive effect of diatom exudates seems highly
399 probable.

400

4 Conclusions

In this study we investigated the photochemical effect of artificial and natural polysaccharide material in aquatic systems on iron speciation and on the production of H_2O_2. Artificial PS caused high photochemical production of H_2O_2, which acts as a strong oxidant for metals and organic matter on the one hand. On the other hand H_2O_2 is formed photochemically via the superoxide intermediate which is capable of reducing Fe(III). We found increased steady state Fe(II) concentrations in illuminated seawater with a high concentration of exudates of *Phaeodactylum tricornutum*. In the dark this effect of artificial PS on ferrous iron was not detectable, suggesting that light-produced superoxide reduces Fe(III) maintaining elevated Fe(II) concentration. In coastal seawater with high content of organic matter originating partly from diatoms a positive effect of the exudates on the bioavailability of iron seems likely. Field studies comparing natural phytoplankton bloom waters with open ocean waters are needed to confirm these photoreduction results and the counteracting effect of H_2O_2 on a daily time scale and as a function of particle size (dissolved, colloidal and particulate fraction).

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6 References

Benner, R., J. D. Pakulski*, et al.* (1992). "Bulk chemical characteristics of dissolved organic matter in the ocean." Science **255**: 1561-1564.

Boyd, P. W., T. Jickells*, et al.* (2007). "Mesoscale iron enrichment experiments 1993–2005: synthesis and future directions." Science **315**: 612-617.

Boye, M. (2001). "Organic complexation of iron in the Southern Ocean." Deep Sea Research I **48**(6): 1477-1497.

Butler, A. (2005). "Marine Siderophores and Microbial Iron Mobilization." BioMetals **18**(4): 369-374.

Cooper, W. J., R. G. Zika*, et al.* (1988). "Photochemical formation of H_2O_2 in natural waters exposed to sunlight." Environ. Sci. Technol. **22**: 1156-1160.

Croot, P. L. and M. Johansson (2000). "Determination of iron speciation by cathodic stripping voltammetry in seawater using the competing ligand 2-(2-Thiazolylazo)-p-cresol (TAC)." Electroanalysis **12(8)**: 565-576.

Croot, P. L. and P. Laan (2002). "Continuous shipboard determination of Fe(II) in polar waters using flow injection analysis with chemiluminescence detection." Analytica Chimica Acta **466**: 261-273.

Croot, P. L., P. Laan*, et al.* (2005). "Spatial and temporal distribution of Fe(II) and H_2O_2 during EisenEx, an open ocean mescoscale iron enrichment." Mar. Chem. **95**: 65-88.

Donat, J. R. and K. W. Bruland (1988). "Direct determination of dissolved Cobalt and Nickel in seawater by differential pulse cathodic stripping voltammetry preceded by adsorptive collection of cyclohexane-1,2-dione dioxime complexes." Anal. Chem. **60**: 240-244.

Falkowski, P. G., R. T. Barber*, et al.* (1998). "Biogeochemical controls and feedbacks on ocean primary production." Science **281**(5374): 200-206.

Ford, C. W. and E. Percival (1965). "The carbohydrates of *Phaeodactylum tricornutum*." J. Chem. Soc.: 7035-7041.

Fujii, M., A. L. Rose*, et al.* (2006). "Superoxide-mediated dissolution of amorphous ferric oxyhydroxide in seawater." Environmental Science & Technology **40**(3): 880-887.

Garg, S., A. L. Rose*, et al.* (2007a). "Superoxide-mediated reduction of organically complexed iron(III): Impact of pH and competing cations (Ca2+)." Geochimica Et Cosmochimica Acta **71**: 5620-5634.

Garg, S., A. L. Rose, *et al.* (2007b). "Superoxide mediated reduction of organically complexed Iron(III): Comparison of non-dissociative and dissociative reduction pathways." Environmental Science & Technology **41**(9): 3205-3212.

Geider, R.J, *et al.* (1994). "The role of iron in phytoplankton photosynthesis and the potential for iron-limitation of primary productivity in the sea." Photosynth. Res. **39**: 275-301.

Gerringa, L. J. A., M. J. A. Rijkenberg, *et al.* (2004). "The influence of solar ultraviolet radiation on the photochemical production of H_2O_2 in the equatorial Atlantic Ocean." J. Sea Res. **51**: 3-10.

