PhD thesis

"The Effect of Acidic Polysaccharides on The Biogeochemistry of Iron in The Marine Environment"

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CHAPTER 1:

Introduction

1 Introduction

In the early 1990's the first IPCC report stated the effect of anthropogenic CO_2 emissions on global warming and John Martin's Iron Hypothesis (Martin and J.H 1990), relating atmospheric dust deposition, a major source of iron to the surface ocean, to the CO_2 concentration in the atmosphere and the last ice age, culminating in the well known sentence "Give me (half) a tanker of iron and I'll give you a new ice age!". Since then, several large-scale in situ Fe fertilisation experiments revealed that in large areas of the ocean, the so called high nutrient low chlorophyll (HNLC) areas, phytoplankton growth is partly limited by depleted Fe conditions (Geider *et al.* 1994; De Baar and Boyed 2000; Boyd *et al.* 2007).

The ocean receives Fe from upwelling, riverine input, melting icebergs, atmospheric dust input, input from anoxic sediments, hydrothermal vents and direct recycling by organisms(Tovar-Sanchez *et al.* 2007). However, in HNLC regions the Fe input to surface waters is very low resulting in Fe limitation of phytoplankton growth.

Fe is an important nutrient for marine phytoplankton (Geider *et al.* 1994; Falkowski *et al.* 1998; Morel and Price 2003), being essential in metabolic reactions like the photosynthetic electron transport and the assimilation of nitrogen. It is also required for the synthesis of chlorophyll (Martin *et al.* 1988; Maldonado *et al.* 1999) as well as for the functioning of the enzyme superoxide dismutase which inhibits the breakdown of chlorophyll by superoxide radicals (Coale 1991).

The thermodynamic stable species of Fe in oxygenated natural seawater is Fe^{3+}, which undergoes rapid hydrolysis at pH 8 (Bruland *et al.* 1991). The Fe hydroxides are only little soluble and precipitate finally as Fe_2O_3. Fe is continuously removed from the surface ocean via hydrolysis and scavenging onto sinking particles (Geider 1999). More than 99% of the remaining dissolved Fe is found to be bound by organic compounds (Rue and Bruland 1995; van den Berg 1995; Nolting *et al.* 1998; Hutchins *et al.* 1999; Croot and Johansson 2000; Boye 2001), which help retain Fe in the surface ocean. These Fe ligands comprise highly specific low molecular weight siderophores (Wilhelm and Trick 1994; Macrellis *et al.* 2001; Butler 2005), less specific protoporphyrins (Nakabayashi *et al.* 2002), hemes (Gledhill 2007), and even less specific molecules to large for transmembrane transport (Macrellis *et al.* 2001). Phytoplankton do not have a ligand specific uptake mechanism like prokaryotes do for siderophores. Instead eukaryotic phytoplankton take up Fe very efficiently (Voelker and Wolf-Gladrow 1999) via ferrireductase, a non specific cell surface enzyme for extracellular Fe reduction, through membrane bound transport proteins and by diffusion across plasma membrane (Croot *et al.* 1999). There are several other mechanisms to make organically bound Fe bioavailable, such as thermal dissolution (Wells and Goldberg 1993), digestion by grazers (Hutchins and Bruland 1994; Barbeau *et al.* 1996) or photochemical redox processes (Waite and Morel 1984; Sunda and Huntsman 1995) using dissolved organic compounds as an electron source (Kuma *et al.* 1992).

The main oxidation pathway of Fe(II) to Fe(III) is the reaction with O_2 or H_2O_2 according to the Haber-Weiss mechanism (Millero *et al.* 1987; Millero and Sotolongo 1989; King *et al.* 1995). In marine systems H_2O_2 functions as a strong oxidant or a reductant (Millero and Sotolongo 1989; Croot *et al.* 2005). Hence, it is important for the cycling of organic compounds and trace metals like Fe (Millero and Sotolongo 1989). H_2O_2 is the most stable intermediate in the reduction of O_2 to H_2O and is mainly produced in the water column by photochemical reactions involving dissolved organic matter (DOM) and O_2 (Cooper *et al.* 1988; Scully *et al.* 1996; Yocis *et al.* 2000; Yuan and Shiller 2001). Light absorbed by DOM induces an electron transfer to molecular oxygen, forming the superoxide anion radical, which undergoes disproportionation to form hydrogen peroxide. Hence light, O_2, H_2O_2 and organic compounds are important factors in the very complex chemistry of Fe in seawater. The oxidation of Fe can be inhibited (Theis and Singer 1974; Miles and Brezonik 1981) or accelerated (Sedlak and Hoigne 1993; Rose and Waite 2002, 2003) in the presence of organic compounds.

A great number of phytoplankton species release carbohydrates into the surrounding water (Myklestad *et al.* 1972; Myklestad *et al.* 1989; Myklestad 1995; Hong *et al.* 1997). Phytoplankton exudates, rich in acidic polysaccharides, account significantly for the dissolved marine organic matter pool especially during bloom events (Aluwihare *et al.* 1997; Aluwihare and Repeta 1999; Benner 2002) and are highly surface active (Mopper *et al.* 1995). These exudates and transparent exopolymer particles (TEP), abiotically formed from these exudates, show high affinity to Th and other trace elements (Santschi 1997; Quigley *et al.* 2001; Guo *et al.* 2002; Quigley *et al.* 2002).

The objective of this PhD project was to investigate the effect of acidic polysaccharides on the biogeochemistry of Fe in seawater. Three main themes were identified where research is required. Firstly, to study the specific effects of polysaccharides on Fe speciation in the light replete upper ocean. Secondly, to deepen our knowledge on influencing factors and the distribution of H_2O_2 – important in the redox chemistry of Fe in the upper ocean. Thirdly, acidic polysaccharides may represent an important fraction of the uncharacterized Fe ligands in the ocean. Reported chemical and biological properties of phytoplankton exudates support their Fe binding potential. The following hypotheses were made:

1.1 Polysaccharides stabilize Fe(II) via complexation

1.2 Fe bound to polysaccharides is released via photochemical processes

2. Phytoplankton exudates enhance the photoproduction of H_2O_2, a major player in the redox chemistry of Fe.

3. Acidic polysaccharides and TEP are strong Fe chelators contributing significantly to the pool of unknown organic Fe-ligands in the ocean, released by diatoms to prevent Fe from precipitating from the surface ocean

Hypotheses 1, 2 and 3 were investigated and results are presented and discussed in chapters 2, 3, and 4, respectively.

CHAPTER 2:

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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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 nmol L^{-1} (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.

1 Introduction

Marine phytoplankton contributes significantly to the CO_2 flux from the atmosphere into the ocean, thus impacting atmospheric CO_2 concentrations (Falkowski *et al.* 1998). Global marine primary productivity shows great spatial and temporal variability, caused primarily by variable light, zooplankton grazing and nutrient distributions. In addition to the macronutrients (PO_4, NO_3), iron is an essential trace element for photo-autotrophic organisms (Geider *et al.* 1994; Falkowski *et al.* 1998; Morel and Price 2003). Several large scale iron fertilization experiments have revealed that in 40% of the surface ocean, the so called High Nutrient Low Chlorophyll (HNLC) areas, iron is at least partially responsible for limitation of phytoplankton growth (Boyd *et al.* 2007). However, iron limitation can occur in coastal areas as well (Hutchins and Bruland 1998) and here the supply of Fe through upwelling and resuspension determine its cycling.

Free hydrated Fe(III) concentrations in seawater are very low ($<10^{-20}$ mol L^{-1}) (Rue and Bruland 1995) and the more soluble Fe(II) is rapidly oxidised (Millero *et al.* 1987; Millero and Sotolongo 1989; King *et al.* 1995; Gonzalez-Davila *et al.* 2005, 2006). Thus concentrations of dissolved Fe in the ocean should be very low. However, over 99% of the dissolved iron in seawater is reported to be bound by organic compounds (Rue and Bruland 1995; van den Berg 1995; Croot and Johansson 2000; Boye 2001) and these ligands can maintain the concentrations typically seen in the ocean (Johnson *et al.* 1997). Iron binding ligands in seawater mainly consist of bacterial siderophores (Macrellis *et al.* 2001; Butler 2005) and possibly planktonic exudates like acidic polysaccharides (PS) (Tanaka *et al.* 1971). Transparent exopolymer particles (TEP), which are rich in acidic polysaccharides, are ubiquitous in the surface ocean (Passow 2002). TEP has been shown to bind ^{234}Th (Passow *et al.* 2006) and are therefore a prime candidate to bind iron.

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

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

Increased photochemical reduction of Fe(III) in the presence of sugar acids has been reported (Kuma *et al.* 1992; Ozturk *et al.* 2004; Rijkenberg *et al.* 2005) but for polysaccharides no such studies have been carried out so far. However, the relative 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 (~20°C) in the laboratory. In experiments 1 and 3 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 UV photo-oxidation, the so called organic-free UVSW (Donat and Bruland 1988), and 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 similar carbon content of ~40% (w/w).

Dissolved diatom exudates were collected as the 0.4 µm filtrate of a senescent culture of *Phaeodactylum tricornutum* grown in f/2 medium which also contained 10 µmol L^{-1} EDTA. Ford and Percival (1965) separated a significant amount of a water-soluble glucan from an aqueous extract of *P. 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 these 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 UVSW). 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 over a 60 min period after the iron addition. The UV light was switched on for the whole experiment right after the addition of iron to the sample bags. Temperature, salinity, oxygen concentration, pH and total iron were measured before the iron addition and at the end of the experiment. H_2O_2 in all organic-free seawater samples was adjusted to an initial concentration of 5 nmol L^{-1} and was measured again at the end of the experiment.

2.3 Analyses

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

Reagents were from Sigma-Aldrich and at least p.a. grade. All solutions were 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 the 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 and Shiller 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 and Bruland 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 illumination large amounts (140-240 nmol L^{-1}) of H_2O_2 were formed due to the addition of 10 mg L^{-1} of polysaccharides to MQ water (Figure 1). The H_2O_2 concentrations in all samples increased linearly with time during the experiment, after the light was switched on. Gum xanthan showed the highest photochemical production of H_2O_2 followed by carrageenan and laminarin, which can be explained by their different absorptivity at <400 nm (Figure 2). The addition of laminarin led to a net accumulation rate of H_2O_2 of 22.5±8.1 nmol L^{-1} h^{-1}, which was twice as high as that for pure MQ water (12.3±9.2 nmol L^{-1} h^{-1}). The H_2O_2 accumulation during illumination of the MQ water was highly variable and probably due to organic matter leaching from the resin of the filter cartridge of the MQ system. However, the DOC concentration in MQ water was <10 µmol L^{-1} (which was the limit of quantitation of the instrument). H_2O_2 accumulation rates of 36.2±3.6 nmol L^{-1} h^{-1} and 43.4±5.2 nmol L^{-1} h^{-1} were determined in samples with added carrageenan and gum xanthan, respectively. The photochemical production of H_2O_2 was thus 3-4 times higher in the presence of carrageenan and gum xanthan compared to pure MQ water. Linear H_2O_2 accumulation rates of similar magnitude have been reported by Cooper *et al.* (1988) and Miller *et al.* (1995) in natural seawater samples. The main structural differences between the molecules of these three PSs are that laminarin has a linear structure of linked glucose monosaccharide units, carrageenan has sulphur containing groups and gum xanthan has a branched structure incorporating uronic acid groups. The PS concentration used in our experiment is equivalent to about 4 mg L^{-1} organic carbon leading to normalised H_2O_2 generation rates of 5.2±1.9 nmol L^{-1} (mg C)$^{-1}$ h^{-1} (laminarin), 9.1±0.9 nmol L^{-1} (mg C)$^{-1}$ h^{-1} (carrageenan) and 10.9±1.3 nmol L^{-1} (mg C)$^{-1}$ h^{-1} (gum xanthan). These values are up to 29 times higher than the rate of 0.38 nmol L^{-1} (mg C)$^{-1}$ h^{-1} reported by Price et al. (1998) for the >8000 Da fraction of natural DOM in the 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 and Cline 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 and Zafiriou 1990). Photochemical production rates of H_2O_2 in the Atlantic Ocean and Antarctic waters range from 2.1 to 9.6 nmol L^{-1} h^{-1} (Obernosterer 2000; Yocis *et al.* 2000; Yuan and Shiller 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 and Zafiriou 1990; Petasne and Zika 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). 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 calculated values can be compared to Fe(II) half-life values of 42 s (UVSW) and 35 s (UVSW+PS) measured during experiment 2. The calculated half-life under O_2 saturation of 82 s deviates by 95 % (UVSW) and 134 % UVSW+PS) respectively from the measurements. The calculated value of 25 s under the ambient H_2O_2 conditions is 40 % (UVSW) and 29 % (UVSW+PS) lower than the measured half-lives. This indicates that the high H_2O_2 concentration had a stronger oxidising effect on Fe(II) than the dissolved O_2 in the samples.

For the natural SW sample the calculated half-life of 43 s under O_2 saturation(Millero *et al.* 1987; Millero and Sotolongo 1989) does not fit the measured data well. The half-life of Fe(II) in the natural SW sample determined via an exponential fit to the measured data (Figure 3) was ~17 times (11.9 min) and with PS added ~19 times (13.3 min) longer than theoretical value. The measured data followed the exponential oxidation curve calculated for the low H_2O_2 concentration of these samples whereas the high O_2 content seemed to not accelerate the measured oxidation of Fe(II).

The DOC content of the natural SW (97 µmol L^{-1}) was almost 10 times higher than of the UVSW. The difference in Fe(II) oxidation between the water types might therefore be due to the stabilisation of Fe(II) against oxidation by natural occurring compounds of the coastal SW (Theis and Singer 1974; Miles and Brezonik 1981; Santana-Casiano *et al.* 2000; Rose and Waite 2003a; Santana-Casiano *et al.* 2004). These results show that the added gum xanthan was not a good model for natural occurring substances stabilising Fe(II) against oxidation. Initial H_2O_2 concentrations also differed appreciably, with 5 nmol L^{-1} H_2O_2 in the natural SW sample and 270 nmol L^{-1} H_2O_2 in the UVSW sample. UV oxidation in UVSW water during removal of natural DOM must have caused the differences in H_2O_2. We calculated Fe(II) oxidation rates due to O_2 and H_2O_2 in our experiment to investigate if the differing rates could have been caused by differing initial H_2O_2 concentrations. From the comparison between our measured and theoretically calculated values we conclude that a strong effect of H_2O_2 on the lifetime of Fe(II) was observed but no effect of gum xanthan was found in this experiment conducted without irradiation. The lower initial H_2O_2 concentrations in the natural SW sample (5 nmol L^{-1} H_2O_2; Figure 3) compared to the UVSW sample (270 nmol L^{-1} H_2O_2; Figure 4) appears to be the major cause for slower Fe(II) oxidation, suggesting that H_2O_2 mainly controls the oxidation of Fe(II).

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

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

Differences in H_2O_2 production during the first hour of irradiation were significant between UVSW with and without exudates. In the UVSW sample with added diatom exudates a linear production rate of 33 nmol L^{-1} h^{-1} H_2O_2 was determined whereas in pure UVSW the respective rate was only 5 nmol L^{-1} h^{-1}. The higher production rate of H_2O_2 in the presence of exudates, suggests increased photochemical production of H_2O_2. UVSW without exudates contained 11 µmol L^{-1} DOC and no measurable total monosaccharides and polysaccharides, whereas UVSW mixed with exudates of *P. tricornutum* contained ~450 µmol L^{-1} DOC, including 0.4 mg glucose eq. L^{-1} (i.e. 13 µmol C L^{-1}) total MS and PS. The DOC- normalised H_2O_2 generation rate of 6.1 nmol L^{-1} (mg C)$^{-1}$ h^{-1} calculated from UVSW with exudates indicates that laminarin-like diatom exudates (Ford and Percival 1965) photochemically produce H_2O_2. However, the high DOC content suggests that there was also other organic matter contributing to the photo-production of H_2O_2.

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

The model uses a constant photoproduction term $k_{h\nu}$[FeL] of ferrous iron, and constant oxidation rates with oxygen (k_{O2}). The oxidation rates with hydrogen peroxide (k_{H2O2}) are assumed to increase linearly with a photoformation rate of 33 nmol L^{-1} h^{-1} as measured in this experiment and initial H_2O_2 concentration are set at 4.6 nmol L^{-1}. The initial Fe(II) concentration [$Fe(II)_0$] is set at 100 nmol L^{-1} Fe(II), the amount added in the experiment, and increases in the model by the constant photoreduction of the FeL complex (where L is either EDTA or diatom exudates or a combination of both). The direct photoreduction of inorganic iron colloids and dissolved ferric iron is also possible (Waite and Morel 1984; Wells and L.M.~Mayer 1991; Wells *et al.* 1991; Johnson *et al.* 1994), but rates for these processes are negligibly low. For both processes together we calculated rates of about 0.004 nmol L^{-1} s^{-1} of Fe(II) for 100 nmol L^{-1} Fe(II) added using the rate coefficients 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 (Eq. 1).

