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Iron-binding ligands in Dutch estuaries are not affected by UV induced photochemical degradation

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Abstract

This study shows that ultraviolet B (UV-B: 280–315 nm) and UV-A (315–400 nm) have no significant influence on the photodegradation of organic Fe(III)-binding ligands in estuarine waters from Marsdiep and Scheldt (The Netherlands). High salinity estuarine seawater from the Marsdiep and Scheldt contains concentrations of organic Fe(III)-binding ligands as high as 24.4 equivalent of nM Fe (eq nM Fe) and ~4.6 eq nM Fe, respectively, with conditional stability constants (K') of $10^{21.0}$ and $10^{20.1}$, respectively. Investigation of the relation between the organically bound iron fraction and the photoproduction of Fe(II) in the estuarine Marsdiep and Scheldt water shows that the concentration of Fe(II) produced was very low (<240 pM). This demonstrates that the major part of the organically complexed Fe(III) is not involved in photoinduced Fe redox cycling. Consequently UV has no influence on the transport of dissolved organically complexed Fe(III) from the estuarine environment to the coastal zone.

Keywords: Estuary; UV; Organic complexation; Fe(II); Ligand; Photochemistry; Reduction; Iron

1. Introduction

The overall dissolved iron (Fe) input of rivers in the world oceans is estimated to be 26×10^9 mol y^{-1} (de Baar and de Jong, 2001). The majority of dissolved Fe in river water exists as small colloid particles (Fox, 1988; Dai and Martin, 1995; Wen et al., 1999). Flocculation of these colloids, due to the change in ionic strength upon mixing of fresh river water with seawater during estuarine mixing, causes a massive removal of the freshly formed particulate Fe (Sholkovitz, 1978; Sholkovitz et al., 1978). In the Scheldt estuary, this flocculation occurs between salinities of 1–5 (Wollast and Peters, 1980).

Organic complexation is an important factor in the biogeochemistry of Fe in estuarine waters, as it maintains iron in the dissolved phase at high salinities beyond the flocculation zone. The dissolved phase will be flushed from the estuary while the non-organically complexed fraction tends to aggregate and adsorb to particles, thereby residing within the internal cycle of the estuary for a longer time (Morris et al., 1986). Thus organically complexed iron of estuarine systems can act as a source of dissolved iron for coastal zone waters (Powell and Wilson-Finelli, 2003a). Despite its importance in the geochemical Fe cycle and despite the fact that primary production can be limited by Fe availabil-

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ity in coastal environments (Glover, 1978; Hutchins and Bruland, 1998; Hutchins et al., 1998; Kirchman et al., 2000; Bruland et al., 2001), data on the organic complexation of Fe in estuaries is scarce.

The chemistry of Fe in the more saline estuarine waters is complex. Dissolved Fe can exist in two different oxidation states, Fe(II) and Fe(III). The Fe(III) is the thermodynamically stable form in oxygenated waters and has a very low solubility in high saline water (Millero, 1998; Liu and Millero, 2002). Fe(III) becomes rapidly hydrolyzed into various Fe(III) oxyhydroxides. The low solubility of Fe oxyhydroxides and the tendency to form colloids contribute to the scarcity of directly bioavailable Fe species in the marine environment (Martin et al., 1995; Powell et al., 1996; SanudoWilhelmy et al., 1996; Wen et al., 1999).

Dissolved Fe(III) occurs for a major part as complexes (Fe(III)L) with strong organic ligands (Gledhill and van den Berg, 1994; Hutchins et al., 1999; Waite, 2001). This organic complexation is normally described in terms of concentration and conditional stability constants (K'_{FeL}) of the apparent ligand(s) for various oceans (Gledhill and van den Berg, 1994; Rue and Bruland, 1995, 1997; van den Berg, 1995; Wu and Luther, 1995; Gledhill et al., 1998; Nolting et al., 1998; Witter and Luther, 1998; Witter et al., 2000b) and coastal seas (Gledhill and van den Berg, 1994; Croot and Johansson, 2000; Macrellis et al., 2001). The conditional stability constants of Fe(III)L complexes range in magnitude between 10^{18} and 10^{23} (Witter et al., 2000a). Although the K'_{FeL} of organic complexes between different oceans may range over 5 orders of magnitude it is not possible to use the K'_{FeL} to identify organic ligands using the K'_{FeL} of known model ligands (Witter et al., 2000a). Instead Witter et al. (2000a) suggested that, based on a comparison of formation- and dissociation rate constants between model ligands and field samples, most unknown ligands in seawater originate from porphyrin and siderophore-like compounds. Porphyrins are molecules that relate to the photosynthetic pigments of autotrophic organisms, notably chlorophyll-a. Siderophores are low-molecular weight, high affinity Fe(III)-binding ligands secreted by e.g. marine cyanobacteria and heterotrophic bacteria under Fe limiting circumstances to bind and transport Fe for uptake (Wilhelm and Trick, 1994; Martinez et al., 2000; Barbeau et al., 2002). Furthermore, Macrellis et al. (2001) found hydroxamate or catecholate Fe-binding functional groups, characteristic for siderophores, present in different size classes of samples taken along the Californian coast. The presence of Fe(III)-binding compounds in the low molecular weight classes (<300 Da) was suggested to be caused by UV induced degradation of larger Fe(III)-binding siderophores in the surface waters.