Gonzalez-Davila, M., J. M. Santana-Casiano, *et al.* (2005). "Oxidation of iron (II) nanomolar with H_2O_2 in seawater." Geochimica et Cosmochimica Acta **69**(1): 83-93.

Gonzalez-Davila, M., J. M. Santana-Casiano, *et al.* (2006). "Competition between O_2 and H_2O_2 in the oxidation of Fe(II) in natural waters." Journal of Solution Chemistry **35**(1): 95-111.

Hellebust, J. A. (1965). "Excretion of some organic compounds by marine phytoplankton." Limnol. Oceanogr. **10**: 192-206.

Hellebust, J. A. (1974). "Extracellular products. In: W. D. P. Stewart, (Ed.), Algal physiology and biochemistry." Blackwell: 838-863.

Hudson, R.J.M, *et al.* (1992). "Investigations of iron coordination and redox reactions in seawater using ^{59}Fe radiometry and ion-pair solvent extraction of amphiphilic iron complexes." Marine Chemistry **38**: 209-235.

Hutchins, D. A. and K. W. Bruland (1998). "Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime." Nature **393**: 561-564.

Johnson, K. S., K. H. Coale, *et al.* (1994). "Iron photochemistry in seawater from the equatorial Pacific." Mar. Chem. **46**: 319-334.

Johnson, K. S., R. M. Gordon, *et al.* (1997). "What controls dissolved iron concentrations in the world ocean?" Marine Chemistry **57**(3 / 4): 137.

King, D. W., H. A. Lounsbury, *et al.* (1995). "Rates and mechanism of Fe(II) oxidation at nanomolar total iron concentrations." Environ. Sci. Technol. **29**: 818-824.

Kuma, K., S. Nakabayashi, *et al.* (1995). "Photoreduction of Fe(III) by hydrocarboxylic acids in seawater." Water Research **29**(6): 1559-1569.

Kuma, K., S. Nakabayashi, *et al.* (1992). "Photoreduction of Fe(III) by dissolved organic substances and existence of Fe(II) in seawater during spring blooms." Mar. Chem. **37**: 15-27.

Laglera, L. M. and C. M. G. Van den Berg (2007). "Wavelength dependence of the photochemical reduction of iron in arctic seawater." Environ. Sci. Technol. **41**: 2296-2302.

Macrellis, H. M., C. G. Trick*, et al.* (2001). "Collection and detection of natural iron-binding ligands from seawater." Marine Chemistry **76**: 175-187.

Miles, C. J. and P. L. Brezonik (1981). "Oxygen consumption in humic-colored waters by a photochemical ferrous-ferric catalytic cycle." Environ. Sci. Technol. **15**(9): 1089-1095.

Miller, W. L., D. W. King*, et al.* (1995). "Photochemical redox cycling of iron in coastal seawater." Mar. Chem. **50**: 63-77.

Millero, F. J. and S. Sotolongo (1989). "The oxidation of Fe(II) with H_20_2 in seawater." Geochim. Cosmochim. Acta **53**: 1867-1873.

Millero, F. J., S. Sotolongo*, et al.* (1987). "The oxidation kinetics of Fe(II) in seawater." Geochim. Cosmochim. Acta **51**: 793-801.

Moffett, J. W. and O. C. Zafiriou (1990). "An investigation of hydrogen peroxide in surface waters of Vineyard Sound with $H_2{}^{18}O_2$ and $^{18}O_2$." Limnol. Oceanogr. **35**: 1221-1229.

Morel, F. M. M. and N. M. Price (2003). "The biogeochemical cycles of trace metals in the oceans." Science **300**: 944-947.

Myklestad, S. M., E. Skanoy*, et al.* (1997). "A sensitive and rapid method for analysis of dissolved mono- and polysaccharides in seawater." Marine Chemistry **56**(3-4): 279-286.

Obernosterer, I. B. (2000). "Photochemical transformations of dissolved organic matter and its subsequent utilization by marine bacterioplankton." PhD thesis: 133 pp.

Ozturk, M., P. L. Croot*, et al.* (2004). "Iron enrichment and photoreduction of iron under UVand PAR in the presence of hydroxycarboxylic acid: implications for phytoplankton growth in the Southern Ocean." Deep Sea Research II **51**: 2841-2856.