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

where

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

t given in [s], k_{hv} and k_{O2} in [s^{-1}], k_{H2O2} in [L nmol^{-1} s^{-1}] and all concentrations given in [nmol L^{-1}].

The parameters k_{O2}, k_{hv}[FeL] and k_{H2O2} were estimated by fitting the model (m_i) to the observed data (d_i), minimizing the root mean squared model-data misfit, scaled by the assumed variance (σ_i^2) of the measurements. If the deviations between model and data are independent and normally distributed, the misfit

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

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

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

(see e.g. D.S. Sivia (2006)). The probability function is well approximated by a multidimensional Gaussian distribution with a maximum value for the best estimated set of parameter values. To obtain an estimate of the variance for this maximum likelihood estimate of the parameters, we also need an estimate of the covariance matrix of the parameters at the minimum of χ^2. This covariance matrix can be estimated as the inverse of the Hessian matrix of χ^2 at the minimum. We can then assume a confidence interval (± one standard deviation) for the best estimates of the parameters, which gives k_{O2} = $(6.0\pm1.2)\cdot10^{-3}$ s^{-1}, k_{H2O2} = $(2.0\pm0.9)\cdot10^{-4}$ L $nmol^{-1}$ $s^{-1}$and $k_{h\nu}$[FeL] = 0.22±0.06 nmol L^{-1} s^{-1}. With this high photoreduction rate the model fits the measured data very well (Figure 7) but the oxidation rates for oxygen and H_2O_2 are 30% lower and 105% higher, respectively, than rates reported by Millero *et al.* (1987; 1989). Holding the oxidation rates k_{O2} and k_{H2O2} fixed at values calculated for the given experimental conditions (22°C, S = 34.2, O_2 saturated, pH 8.1) according to Millero *et al.* (1987; 1989) the model-data misfit becomes somewhat larger and the model requires a slightly higher Fe(II) photoproduction term $k_{h\nu}$[FeL] of about 0.24±0.01 nmol L^{-1} s^{-1} to fit the measured data (Figure 7). The larger error margins when fitting all three parameters, compared to fitting only the photoreduction rate, is explained by the strong correlation between the estimates of k_{H2O2} and of $k_{h\nu}$[FeL], meaning that the data can be represented almost equally well with different combinations of these two parameters.

The estimated photoproduction rates of Fe(II) are about 50 times higher than the photoreduction rate of inorganic colloidal and dissolved iron calculated before, independent of whether we assume the oxidation rate coefficients by Millero et al. (1987; 1989) or Johnson et al. (1994) . This indicates high photoreduction of Fe(III) mediated by the added organic material. This high reduction of Fe(III) could have resulted either from direct photoreduction of the FeL or indirectly via light induced (see absorbance spectra Figure 2) formation of superoxide (DOM + hν → DOM*; DOM* + O_2 → DOM^+ + O_2^-; and Fe(III) + O_2^- → Fe(II) + O_2) and the subsequent reduction of ferric iron (King *et al.* 1995; Voelker and Sedlak 1995; Rose and Waite 2005; Fujii *et al.* 2006; Rose and Waite 2006; Waite *et al.* 2006; Garg *et al.* 2007b, a).

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

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

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 *P. 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 the dissolved, colloidal and particulate fraction.

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7 Figures

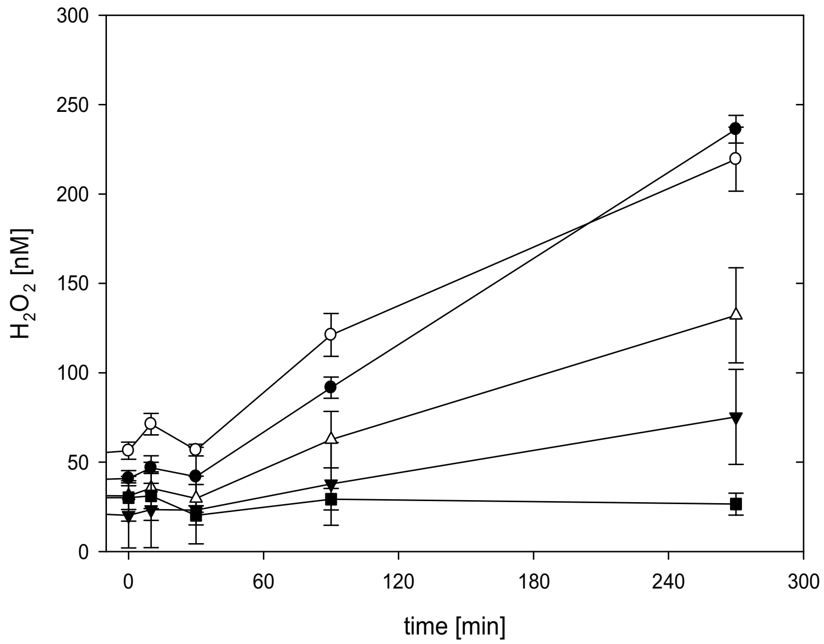

Figure 1: Photogeneration of H_2O_2 during 270 min of irradiation of a 10 mg L^{-1} solution of laminarin (open triangle), carrageenan (open circle), gum xanthan (filled circle) and of pure MQ water (filled triangle) and the mean of all 4 dark controls (filled squares)

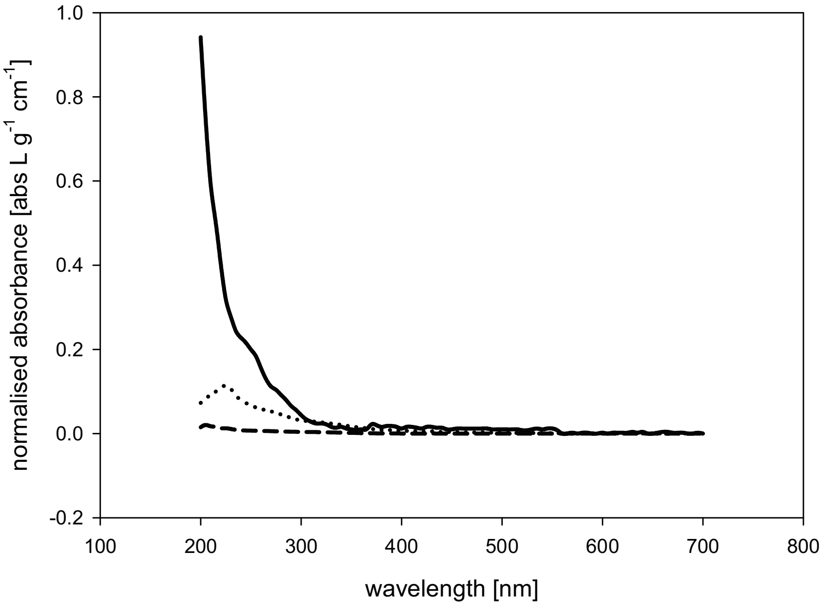

Figure 2: Absorbance spectra (normalised absorbance for 1 g polysaccharide L^{-1} in a 5 cm cuvette) of the polysaccharides laminarin (dashed line), carrageenan (dotted line), gum xanthan (solid line) dissolved in MQ water and filtered over 0.2 µm membrane.

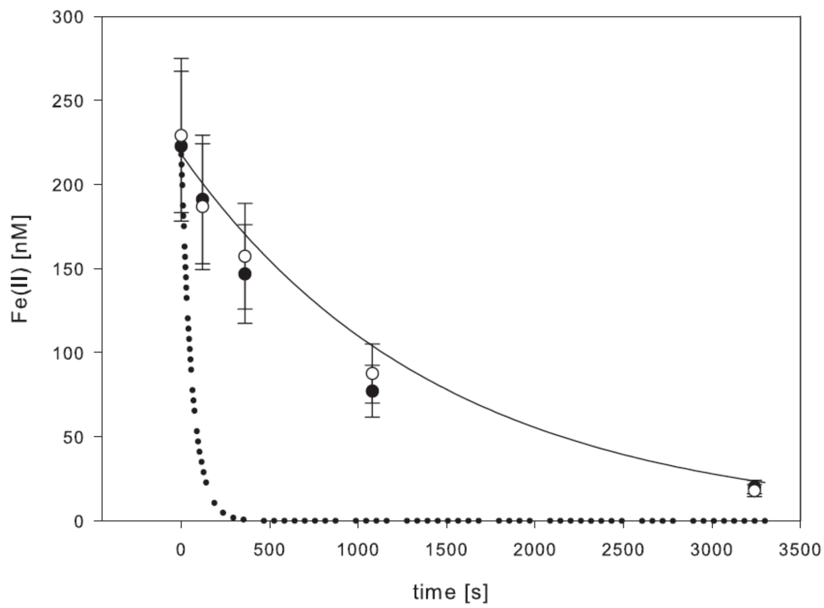

Figure 3: Dark oxidation of 218 nmol L^{-1} Fe(II) in natural SW (filled circles) and natural SW with PS added (open circles). Model results of oxidation of Fe (II) under O_2 saturation (dotted line) and in the presence of 5 nmol L^{-1} H_2O_2 (solid line) at pH 8.4, S = 34.1, 18°C are also depicted.

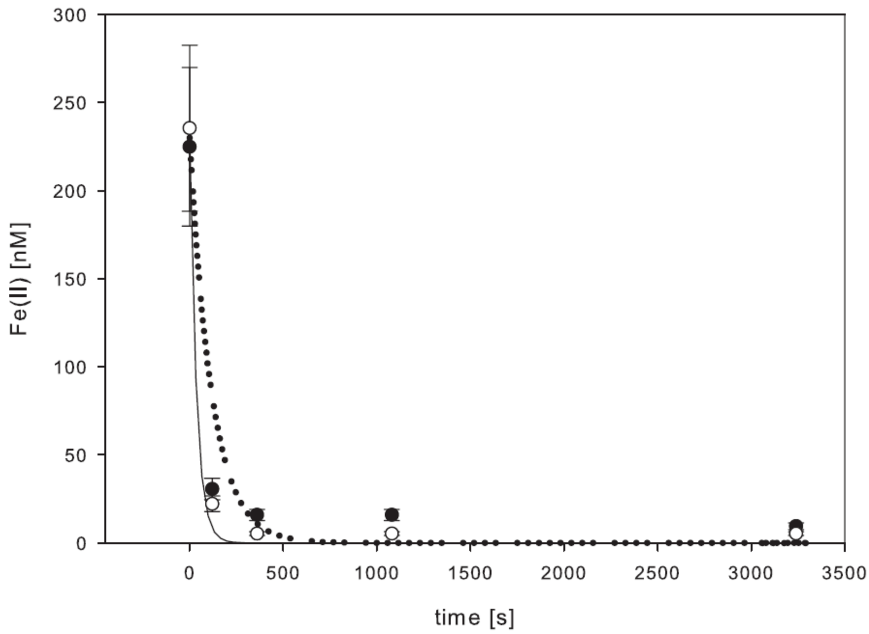

Figure 4: Dark oxidation of 230 nmol L^{-1} Fe(II) in UVSW (filled circles) and UVSW with PS added (open circles). Model results of oxidation of Fe (II) under O_2 saturation (dotted line) and in the presence of 270 nmol L^{-1} H_2O_2 (solid line) at pH 8.3, S = 34.1, 17°C are also depicted.

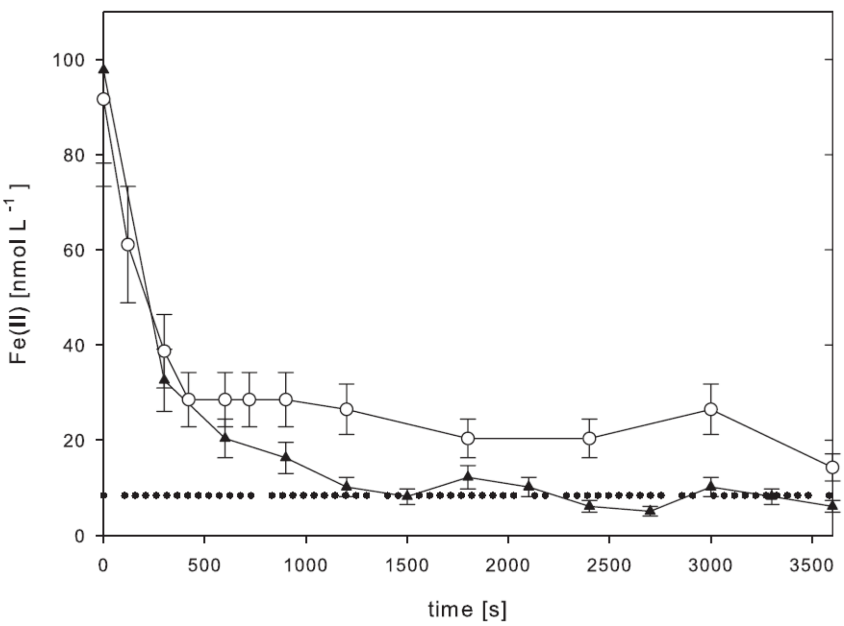

Figure 5: Oxidation of Fe(II) in pure UVSW (triangles) and in UVSW with added diatom exudates (circles) (22°C, S = 34.2, O_2 saturated, pH 8.1, UVB = 0.3 W m^{-2}, UVA = 17.6 W m^{-2}, PAR = 3.8 W m^{-2}). The dotted line depicts the detection limit.

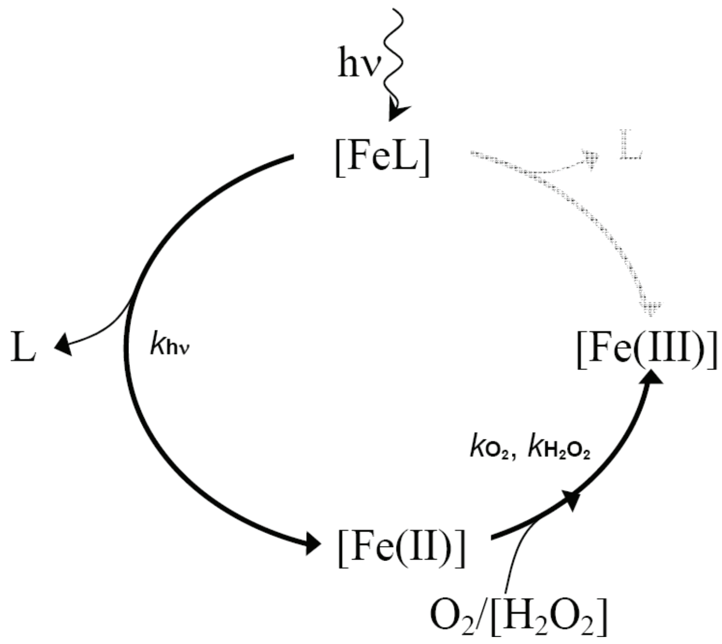

Figure 6: Schematic photoredox cycle for FeL describing the Fe cycling in experiment 3 adapted from Sunda and Huntsman (2003)

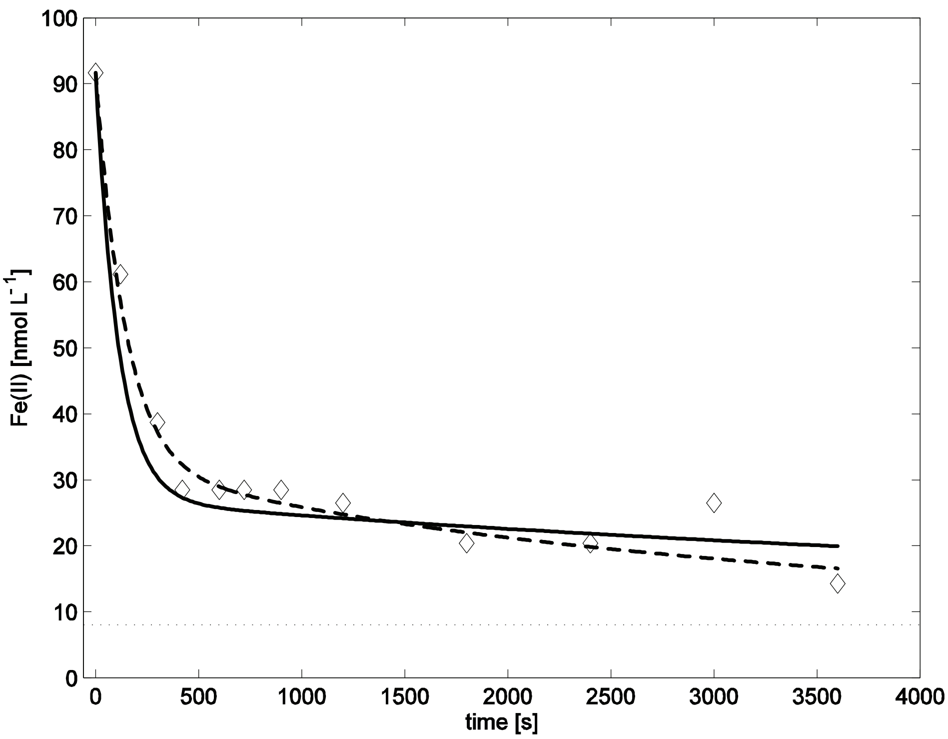

Figure 7: Best curve fits for measured data (experiment 3) of the oxidation of Fe(II) in UVSW (22°C, pH 8.1) with added diatom exudates (diamonds) using fix oxidation rates calculated according to Millero *et al.* (1987; 1989) and the best estimate for the photoproduction term (solid line) and using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

CHAPTER 3:

Identifying the processes controlling the distribution of H_2O_2 in surface waters along a meridional transect in the eastern Atlantic.

