UNIVERSIDAD DE LAS PALMAS
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TESIS DOCTORAL

Chemistry of Fe(II) in the presence of organic exudates from phytoplankton and of copper in seawater

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Y para que así conste, y a efectos de lo previsto en el Artº 73.2 del Reglamento de Estudios de Doctorado de esta Universidad, firmo la presente en Las Palmas de Gran Canaria, a 27 de Mayo de 2011.

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Chemistry of Fe(II) in the presence of organic exudates from phytoplankton and of copper in seawater

Química del Fe(II) en presencia de exudados orgánicos de origen fitoplanctónico y de cobre en agua de mar

Tesis Doctoral presentada por D. Aridane González González para obtener el grado de Doctor por la Universidad de Las Palmas de Gran Canaria.

Dirigida por Dra. Dª. J. Magdalena Santana Casiano
Dr. D. Melchor González Dávila

La Directora **El Co-Director** **El Doctorando**

A mis padres y
mis hermanos.

A Saray y
Juan Antonio.

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We must not forget that when radium was discovered no one knew that it would prove useful in hospitals. The work was one of pure science. And this is a proof that scientific work must not be considered from the point of view of the direct usefulness of it. It must be done for itself, for the beauty of science, and then there is always the chance that a scientific discovery may become like the radium a benefit for humanity.

Marie Curie (1867 - 1934), Lecture at Vassar College, May 14, 1921

ABSTRACT

The oxidation of Fe(II) has been studied in seawater in the presence of high nutrient concentrations, organic exudates and copper. In addition, the Fe(II) oxidation has been studied in a wide range of physical-chemistry properties, as the nutrient concentration, pH, temperature and salinity.

The experimental results obtained in the present PhD Thesis demonstrated the fragility of the Fe(II) chemistry in the marine environment, showing a strong interaction with the most common nutrients (nitrates, phosphates and silicates) which are usually used in laboratory experiments. These nutrients are also found at higher concentrations in coastal waters under eutrophication conditions. Silicate ($Si(OH)_4$) is the main responsible of the increment on the Fe(II) oxidation rate constant measured in these studies.

The role of the organic exudates, excreted by phytoplankton species, on the oxidation of Fe(II) was carried out for two different species, *Phaeodactylum tricornutum* and *Dunaliella tertiolecta*. For both cases, the sum of the organic exudates excreted produced the decreasing of the Fe(II) rate constant in seawater, indeed as a function of pH, temperature and salinity. In addition, this retarding has a lineal dependence with the cellular density in the experimental culture.

For all the studied cases, a kinetic model was developed in order to explain the experimental results. These models can help to understand the speciation of Fe(II) and the contribution of each Fe(II) species to the overall rate constant, under the experimental conditions taken into account in the present work. From these models can be extracted that $Fe(H_3SiO_4)^+$ species plays an important role both in the speciation and the oxidation process.

Furthermore, the organic ligands considered in these models also play a crucial role in the Fe(II) speciation and fractional contributions of the Fe(II) organically complexed to the overall rate constant.

The competition between Fe(II) and copper has also been studied in this PhD Thesis. The oxidation of Cu(I) was studied previously in seawater at nanomolar concentrations. In this case, the oxidation of Fe(II) showed a strong dependence with the Cu(II) concentration in solution, as a function of pH, bicarbonate concentration and hydrogen peroxide concentration where the Fe(II) rate constant always increased with the Cu(II) concentration.

RESUMEN

La oxidación de Fe(II) en agua de mar ha sido estudiada en presencia de altas concentraciones de nutrientes, exudados orgánicos y de cobre. Además, la oxidación de Fe(II) ha sido estudiada en un amplio rango de propiedades físico-químicas, como son las concentraciones de nutrientes, el pH, la temperatura y la salinidad.

Los resultados experimentales obtenidos en la presente Tesis Doctoral evidencian la fragilidad de la química del Fe(II) en el medio marino, mostrando una fuerte interacción con los nutrientes mayoritarios (nitratos, fosfatos y silicatos) comúnmente utilizados en estudios de laboratorio, también presentes en altas concentraciones en zonas costeras. De los tres nutrientes estudiados, el $Si(OH)_4$ es el principal responsable del incremento observado en la constante de velocidad de oxidación para el Fe(II).