Photochemical (-induced) reactions of Fe(III)-siderophore complexes can result in the decomposition of the siderophores and the concomitant photoreductive production of Fe(II) in sunlit waters (Barbeau et al., 2001). Furthermore, organic Fe(III)-binding ligands can increase or decrease Fe(III) photoreducibility by affecting Fe(III) aggregation and by binding Fe(III) resulting in photostable complexes (Rijkenberg et al., submitted for publication). Iron(II), although in itself well soluble in seawater, becomes rapidly oxidized by O_2 and H_2O_2 (Millero et al., 1987; Millero and Izaguirre, 1989; Millero and Sotolongo, 1989; King et al., 1991, 1995; King, 1998) but at the same time Fe(II) is thought to be an important chemical species well available for uptake by biota (Anderson and Morel, 1980; Takeda and Kamatani, 1989; Auclair, 1995; Croot et al., 2001). The redox cycling of (colloidal) iron hydroxides and iron(III) chelates initiated by photochemical processes is often mentioned as an important mechanism by which iron bioavailability for phytoplankton is enhanced by increasing the concentration of reactive inorganic species of Fe(II) and Fe(III) (Anderson and Morel, 1982; Finden et al., 1984; Rich and Morel, 1990; Wells and Mayer, 1991; Johnson et al., 1994; Miller and Kester, 1994; Sunda and Huntsman, 1995; Barbeau et al., 2001).

There is still much controversy about the importance of photodegradation and subsequent Fe(II) production of organically complexed Fe(III) in the natural environment. Siderophores may be photodegradable, but until recently none of the depth profiles of Fe(III) chelators reported (Gledhill and van den Berg, 1994; Rue and Bruland, 1995; Wu and Luther, 1995) exhibit shallow mixed layer minima or any other features which would suggest a surface/photochemical sink (Moffett, 2001). Yet, Boyé et al. (2001) recently reported depth profiles, where lower concentrations of organic Fe(III)-binding ligands in Southern Ocean surface waters suggest photodegradation. Furthermore, Powell and Wilson-Finelli (2003b) reported photodegradation of organic Fe(III)-binding ligands at the timescale of hours in the Gulf of Mexico.

Photodegradation of organically bound Fe(III) will influence the biogeochemical cycle of iron and could increase its biological availability and reactivity. Here we report experiments performed with high saline estuarine Marsdiep and Scheldt waters to investigate the influence of light on the presence of organic Fe(III)-binding ligands. Furthermore, we show that although a

high concentration organic Fe(III)-binding ligands is present in the Marsdiep and Scheldt water, upon irradiation the Fe(II) production is relatively low as compared with the fraction of Fe(III) bound to organic ligands.

2. Materials and methods

2.1. Samples

Marsdiep water (0.2 μm filtered) was collected at the 8th of May 2003 at 11:50 from the NIOZ jetty during high tide (Fig. 1). The salinity was 29.1 versus the practical salinity scale, and the DOC concentration was 298 ± 14.6 μM C. The Marsdiep water was collected in an acid cleaned 20 l carboy using a portable flow bench (Interflow) and acid cleaned tubing. The carboy was stored in the dark at 4 °C. The collected Marsdiep water was used within a week after collection.

Scheldt water (0.2 μm filtered) was collected in the Westerschelde near Vlissingen at the 5th of April 2001 (Fig. 1). Surface seawater was pumped into an overpressurized class 100 clean air container using a Teflon diaphragm pump (Almatec A-15, Germany) driven by a compressor (Jun-Air, Denmark, model 600-4B) connected via acid-washed braided PVC tubing to a torpedo towed at approximately 3 m depth alongside the ship (Navicula, Royal NIOZ). The seawater was filtered in-line by a (Sartorius Sartobran filter capsule 5231307H8) with a cut-off of 0.2 μm. The <1 kDa truly dissolved or "soluble" Fe fraction was filtered using an acid-cleaned Amicon SP60 cartridge and a peristaltic pump of Watson Marlow (604S/R). The salinity of the Scheldt water was 26 and the DOC concentration was 217.6 μM. The collected Scheldt water was used the next day for the deck-incubations.

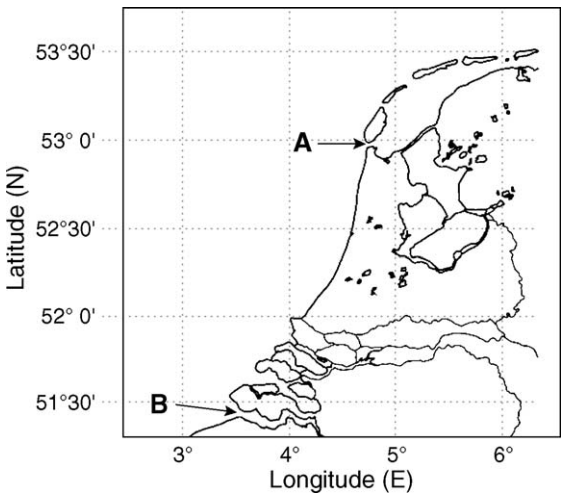

Fig. 1. The coastline of the Netherlands. Samples were taken in the Marsdiep (A) and in the Westerschelde (Scheldt) (B).

2.2. Experimental

The influence of irradiance on the organic Fe(III)-binding ligands in Marsdiep water was investigated in the laboratory. Before use, acid-cleaned UV transparent polymethylmetacrylate (PMMA) bottles (Steeneken et al., 1995) were conditioned overnight with Marsdiep water (0.2 μm filtered) at 15 °C, the temperature of the water in the field at the time of collection. The experiments were performed in a temperature controlled class 100 clean container. The pH of the Marsdiep samples was 8.14 ± 0.006 (Metrohm 713 pH meter). To investigate the influence of UV on the photodegradation of organic Fe(III)-binding ligands a PMMA bottle containing Marsdiep water was placed in the light and another PMMA bottle containing Marsdiep water was placed in the dark. Samples were taken at $t=0$ and $t=18.5$ h. The samples were frozen and used within a week for competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV) to determine the concentration and the log K' of the organic Fe(III)-binding ligands (equivalents of Fe(III)-binding places).