Sebastian Steigenberger and Peter L. Croot

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Identifying the processes controlling the distribution of H_2O_2 in surface waters along a meridional transect in the eastern Atlantic

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[1] Hydrogen peroxide (H_2O_2) is an important oxidant for many bio-relevant trace metals and organic compounds and has potential as a tracer for mixing in near surface waters. In this study we combine H_2O_2 and bio-optical measurements with satellite data for a meridional transect from 46°N to 26°S in the eastern Atlantic in order to determine the key processes affecting its distribution. Surface H_2O_2 ranged from 21–123 nmol L^{-1}, with maximum inventories (0–200 m) of 5.5–5.9 mmol m^{-2} found at 30°N and 25°S. Analyses showed a strong positive correlation of surface H_2O_2 with daily irradiances and recent precipitation, though poor correlations with CDOM suggest sunlight is the limiting reactant for H_2O_2 formation. Vertical distributions of H_2O_2 were controlled by a combination of mixing processes and phytoplankton activity. The present study highlights processes controlling global H_2O_2 distributions and points towards the development of parameterization schemes for prediction via satellite data. **Citation:** Steigenberger, S., and P. L. Croot (2008), Identifying the processes controlling the distribution of H_2O_2 in surface waters along a meridional transect in the eastern Atlantic, *Geophys. Res. Lett.*, *35*, L03616, doi:10.1029/2007GL032555.

1. Introduction

[2] In marine systems H_2O_2 functions as a strong oxidant or a reductant and thus it is important for the cycling of organic compounds and trace metals like Fe [*Millero and Sotolongo*, 1989]. H_2O_2 is the most stable intermediate in the four-electron reduction of O_2 to H_2O and is mainly produced in the water column by photochemical reactions involving dissolved organic matter (DOM) and O_2 [*Yuan and Shiller*, 2001]. Light absorbed by DOM induces an electron transfer to O_2 forming O_2^-, which undergoes disproportionation to form H_2O_2. Typical open ocean H_2O_2 profiles show an exponential decrease from a surface maximum consistent with the downwelling irradiance. Maximum concentrations of 300 nmol L^{-1} have been reported in Equatorial and Tropical regions with high DOM concentrations as for the Amazon plume [*Croot et al.*, 2004]. In regions with low DOM and low sunlight, surface H_2O_2 levels are much lower with Southern Ocean values of 10–20 nmol L^{-1} [*Croot et al.*, 2005].

[3] Another potential source for H_2O_2 in surface seawater is from precipitation which preferentially removes H_2O_2 from the atmosphere during rain events [*Cohan et al.*, 1999], consequently the atmospheric input at the equator and in the Inter Tropical Convergence Zone (ITCZ) is high [*Croot et al.*, 2004; *Weller and Schrems*, 1993; *Yuan and Shiller*, 2000] compared to areas with less precipitation. H_2O_2 in the ocean is also produced biologically by phytoplankton [*Palenik and Morel*, 1988]. While photochemical production is considered the dominant pathway for H_2O_2 formation, in a few cases in the Southern Ocean, distinct H_2O_2 maximums at depth, corresponding to the chlorophyll maximum, suggest a significant biological source of H_2O_2 [*Croot et al.*, 2005].

[4] Removal pathways also determine H_2O_2 concentrations in the water column and include the Catalase and Peroxidase activity of phytoplankton [*Moffett and Zafiriou*, 1990] along with redox reactions with reduced metals (e.g. Fe(II) and Cu(II)) [*Millero and Sotolongo*, 1989; *Moffett and Zika*, 1987]. The 'dark decay life-time' of H_2O_2 can vary from hours to weeks in the ocean [*Petasne and Zika*, 1997], but typically is around 4 days in the open ocean [*Plane et al.*, 1987]. Overall, the decay rate of H_2O_2 is apparently controlled by several factors including H_2O_2 concentration, colloid concentration, bacteria/cyanobacteria numbers and temperature, which controls enzymatic decay [*Wong et al.*, 2003; *Yuan and Shiller*, 2001]. Due to its short lifetime H_2O_2 shows potential as a tracer for recent vertical mixing activity [*Johnson et al.*, 1989].

[5] In the present study we compare H_2O_2 profiles with physical and bio-optical measurements and available satellite data to determine the major processes controlling the distribution of H_2O_2 in the upper ocean along a meridional transect in the eastern Atlantic.

2. Methods

2.1. Sampling

[6] Samples were collected during the GEOTRACES cruise, ANTXXIII-I from 14 October to 17 November 2005 on board the German research vessel *R. V. Polarstern* on a transect between Bremerhaven and Cape Town. Six to seven depths were sampled for H_2O_2 from the upper 200 m at 19 stations (Figure 1), at local noon, using Niskin bottles on a standard CTD rosette. All analytical work was carried out in an AirClean class 100 laminar flow clean bench. Chlorophyll and chromophoric dissolved organic matter (CDOM) were measured in samples collected from the same CTD/rosette cast.

[7] By sampling only at local noon we were unable to examine the importance of the solar driven diel cycle in H_2O_2, by which variations of up to 40 nmol L^{-1} H_2O_2 have been reported with maxima in the afternoon or early evening [*Yuan and Shiller*, 2001; *Zika et al.*, 1985a; *Zika*

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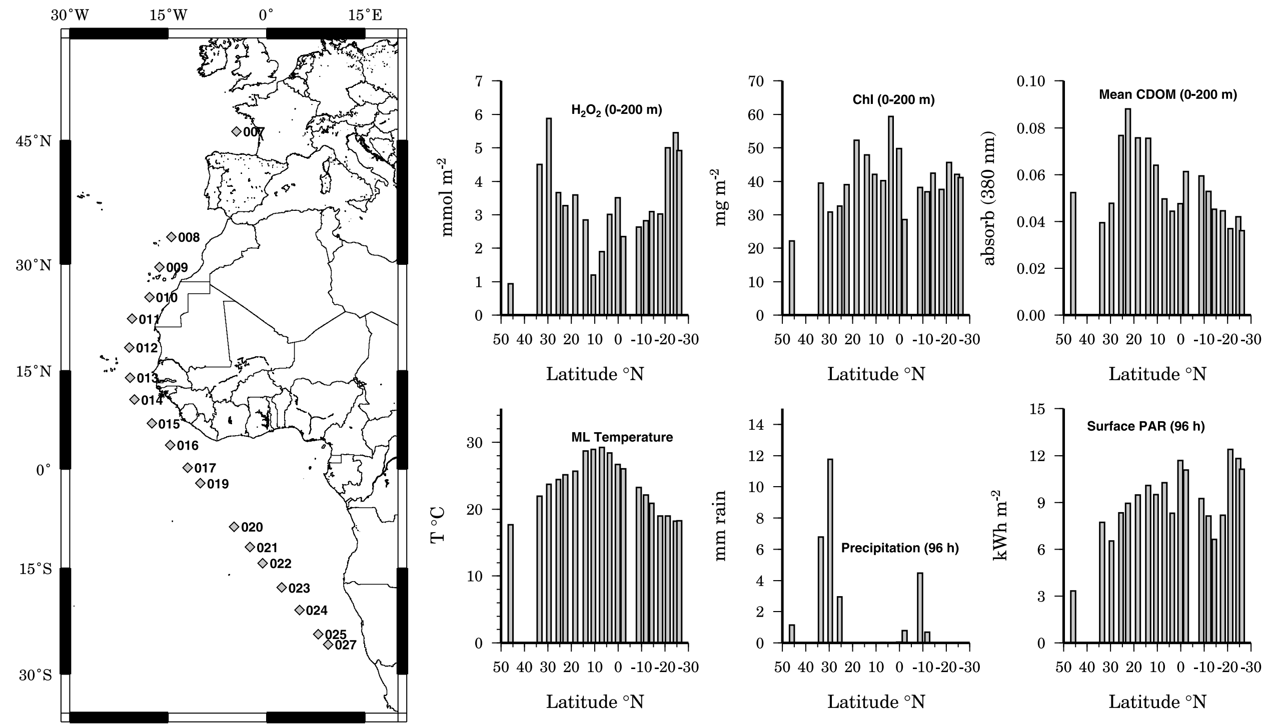

Figure 1. (left) ANTXXIII-1 cruise transect with location of the sampling stations. (right) Representative latitudinal distributions of some of the analysed parameters along the ANTXXIII-1 cruise transect. Clockwise from top left: H_2O_2 inventory (0–200 m), chlorophyll inventory (0–200 m), mean CDOM in the upper 200 m, surface PAR for the preceding 96 hours (source: HelioClim-2 database), precipitation for the preceding 96 hours (source: TRMM data), and mixed layer temperature.

et al., 1985b]. In the present work by sampling at the local noon, we are able to provide a valid comparison between stations along the transect but it is clear more work on the diel cycling of H_2O_2 is required.

2.2. H_2O_2 Measurements in Surface Waters

[8] Samples were drawn into 100 mL low density brown polyethylene bottles which were impervious to light. Unfiltered samples for H_2O_2 were analyzed within 1–2 h of collection using a flow injection chemiluminescence (FIA-CL) reagent injection method [*Yuan and Shiller*, 1999] as described by *Croot et al.* [2004]. Five replicates of each sample were analyzed with a typical precision of 2–3% in the concentration range of 2–120 nmol L^{-1} and a detection limit (3σ) of typically 0.6 nmol L^{-1}.

2.3. Measurement of the Natural Light Field Within the Upper Water Column

[9] A freefalling spectroradiometer (SPMR, Satlantic) was deployed for measuring the natural light field within the upper water column (down to 150–200 m). The spectral downwelling irradiance was measured at 13 wavelengths covering a spectral range from 339–682 nm.

2.4. Photosynthetically Active Radiation (PAR) Data

[10] Hourly sub-surface PAR (400–700 nm) estimates for the sampling period were obtained from the HelioClim-2 database (http://www.soda-is.com/eng/services/service_invoke/gui.php) which is constructed from METEOSAT data using the Heliosat-2 method [*Rigollier et al.*, 2004].

2.5. Measurement of Chlorophyll and CDOM Within the Upper Water Column

[11] The samples were filtered to collect the particulate matter and then stored in liquid nitrogen. Samples were analyzed post-cruise with HPLC (High Performance Liquid Chromatography) by R. A. Reynolds and D. Stramski (Scripps Institution of Oceanography, U.S.). Spectral absorption measurements of CDOM at 326 and 380 nm were made onboard the ship by R. Röttgers (GKSS Research Centre, Germany) using PSICAM [*Rottgers and Doerffer*, 2007].

2.6. Other Parameters

[12] Salinity, temperature and transmission were measured via a CTD (SBE 911plus, Sea-Bird Electronics). The integrated (over 3 h) precipitation data in mm were obtained from NASA TRMM (Tropical Rainfall Measuring Mission) product 3B42 using the GIOVANNI web-interface (http://daac.gsfc.nasa.gov/techlab/giovanni/).

2.7. Statistics

[13] A Spearman rank test was performed on the data which yielded pairwise correlation coefficients (ρ) between the parameters. All statistical analyses were done with SigmaStat 3.1 (Systat Software Inc.).

3. Results

3.1. Latitudinal Patterns of H_2O_2, Irradiance, SST, Chlorophyll/CDOM, and Precipitation During ANT XXIII-1

[14] A transect from 46°N to 26°S across the Atlantic covers a wide range of upper ocean environments [*Sarthou*

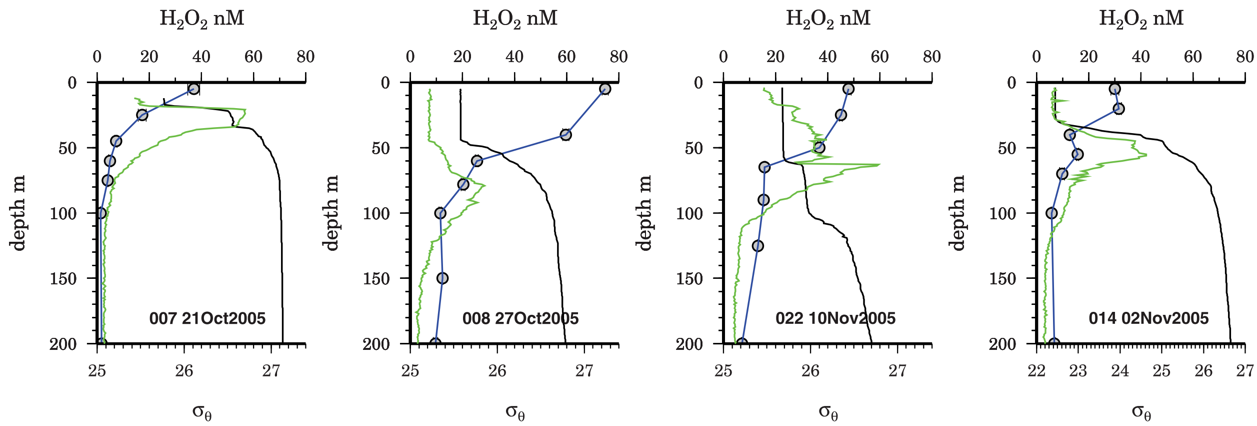

Figure 2. Vertical profiles of H_2O_2 (black circles on blue line), density σ_θ (black line), and chlorophyll fluorescence (green line, arbitrary fluorescence units where the x-axis spans from 0 to 1) showing (left) the characteristic exponential decrease (representing Stn PS 69/007, 10–13, 15–20, 24–27), (middle left) the effect of 7 mm precipitation (previous 96 h accumulated, PS 69/008), (middle right) the effect of vertical mixing (representing Stn 21–23), and (right) coinciding peaks in chlorophyll fluorescence and H_2O_2 (representing Stn 9, 14).

et al., 2003]. Surface chlorophyll concentrations ranged from 0.09–0.29 μg L^{-1}, the sea surface temperature (SST) ranged from 18–29°C with H_2O_2 inventories (0–100 m) ranging from 0.9–5.9 mmol m^{-2} (Figure 1). Highest H_2O_2 inventories between 5.5–5.9 mmol m^{-2} were found at stations 009 (30°N) and 025 (24°S), consistent with the earlier findings of *Yuan and Shiller* [2005], lowest inventories of 0.9–1.29 mmol m^{-2} at stations 007 (46°N) and 014 (10°N) (Figure 1). The highest integrated (0–200 m) chlorophyll values of 50–60 mg m^{-2} were found off Mauritania (Stn PS 69/012) and near the Equator (Stn 016) (Figure 1). CDOM absorbance, averaged over 0–200 m depth, was highest off north–west Africa (Stn 010–014) (Figure 1).

[15] Both the measured instantaneous PAR and the temperature of the mixed layer (Figure 1) reached maximum values near the Equator at station 016 and 15 respectively and decreased until station 021. Significant precipitation events (5–12 mm) occurring in the preceding 4 days before station occupation were detected for the stations off Morocco (Stn 008–009) and in the South Atlantic (Stn 021) (Figure 1).