Passow, U. (2002). "Transparent exopolymer particles (TEP) in aquatic environments." Progress in Oceanography **55**: 287-333.

Passow, U., J. Dunne*, et al.* (2006). "Organic carbon to ^{234}Th ratios of marine organic matter." Mar. Chem. **100**: 323-336.

Petasne, R. G. and R. G. Zika (1997). "Hydrogen peroxide lifetimes in south Florida coastal and offshore waters." Mar. Chem. **56**: 215-225.

Price, D., R. F. C. Mantoura*, et al.* (1998). "Shipboard determination of hydrogen peroxide in the western Mediterranean sea using flow injection with chemiluminescence detection." Analytica Chimica Acta **377**: 145-155.

Rice, P. J., B. E. Lockhart*, et al.* (2004). "Pharmacokinetics of fungal (1–3)-β-Image-glucans following intravenous administration in rats." International Immunopharmacology **4**(9): 1209-1215.

Rijkenberg, M. J. A., A. C. Fischer*, et al.* (2005). "The influence of UV irradiation on photoreduction of iron in th Southern Ocean." Mar. Chem. **93**: 119-129.

Rijkenberg, M. J. A., L. J. A. Gerringa*, et al.* (2006). "Enhancement and inhibition of iron photoreduction by individual ligands in open ocean seawater." Geochimica Et Cosmochimica Acta **70**(11): 2790-2805.

Rose, A. L. and D. Waite (2006). "Role of superoxide in the photochemical reduction of iron in seawater." Geochimica Et Cosmochimica Acta **70**(15): 3869-3882.

Rose, A. L. and T. D. Waite (2002). "Kinetic model for Fe(II) oxidation in seawater in the absence and presence of natural organic matter." Environ. Sci. Technol. **36**: 433-444.

Rose, A. L. and T. D. Waite (2003a). "Effect of Dissolved Natural Organic Matter on the Kinetics of Ferrous Iron Oxygenation in Seawater." Environ. Sci. Technol. **37**: 4877-4886.

Rose, A. L. and T. D. Waite (2003b). "Kinetics of iron complexation by dissolved natural organic matter in coastal waters." Marine Chemistry **84**(1-2): 85-103.

Rose, A. L. and T. D. Waite (2005). "Reduction of organically complexed ferric iron by superoxide in a simulated natural water." Environmental Science & Technology **39**(8): 2645-2650.

Rue, E. L. and K. W. Bruland (1995). "Complexation of iron(III) by natural organic ligands in the central North Pacific as determined by a new competitive ligand equilibrium / adsorptive cathodic stripping voltammetric method." Marine Chemistry **50**: 117-138.

Santana-Casiano, J., M. G.-D. Vila*, et al.* (2000). "The effect of organic compounds in the oxidation kinetics of Fe(II)." Marine Chemistry **70**(1-3): 211-222.

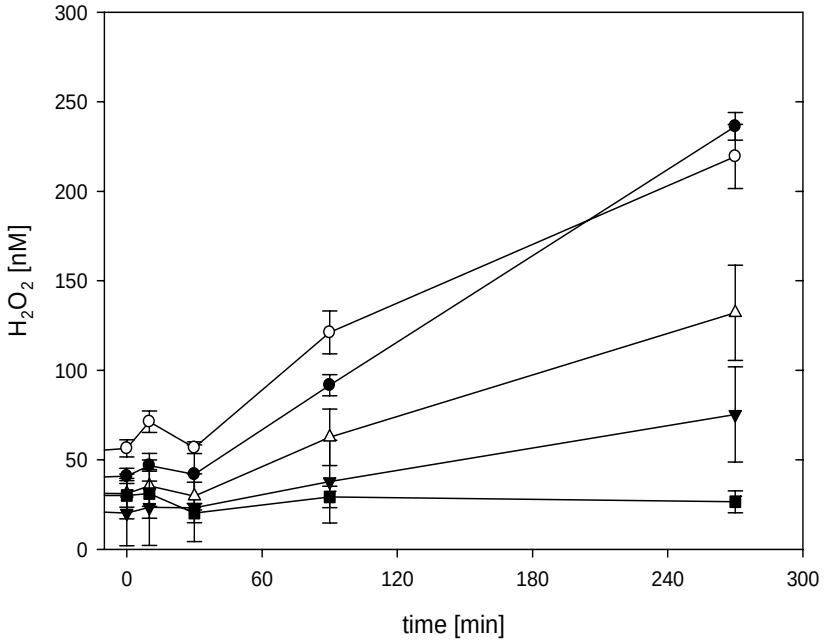
Santana-Casiano, J. M., M. Gonzalez-Davila*, et al.* (2004). "The oxidation of Fe(II) in NaCl-HCO_3- and seawater solutions in the presence of phthalate and salicylate ions: a kinetic model." Mar. Chem. **85**(1-2): 27-40.