El efecto que desempeñan los compuestos orgánicos exudados por el fitoplancton se estudió para dos especies, *Phaeodactylum tricornutum* y *Dunaliella tertiolecta*. En ambos casos se observó que la mezcla de los exudados orgánicos producían una disminución de la velocidad de oxidación de Fe(II) en agua de mar, siendo función del pH, la temperatura y la salinidad. Además, esta disminución describió una importante dependencia lineal con la densidad celular existente en el cultivo experimental.

En todos los casos estudiados se desarrolló un modelo cinético teórico que describiera los resultados experimentales, con el que se puede conocer la especiación del Fe(II) y la contribución de cada una de las especies de Fe(II) a la velocidad total del proceso de oxidación, bajo las condiciones experimentales consideradas en cada estudio. De estos modelos

se extrae que la especie $Fe(H_3SiO_4)^+$ juega un papel importante en condiciones de altas concentraciones de silicatos, tanto en la especiación como en la velocidad del proceso. Pero además, los compuestos orgánicos que se han considerado en dicho modelo, juegan un papel fundamental, tanto en la especiación de Fe(II) como en la contribución de las especies complejadas orgánicamente de Fe(II) al proceso global de oxidación.

El efecto competitivo entre Fe(II) y cobre ha sido también estudiado en esta Tesis Doctoral, para lo que tuvo que ser estudiado previamente la cinética de oxidación de Cu(I) a nivel nanomolar en agua de mar. En este caso, se obtuvo una fuerte dependencia de la velocidad de oxidación de Fe(II) con la concentración de Cu(II) en la disolución. En todos los casos, tanto en función del pH, la concentración de bicarbonato como la concentración de peróxido de hidrógeno, la velocidad de oxidación de Fe(II) aumentó con la concentración de Cu(II).

Los resultados obtenidos en esta Tesis Doctoral permiten profundizar en la química del Fe(II) en agua de mar, pero además abre numerosas puertas para incrementar el conocimiento del ciclo biogeoquímico del Fe(II) en aguas naturales.

THESIS PREVIEW

The present PhD thesis was carried out at the Universidad de Las Palmas de Gran Canaria, in the QUIMA group of the Chemistry Department. The Thesis is entitled "***Chemistry of Fe(II) in the presence of organic exudates from phytoplankton and of copper in seawater***". This thesis compiles a total of five articles framed within the research projects CTM2006-09857, CTM2009-12526 and CTM2010-19517-MAR. The project was made possible by the FPI grant with reference number BES-2007-15776. The PhD Thesis was supervised by Drs. J. Magdalena Santana Casiano and Melchor González Dávila.

This PhD Thesis is structured into a general introduction that explains the most relevant aspects of Fe(II) chemistry in the ocean and the need for studying the interaction between Fe(II) and organic exudates excreted by microalgae in marine environments. Chapters start with the study of the Fe(II) oxidation in seawater at high nutrient concentration (nitrate, phosphate and silicate), because this is the seawater media that was used to culture the species selected in this Thesis (*Phaeodactylum tricornutum* and *Dunaliella tertiolecta*). The effect of the organic exudates excreted by microalgae species was studied as a function of pH, temperature and ionic strength (salinity). Finally, the study of the competitive process between Fe(II) and copper in seawater is presented. The oxidation of Cu(I) in seawater at nanomolar levels was first characterized due to the lack of previous work at these levels.

In order to comply with the requirements established by the Universidad de Las Palmas de Gran Canaria for PhD Thesis (BOULPGC. ART. 2 Cap. 1, 5 November 2008), a Spanish summary containing more than 50 pages is included.

PRESENTACIÓN DE LA TESIS

La presente Tesis Doctoral ha sido desarrollada en la Universidad de Las Palmas de Gran Canaria, dentro del grupo de investigación QUIMA del Departamento de Química, en la Facultad de Ciencias del Mar, titulada "***Química del Fe(II) en presencia de exudados orgánicos de origen fitoplanctónicos y de cobre en agua de mar***". La Tesis ha sido elaborada a partir de la recopilación de cinco trabajos de investigación englobados dentro de los proyectos CTM2006-09857, CTM2009-12526 y CTM2010-19517-MAR. Además ha sido posible gracias a la beca FPI con referencia BES-2007-15776. La Tesis Doctoral ha sido dirigida por los Doctores J. Magdalena Santana Casiano y Melchor González Dávila.