3.2. Vertical Distribution of H_2O_2, Light, and Chlorophyll/CDOM During ANT XXIII-1

[16] The vertical profiles of H_2O_2 show the typical exponential decrease in the upper 50–100 m (Figure 2). At most of the stations H_2O_2 concentrations below 100 m depth were <10 nmol L^{-1}. Lowest surface concentrations (<30 nmol L^{-1}) were found in the Bay of Biscay (Stn PS 69/007) and at station 014 (10°N) coinciding with cloudy conditions. Maximum concentrations (123 nmol L^{-1}) were recorded in the southern Angola Basin (Stn 025) under clear skies. The vertical distribution of H_2O_2 in the Angola Basin (i.e. Stn PS 69/021-023) showed a deviation from the expected exponentially decrease, with almost constant H_2O_2 concentrations for the upper 50 m, followed by a strong decrease towards 60–80 m (Figure 2). At stations 009 and 014 small increases in H_2O_2 concentrations anomalous from the normal exponential pattern coincided with both the chlorophyll and CDOM maximums.

[17] Surface chlorophyll was elevated in the Bay of Biscay (Stn 007), off Mauritania (Stn 012), and in the Angola Basin (Stn 023). Vertical chlorophyll profiles showed subsurface maxima and sharp decreases with depth. Maximum surface absorbance of CDOM at 380 nm was observed in the Bay of Biscay (Stn 007) and off Mauritania (Stn 012). CDOM absorbance was low at the surface and reached highest values at the chlorophyll maximum and stayed high until 200 m depth. The average euphotic depth (1% PAR, z_{eu}) was 70 m (range 45–101 m) along the transect.

3.3. Statistical Analyses

[18] See Table 1. The vertical distribution of H_2O_2 was strongly correlated, as expected, with irradiance and was strongest at 442 nm (ρ = 0.86 p < 0.05 df = 56) and to a lesser extent with both PAR and UVA (340 nm). The vertical distribution of CDOM and H_2O_2 revealed a modest negative correlation (ρ = −0.57 p < 0.05 df = 72). H_2O_2 and chlorophyll depth distributions in turn were weakly correlated (ρ = 0.32 p < 0.05 df = 75). The depth of the chlorophyll maximum and the depth of maximum CDOM absorbance correlated strongly (ρ = 0.76 p < 0.05 df = 15, Stn 021–023 excluded due to strong mixing in the upper water column). A further strong correlation existed between vertical profiles of H_2O_2 and temperature (ρ = 0.75 p < 0.05 df = 131).

[19] A modest negative correlation was calculated for H_2O_2 inventories over the MLD and the average temperature over the MLD (ρ = −0.56 p < 0.05 df = 18), which becomes very strong (ρ = −0.96 p < 0.05 df = 11) excluding the stations of significant precipitation (Stn 7–10, 19–21). Strongly negative correlated were surface H_2O_2 concentrations and surface chlorophyll concentrations (ρ = −0.75 to −0.92). Modest and strong correlations (ρ = 0.66 and −0.74) were found between PAR and UVA irradiation respectively and surface H_2O_2 concentrations. Precipitation

Table 1. Results of Spearman Rank Analysis of Correlations Between H_2O_2 and Potentially Important Parameters[a]

	Irradiance						
	PAR	340 nm	442 nm	Water Temperature	Chlorophyll, HPLC	CDOM, absorbance at 380 nm	Precipitation ≥ 1mm
	H_2O_2 Surface Data						
ρ = …	*0.28(0.66)*	*0.40(**0.74**)*	*0.29(0.48)*	−0.22 (−0.44)	**−0.75 (−0.92)**	*−0.28 (−0.11)*	**0.86**
p = …	*0.25(<0.05)*	0.08(***<0.05***)	*0.22(0.11)*	0.37 (0.14)	**<0.05(<0.05)**	*0.24 (0.72)*	**<0.05**
df = …	*18(11)*	18(***11***)	*18(11)*	18 (11)	**18 (11)**	*18 (11)*	**6**
	H_2O_2 Inventories						
				−0.56(**−0.96**)[b]	*0.20 (0.01)*[c]	*−0.38 (−0.40)*[d]	
	—	—	—	< 0.05(**<0.05**)	*0.4 (0.96)*	*0.11(0.19)*	—
				18 (**11**)	*18 (11)*	*18 (11)*	
	H_2O_2 Depth profiles						
	0.71 (0.72)	**0.72(0.72)**	**0.83(0.86)**	**0.75** (0.65)	0.32 (0.25)	−0.57 (−0.52)	
	<0.05 (<0.05)	**<0.05(<0.05)**	**<0.05(<0.05)**	**<0.05** (<0.05)	<0.05 (0.09)	<0.05 (<0.05)	—
	63 (40)	**69 (42)**	**84(56)**	**131** (82)	75 (47)	72 (47)	

[a]Correlations measured are surface concentrations, water column inventories, and discrete concentrations at depth. Values in brackets were determined excluding stations of significant precipitation (Stn 7–10, 19–21).
[b]Temperature averaged over MLD, H_2O_2 integrated over MLD.
[c]Integrated over 0–200 m.
[d]CDOM absorbance averaged over 0–200 m, H_2O_2 integrated over 0–200 m.

and surface H_2O_2 values correlated strongly ($\rho = 0.86$ $p < 0.05$ df = 6) at the seven stations affected by rain. Excluding stations of significant rainfall generally increased the strength of the correlations in the surface data.

[20] Estimated noon sub-surface PAR values from the HelioClim-2 database were in good agreement with the measured instantaneous PAR ($\rho = 0.70$ $p < 0.05$ df = 18). Excluding the stations of significant precipitation (Stn 7–10 and 19–21) the sub-surface PAR estimates integrated over 24, 48 and 96 h before sampling correlated modestly with surface H_2O_2 ($\rho = 0.66$–0.84 $p < 0.05$ df = 11).

4. Discussion

[21] Our results show that the distribution and inventories of H_2O_2 observed during ANT XXIII-1 were affected by several factors related to variations in the sources, sinks and physical mixing at each location. H_2O_2 inventories were strongly negatively correlated to the SST in line with expected temperature effects on enzymatic sinks for H_2O_2 [*Wong et al.*, 2003]. Inventories of H_2O_2 decreased equatorwards in contrast to SST and daily PAR irradiance (Figure 1). The highest observed H_2O_2 inventories (0–100 m) were found at the start and near the end of the cruise (Figure 1) possibly reflecting a balance between photo-formation and temperature-dependent loss processes. The strong negative relationship between H_2O_2 and chlorophyll at the surface is probably related to increased Catalase and Peroxidase activity arising from increased phytoplankton biomass [*Moffett and Zafiriou*, 1990]. Latitudinal decreases in H_2O_2 suggest that sink terms are more sensitive to temperature than the photo-formation of H_2O_2 is to irradiance, a finding consistent with recent studies in the North–West Pacific [*Yuan and Shiller*, 2005]. Inventories of H_2O_2 decreased by 49% from station PS 69/009 to 016 despite an increase in irradiance of 49% (Figure 1), this may arise from the simultaneous increase in SST by 25% (Figure 1). However, there is a strong correlation of surface H_2O_2 and UV irradiance (340 nm) underlining the importance of UV radiation for the photo-formation of H_2O_2 [*Gerringa et al.*, 2004]. The modest to strong positive correlation of surface H_2O_2 with the preceding 24, 48 and 96 h total sub-surface PAR flux indicates that, away from areas of precipitation, there is a clear connection to the "light history" at each station. Since instantaneous irradiation data can not solely explain distribution patterns of H_2O_2 in seawater, databases such as HelioClim-2 are a useful new tool for estimating the "light history" in the Ocean.

[22] The vertical distribution of H_2O_2 was dominated by irradiance (Table 1) with most stations showing a characteristic exponential decrease (Figure 2). The correspondence of the chlorophyll and CDOM maximum with the local increases in H_2O_2 009 and 014 (Figure 2) suggest either; production of H_2O_2 by phytoplankton [*Croot et al.*, 2005; *Palenik and Morel*, 1988] or from fresh photo-labile phytoplankton exudates. Station 014 (Figure 2) shows the inverse shape of the depth profiles of CDOM and H_2O_2 causing the modest negative correlation between these parameters (Table 1) which is related to photobleaching of CDOM in surface waters [*O'Sullivan et al.*, 2005].

[23] At Station 008, high surface H_2O_2 levels show the effect of precipitation (Figures 1 and 2) on H_2O_2 levels. Although the number of rain events was small they correlated strongly with H_2O_2 surface concentrations (Table 1) confirming that rain is an important source for H_2O_2 to the surface ocean [*Croot et al.*, 2004]. In the Angola Basin (Stn 021–23), strong mixing in the upper water column with the euphotic depth (z_{eu}) and the MLD roughly equivalent, resulted in significantly higher H_2O_2 concentrations at depth than at other locations (Figure 2), highlighting the importance of upper ocean mixing processes [*Johnson et al.*, 1989] on H_2O_2 distribution. It should be noted however that strong mixing has no apparent effect on H_2O_2 inventories. The strong correlation between vertical profiles of temperature and H_2O_2 (Table 1) is not unexpected as both parameters are forced by sunlight and have similar vertical distributions, in our study region, with highest values at the surface and decreasing values with depth. Increased temperatures should shorten the lifetime of H_2O_2 [*Moffett and Zika*, 1987; *Wong et al.*, 2003] which is seen in the negative correlation between H_2O_2 inventories and SST.

5. Conclusion

[24] Measurements of H_2O_2 during ANT XXIII-1 show a number of factors influenced its distribution. The latitudinal

patterns observed indicate irradiance, water temperature and recent precipitation as key controls. Vertical distributions of H_2O_2 were strongly controlled by photo-formation and mixing processes in the upper water column. The recent irradiation history and phytoplankton activity appear to be the key sources and sinks in determining the observed H_2O_2 levels, with CDOM playing a minor role suggesting sunlight is the key limiting reactant in the formation of H_2O_2 in the Tropical and Sub-Tropical surface ocean. This work points to the future possibilities of developing satellite based estimations of H_2O_2 in the global ocean.

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CHAPTER 4:

Characterization of phytoplankton exudates and phytagel in relation to their complexing capacity of copper, cadmium and iron

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Characterization of phytoplankton exudates and phytagel in relation to their complexing capacity of copper, cadmium and iron

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Abstract

The goal of this work was to determine electrochemically, the complexing capacity to copper (CCu) and cadmium (CCd) of exudates released by cultures of two marine diatoms *Thalassiosira weissflogii* and *Skeletonema costatum*, as well as the coccolithophore *Emiliana huxleyi*. The study also aimed to determine compounds responsible for the complexation of Cu and Cd by these exudates. In particular, reduced sulphur species (RSS), surface active substances (SAS) and thio/amino groups were studied. These measurements were combined with colorimetric analyses of transparent exopolymer particles (TEP) and carbohydrates to investigate their role in the complexation of Cu and Cd. Phytagel, carrageenan, laminarin and alginic acid were analysed as model substances, where the former was analyzed for complexation of Cu and Cd and the three latter for complexation of iron. In these experiments, solutions of polysaccharides were titrated with the relevant trace metal in order to determine the apparent stability constant of the resulting complexes.

The organic matter released by all three phytoplankton cultures and the polysaccharide phytagel complexed Cu and to a lesser extent Cd. However, Cd complexes showed higher apparent stability constants and consequently the exudates and phytagel bound Cd more specifically than Cu. Sulphur-rich “glutathione” type ligands were found in all phytoplankton samples and were possibly responsible for the complexation of Cu. The correlation of monosaccharides with the complexing capacity to Cd, indicated that in the phytoplankton samples these compounds bound Cd. TEP, SAS and polysaccharides did not appear to be primarily responsible for the complexing properties of the phytoplankton samples. No specific iron binding properties of laminarin, carrageenan, phytagel and alginic acid could be found probably partly due to limitations of the applied electrochemical methods. However, these measurements confirmed that the analysed model polysaccharides are highly surface active.

INTRODUCTION

Phytoplankton contribute quantitatively and in terms of reactivity significantly to the pool of dissolved organic matter in the ocean (Benner et al., 1992; Aluwihare et al., 1997; Aluwihare & Repeta, 1999; Benner, 2002). A significant fraction of marine organic matter acts as ligands for trace metals. In surface waters > 99% of copper (Cu), cadmium (Cd), or iron (Fe) exist complexed to organic ligands (Bruland, 1992; Buck and Bruland, 2005; Buckley and van den Berg, 1986; (Rue and Bruland, 1995; van den Berg, 1995). Phytoplankton have the capability of producing such metal complexing ligands (Bruland et al., 1991), and consequently phytoplankton composition and activity may control trace metal speciation, e.g. (Muller et al., 2003). However, ligands that bind different metals differ physico-chemically, and temporal and spatial variability of the speciation of any one metal is also high, e.g. (Blake et al., 2004). Due to this complexity, the chemical structure of metal binding ligands remains largely unknown. However, different substance classes, including sulfur rich substances like thiols, surface active substances (SAS), transparent exopolymer particles (TEP) and polysaccharides may be of major importance as potential ligands.

Sulfur-groups have a very strong affinity to most heavy metals and the sulfur metal complex is very stable. Sulfur containing compounds, like glutathione (GSSG, a tripeptide) and other thiols are important ligands found in phytoplankton cultures and in natural seawater (Leal et al., 1999; Laglera and van den Berg, 2003; 2006; Dryden et al., 2006). Glutathione has been shown to be an especially important compound of the ligands responsible for the nearly complete complexation of Cu in seawater (Ross et al., 2003).

SAS include a variety of organic substances (proteins, polysaccharides, humic type substances) which possess hydrophobic (e.g. fatty acids chains, aromatic rings, hydrocarbons) and hydrophilic functional groups (e.g. NH_2 , COOH, OH, SH) and therefore participate in electrostatic and hydrophopic interactions (Ćosović, 2005). SAS act as metal ligands and bind

Cu well (Plavšić et al., 2006). Operationally defined, SAS accumulate at phase boundaries e.g. in nature at the seawater – atmosphere or particle surface – seawater boundary (Liss and Duce, 1997). Thus SAS that act as ligand concentrate the respective trace metal on surfaces, determining the partitioning of the trace metal.

Specific polysaccharides common in the ocean, e.g. alginic acid or carrageenan, have been shown to bind metal ions (Gimenez et al., 1995; Kim et al., 1995). Acidic PS and especially S-rich PS, rather than the more abundant neutral ones are thought to be primarily responsible for the binding of trace elements (Santschi et al., 2006). TEP are rich in acidic polysaccharides (uronic acids or PS with sulfated or phosphorelated acidic groups) that make them extremely surface active (Mopper et al., 1995; Zhou et al., 1998). Specifically the high affinity of acidic PS to thorium and trace metals like iron, have been shown (Honeyman & Santschi, 1991; Quigley et al., 2002; Passow et al., 2006). By binding trace elements (Quigley et al., 2001; Guo et al., 2002), both as TEP and as dissolved TEP-precursors that form TEP abiotically (Passow, 2000), the PS determine the biochemical cycling of these trace substances (Verdugo et al., 2004; Santschi et al., 2006; Scoullos et al., 2006) and their bioavailability.

Nevertheless, our knowledge of phytoplankton derived ligands for trace elements is scarce, partially because of the high complexity of their chemical structure, and partially because these type of investigations are methodologically challenging (e.g. separation and preconcentration).

Electrochemical methods allow the determination of the complexing capacity of trace metal ions, as well as the characterization of organic matter possibly responsible for binding trace metals. Such methods include: 1) a titration that determines the complexing capacity for Cu and Cd ions in natural seawater using the static mercury drop electrode (SMDE)(Plavšić et al., 1982 ; Plavšić, 2003; Scoullos et al., 2004; Plavšić et al., 2006) ; 2) the constant current potentiometric analysis (CPSA), which detects amino- and/or thio- groups (Ciglenečki et al., 2000; Ciglenečki et al., 2003) a voltammetric method to determine the concentration of reduced sulfur species (RSS) (Ciglenečki and Ćosović, 1996) and 4) a voltammetric method to determine the concentration and type of surface active substances (SAS) adsorbed on the mercury electrode (Ćosović, 1985; Kozarac et al., 1989; Plavšić et al., 1990). 5) The iron binding strength of the model substances phytagel, carrageenan, laminarin and alginic acid was investigated using competitive ligand exchange cathodic stripping voltammetry (CLE-CSV) as described by Croot et al. (2002). 6) Square wave voltammetry (SWV) scans were performed, to identify Fe complexes of carrageenan, laminarin and alginic acid. Colorimetric methods measuring concentrations of transparent exoploymer particles (TEP) and carbohydrates (polysaccharide and monosaccharide) provide a different approach to characterizing marine organic matter potentially important for binding of trace metals. Electrochemical and colorimetrical methods characterizing marine organic matter, have never been really compared, except in the paper dealing with a mucilage event in the coastal sea (Scoullos et. al. 2006) in which a similar approach was applied.