Scully, N. M., D. J. McQueen*, et al.* (1996). "Hydrogen peroxide formation: The interaction of ultraviolet radiation and dissolved organic carbon in lake waters along a 43-75 degrees N gradient." Limnol. Oceanogr. **41**(3): 540-548.

Sedlak, D. L. and J. Hoigne (1993). "The role of copper and oxalate in the redox cycling of iron in atmospheric waters." Atmospheric Environment **27A**(14): 2173-2185.

620 Sivia, D. S. (2006). "Data Analysis, A Bayesian Tutorial." 246 pp.
621
622 Stookey, L. L. (1970). "Ferrozine - a new spectrophotometric reagent for iron." Anal.
623 Chem. **42**(7): 779-781.
624
625 Sunda, W. and S. Huntsman (2003). "Effect of pH, light, and temperature on Fe-
626 EDTA chelation and Fe hydrolysis in seawater." Mar. Chem. **84**: 35-47.
627
628 Tanaka, Hurlburt*, et al.* (1971). "Application of Algal Polysaccharides as *in vivo*
629 Binders of Metal Pollutants." Proceedings of the International Seaweed Symposium **7**:
630 602-604.
631
632 Theis, T. L. and P. C. Singer (1974). "Complexation of Iron(ll) by organic matter and
633 its effect on Iron(ll) oxygenation." Environ. Sci. Technol. **8**: 569-573.
634
635 van den Berg, C. M. G. (1995). "Evidence for organic complexation of iron in
636 seawater." Marine Chemistry **50**: 139-157.
637
638 Viollier, E., P. W. Inglett*, et al.* (2000). "The ferrozine method revisited: Fe(II)/Fe(III)
639 determination in natural waters." Applied Geochemistry **15**(6): 785-790.
640
641 Voelker, B. M. and D. L. Sedlak (1995). "Iron reduction by photoproduced
642 superoxide in seawater." Mar. Chem. **50**: 93-102.
643
644 Waite, T. D. and F. M. M. Morel (1984). "Photoreductive dissolution of colloidal iron
645 oxides in natural waters." Environmental Science & Technology **18**: 860-868.
646
647 Waite, T. D., A. L. Rose*, et al.* (2006). "Superoxide-mediated reduction of ferric iron
648 in natural aquatic systems." Geochimica Et Cosmochimica Acta **70**(18): A681-A681.
649
650 Wells and M. L. a. L.M.~Mayer (1991a). "The photoconversion of colloidal iron
651 oxyhydroxides in seawater." Deep-Sea-Research A **38**: 1379-1395.
652
653 Wells, M., L. Mayer*, et al.* (1991b). "The photolysis of colloidal iron in the oceans."
654 Nature **252**: 248-250.
655
656 Yocis, B. H., D. J. Kieber*, et al.* (2000). "Photochemical production of hydrogen
657 peroxide in Antarctic Waters." Deep Sea Research I **47**(6): 1077-1099.
658
659 Yuan, J. and A. M. Shiller (1999). "Determination of subnanomolar levels of
660 hydrogen peroxide in seawater by reagent-injection chemiluminescence detection."
661 Analytical Chemistry **71**(10): 1975-1980.
662
663 Yuan, J. and A. M. Shiller (2001). "The distribution of hydrogen peroxide in the
664 southern and central Atlantic ocean." Deep Sea Research II **48**: 2947-2970.
665
666 Zepp, R. G. and D. M. Cline (1977). "Rates of direct photolysis in aquatic
667 environment." Environ. Sci. Technol. **11**(4): 359-366.
668