The influence of irradiance on the organic Fe(III)-binding ligands in Scheldt water was performed by means of deck incubations. The incubations were performed using acid cleaned PMMA and pre-conditioned bottles in UV transparent PMMA incubators. The bottles, incubated between 8:45 and 22:40 at the 7th of April 2001, were kept at a constant ambient temperature of 12 °C using running seawater from the ship's pumping system. The pH of the Scheldt water was 8.8 (Metrohm 713 pH meter). Sampling was performed in a laminar flow bench in a class 100 clean container. Samples for the determination of the concentration of organic iron binding ligands and their log K' were immediately frozen. Stored samples for dissolved iron were acidified with 100 μl 3× quartz distilled HCl per 100 ml sample (pH = 2).

Investigation of the photoproduction of Fe(II) was performed in a temperature controlled class 100 clean container. The PMMA bottles were pre-conditioned with the water as used during the experiments and at the temperatures as used during the experiments. Water was continuously stirred using a magnetic induced, Teflon stirring bean during the experiment. An experiment typically started with measuring the background signal in the dark, after which the lamps were switched

on. During 90–120 min the production of Fe(II) was followed. When enough sample volume was available, the oxidation of Fe(II) was followed for 30 min after having turned off the light.

2.3. Light

Philips UV-B (TL-12), UV-A (TL′ 40W/05) and VIS (TL′D 36W/33) lamps were used to simulate the solar spectrum in the experiments with Marsdiep water. Spectral conditions were measured using a MACAM Spectroradiometer SR9910 with a small spherical 4π sensor. All sides of the box shaped PMMA bottle, except the top, were covered with black plastic to prevent focussing effects. Spectra were recorded from 280–700 nm.

Previously Fe(III) siderophore complexes have been shown to absorb light in the high UV-B and low UV-A range (Barbeau et al., 2001, 2003). Therefore, we used mainly UV-B and UV-A lamps during the incubation, but VIS lamps were included as well. Table 1 shows the doses of UVB, UVA and VIS received by the Marsdiep water during the incubation. The doses of solar UVB and UVA as measured by a hyperspectral radiometer (280–680 nm) containing a cosine collector (TriOS Optical Sensors) at the roof of the Royal NIOZ at 300 m from the Marsdiep sample station, and the dose of solar VIS as measured with TriOS Ramses ACC irradiation meter with cosine collector at the Marsdiep sample station, for the 8th of May 2003, are included for comparison. During the experiments an UVB irradiance dose 4 times higher, an UVA dose 3 times lower and a VIS dose 2200 times lower than the dose which estuarine seawater could receive during a day at the water surface (8th May 2003) were used (Table 1). Because we hereby ignored the effect of mixing and the strong light attenuation in these estuarine waters the applied UVB dose and probably also UVA irradiance are likely higher than in situ UVB and UVA doses.

Table 1
The doses of UV-B, UV-A and VIS irradiance received by the coastal Marsdiep water during the incubation of 18.5 h

Wavelength region	Incubation (kJ m^{-2})	Solar irradiance 8th of May (kJ m^{-2})
UV-B	204.39	48.02
UV-A	377.04	1170.71
VIS	919.24	1.98 10^6

As comparison the doses of solar UV-B and UV-A irradiance detected nearby (within 300 m) at the Royal NIOZ during a whole day (14.75 h of daylight) are given.

A PUV 500 surface sensor (Biospherical Instruments) was used to measure the irradiance during the deck-incubation of the Scheldt seawater. The PUV measured irradiance between 9:15 and 16:05 (local time, 7th of April 2001). The irradiance values, given as dose, were measured at 305 nm (0.14 kJ m^{-2} nm^{-1}), 320 nm (3.09 kJ m^{-2} nm^{-1}), 340 nm (6.04 kJ m^{-2} nm^{-1}), 380 nm (7.91 kJ m^{-2} nm^{-1}) and VIS (400–700 nm, 20.49 E m^{-2}). The deck-incubations of the Scheldt seawater were performed between 8:45 and 22:40 so the actual dose of irradiance received by the Scheldt sample was somewhat higher than measured with the PUV.

2.4. Competitive ligand exchange-adsorptive cathodic stripping voltammetry

Determination of the organic speciation of iron in the Marsdiep and Scheldt water was performed using CLE-ACSV. The 2-(2-Thiazolylazo)-*p*-cresol (TAC) (Aldrich, used as received) reagent was used as competing ligand (Croot and Johansson, 2000). All solutions were prepared using 18.2 MΩ nanopure water. The equipment consisted of a μAutolab voltammeter (Ecochemie, Netherlands), a static mercury drop electrode (Metrohm Model VA663), a double-junction Ag/saturated AgCl reference electrode with a salt bridge containing 3 M HCl and a counter electrode of glassy carbon. The titration was performed using 0.01 M stock solution of TAC in 3× quartz distilled (QD) methanol, 1 M boric acid (Suprapur, Merck) in 0.3 M ammonia (Suprapur, Merck) (extra cleaning by the addition of TAC after which TAC and $Fe(TAC)_2$ was removed with a C18 SepPak column) to buffer the samples to a pH of 8.05 and a 10^{-6} M Fe(III) stock solution acidified with 0.012 M HCl (3× QD). Aliquots of 15 ml were spiked with Fe(III) up to final concentrations between 0 and 20 nM and allowed to equilibrate overnight (>15 h) with 5 mM borate buffer and 10 μM TAC. The concentration $Fe(TAC)_2$ in the samples was measured using the following procedure: i) removal of oxygen from the samples for 200 s with dry nitrogen gas, after which a fresh Hg drop was formed, ii) a deposition potential of −0.40 V was applied for 30–60 s according to the sample measured, the solution was stirred to facilitate the adsorption of the $Fe(TAC)_2$ to the Hg drop, iii) at the end of the adsorption period the stirrer was stopped and the potential was scanned using the differential pulse method from −0.40 to −0.90 V at 19.5 mV s^{-1} and the stripping current from the adsorbed $Fe(TAC)_2$ recorded.