La Tesis Doctoral está estructurada en una introducción general, que explica los aspectos más relevantes de la química del Fe(II) en el océano y la necesidad de realizar estudios de interacción entre Fe(II) y los exudados orgánicos excretados por especies de microalgas en el medio marino. Los capítulos comienzan estudiando la oxidación de Fe(II) en agua de mar a altas concentraciones de nutrientes (nitratos, fosfatos y silicatos), ya que éste será el agua de mar de referencia utilizada para cultivar las especies seleccionadas en esta Tesis (*Phaeodactylum tricornutum* y *Dunaliella tertiolecta*). El efecto de los exudados orgánicos excretados por las especies de microalgas se estudió en función del pH, la temperatura y la fuerza iónica (salinidad). Finalmente, se estudió el efecto competitivo entre Fe(II) y cobre en agua de mar, para lo que se estudió en primer lugar la oxidación de Cu(I) en agua de mar a niveles nanomolares de concentración, ya que no existen estudios a estos niveles de concentración en la bibliografía.

Con el objeto de cumplir los requisitos establecidos por el Reglamento de Elaboración, Tribunal, Defensa y Evaluación de Tesis Doctorales de la Universidad de Las Palmas de Gran Canaria (BOULPGC. Art. 2 Cap. 1, 5 de Noviembre de 2008), se ha procedido a escribir un resumen de más de 50 páginas en Español.

CONTENTS / ÍNDICE

CHAPTER I:

Introduction

INTRODUCTION

Iron is a crucial element in the environment because it is an essential micronutrient for the life of organisms, playing an important role for the utilization and production of gases related to climate change such as CO_2 or dimethylsulfoxide (Bowie et al., 2002). There is a great paradox about iron in oceans because it is the fourth most abundant element on Earth, but it is a trace element in oceanic waters at subnanomolar levels. Therefore, knowledge of physical-chemistry processes where iron is present in the ocean is fundamental. Some aspects must be investigated deeply (Wells et al., 1995), such as its distribution in the ocean (de Baar and de Jong, 2001) and its interaction with biota (Geider, 1999). Furthermore, iron is present in the ocean as two different oxidation stages, Fe(II) and Fe(III) (Sunda, 2001), adding further difficulties to the study of the biogeochemical cycle of iron in natural waters.

This introduction has been structured into a number of sections, which will help to understand the biochemistry of iron in natural waters and to analyze and discuss the experimental results obtained in the PhD Thesis at hand.

1.1. Sources of iron for the oceans

In natural waters, the concentration of iron is strongly linked to the continental sources. The mean inputs of iron to seawater are rivers, atmospheric depositions, hydrothermal sources and sediments. Therefore, iron concentrations increase close to coastal areas, where the continental influences are more significant (Bowie et al., 2002). Accordingly, the

variability of the iron sources in respect to the geographic locations causes the biogeochemistry of iron to depend on each basin and its properties (de Baar and de Jong, 2001).

In the following, each of the sources of iron for the oceans will be described:

- *River inputs:*

Input of iron and other trace elements from rivers has been reported by Chester (1990). River inputs are irregularly located, above all in the Atlantic Ocean, where the most important rivers are the Amazon, Orinoco, Congo and Mississippi.

Iron inputs from river discharges to the seawater are predominantly as particulated and dissolved Fe. The amount of particulated Fe from rivers is around 500 times higher than the amount of dissolved Fe (Chester, 1990). This amount of particulated Fe is practically negligible in ocean waters because the majority of this iron precipitates in delta or discharge regions (de Baar and de Jong, 2001).

Dissolved Fe also includes colloidal Fe (Fox, 1988), and when it reaches the surface waters can flocculate due to the difference in ionic strength between both media. Organic matter that is transported from the river can yield the iron complexation and the formation of particles (Fox, 1988). Therefore, the most important sink of particulated Fe, from river waters, is the deposition in delta regions, although a fraction can be transported to oceanic basins through transport processes or turbidity currents in deep zones and submarine canyons.

- *Atmospheric deposition:*

Atmospheric deposition probably represents the dominant iron source for surface oceans (Duce and Tindale, 1991). Iron inputs from the atmosphere can be of two types, dry deposition (70%) and wet deposition (30%) (Jickells and Spokes, 2001). Dry deposition introduces large quantities of dissolved iron to surface waters even higher than from river inputs. In this case, iron is predominantly as Fe(III).

From the perspective of Fe(II), the most important contribution comes from wet deposition because it has an acid pH (pH=4-7) and a considerable fraction of dissolved Fe(II) can be measured in surface waters (Millero et al., 1995a). In addition, rainwater is one of the most important sources of hydrogen peroxide for the ocean (Kieber et al., 2001), participating in the Fe(II) oxidation processes in seawater. Rainwater also contributes to the input of organic ligands capable of complexing Fe(II), even after its deposition in the seawater (Willey et al., 2008).