669 **7 Figures**



670

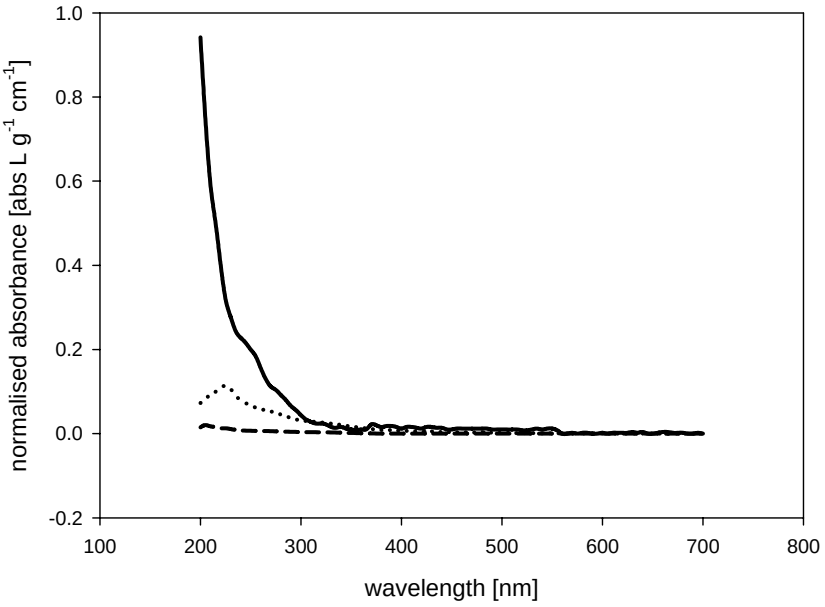
671 Figure 1: Photogeneration of H_2O_2 during 270 min of irradiation of a 10 mg L^{-1}

672 solution of laminarin (open triangle), carrageenan (open circle), gum xanthan (filled

673 circle) and of pure MQ water (filled triangle) and the mean of all 4 dark controls

674 (filled squares)

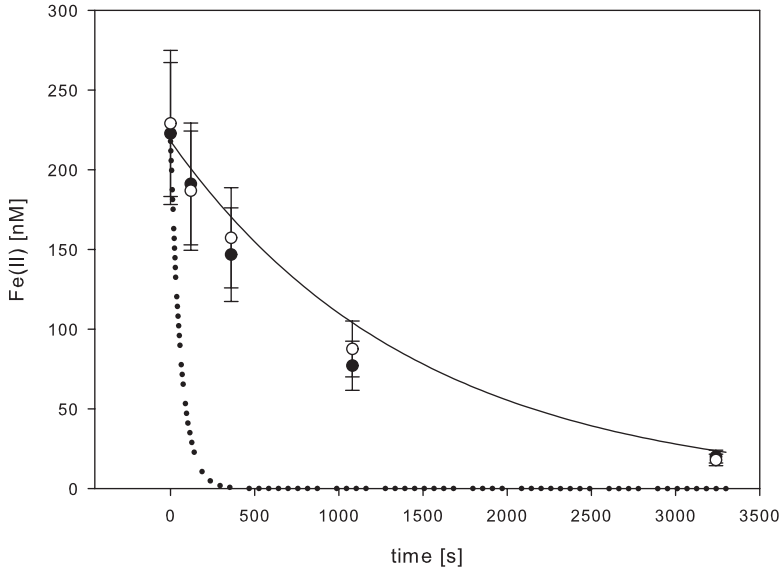
675



676

677 Figure 2: Absorbance spectra (normalised absorbance for 1 g L^{-1} and 5 cm cuvette) of
678 laminarin (dashed line), carrageenan (dotted line), gum xanthan (solid line) dissolved
679 in MQ water and filtered over 0.2 µm membrane