The ligand concentration and the conditional stability constants were calculated using the non-linear fit of the Langmuir isotherm (Gerringa et al., 1995).

2.5. Iron(II) analysis

Concentrations of Fe(II) were followed using an automated flow injection analysis system employing a luminol-based chemiluminescence detection of Fe(II) (Seitz and Hercules, 1972; King et al., 1995; O'Sullivan et al., 1995). An alkaline luminol solution is mixed with the Fe(II) sample in a spiral shaped flow cell in front of a Hamamatsu HC135 photon counter. At pH 10, Fe(II) is rapidly oxidized by oxygen on a millisecond timescale causing the oxidation of luminol and producing blue light (Xiao et al., 2002).

Samples were transported in-line from the PMMA bottle to a sample loop. Then the sample was, by introducing it in a pure water carrier (18.2 MΩ nanopure water), transported into the flow cell every 93 s.

The complete analytic system was built inside a light-tight wooden box. The luminol reagent and the carrier were kept in light-tight bags (as used for storage of photographic films). The tubing was covered by aluminum foil and the tubing of the peristaltic pump was shaded by black plastic.

A 15 mM 5-amino-2,3-dihyfro-1,4-phthal-azinedione (Luminol) (SIGMA) stock solution was prepared weekly in 20 mM Na_2CO_3. The 50 μM luminol reagent solution was made in 0.5 M NH_3 (Suprapur, Merck) and 0.1 M HCl (Suprapur, Merck). The luminol reagent solution was stored in the dark for at least 24 h before use to ensure that the reagent properties had stabilized. An 0.01 M Fe(II) stock was prepared monthly by dissolving ferrous ammonium sulfate hexahydrate $(Fe^{(II)})(NH_4SO_4)_2 \cdot 6H_2O$ (Baker Analyzed, reagent grade) in 0.012 M (3× QD) HCl. Working solutions were prepared daily. All Fe(II) stock solutions were refrigerated in the dark at 4 °C when not in use.

Calibration was performed by the standard addition of known concentrations of Fe(II) to the sample matrix. The standard addition for the experiments with Scheldt water was performed in Southern Ocean seawater because of a lack of sample. The time delay between Fe(II) addition and measurement caused an oxidation effect. This oxidation effect was accounted for by extrapolating the data back to time zero using the fact that Fe(II) oxidation in seawater very closely approximates pseudo-first-order kinetics.

2.6. Total dissolved Fe analysis

Dissolved iron, defined as the Fe fraction passing an 0.2 μm filter, was determined using flow injection analysis with luminol chemiluminescence and H_2O_2 (de Jong et al., 1998). Given the very low Fe(II) concentrations, the values for total dissolved Fe are almost completely representing the Fe(III) state, which in turn is virtually completely consisting of both Fe-organic complexes and Fe-colloids, i.e. free truly dissolved inorganic Fe(III) is deemed rare if not negligible.

3. Results and discussion

3.1. Iron speciation of the Marsdiep and Scheldt estuary samples

Marsdiep water was sampled during the final stage of a phytoplankton spring bloom with low cell numbers caused by an early depletion of the nutrients phosphate and nitrate. The bloom developed from the 16th of March until the 15th of May, 2003. The Marsdiep water contained 25.4 nM dissolved iron and 24.4 eq nM Fe of dissolved ligands with a log K'_{FeL} of 21.0 ± 0.18 ($n=4$). Some 4 nM Fe is present as TAC-labile Fe (TAC-labile Fe: Fe labile with respect to TAC is the Fe being bound by 10 μM TAC after >15 h equilibration) (Table 2). Samples from the Scheldt estuary contained concentrations of dissolved Fe of 12–15 nM and a relatively high concentration of TAC-labile Fe: 11–12 nM. The Fe(III)-binding ligand concentration was 4.6 eq nM Fe with a log K'_{FeL} of 20.1 ($n=1$) (Table 2).

Similar values for the log K'_{FeL} in coastal waters were reported by Rose and Waite (2003) using a kinetic approach and by Powell and Wilson-Finelli (2003a) using CLE-ACSV. Croot and Johansson (2000) found similar values for the log K' and the initial concentration of TAC-labile Fe during an algal bloom at Valö, Sweden. However, Gobler et al. (2002) found extremely high conditional stability constants, log K' ~23, for organic iron binding ligands at similar salinities in the Peconic Estuary (Long Island, USA).

3.2. TAC-labile Fe concentrations and their response to irradiance

In open ocean waters the concentration of ligands are always higher than the concentration of dissolved Fe (Gledhill and van den Berg, 1994; Rue and Bruland, 1995; van den Berg, 1995). This means that after equilibration (>15 h) all dissolved iron is complexed by organic ligands and not available for TAC. Titration with Fe(III) results typically in low concentrations of TAC-labile Fe for the first few Fe(III) additions in open ocean water (Fig. 2).

The curvature in the titration curves of Marsdiep and Scheldt water (Figs. 2 and 3) indicating competition

Table 2
Dissolved Fe, TAC-labile Fe, total ligand concentration and the logarithm of the conditional stability constant (K') for the Marsdiep and Scheldt samples and the samples of Marsdiep water that were UV destructed

Samples	$[Fe]_{dissolved}$ (nM)	[TAC-labile Fe](nM)	$[L]_{total}$ (nM)	±95% confidence interval	log K'_{FeL}	±95% confidence interval
Marsdiep samples						
Dark ($t=0$)	25.39	4.30	22.9	0.78	21.23	0.27
Light ($t=0$)	25.42	4.46	25.9	0.82	20.83	0.11
Dark ($t=18.5$ h)	25.21	3.49	23.5	0.55	21.37	0.22
Light ($t=18.5$ h)	25.59	3.52	25.1	0.84	21.17	0.19
Scheldt samples						
Dark	12.34	11.04	4.2	2.62	20.09	1.04
Light	14.68	11.62	5.0	1.36	20.56	1.10
UV destruction						
A ($t=0$)	26.09	6.01	23.6	1.32	20.89	0.26
B ($t=0$)	26.61	6.15	22.7	1.41	21.03	0.40
A ($t=10$ h)	18.55	2.75	18.0	0.79	21.42	0.34
B ($t=10$ h)	18.38	2.39	17.9	0.51	21.41	0.21