- *Marine sediments:*

Marine sediments are another kind of iron source for the ocean. This fact has been demonstrated due to the increase of iron concentration near the continental margins (Chester, 1990; Bowie et al., 2002). More than half of the iron in sediments is present as iron oxides and organic matrixes. Generally, the higher Fe(II) concentrations are explained as a consequence of the diagenesis process (Davison et al., 1991; Lovely, 1991; Burdige, 1993; Canfield et al., 1993; Postma, 1993; van Capellen and Wang, 1996).

- *Hydrothermal sources:*

Hydrothermal circulation across the ocean basin generates inputs of reduced metals, as Fe and Mn, which precipitate when they arrive in ocean waters because of the gradient of temperature and form oxy-hydroxides (Campbell, 1991; German et al., 1991). This process allows the formation of ferromanganese deposits in zones near its emission. The Fe(II) emitted is rapidly oxidized and the net input of iron from hydrothermal sources is negligible compared to others such as diagenesis, rivers and atmospheric depositions.

The biogeochemical cycle of iron in the ocean is shown in Figure 1-1. The most important sources for surface waters are rivers and/or atmospheric depositions, as described above. Sediments only contribute notably in deep waters when the iron can come back to the ocean circulation.

According to Figure 1-1, organic matter plays a key role on iron chemistry in natural waters. The organic matter can come from rivers, rainwater, or be produced directly in the ocean. In addition, oxygen reactive species (O_2^- and H_2O_2) are produced from solar radiation and photodegradation of organic matter (Zika et al., 1985a; Moore et al., 1993). This process also produces the rupture of metal-ligand complexes and releases Fe(II) and Fe(III) to the seawater, where Fe(III) is highly insoluble. Fe(III) is common in colloidal fraction and organically complexed (99%) (Gledhill and van den Berg, 1994; Donat and Bruland, 1995; Wu and Luther, 1995; Bergquist et al., 2007).

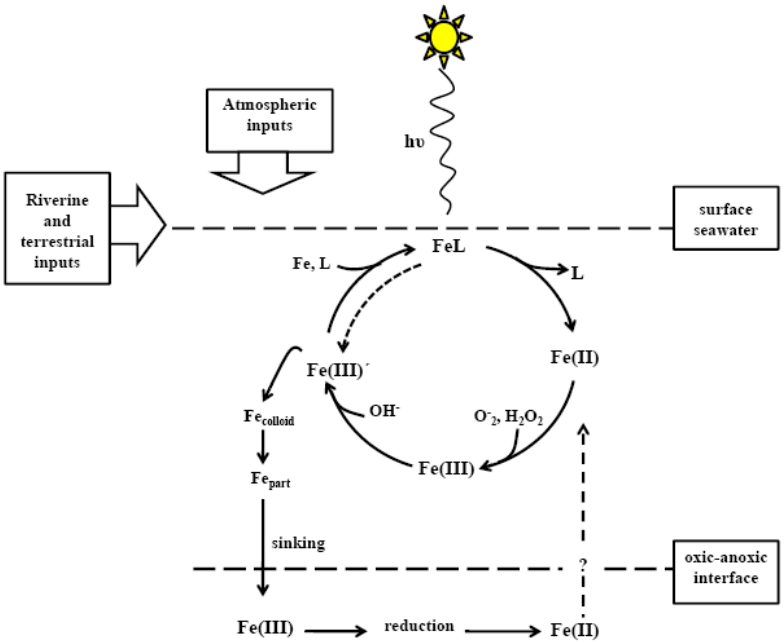

Figure 1-1. The biogeochemical cycle of iron in the ocean, modified from (Barbeau, 2006).

The vertical profile of iron has a nutrient-type profile in open ocean waters, where the atmospheric inputs are not important (Johnson et al., 1997), except in some geographic locations where atmospheric deposition introduces important amounts of dust to the surfaces, as the North-East Atlantic Ocean (Duce and Tindale, 1991). Furthermore, iron can also be involved in adsorption processes, modifying its profile in the water column (Bruland et al., 1994; de Baar and de Jong, 2001).