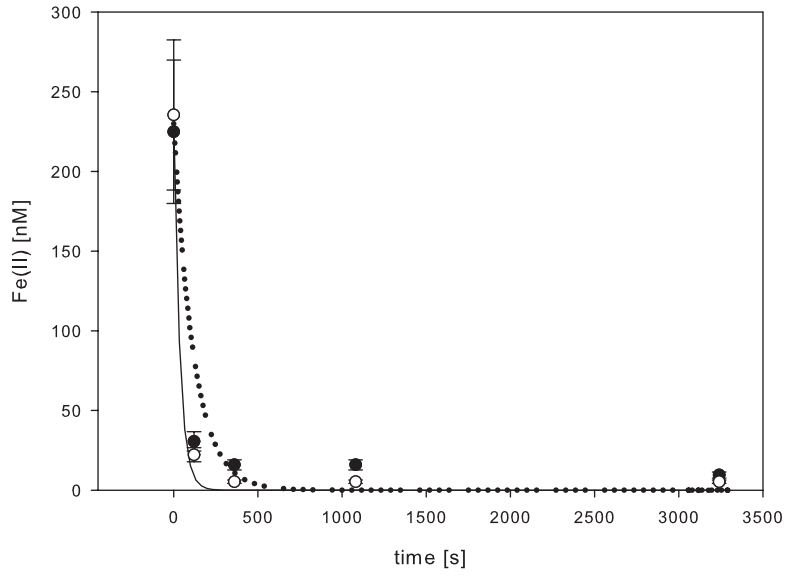
680



681

682 Figure 3: Dark oxidation of 218 nmol L^{-1} Fe(II) in natural SW (filled circles) and
683 natural SW with PS added. Model results of oxidation of Fe (II) under O_2 saturation
684 (dotted line) and in the presence of 5 nmol L^{-1} H_2O_2 (solid line) at pH = 8.4, S = 34.1,
685 18 °C are also depicted

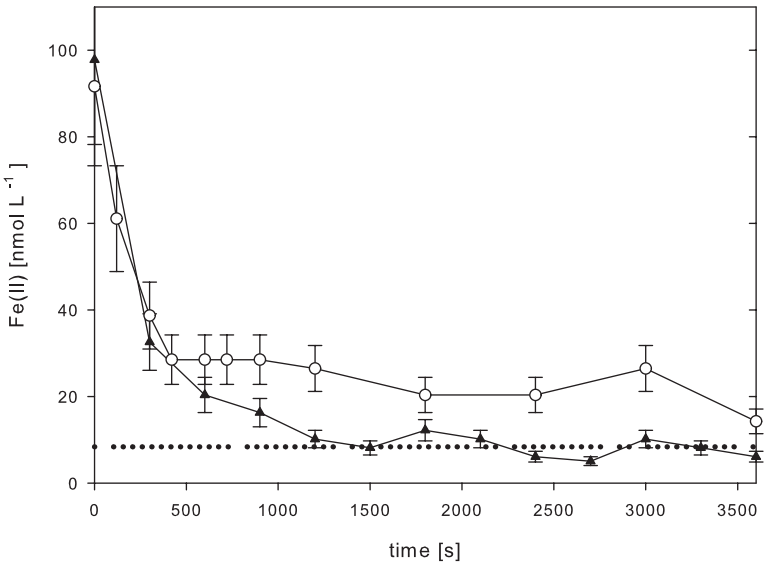
686



687

688 Figure 4: Dark oxidation of 230 nmol L^{-1} Fe(II) in UVSW (filled circles) and UVSW
689 with PS added. Model results of oxidation of Fe (II) under O_2 saturation (dotted line)
690 and in the presence of 270 nmol L^{-1} H_2O_2 (solid line) at pH = 8.3, S = 34.1, 17 °C are
691 also depicted

692



693

694 Figure 5: Oxidation of Fe(II) in pure UVSW (triangles) and in UVSW with added

695 diatom exudates (circles) (22 °C, S = 34.2, O_2 saturated, pH = 8.1, UVB = 0.3 W m^{-2},