The $[Fe]_{dissolved}$ is the fraction iron determined in 0.2 μm filtered seawater as measured by flow injection analysis using luminol chemiluminescence (de Jong et al., 1998). The TAC-labile Fe is the labile iron concentration recoverable by adding 10 μM TAC, allowing the sample to equilibrate overnight, this value is an average of the first few values of the titration. The $[L]$, the concentration of Fe(III)-binding ligand, and log K'_{FeL}, the conditional stability constant of the iron binding ligand expressed with respect to Fe^{3+} , and their 95% confidence interval were estimated using non-linear least squares analysis (Systat), the method of Wilkinson (Wilkinson, 1961; Gerringa et al., 1995).

between the natural ligands and the added concentration TAC revealed the presence of strong ligands. The concentration of TAC-labile Fe of titrations with estuarine seawater was higher then observed for titrations of open ocean water. Croot and Johansson (2000) reported TAC-labile Fe values between of ~6.2 nM during a bloom and ~4.6 nM after a bloom (10 μM TAC, overnight equilibration) at Valö, Sweden. The TAC-labile Fe concentrations determined during this study were between 3.5 and 4.5 nM for Marsdiep water and between 11 and 12 nM for water sampled in the Scheldt estuary.

The high TAC-labile Fe concentrations are explained by the existence of weak Fe(III)-binding compounds in the coastal seawater samples. These weak Fe(III)-binding ligands cannot compete with TAC and lose their Fe(III) to TAC (Gerringa et al., submitted for publication). Furthermore, in competition with the strong Fe(III)-binding ligands, TAC is able to catch some of the Fe(III) initially bound by the natural strong ligands and so creating a pool of iron-free ligands which were subsequently titrated (Gerringa et al., submitted for publication). This explains why the titration shows a

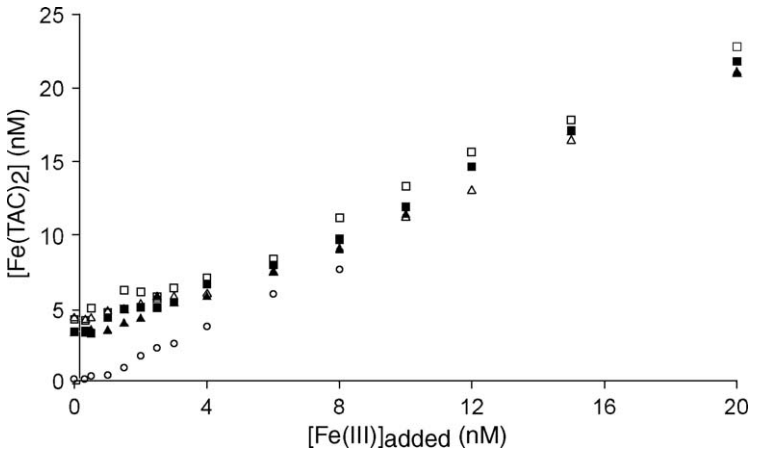

Fig. 2. The CLE-ACSV Fe titration data from the Marsdiep and from Southern Ocean seawater (○). The titration curves for the coastal Marsdiep water were determined before and after the sample received 18.5 h irradiance or darkness: $t=0$ light (Δ), $t=0$ dark (□), $t=18.5$ h light (▲) and $t=18.5$ dark (■).

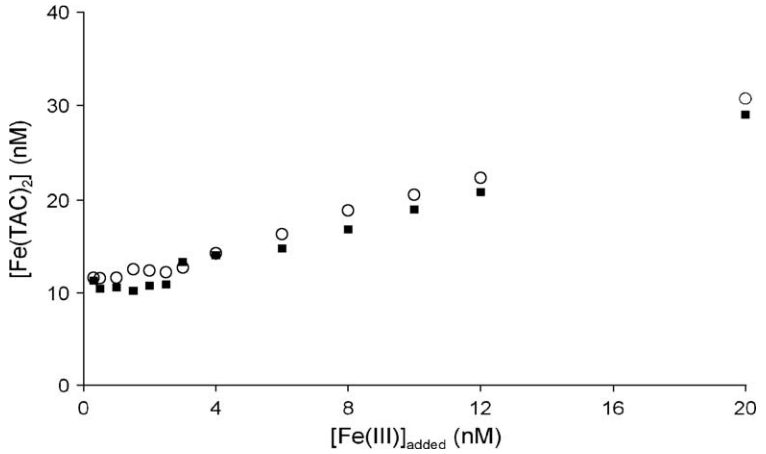

Fig. 3. The CLE-ACSV Fe titration data from the Westerschelde. The titration curves for the Scheldt water were determined after a sample received 12 h of daylight in spring (7th of April, 2001) (○) and after being incubated for 12 h in the dark (■).

curve in the presence of Fe(III) bound by weaker ligands.

Comparing 1 kDa filtered Scheldt samples with 0.2 μm filtered Scheldt samples showed us that the initial TAC-labile Fe fraction decreased with filter pore size, indicating that at least part of the TAC-labile Fe fraction had a relationship with the colloidal iron fraction (Fig. 4).