The goal of this study was to characterize the complexing capacity of organic matter released by phytoplankton to copper (Cu) and cadmium (Cd) and characterize the organic matter responsible for the complexation. The above mentioned electrochemical methods are combined with colorimetrical analysis of transparent exopolymer particles (TEP) and carbohydrates released by phytoplankton to investigate if TEP, carbohydrates, reduced sulfur species (RSS), thio/amino groups (CPSA) and surface active substances (SAS) play a central role for the complexing of Cu and Cd. The complexing capacity for copper and cadmium was investigated in three marine phytoplankton cultures, of the diatom *Thalassiosira weissflogii* and *Skeletonema costatum*, as well as the coccolithophore *Emiliana huxleyi*. Organic matter in all three cultures was characterized in parallel and related to the complexing capacity.

Phytagel was used as a model substance. The complexing properties of several polysaccharides (PS) - alginic acid, laminarin, carrageenan (sulphurous polysaccharide)– were investigated using the square wave voltammetry (SWV) and competitive ligand exchange cathodic stripping voltammetry (CLE-CSV).

MATERIALS AND METHODS

The complexing capacities of the organic material to copper and cadmium (Co, Cd) were determined in the three cultures and three size fractionated phytagel solutions. The organic substances in these samples were characterized by measuring the concentrations of reduced sulfur species (RSS), amino/thio groups (CPSA), surface active substances (SAS), TEP and different components of carbohydrates. All metal additions and respective measurements were conducted in triplicate, except when noted otherwise.

Preparations of samples

Marine diatom cultures.

Cultures of the two marine diatoms, *S. costatum* and *T. weissflogii* and the coccolitophore *Emiliania huxleyi* were grown in f/2 media at 15°C, under a 16: 8 light: dark cycle at 30 to 40 μmol m^{-2} s^{-1} light . The f/2 media (Guillard and Ryther, 1962) was based on filtered (0.45 μm nitrocellulose membrane, Millipore) seawater from the North Sea. The whole cultures were collected and used in their stationary phase at cell concentrations of app. $5x10^6$ cells/L. At this time cells had released large amounts of organic substances into the water, many of which may act as ligands for copper or cadmium. The cultures were used either undiluted and/or after dilution with a NaCl – Milli-Q solution (0.55 mol L^{-1}) at a dilution factor of 1: 5 by volume (10 mL culture in 50 mL total volume).

Solutions of phytagel.

Phytagel, which is an agar substitute produced from a bacterial substrate composed of glucuronic acid, rhamnose and glucose, was used as a model polysaccharide compound. Phytagel (Sigma), has an average molecular weight of 1000 kDa and produces a clear colorless gel in seawater. A stock solution of 1g/L of phytagel was prepared in Milli-Q water and was homogenized in an ultrasonic bath. An unfiltered solution as well as two filtrates (0.2 and 0.7 µm, respectively, Millipore) of phytagel were analyzed electrochemically. For SWV scans and CLE-CSV laminarin, carrageenan and alginic acid were dissolved in artificial seawater containing 0.55 mol L^{-1} NaCl and adjusted to pH 8.

Analysis

Complexing capacity determinations with Cu and Cd.

Differential pulse anodic stripping voltammetry (DPASV) is considered particularly suitable for measuring Cu and Cd concentrations in seawater (Plavšić et al., 1982; Ellwood, 2004, Scoullos et al., 2006) and was used for the determination of the respective complexing capacities, CCu and CCd, as well as the corresponding apparent (i.e. conditional) stability constants (K_{app}) (Ružić, 1982; van den Berg, 1982). The complexing capacity of a solution is defined as the amount of unknown ligand present, which can transform a trace metal from its detectable to an undetectable complexed form (Ruzic, 1982)

The complexing capacity values were determined by direct titration of 25 mL of sample with copper or cadmium ions. Figure 3 illustrates the determination of the complexing capacity using the example of *S.costatum*. Figure 3a presents results of the anodic stripping voltammetric waves for copper ion titration, where increasing amounts of copper ions (8 – 260 nmol L^{-1} Cu) were added to a diluted culture (1: 5) of *S.costatum*. Upon additions of copper, the Cu peak increased. In Figure 3b the concentrations of the added copper ions is depicted on the x axis vs. the retrieved (measured) copper ion concentrations for each addition of Cu ion to pure electrolyte (0.55 mol L^{-1} NaCl) or to the *S. costatum* culture. The difference between the intercepts of the two lines on the x-axis gives the approximate value of the copper complexing capacity (CCu). The exact value of the CCu can be calculated from the regression of the Cu ion measured (on x-axis) vs. the Cu ion measured normalized to the complexed Cu as shown in Figure 3c. The concentration of complexed Cu was calculated from $Cu_{total} – Cu_{measured}$. The inverse of the slope gives the CCu while K_{app} (apparent stability constant) is obtained from the intercept on the y-axis (Ružić, 1982; van den Berg, 1982). Every addition of metal ions to the sample solution was measured three consecutive times. The complexing capacities of the diluted and undiluted cultures were identical, indicating that this parameter is independent of dilution.

Identification of Fe complexes of laminarin, carrageenan and alginic acid using SWV.

Scans were performed in the region of -0.2 to -1.8 V methods' upper limit determined by the oxidation of Hg and lower limit by the formation of H_2 using a hanging mercury drop electrode without a deposition step, since uncomplexed Fe can not be accumulated electrochemically on the mercury. Omitting a depositioning step requires working with high Fe concentrations (10^{-6} mol L^{-1}) as the sensitivity is low.

Determination of apparent stability constants of Fe complexes of laminarin, carrageenan and alginic acid using CLE-CSV.

The Fe binding strength of the dissolved PS was investigated using CLE-CSV as described by Croot et al. (2000). In short, each PS containing solution was titrated with Fe and the portion of Fe not complexed by the PS but bound to the competing ligand 2-(2-Thiazolylazo)-p-cresol (TAC) was measured. TAC is an Fe ligand of a known stability constant. It was added to each sample in a known concentration. Fe reaches an equilibrium distribution between the PS and TAC according to the concentration of each constituent and the stability constant of each complex. The Fe-TAC complex can be measured electrochemically, as it induces a current when reduced on the electrode, whereas the potential Fe-PS complex cannot. This method has a detection window of conditional stability constants (K') of competing Fe ligands which ranges from log K'_{FeL}= 21.4 to 23.4 (for 10 μmol L^{-1}TAC), depending on the concentration of added TAC.

The titration curve (i.e. added Fe vs. reduction current) can be evaluated by fitting a model of the competitive equilibrium in the solution, hence retrieving the values of the unknown parameters, i.e. the stability constant of the Fe-PS complex and the concentration of PS. The general pattern of a titration curve consist of an initial non-linear phase until the competing ligand (e.g. PS) is saturated with Fe, followed by a linear phase titrating only TAC, which is added to the sample in excess over the concentration of competing ligand and added Fe.

Determination of reduced sulfur species (RSS).

RSS were determined by square wave cathodic stripping voltammetry (SWV; Ciglenečki and Ćosović 1996). Measurements were performed with a μ-Autolab analyzer (Electrochemical Instruments, Eco Chemie) connected to a 663 VA stand (Metrohm), with an SMDE (static mercury drop electrode) as the working electrode. The reference electrode was an Ag/AgCl (3 mol L^{-1} KCl) electrode connected to the solution via an electrolyte bridge. A platinum electrode served as the auxiliary electrode. Electrochemical determination of RSS is based on the reaction between sulfur and the mercury electrode. Measurements were conducted before and after purging the 50 mL solution of phytagel or culture with N_2 gas to determine if some fraction of the RSS was present as sulfides. The samples were measured immediately after preparation and as purgable S species were removed after the first scan, replicate measurements of the initial pre-purge values were not performed. After accumulation of RSS on the electrode surface at a deposition potential of E = –0.20 V (vs. Ag/ AgCl) from a stirred solution, we ran potential scans in the negative direction (up to E = –1.00 V vs Ag/AgCl). HgS reduction peaks that are characteristic for many RSS were recorded (Ciglenečki and Ćosović, 1996). Then the solution was acidified (pH 2.0), purged with N_2 and after that the pH was readjusted back to pH 8.0 and, the RSS signal recorded again. Samples were not readjusted to exactly the original pH. Readjustment was done in the electrochemical cell, so approximate concentrations either of HCl or NaOH were added according to the previous experiment. As a consequence the signal of RSS as seen in the voltammogram differed slightly between the original and pH adjusted sample.

Sulfur species concentration is expressed as equivalents of glutathione (GSSG eq.), determined from the calibration with glutathione. Glutathione was chosen for calibration because of its electrochemical similarity with the sulfur species observed in the three cultures, i.e. the half –wave potential of appearance and behavior upon pH changes were similar (Ciglenečki and Ćosović, 1996).

Constant-current chronopotentiometric stripping analysis (CPSA) for the determination of amino/thio groups.

Constant-current CPSA produces well-resolved current peaks, 'presodium' currents with the static mercury drop electrode (SMDE) that are characteristic for proteins and other compounds with –SH and $–NH_2$ groups (Tomschik et al., 1999; Mader et al., 2001). Accumulation of the catalytically active compound at the SMDE polarized to a potential E = -0.20 V, was achieved by stirring the solution for 60 s (accumulation time, t_a). After a quiescent period of 10 s, a constant stripping current of I = –1 μA intensity was passed through the electrolytic circuit, and constant-current CPSA curves were recorded. These measurements were carried out in samples previously purged with N_2

Surface-active substances (SAS).

SAS were determined with phase-sensitive alternating current voltammetry (Ćosović and Vojvodić, 1987). This electrochemical method measures the capacitive current (i.e. the current arising from adsorption processes, measured out-of-phase with the applied potential) separately from the faradaic current (originating from redox processes, measured in-phase with the applied potential). Out-of-phase measurements have found wide application in the study of organic substances with surface-active properties in marine and freshwater systems. The decrease in the capacitive current in the presence of surface-active organic material below the value for the pure electrolyte solution indicates the amount of this material adsorbed onto the electrode (and can be expressed quantitatively by an equivalent amount of a selected SAS, e.g. Triton-X-100). The shape of the voltammetric curves recorded (i.e. current vs. potential) is characteristic for the substance investigated. Samples were calibrated against the nonionic SAS, Triton-X-100. Prior to measurements, samples were thoroughly homogenized by stirring.

TEP determinations.

TEP (transparent exopolymer particles) were analyzed colorimetrically (Passow and Alldredge, 1995). Six replicate samples of 50 mL each were filtered onto 0.4 µm polycarbonate filters (Poretics) and stained with Alcian blue. Gum Xanthan was used as a calibration standard and results are expressed as Gum Xanthan equivalent per Liter (GX eq. L^{-1}).

Carbohydrate determinations.

Carbohydrates (polysaccharides plus monosaccharides) were determined for the whole sample (dissolved + particulate fraction) and for the dissolved fraction (< 0.4 µm) according to Myklestadt (1997). Between 10 and 20 mL sample were filtered through a 0.4 µm filter for the determinations of dissolved carbohydrates. Total (dissolved + particulate) and dissolved MS (monosaccharides), respectively were determined in 1 mL each (triplicates) of unfiltered and in 1 mL each (triplicates) of 0.4 µm filtered sample. Four mL of filtered and unfiltered sample were hydrolyzed and the total carbohydrate content was measured. The PS (polysaccharide) concentration was determined by subtracting the MS concentration from the total carbohydrate concentration. This method, which measures both, neutral and charged carbohydrates, subjects the saccharides to an oxidation reaction at alkaline pH, during which Fe^{3+} is reduced to Fe^{2+}. The Fe^{2+} is then determined colorimetrically after condensation with the cromogen 2,4,6-tripyridyl-s-triazine (TPTZ) and formation of the purple color of $Fe(TPTZ)_2{}^{2+}$.

RESULTS AND DISCUSSION

Complexing capacity and stability constant for Cu and Cd complexation

The results of the complexing capacity measurements for Cu and Cd for the three phytoplankton cultures and the model substance phytagel are presented in Table 1. The complexing capacity for copper ions was highest in the *T.weissflogii* (1.14 µ mol L^{-1}) and the *S. costatum* cultures (1.06 µmol L^{-1}) while it was an order of magnitude lower (0.14 µmol L^{-1}) in the culture of *E.huxleyi.* In the culture of *E.huxleyi* the complexing capacity for cadmium ions was, however, twice that of those of *T.weissflogii* and the *S. costatum* (0.04 vs. 0.02 µmol L^{-1} and 0.02 µmol L^{-1}, respectively). Appreciably lower CCd values compared to CCu values are common (Scoullos et al., 2004; Scoullos et al., 2006). Complexing capacities for both Cu and Cd were lower in the 5 mg L^{-1} phytagel solution than in those stemming from phytoplankton cultures. In both diatom cultures, the apparent stability constant for Cd was higher than that for Cu, indicating more specific binding places for Cd ions compared to Cu ions. More specific binding for Cd is also supported by the lower complexing capacity for Cd, as more specific sites are rarer (Gordon et al., 2000; Laglera and Berg, 2003).

A CCu of 0.33 µmol L^{-1} was obtained for the unfiltered phytagel solution, whereas the CCu after filtration through either 0.2 µm or 0.7 µm was 73% lower at 0.09 µmol L^{-1}, suggesting that particles larger 0.7 µm contributed most significantly to binding Cu. Natural marine Cu-complexing ligands have been found to belong to 50% to substances between 1 and 10 kDa (Wells et al., 1998b; Wen et al., 1999), implying colloidal aggregation or gel formation of Cu complexing ligands or adsorption of Cu to gel particles in our phytagel solution. Our analysis method can't differentiate between complexation and adsorption onto particles. Alternatively the complexing substances absorbed to the filter thus retaining them although their molecular weight should have allowed them to pass.

Cd has been detected mainly complexed to organic matter in the low molecular weight fraction < 1k Da (Grzybowski, 2000; Wells et al., 1998a). In our phytagel solution filtration (by either 0.2 µm or 0.7 µm) reduced the CCd by only 33%, indicating that in our phytagel solution Cd complexed mostly with substances passing a 0.2 µm filter. Clearly ligands complexing Cu and Cd differed even in our model phytagel solution.

Identification of Fe complexes of laminarin, carrageenan and alginic acid using SWV

Initial scans performed in artificial seawater containing only 0.5 mmol L^{-1} BisTris buffer (pH 8) revealed a peak at -1.4 V which increased linearly (Fig. 1a) when adding Fe (up to 15 µmol L^{-1}). The sensitivity was 1.4 nA $µmol^{-1}$ L . Apart from buffering the pH, Bistris buffer also slightly complexes Fe and prevents hydrolysis and precipitation of Fe (Taylor et al., 1994). Hydrolysis and formation of less soluble Fe hydroxides shows a quadratic or cubic relationship to the Fe concentration and is even more sensitive to increasing pH. This explains further scans of artSW, without BisTris buffer present, which did not show any Fe peak at pH 6-8. However, lowering the pH to 4 resulted in a peak at -1.6 to -1.7 V, which changed with changing Fe concentrations, but not systematically.

SWV scans (Fig. 1b) of laminarin (2 g L^{-1}) and alginic acid (2 g L^{-1}) dissolved in artificial seawater at pH 8 also showed no peak from -0.2 to -1.8 V, i.e. there was no dissolved Fe species present that could be reduced. At pH 4-8 carrageenan (0.1 g L^{-1}) caused spike like peaks at -0.5 and -0.75 V (Fig. 1b) which can be related to the sulphur contained in the side chains of this mucopolysaccharide. No peaks at -1.4 to -1.7 V could be detected.

Determination of apparent stability constants of Fe complexes of laminarin, carrageenan and alginic acid using CLE-CSV.

The titration data of all analysed PS over a range of 0.02 to 0.5 mg L^{-1} did not show a curvature (Fig. 2). Consequently, no stability constant could be determined. Even reducing the TAC concentration by 50%, to lower the detection window and shift the equilibrium more towards the Fe-PS complex, showed very similar results. The titration data showed generally a lower slope (Fig. 2) than the reference sample, which was UV treated organic-free seawater (UVSW).

The titrations of PS solutions of up to 0.5 mg L^{-1} did not yield any K' values, indicating that these PS bind Fe only weakly (log K'< 21.4) if at all. Confirming the results from the SWV measurements that did not show any specific Fe complexation either. However, the inability to determine these K' values may have been due to the fact that the sensitivity of the method was hindered by the PS themselves.