696 UVA = 17.6 W m^{-2}, PAR = 3.8 W m^{-2}). The dotted line depicts the detection limit.

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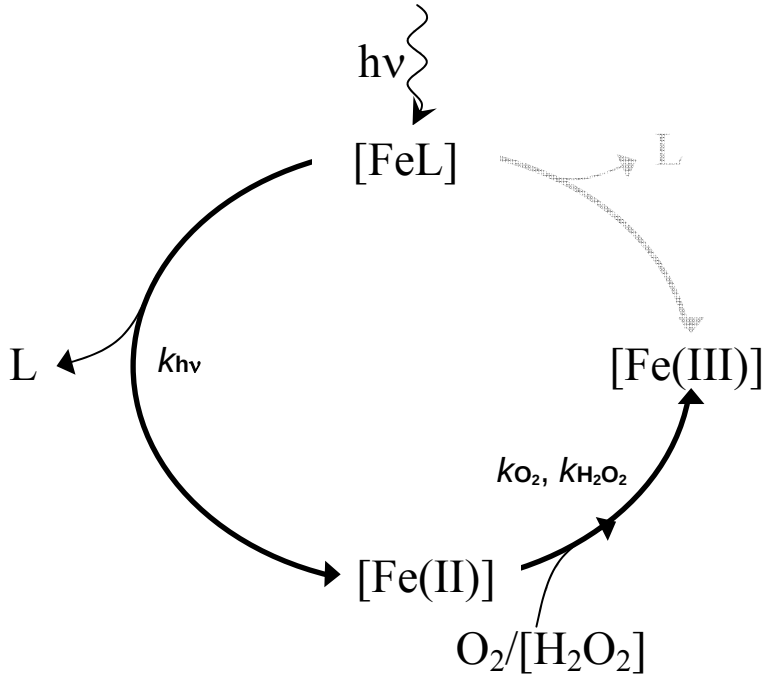

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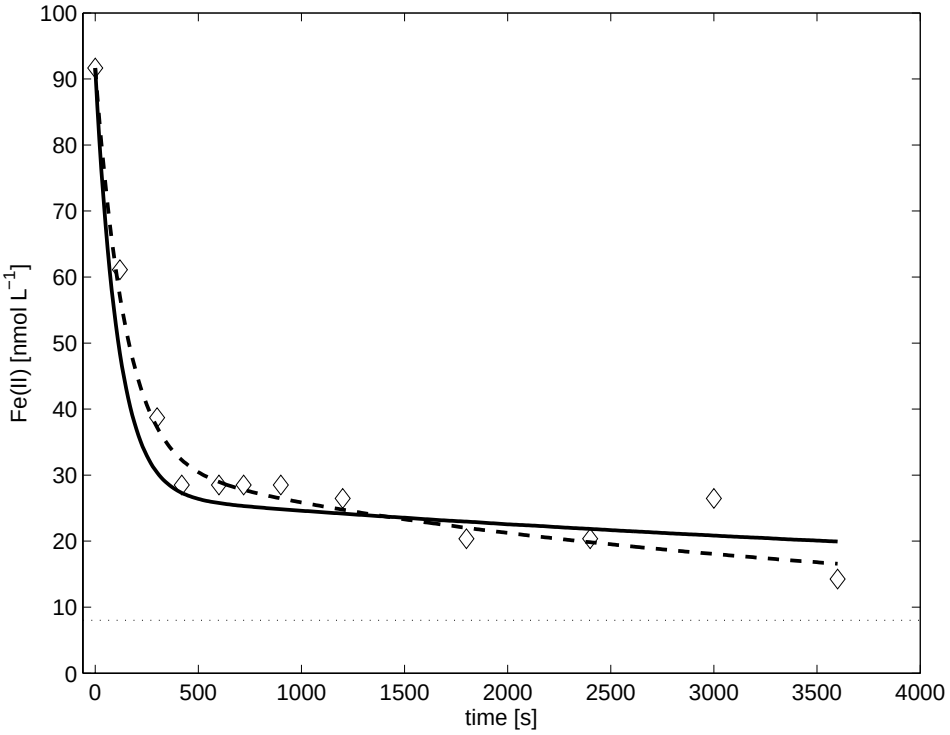
702

703 Figure 6: Schematic photoredox cycle for FeL describing the Fe cycling in experiment

704 3 adapted from Sunda and Huntsman (2003)

705



706

Figure 7: Best curve fits for measured data (experiment 3) of the oxidation of Fe(II) in UVSW (22 °C, pH = 8.1) with added diatom exudates (diamonds) using fix oxidation rates calculated according to Millero *et al.* (1987; 1989) and the best estimate for the photoproduction term (solid line) and using the best parameter estimates for all three parameters (dashed line) the dotted line shows the detection limit