The TAC-labile iron concentration in Marsdiep water decreased in time independent of the irradiance treatment (Fig. 5). Marsdiep water incubated in the dark for 18.5 h showed the same decrease in TAC-labile Fe as Marsdiep water incubated during 18.5 h in the light. This time-dependent decrease was probably caused by degradation of weak organic iron binding ligands or by Fe(III)-binding places related to colloidal material subject to processes as aging (Cornell and Schwertmann, 1996), adsorption of organic material (Kreller et al., 2003) and aggregation (Wells and Goldberg, 1993; Hunter et al., 1997).

3.3. The influence of UV on the organic Fe(III)-binding ligands

Irradiation with UVC (254 nm) during UV-destruction of the Marsdiep water resulted in a 22% decrease in the concentration of Fe(III) chelators. Also a decrease in the dissolved Fe concentration was observed (Fig. 6A, Table 2). The high UV irradiance inducing the degradation of the Fe(III) chelators and increasing the concentration of free iron in the Marsdiep water probably caused an increase in inorganic Fe(III), this next resulting in the precipitation of particulate iron to the

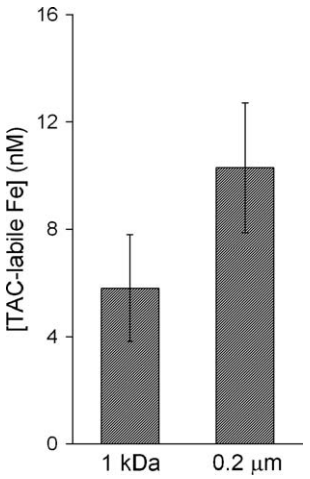

Fig. 4. The TAC-labile Fe concentrations for two size fractions of Scheldt water; <1000 Da filtered ($n=3$) and <0.2 μm filtered ($n=4$).

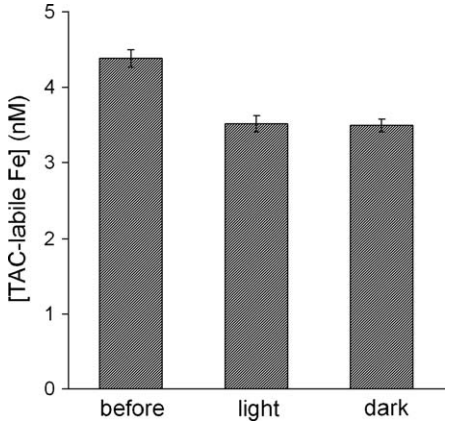

Fig. 5. The TAC-labile Fe values determined for Marsdiep water, before and after incubation during 18.5 h in the light or in the dark.

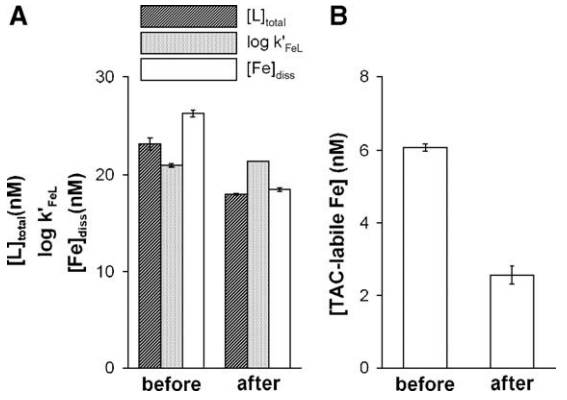

Fig. 6. (A) The total concentration of Fe(III)-binding ligand, log K'_{FeL} and the concentration dissolved Fe of Marsdiep water before and after 10 h of UV destruction. (B) The TAC-labile Fe concentration before and after 10 h of UV destruction of the Marsdiep water. The error bars give the standard errors ($n=2$).

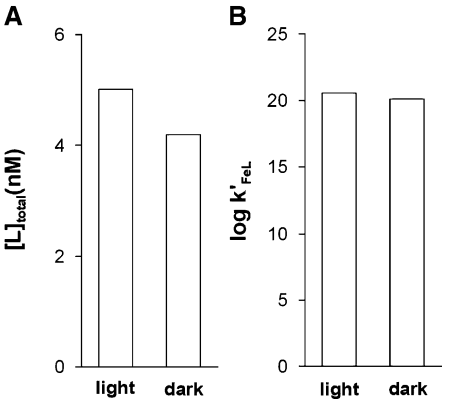

Fig. 8. (A) The total concentration of Fe(III)-binding ligand and (B) log K'_{FeL} of Scheldt water after 12 h of natural sunlight or 12 h in the dark.

bottom of the quartz tubes. Also the concentration TAC-labile Fe decreased during the UV-exposure (Fig. 6B, Table 2). This indicates that particularly the weaker ligand class is UV degraded. The use of a high, unnatural UVC irradiance level, shows the Fe chemical effects of photoinduced degradation of Fe(III)-binding ligands.

However, exposure of estuarine water sampled in the Marsdiep with simulated solar irradiance showed no significant decrease in the concentration of organic Fe(III)-complexing ligands or change in the log K'_{FeL}, (Figs. 2 and 7). Additionally, organic Fe(III)-complexing ligands in Scheldt water ($S=26$) showed no decrease in concentration after incubation under natural sunlight for a whole day as compared to incubation in the dark (Figs. 3 and 8).

Our results are consistent with the absence of vertical gradients of Fe(III) chelators in open ocean waters, i.e. the absence of minima within the shallow mixed layer minima, or any other feature which would suggest a surface/photochemical sink (Gledhill and van den Berg, 1994; Rue and Bruland, 1995; Wu and Luther, 1995; Moffett, 2001). On the other hand, it was reported recently that a weak Fe(III)-binding ligand class (log K' 20–22, <1 kDa) in the Gulf of Mexico is being photodegraded (Powell and Wilson-Finelli, 2003b). Powell and Wilson-Finelli (2003a) demonstrated that organic Fe(III)-complexing ligands change little in the surface samples of the stratified Mississippi plume. Yet, they explained the steady presence of the ligands with the continuous production of new ligands replacing the photodegraded organic Fe(III)-binding ligands. Production of organic Fe(III)-binding ligands to compensate for the photodegraded ligands cannot explain the constant Fe(III)-binding capacity in our experiments because phytoplankton and the majority of bacteria were filtered out.