Characterization of organic matter in culture media

Concentrations of sulfur species: All three cultures contained different concentrations of sulfur (Table 1), with by far the highest concentrations in the media stemming from *T. weissflogii*. Some fraction of the RSS in this culture must have been present as sulfides, as indicated by the fact that some part of the sulfur species was purged with N_2 gas. The original SWV voltammogram for *T. weissflogii* exudates was almost halved upon acidification, purging with N_2 and readjustment to pH 8.1 (Fig. 4a). This decrease in sulfur was due to the presence of sulfur species that were purged with N_2 gas. The peak potential after the readjusting of the pH is a little bit shifted towards more negative potential (from -0.6 to -0.65), because the reaction is very sensitive to small changes in pH (Ciglenečki and Ćosović, 1996). The original square wave voltammogram for the *S. costatum* culture (Fig. 4b) remained the same upon acidification to pH 2, purging with N_2, and readjustment of pH back to 8.1 indicating the presence of sulfur species that could not be purged by N_2 gas only. The culture of *E. huxleyi* (not shown) behaved the same as the culture of *S. costatum*, i.e. the voltammetric sulfur peak did not change upon acidification and purging with N_2, indicating the absence of purgeable sulfur species. Assuming that about half of the sulfur in the *T. weissflogii* culture was purgeable, the concentration of organic, non purgeable sulfur was still highest in the culture of *T. weissflogii* compared to *S. costatum* and *E. huxleyi*. These non-purgeable sulfur species of all three cultures electrochemically resembled glutathione, i.e. the half –wave potential, the shape of the voltammogram and behavior upon pH changes were similar (Ciglenečki and Ćosović, 1996), suggesting this substance class to be abundant in all three cultures. Glutathione and other thiols are present in surface waters, and are known to be released by phytoplankton, including *E. huxleyi* (Dupont and Ahner, 2005) and *T. weissflogii* (Tang et al., 2005) when exposed to elevated concentrations of Cu or Cd.

Presence of NH_2 and/or sulfur groups: The CPSA method determines the presence of NH_2 or sulfur containing groups, that may be present in different macromolecular compounds e.g. in amino acids, proteins, polysaccharides (Tomschik et al., 1999; Mader et al., 2001; Ciglenečki et.al., 2003). Of our cultures and the phytagel, only the *S. costatum* culture showed the characteristic "presodium" wave (Fig. 5) indicating the catalytic effect of $–NH_2$ and/or sulfur groups. Voltammetric peaks 5 and 6 (Fig. 5) were obtained by prolonged accumulation (180 s and 300 s) of *S. costatum* and are situated at the potential of ~ -1.7 V. For shorter accumulation times the peaks were not so pronounced. The appearance of the voltammetric current peak at ~ -1.7 V vs. ref. Ag/AgCl electrode indicates the presence of $–NH_2$ groups, while for PS with sulfur groups, such as carrageenans, a more positive catalytic voltammetric peak would have been observed (at -1.4 V to-1.5 V) (Plavšić and Ćosović, 1998; Ciglenečki et al., 2003). It is known that in *S.costatum* culture during the onset and development of the bloom 60-80 % of total complexing agents exuded could be ascribed to "protein-like" compounds which could explain the observed voltammetric peak at – 1.7 V (Lorenzo et al., 2007).

Surface active substances: The characteristic ac (alternating current) out of phase voltammetric curves for the three phytoplankton cultures and the model compounds phytagel, laminarin, carrageenan and alginic acid showed the suppression of the capacity current in comparison to the capacity current of the pure electrolyte (0.55 mol L^{-1} NaCl) (Fig. 6). This difference in the shape of the curves of the electrolyte compared to the phytoplankton cultures indicate the presence of natural, heterodispersed polysaccharids in the cultures (i.e. at negative potentials (~-1.6 to -1.8 V) the ac voltammetric curves of a cultures are not smooth like for the electrolyte solution) (Plavšić et al., 1990). The ac scans of solutions of phytagel, laminarin, carrageenan and alginic acid showed a pronounced desorption wave at a potential range from ~-0.7 V to -1.4 V, which is characteristic of synthetic surfactants, like Triton-X-

100 or sodium dodecyl sulfate, although some SAS of natural origin like fatty acids may also exhibit pronounced desorption waves (Ćosović, 1985).

The highest concentration of SAS in the phytoplankton samples was observed in the culture of *E .huxleyi* (0.26 mg L^{-1} eq. Triton-X-100) followed by *S. costatum* (0.24 mgL^{-1} eq Triton-X-100) and *T. weissflogii* (0.18 mg L^{-1} eq. Triton-X-100) (Table 1, Fig. 6A). The same amount of SAS was also measured in *E. huxleyi* in stationary phase by Ciglenečki and Ćosović (1996). The camparison of phytagel, laminarin, carrageenan and alginic acid (Fig. 6B) showed highest surface activity for laminarin, decreasing via phytagel, alginic acid and carrageenan.

TEP and carbohydrates: The total (dissolved + particulate) carbohydrate (polysaccharide + monosaccharide) concentration ranged between 1.2 and 3.6 mg glucose equivalent L^{-1}, of which 53%, 57%, 79% and 98% belonged to the dissolved (< 0.4 μm) pool in phytagel, *T. weissflogii, E. huxleyi* and the *S. costatum*, respectively (Fig. 7). Less than 40% of the carbohydrates were polysaccharides in *E. huxleyi* and *S. costatum*, whereas polysaccharides dominated the carbohydrate pool in *T. weissflogii* (> 60%) or the phytagel solution (> 80%). Polysaccharide concentrations ranged between 0.5 and 2.5 mg glucose eq. L^{-1} in the cultures and phytagel solution, with higher values in the unfiltered compared to the 0.4 μm prefiltered samples (Fig. 7). Concentrations of dissolved polysaccharides were highest in *T. weissflogii* (1.3 mg glucose eq. L^{-1}), followed by *E. huxleyi* (0.8 mg glucose eq. L^{-1}) and *S. costatum* (0.7 mg glucose eq. L^{-1}) and lowest in phytagel (0.3 mg glucose eq. L^{-1}). Particulate polysaccharides were highest in *T. weissflogii* and phytagel and almost absent in *S. costatum* (Fig. 7).

TEP concentrations were highest in the phytagel solution (6500 mg xanthan eq L^{-1}), followed by the media of *E. huxleyi* (2300 mg xanthan eq L^{-1}) and *T. weissflogii* (1000 mg xanthan eq L^{-1}) with the lowest concentration in culture media of *S. costatum* (400 mg xanthan eq L^{-1}) and reflected neither the pattern of particulate polysaccharide, nor that of SAS (Fig. 7).

Characteristics of DOM and its relationship to Cu- and Cd-complexing capacity

We characterized the organic material, which could be responsible for the binding of Cu or Cd, by measuring sulfur content, SAS, poly- and monosaccharides (in their particulate and dissolved phase) and TEP. Each of these measurements characterizes a certain fraction of DOM, but none of these fractions are well characterized and the degree of overlap between these pools is unknown.

Our data suggest that in our cultures and phytagel, TEP concentration was not correlated with either the carbohydrate fractions or with SAS or sulfur concentrations. The lack of a correlation with polysaccharides confirms that acidic polysaccharides are a varying fraction of total polysaccharides. The lack of a correlation between TEP and SAS suggests that not all TEP are surface active and that other substances besides TEP are responsible for the binding of trace elements. TEP are rich in sulfur (Zhou et al., 1998), but our samples clearly contained other substances rich in sulfur as TEP and sulfur concentrations showed no correlation. The correlation between PS and sulfur (PS < 0.4 µm: $r = 0.94$, $p < 0.01$, $n = 3$, PS > 0.4µm: $r = 0.91$, $p < 0.05$, $n = 3$) suggests that a significant fraction of this total amount of sulfur was associated with non acidic polysaccharides. Electrochemical characterizations suggested glutathione type substances to be responsible for the high sulfur content.

SAS were negatively correlated both with dissolved PS ($r = 0.96$, $p < 0.001$, $n = 4$) and sulfur content ($r = 0.99$, $p < 0.0005$, $n = 3$), implying that SAS in our samples consisted of sulfur-poor substances other than polysaccharides. SAS generated by phytoplankton can be rich in proteinaceous substances (Gašparović et al., 2007).

Although our RSS measurements suggest the presence of glutathione type substances in all three cultures, exudates of all three cultures differed in their other characteristics. A comparison between them shows that *T. weissflogii* cultures contained large amounts of both, purgeable and non-purgeable sulfur, high concentrations of polysaccharides (both dissolved and particulate), but relatively low TEP concentrations. The culture of *S. costatum* was characterized by fairly high concentrations of non-purgeable sulfur, and low concentrations of particulate carbohydrates (both polysaccharides and monosaccharides) and TEP, whereas CCu was similarly high. The presence of NH_2 groups further characterized *S. costatum* exudates. Assuming the main CCu ligand in both diatom cultures was the same, it was not measured as TEP and did not contain NH_2 groups, but could have been included in determinations of RSS, SAS and dissolved polysaccharides. Organic matter of *E. huxleyi*, which had a relatively high CCd was characterized by high concentrations of monosaccharides (both particulate and dissolved) and relatively low sulfur content.

Assuming the main respective Cu or Cd binding ligand in the three cultures and in the phytagel solution belonged to the same substance group and contributed significantly to that group, a relationship between the complexing capacity and the concentration of that substance group is expected. In our experiments however, the concentration of TEP was not correlated to the binding capacity or the stability constants for Cu or Cd. SAS, another group of potential trace metal ligands, were also not correlated to either CCu or CCd. So both TEP and SAS appeared not to have been responsible for the observed complexing capacities of Cu or Cd. Carbohydrates (poly- or monosaccharides) could also not explain the observed binding capacities for Cu or Cd, except that the monosaccharide concentration was positively correlated to CCd (MS < 0.4 µm: $r = 0.95$, $p < 0.005$, $n = 4$, MS > 0.4 µm: $r = 0.85$, $p < 0.05$, $n = 4$). Thus, the data suggest that MS were primarily responsible for the complexing capacity of Cd in all samples. A glutathione-type ligand was found in all three cultures. Assuming that about half of the sulfur in the culture of *T. weissflogii* belonged to this ligand (half was purgeable) a significant (RSS *T. weissflogii*: 90 nmol L^{-1} GSSG eq., $r = 0.94$, $p < 0.01$, $n = 3$) positive relationship between RSS and CCu was found, indicating that this glutathione-type ligand could be responsible for the binding of Cu in all three cultures.

The apparent absence of significant amounts of ligands in TEP or SAS could have several reasons. Either, no metal binding chemical groups were present in these substances or, alternatively, potential metal binding groups made up only a small fraction of TEP or SAS, which effect was lost in the noise of the bulk measurement. Both TEP and SAS are operationally defined groups of organic substances, containing a large variety of chemically different molecules not yet characterised.

CONCLUSION

The three phytoplankton cultures and the solution of phytagel, used as a model polysaccharide, complexed copper ions and to a lesser extent also cadmium ions. The apparent stability constants were higher for cadmium ions than for Cu showing that Cd binding places are more specific. No apparent stability constant for iron could be determined for laminarin, carrageenan and alginic acid indicating low values of log $K' < 21.4$. Nevertheless these polysaccharides showed high surface activity which agrees with reported abiotical formation of TEP from acidic polysaccharides (Passow, 2002). Some ligands in the phytoplankton cultures belonged to the glutathione-type. These might have been responsible for the binding of Cu. Monosacchride concentrations correlated with CCd, implying that the Cd ligand belonged to this group of molecules. None of the other measured substance classes (TEP, SAS, polysaccharides) assumed to be potentially important ligands, could be identified as being primarily responsible for the complexing properties of any culture. This suggests a high variability in space and time with regards to the chemical substances responsible for the binding. The absence of correlations between the complexing capacity and TEP, SAS or carbohydrates in our measurements contradict the believe that these substance groups contain a major fraction of trace metal binding ligands in seawater.

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FIGURE CAPTIONS

Fig.1. Square wave voltammograms showing A) Fe additions to a 0.5 mmol L^{-1} BisTris solution in artificial seawater at pH 8 and B) scans of solutions of polysaccharides in artificial seawater at pH 8, 10-30 µmol L^{-1} Fe added, carrageenan shows specific sulphur peaks at -0.5 to -0.75 V.

Fig.2. CLE-CSV data of three polysaccharides titrated with iron, showing decreased sensitivity compared to UVSW, but no apparent stability constants could be derived.

Fig.3. The complexing capacitiy for Cu by *S.costatum* (1:5 diluted): A) Voltammograms of the Cu ions additions to 0.55 mol L^{-1} NaCl (additons of Cu ions are indicated). B) The data presented as concentrations of Cu ions added vs. Cu ion retrieved (measured). C) Results from B as free Cu ions retrieved vs. Cu ions retrieved/ complexed Cu ions.

Fig.4. A SWV voltammograms for A) *T.weissflogii* and B) *S.costatum* at pH 8.1 (solid line) and after acidification, purging with N_2 gas and readjustment the pH back to ~8.1 (dotted line). Experimental conditions: A) and B) Deposition potential, Ed = -0.2 V; deposition time t_d = 120 s; amplitude = 25 mV; frequency = 100 s^{-1}.

Fig.5. CPSA voltammogram of *S. costatum*. Experimental conditions: Deposition potential, Ed = -0.2 V; constant current applied = 1 µA; max. time of measurement = 5 s; depositions times (t_d) as shown in the figure.

Fig.6. ac voltammograms (potential vs. current curves) of phytoplankton cultures, phytagel ($5mg\ L^{-1}$) and a $0.55\ mol\ L^{-1}$ NaCl solution. Experimental conditions: Potential of deposition, Ed = -0.6 V ; time of deposition, t_d = 60 s; amplitude = 10 mV.

Fig.7. Concentrations of particulate and dissolved carbohydrates (= polysaccharide + monosaccharide), polysaccharides, monosaccharides, TEP, SAS and reduced sulfur species in phytagel ($5mg\ L^{-1}$) and in phytoplankton cultures of *S.costatum*, *T. weissflogii* and *E.huxleyi*.

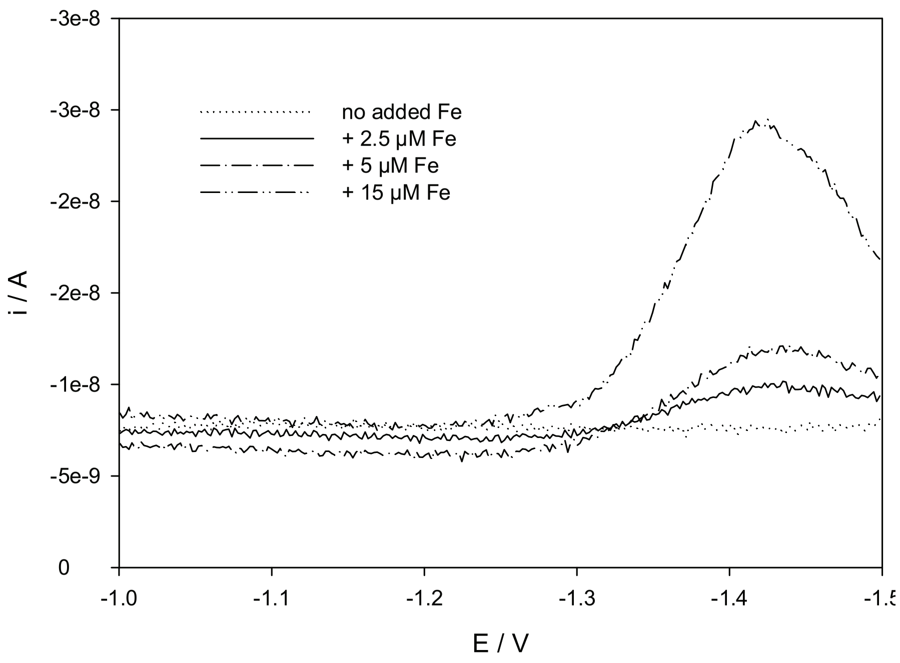

-3e-8
-3e-8
-2e-8
-2e-8
-1e-8
-5e-9
0
i / A
no added Fe
+ 2.5 µM Fe
+ 5 µM Fe
+ 15 µM Fe
-1.0
-1.1
-1.2
-1.3
-1.4
-1.5
E / V