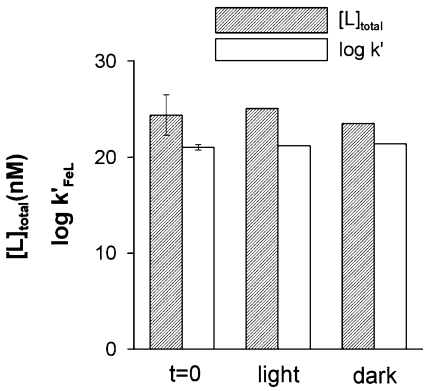

Fig. 7. The total concentration of Fe(III)-binding ligand and log K'_{FeL} for Marsdiep water at $t=0$ ($n=2$) and before and after 18.5 h in the light ($n=1$) or in the dark ($n=1$).

Macrellis et al. (2001) had suggested larger iron(III)-binding siderophores to be photodegraded to smaller Fe(III)-binding molecules. This has been postulated to explain the detection of molecules with siderophore-like characteristics in a size class smaller than the defined size range for siderophores (300–1000 Da) in surface waters (Macrellis et al., 2001). Reduction of Fe(III) with concomitant photodegradation of the siderophore and a significant drop in Fe(III)-binding strength was also shown by Barbeau et al. (2001). The reason that we did not observe photodegradation of the Fe(III)-binding capacity could be that the overall input of photosensitive Fe(III) chelators was low. Related to this, the photoreactive fraction could have already have been quickly photodegraded during river transport or in the shallow well mixed coastal seawaters. Estuarine waters contin-

uously receive new organic material originating from river input and biological activity. The fraction of photodegradable Fe(III) chelators within this organic material appears to be low.

3.4. Fe(II) photoproduction in Marsdiep and Scheldt water

Although there was no significant influence of light on the photodegradation of the Fe(III) chelators, the Fe(II) was still photoproduced (Fig. 9). The photoproduction of Fe(II) in Marsdiep water was investigated under three different optical treatments (Fig. 9). The 0.2 μm and 1 kDa filtered Scheldt water was only irradiated with the total spectrum (280–700 nm) (Fig. 10).

The Fe(II) concentration in the dark for Marsdiep water was 43 ± 9 pM Fe(II) and for the Scheldt water 57 ± 4 pM Fe(II). Comparison of the Fe(II) production of the Marsdiep and Scheldt water receiving the same optical treatment showed a "steady state" value of 177 ± 8 pM for the 0.2 μm Marsdiep water and 239 ± 4 pM and 131 ± 8 pM for 0.2 μm and 1 kDa filtered Scheldt water, respectively.

We have no knowledge about the nature and concentrations of the photoreactive Fe(III) species but these experiments show that the concentrations Fe(II) formed during irradiation experiments were very low. The determined concentrations of Fe(III)L were a factor of 100 higher than the concentration Fe(II) for the Marsdiep water and a factor of 20 higher than the concentration of Fe(II) in the Scheldt water. Using the dataset of Fe(II) concentrations in Marsdiep water under irradiance of the total spectrum (280–700 nm, Fig. 9) we roughly calculated the Fe(III) photoreduction rate necessary to compensate the Fe(II) oxidation rate so that it results in the measured Fe(II) concentrations. We assumed the Fe(II) oxidation rate to be first order and calculated the oxidation using only the first 4 data points after turning the light off. Furthermore, we assumed that the oxidation rate did not change during the experiment and we ignored the existence of an initial dark Fe(II) concentration of 0.035 nM. Using the "steady state" Fe(II) concentration of 0.17 ± 0.01 nM Fe(II), this exercise resulted in an average Fe(II) oxidation rate of 1.07 ± 0.11 nM h^{-1} and an average Fe(III) photoreduction rate of 1.24 ± 0.12 nM h^{-1}. The Marsdiep water contained 1 nM dissolved Fe(III) not complexed by strong organic Fe(III)-binding ligands and possibly some dissolved uncomplexed Fe(III) within the uncertainty of the concentration strong organic Fe(III)-binding ligands. Therefore, the measured Fe(II) concentration could be accounted for without using the strong organically complexed Fe(III)

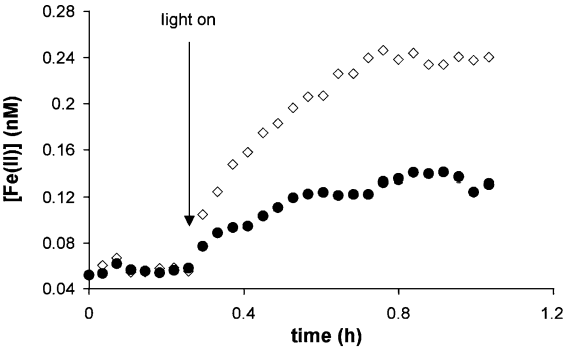

Fig. 10. The concentration Fe(II) in Scheldt water formed upon irradiation of 0.2 μm filtered (◇) and 1 kDa filtered (●) water with irradiance between the wavelengths 280 and 700 nm.

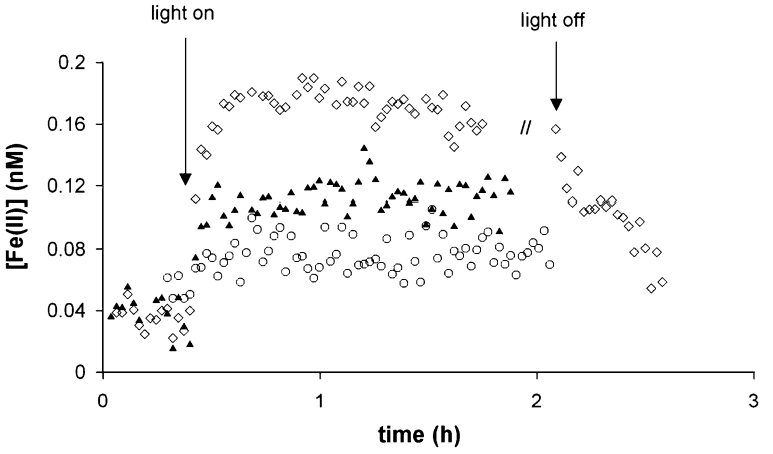

Fig. 9. The concentration of Fe(II) formed during irradiation of Marsdiep water with three different wavelength regions: 280–700 nm (◇), 320–700 nm (▲) and 360–700 nm (○).

pool. This suggests that the majority of the organic complexed iron is not involved in the photoproduction of Fe(II).

The Fe(II) photoproduction in Marsdiep water showed the typical pattern seen during experiments with inorganic iron colloids (Waite and Morel, 1984; Wells and Mayer, 1991). The pattern observed when the light is on, namely a rapid increase until a maximum, followed by a slow decrease in the Fe(II) concentration during further continued irradiance, has been reported before in experiments using colloidal Fe (Wells and Mayer, 1991; Miller et al., 1995; Emmenegger et al., 2001). Wells and Mayer (1991) found that the photoconversion rates diminished with continued irradiation when ferrihydrite was irradiated. Waite and Morel (1984) found the same effect for the photodissolution rate of lepidocrocite. One explanation for the decreasing Fe(II) concentrations during irradiance could be an increase in the Fe(II) oxidation rate. However, Emmenegger et al. (2001) found this effect only after UV digestion of lake water (Melchsee, Switzerland) what could suggest that this decreasing Fe(II) concentration during irradiance is the result of the presence of a limited amount of chromophoric colloidal Fe species available for photoreduction (Wells and Mayer, 1991; Kimball et al., 1992; Rijkenberg et al., 2005). The occurrence of this similar pattern in the production of Fe(II) with time suggests that the produced Fe(II) could originate from a colloidal Fe fraction. Our results support this suggestion clearly when UVB was included in the irradiance spectra offered.

Moreover, we found that the production of Fe(II) was lower in the truly dissolved fraction (1 kDa filtered) of Scheldt water, 131 ± 8 pM Fe(II) compared to the Fe(II) produced in the dissolved fraction (0.2 μm filtered), 239 ± 4 pM Fe(II). This means that part of the Fe(II) produced results from a photoreactive colloidal Fe fraction. We cannot conclude that the Fe(II) formed in the truly dissolved Scheldt water fraction is the result of a photoreactive organic complexed iron or of an inorganic truly soluble fraction, because colloids could have formed in this sample during sample handling in the laboratory. Enclosing a sample of (coastal) seawater in a container causes a decrease in the concentration of soluble iron and an increase in the concentration of particulate iron (Lewin and Chen, 1973). Another effect possibly increasing the colloidal iron fraction is the observation that iron is less soluble in warmer waters (Kuma et al., 1996). The colloidal iron fraction could increase when the estuarine seawater was kept in a warmer laboratory environment during various stages of the process.

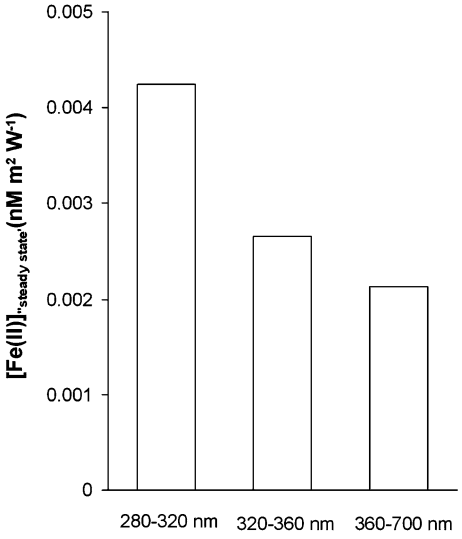

Fig. 11. The mean Fe(II) concentration in Marsdiep at steady state/highest point due to the wavelength regions 280–320, 320–360 and 360–700 nm normalized to 1 W m^{-2}.

Changing the wavelength range of the optical treatments of the Marsdiep water showed that less Fe(II) was formed upon excluding the lower wavelength regions UVB and UVA from the optical treatment. Comparing the values for the steady state normalized to 1 W m^{-2} between the different wavelength regions shows us that UV-B produces most Fe(II), followed by UV-A and VIS, respectively (Fig. 11).

4. Summary and conclusion

We conclude from the present study that UV does not significantly photodegrade the organic Fe(III)-binding ligands in the waters of the Marsdiep and the Scheldt estuary. The UV light has no influence on the organically complexed Fe(III) fraction and will not influence the transport of the dissolved organically complexed Fe(III) phase to the coastal zone.

The concentrations of Fe(II) formed during irradiation experiments were very low (<240 pM). The concentrations of organic Fe(III)-binding ligands are in the order of 24.4 nM for the Marsdiep and 4.6 nM for the Scheldt. Thus, the majority of the organically complexed iron in the Marsdiep water is not involved in the photoproduction of Fe(II) and is not being photodegraded during the process of photoreduction of Fe(III) bound to organic ligands. The pattern of Fe(II) production resembled the pattern observed during irradiation experiments with inorganic colloidal iron like ferrihydrite and goethite (Waite and Morel, 1984; Wells and Mayer, 1991) or amorphous iron oxyhydroxides

(Rijkenberg et al., submitted for publication), suggesting that it might be a colloidal Fe fraction responsible for the photoproduced Fe(II).

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