Fig 1a

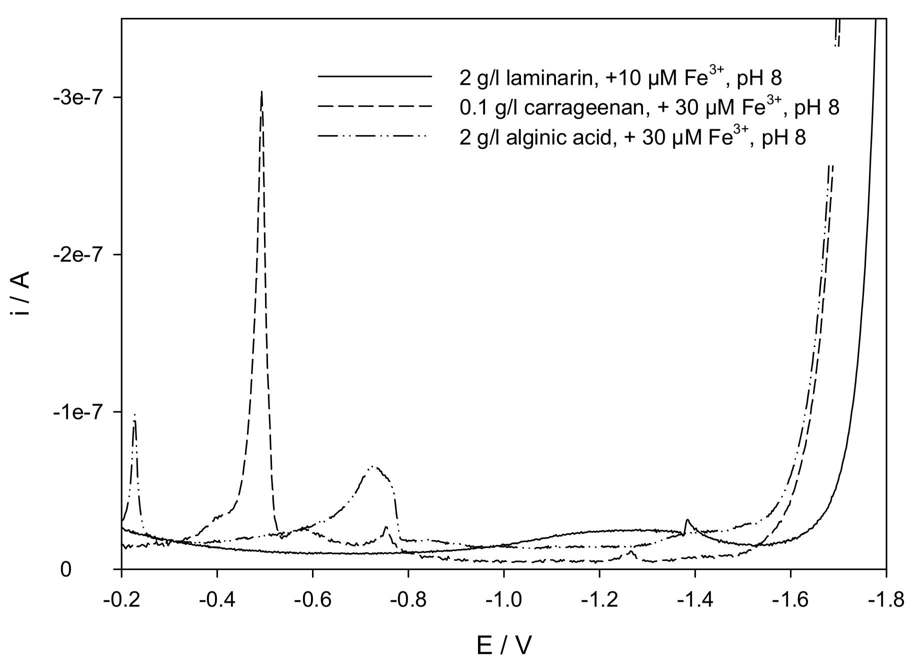

-3e-7
-2e-7
-1e-7
0
i / A
2 g/l laminarin, +10 µM Fe3+, pH 8
0.1 g/l carrageenan, + 30 µM Fe3+, pH 8
2 g/l alginic acid, + 30 µM Fe3+, pH 8
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
E / V

Fig 1b

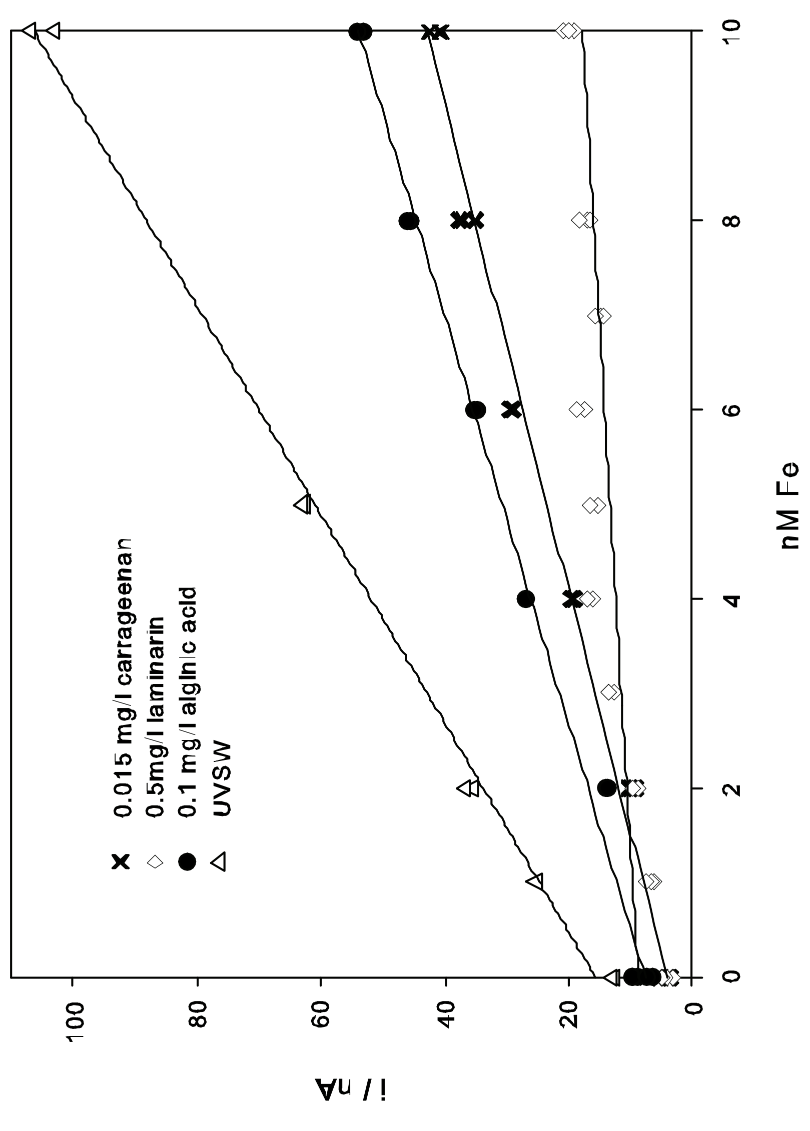

0.015 mg/l carrageenan
0.5mg/l laminarin
0.1 mg/l alginic acid
UVSW
i / nA
nM Fe
0
20
40
60
80
100
0
2
4
6
8
10

Fig 2

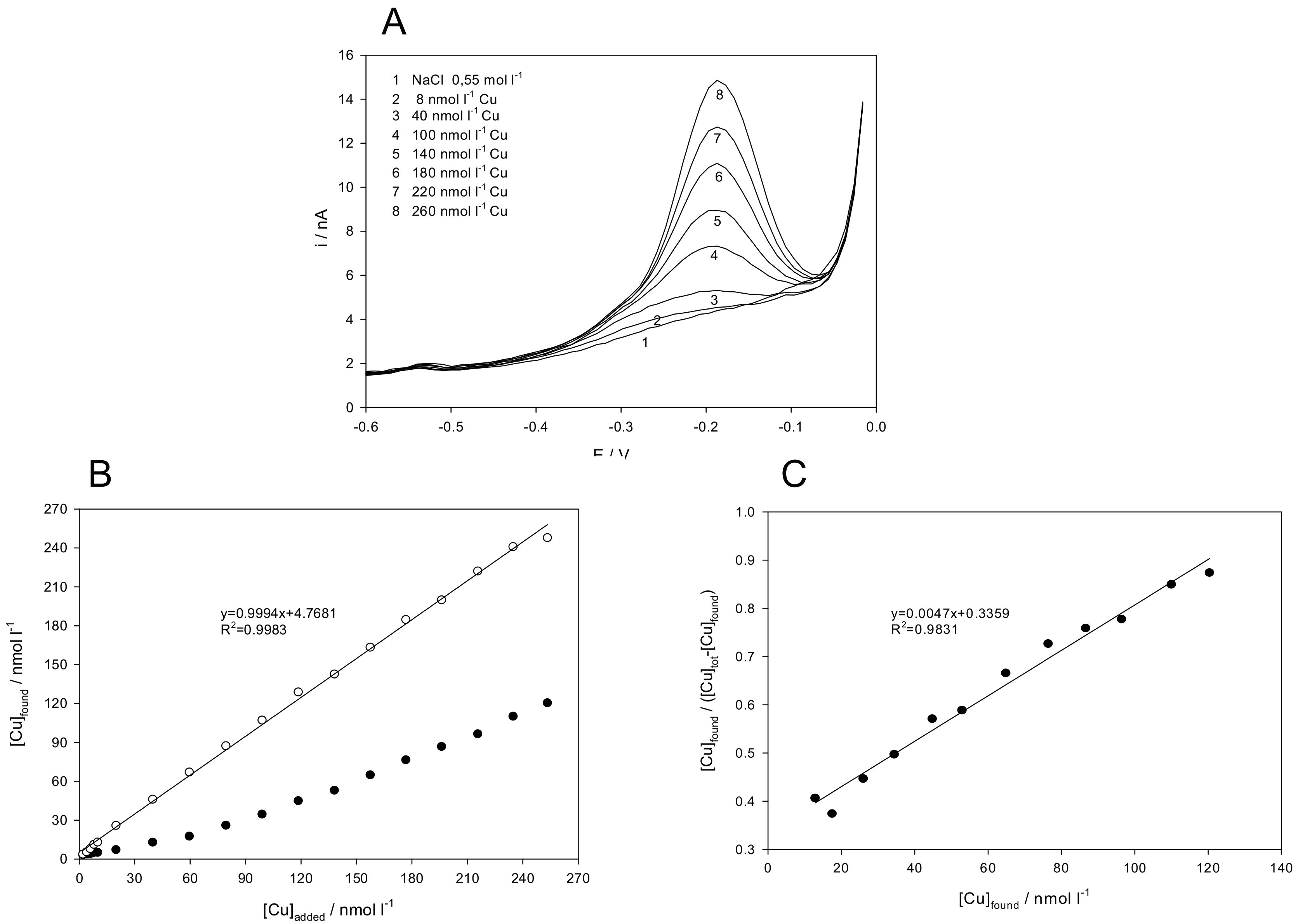
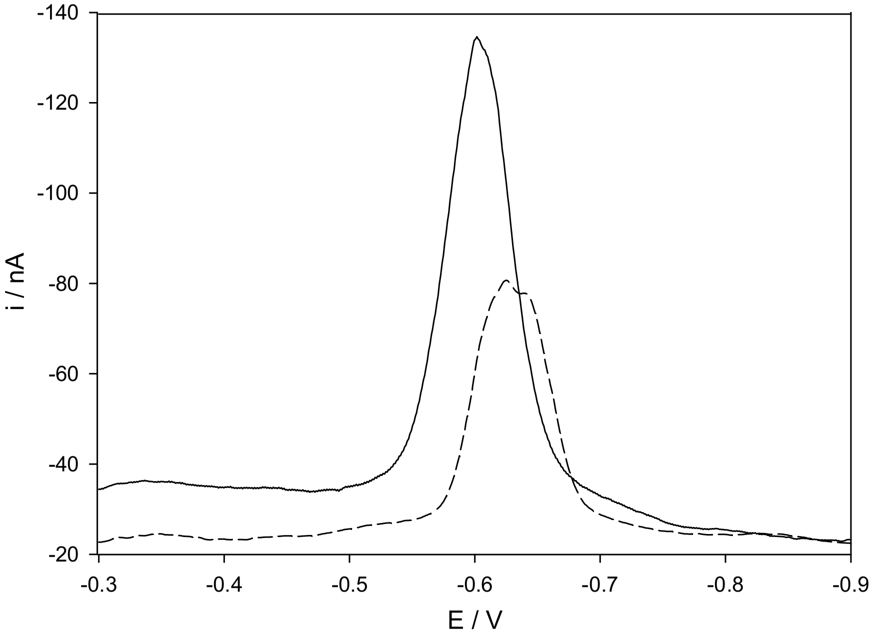

A
i / nA
E / V
1 NaCl 0,55 mol l-1
2 8 nmol l-1 Cu
3 40 nmol l-1 Cu
4 100 nmol l-1 Cu
5 140 nmol l-1 Cu
6 180 nmol l-1 Cu
7 220 nmol l-1 Cu
8 260 nmol l-1 Cu
B
[Cu]found / nmol l-1
[Cu]added / nmol l-1
y=0.9994x+4.7681
R2=0.9983
C
[Cu]found / ([Cu]tot-[Cu]found)
[Cu]found / nmol l-1
y=0.0047x+0.3359
R2=0.9831

Fig 3


-140
-120
-100
-80
-60
-40
-20
i / nA
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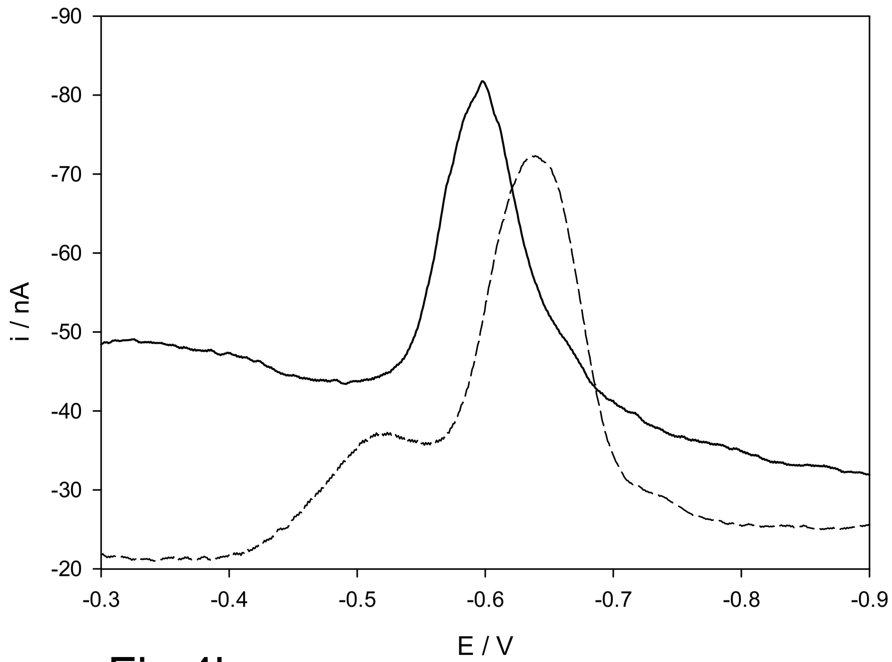
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
E / V

Fig 4a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-70
-60
-50
-40
-30
-20
i / nA
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-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
E / V

Fig 4b

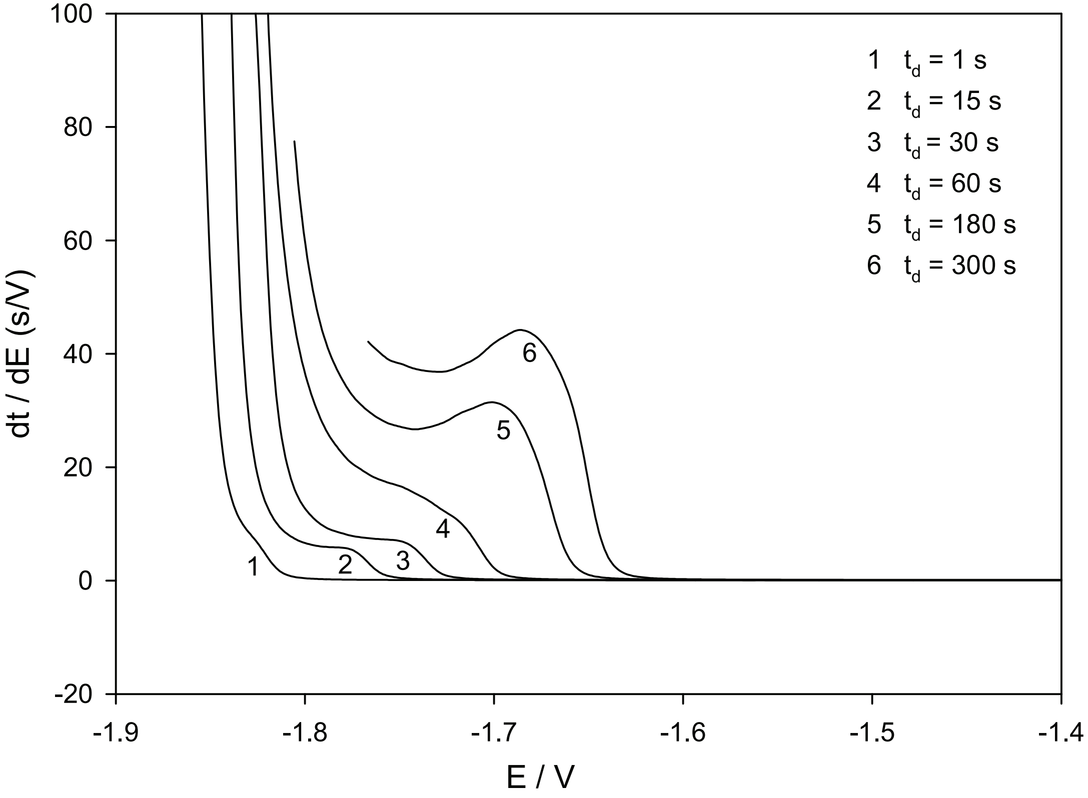

100
80
60
40
20
0
-20
dt / dE (s/V)
-1.9
-1.8
-1.7
-1.6
-1.5
-1.4
E / V
1 t_d = 1 s
2 t_d = 15 s
3 t_d = 30 s
4 t_d = 60 s
5 t_d = 180 s
6 t_d = 300 s
1
2
3
4
5
6

Fig 5

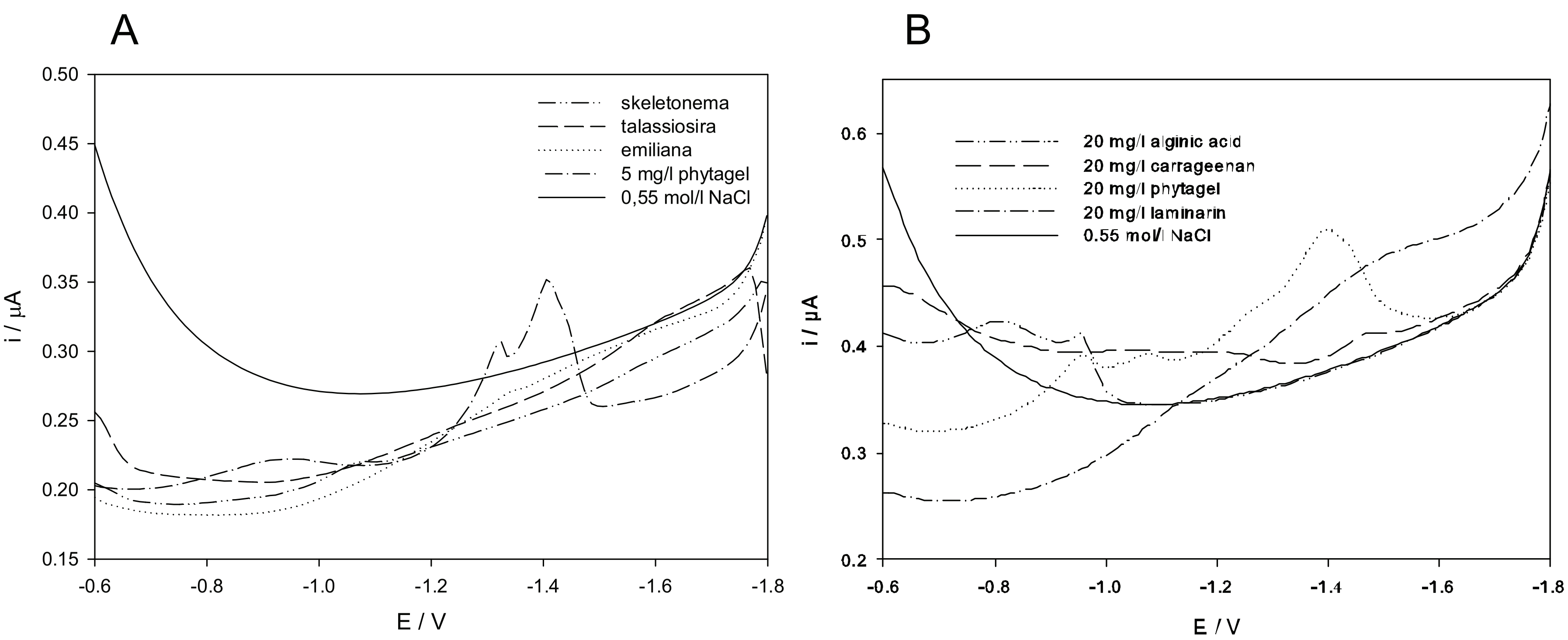
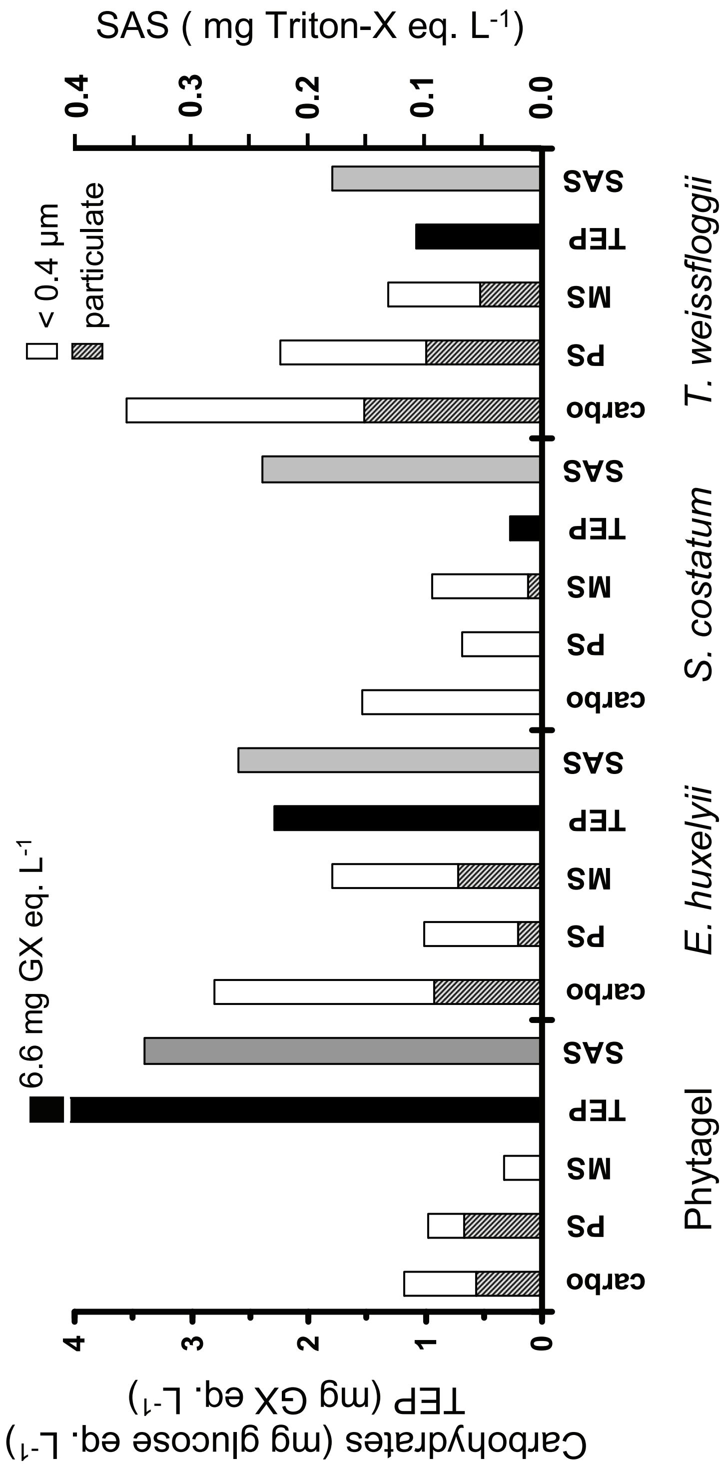

A
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
i / µA
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
E / V
skeletonema
talassiosira
emiliana
5 mg/l phytagel
0,55 mol/l NaCl
B
0.6
0.5
0.4
0.3
0.2
i / µA
E / V
20 mg/l alginic acid
20 mg/l carrageenan
20 mg/l phytagel
20 mg/l laminarin
0.55 mol/l NaCl

Fig 6



SAS (mg Triton-X eq. L-1)
0.4
0.3
0.2
0.1
0.0
< 0.4 µm
particulate
6.6 mg GX eq. L-1
carbo
PS
MS
TEP
SAS
Phytagel
E. huxelyii
S. costatum
T. weissfloggii
4
3
2
1
0
TEP (mg GX eq. L-1)
Carbohydrates (mg glucose eq. L-1)

Fig 7

Table 1. Complexing properties and SAS in samples of the cultures of *Skeletonema costatum, Thalassiosira weissflogii, Emiliana huxleyi* and phytagel, a model substance.

Samples	Cu_T (nM)	CCu[1] (μM)	Log $K_{app\,Cu}$[2]	Cd_T (nM)	CCd[1] (μM)	Log $K_{app\,Cd}$[2]	SAS[3] (mg/L eq. Triton-X-100)	CPSA[4]	Sulphur species(as eq. GSSG[5] nM)
Skeletonema costatum	23.80	1.06	6.45	16.45	0.022	8.73	0.24	yes	72
Thalassiosira weissflogii	26.60	1.14	6.18	14.85	0.020	8.83	0.18	no	200
Emiliana huxleyi	16.21	0.14	7.25	12.59	0.043	7.28	0.26	no	47
Phytagel (5 mg/L)	1.08	0.33	6.65	2.92	0.006	9.19	0.34	no	-
Phytagel (5 mg/L)									
0.7 μm	3.86	0.09	7.38	1.13	0.004	8.70	-	no	-
0.2 μm	1.83	0.09	7.47	1.07	0.004	8.75	0.21	no	-

[1]Apparent complexing capacity for cooper and cadmium ions; [2]Apparent stability constant; [3]Surface Active Substances; [4]Constant current potentiometric stripping analysis-catalytic effect of NH_2 or/and SO_4^- groups-appearance of the "pre"-sodium wave; [5]glutathione; (-) not measured

<u>CHAPTER 5:</u>

Summary and conclusions

5 Summary and conclusions

5.1 Identifying the processes controlling the distribution of H_2O_2 in surface waters along a meridional transect in the Eastern Atlantic

In the present study H_2O_2 profiles were compared with physical and bio-optical measurements and available satellite data to determine the major processes controlling the distribution of H_2O_2 in the upper ocean along a meridional transect in the Eastern Atlantic. The measurements showed that a number of factors influenced H_2O_2 distribution. The latitudinal patterns observed identified irradiance, water temperature and recent precipitation as key controls. Vertical distributions of H_2O_2 were strongly controlled by its photoformation and mixing processes in the upper water column. The recent irradiation history and phytoplankton activity appear to be the major sources and sinks determining the observed H_2O_2 levels, with CDOM playing a minor role. This suggests sunlight is the key limiting reactant in the formation of H_2O_2 in the Tropical and Sub-Tropical surface ocean.

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

In this study we investigated the photochemical effect of artificial and natural polysaccharide material in aquatic systems on iron speciation and production of H_2O_2. Artificial PS caused high photochemical production of H_2O_2, which on the one hand acts as a strong oxidant for metals and organic matter and on the other hand, is formed photochemically via the superoxide intermediate which is capable of reducing Fe(III). Increased steady state Fe(II) concentrations were found in illuminated seawater with high concentrations 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.

5.3 Characterization of phytoplankton exudates and polysaccharides in relation to their complexing capacity of copper, cadmium and iron

The goals of this study were to determine electrochemically, the complexing capacity of organic matter released by cultures of two marine diatoms *Thalassiosira weissflogii* and *Skeletonema costatum*, as well as the coccolithophore *Emiliana huxleyi* to copper (CCu) and cadmium (CCd) as well as to determine the organic matter responsible for the complexation. In particular, the constituents of organic matter whose effects were investigated were reduced sulphur species (RSS), of surface active substances (SAS), thio/amino groups, transparent exopolymer particles (TEP) and carbohydrates. Phytagel, carrageenan, laminarin and alginic acid were analysed as model substances. The organic matter released by all three phytoplankton cultures and the polysaccharide phytagel complexed Cu and to a lesser extent Cd. However, Cd complexes showed higher apparent stability constants and consequently the exudates and phytagel bound Cd more specifically than Cu. Sulphur-rich "glutathione" type ligands were found in all phytoplankton samples and were possibly responsible for the complexation of Cu. The correlation of monosaccharides with the complexing capacity to Cd, indicated that in the phytoplankton samples these compounds bound Cd. TEP, SAS and polysaccharides did not appear to be responsible for the complexing properties of the phytoplankton and no specific iron binding properties of laminarin, carrageenan and alginic acid could be found, probably partly due to limitations of the applied electrochemical methods. However, these measurements confirmed that the analysed model polysaccharides are highly surface active.

5.4 Conclusions

The findings of the experimental work confirmed hypothesis 1.1 “Algal exudates stabilise Fe(II)”, but only under UV irradiation, probably due to reduction of Fe by superoxide. Hypothesis 1.2 “Fe bound to polysaccharides is released via photochemical processes” could not be confirmed, as it appeared that polysaccharides did not bind iron. Despite that, the photochemical production of H_2O_2 via the superoxide radical, which presumably reduced Fe, was enhanced in the presence of polysaccharides and diatom exudates, respectively.

The in situ measurements of H_2O_2 along a meridional transect in the Eastern Atlantic partly confirmed hypothesis 2 “Phytoplankton exudates enhance the photoproduction of H_2O_2, a major player in the redox chemistry of Fe”, as the vertical distributions of H_2O_2 were controlled by phytoplankton activity, but the correlations with CDOM were poor.

Hypothesis 3. “Acidic polysaccharides and TEP are strong Fe chelators contributing significantly to the pool of unknown organic Fe-ligands in the ocean, released by diatoms to prevent Fe from precipitating from the surface ocean” could not be confirmed and no specific Fe complexation was found. Nevertheless, methodological limitations seemed to have obstructed a satisfactory conclusion.

CHAPTER 6:

Future work

6 Future work

This three year project gave significant new insights into the effect of polysaccharides and diatom exudates on the photochemical H_2O_2 formation and on the Fe(II) oxidation kinetics as well as the widely assumed potential contribution of these compounds to the pool of Fe ligands. As a result, this work has deepened our knowledge of iron biogeochemistry in the marine environment. Nevertheless, it is necessary to extend this work to draw further conclusions about this topical subject.

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

In order to extend the laboratory results of this study to natural environments which have at least 100 times lower total iron concentrations, measurements over a range of pH, ligand concentrations, iron concentrations, and light fluxes have to be made. These data will also provide the mechanistic details for a more advanced modeling of Fe(III) speciation, the influence of Fe(II) oxidation on H_2O_2 reduction, the influence of superoxide concentrations on Fe(III) and Fe(II) redox kinetics and changes in Fe(II) oxidation stoichiometry as a function of total iron concentration. This will be a valuable contribution to our mechanistic understanding of the redox chemistry of iron in seawater. Furthermore, in situ measurements of photoproduction of H_2O_2, TEP and polysaccharide concentrations, iron speciation and lifetime of Fe(II) in presence of natural phytoplankton exudates during bloom events will help to estimate the importance of polysaccharides and diatom exudates in the redox cycling of Fe.

6.2 Identifying the processes controlling the distribution of H_2O_2 in surface waters along a meridional transect in the Eastern Atlantic

To get a broader picture of the H_2O_2 distribution on a global scale, more surveys in different oceanic regions and at different times in the year are needed. Parallel monitoring of biological, chemical and physical parameters like chl. a, TEP/PS and CDOM, water temperature and irradiance will reveal the processes controlling global H_2O_2 distributions. This could lead to the development of parameterization schemes for the prediction of H_2O_2 distribution from satellite data, which would be an invaluable contribution to the modelling of the marine Fe biogeochemistry.

6.3 Characterization of phytoplankton exudates and polysaccharides in relation to their complexing capacity of copper, cadmium and iron

The results of this study were affected by limitations of the electrochemical method. The necessary high iron concentrations enhanced the hydrolysis which affected the sensitivity and reproducibility of the measurements. The high surface activity of the polysaccharides also led to a decreased sensitivity which made satisfactory results difficult. The detection limit of this method also seemed to be too high.
A different reasonable approach could aim to determine the bioavailability of iron in presence of polysaccharides and phytoplankton exudates. This can be done by means of growth and uptake experiments with radioactive ^{55}Fe in natural phytoplankton populations.

Room for more research remains!

<u>CHAPTER 7:</u>

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CHAPTER 8:

Appendix

8 List of contributions to the following publications and manuscripts:

Manuscript I (Chapter 2): ***The role of polysaccharides and diatom exudates in the redox cycling of Fe and the photoproduction of hydrogen peroxide in coastal seawaters.***

This submitted manuscript is based on a three months research fellowship at the National Oceanography Centre, Southampton. After my successful application to the BIOTRACS panel at NOCS (Southampton, UK) I set up the respective methods and carried out the described experimental work in the laboratory to test the hypotheses in my submitted proposal. I interpreted the results together with Peter Statham. The modeling was done with support by Christoph Voelker (AWI, Bremerhaven). I wrote the manuscript.

Manuscript II (Chapter 3): ***Identifying the processes controlling the distribution of H_2O_2 in surface waters along a meridional transect in the Eastern Atlantic.***

For this publication I performed the measurements of H_2O_2 aboard RS Polarstern. I evaluated the data and also the ship's CTD dataset of water temperature, salinity, chl. fluorescence and light transmission. I determined the precipitation at the sampling stations using satellite data and I analysed data of CDOM, chl.a and irradiance provided by other workgroups. Finally I made the statistical analyses of all these parameters and wrote the manuscript, which was then submitted and published in Geophysical Research Letters.

***<u>Manuscript III (Chapter 4):</u>* Characterization of phytoplankton exudates and polysaccharides in relation to their complexing capacity of copper, cadmium and iron.**

The CLE-CSV method and the UV photooxidation system in the clean room at AWI (Bremerhaven, Germany) were set up by me from scratch. I carried out the electrochemical measurements regarding iron (SWV, titrations) which are presented in this manuscript. A part of this work was done by me at RBI (Zagreb, Croatia) in order to intensify the cooperation with the scientists at this institute known for their outstanding knowledge in electrochemistry. Additionally I grew the respective phytoplankton cultures and determined the TEP and mono-/polysaccharide concentrations in these samples. I also made ac voltammetric measurements to assess the surface activity of several artificial polysaccharides. Finally I wrote the paragraphs concerning iron complexation for this manuscript.