A typical profile of total dissolved iron in the North Atlantic Ocean (Figure 1-2) shows that there is a minimum of iron in surfaces waters because it is related with the organisms and it is used by bacteria and phytoplankton. Within the aphotic zone, iron undergoes microbial remineralization (Redfield et al., 1963). In addition, there can be a maximum of iron near the

thermocline, which sometimes coincides with a maximum of chlorophyll *a*, and can be explained due to the organic complexation process, either active or passive excretion of specific iron ligands, as siderophores or porphyrins, due to the growth of biota (van den Berg, 1995; Rue and Bruland, 1997; Hutchins et al., 1999a). Another explanation can be that this maximum of iron coincides with the maximum of chlorophyll *a* due to the regeneration of iron from the degradation of organic matter, or from the complexation with organic ligands excreted from the ingestion of microorganisms by the zooplankton (Hutchins and Bruland, 1994; Barbeau and Moffett, 1998). This cycle is completed when the iron returns to surface waters due to upwelling and turbulent mixing. In addition, the vertical profile of iron is highly altered by the external inputs (Bowie et al., 2002). It must be highlighted that Fe(II) can also present a maximum at 20 and 50 m, related to the stabilization with organic ligands (Bowie et al., 2002; Boye et al., 2006). Fe(II) concentrations are negligible at depths below 200 m, increasing only close to basins due to the sediment sources.

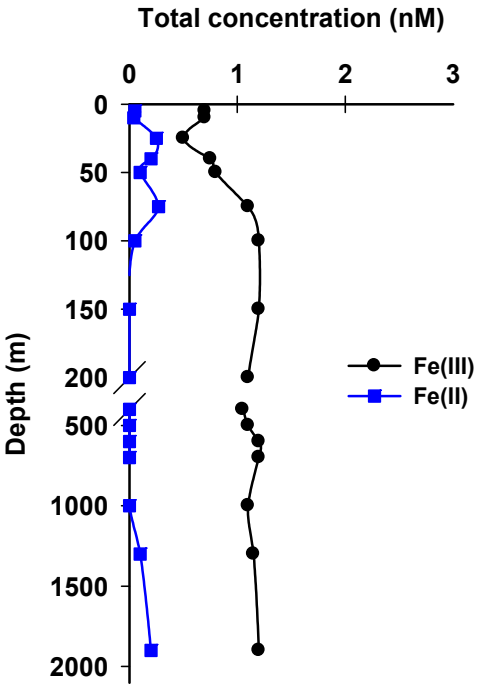

Figure 1-2. Typical profile of total dissolved Fe(II) and Fe(III) in the East Atlantic Ocean (Boye et al., 2006).

1.2. Iron utilization by marine organisms

Once iron is in surface waters, it can be used by organisms. Iron is an essential micronutrient for life, growth and metabolism. Table 1-1 shows the main enzymes and processes where iron is involved with marine organisms.

Table 1-1. Biochemical processes where Fe is involved within organisms (Stumm and Morgan, 1996).

Metal	Enzyme	Functions
Fe	Cytochrome f	Photosynthetic electron transport
	Cytochrome b and c	Electron transport in respiration and photosynthesis
	Ferrodoxin	Electron transport in photosynthesis and nitrogen fixation
	Iron-Sulphur proteins	Photosynthetic and respiratory electron transport
	Catalase	H_2O_2 breakdown to H_2O and O_2
	Peroxidase	H_2O_2 reduction to H_2O
	Chelatase	Porphyrin and phycobilin protein synthesis
Fe/Mo	Nitrogenase	Nitrogen fixation
	Nitrate and Nitrite reductases	Nitrate reduction to ammonia
Fe/Mn	Superoxide dismutase	Disproportionation of $O_2^{\cdot-}$ radicals to O_2 and H_2O_2

Iron participates actively in photosynthesis, respiration and nitrogen fixation. Iron takes also part in the porphyrin and phycobilin synthesis; both of them are key in the formation of heme groups. In addition, iron is involved in the detoxification of reactive oxygen species ($O_2^{\cdot-}$ and H_2O_2). Therefore, these processes cause iron, in spite of being a micronutrient, to be of special interest for organisms (Sunda and Huntsman, 1997).

There is evidence that in the ocean there are internal sources of iron related to its micronutrient condition, as grazing and excretion from zooplankton, viral lysis and bacterial remineralization. All of them release a mix of iron compounds into the seawater which can affect the chemistry of metal in solution (Hutchins et al., 1993).

Several studies have shown that marine phytoplankton is able to access to chelated iron (Glober et al., 1997; Hutchins et al., 1999b; Poorvin et al., 2004; Kustka et al., 2005). Laboratory studies support the idea that

unchelated Fe(III) is highly available for uptake and that it is an important source of the Fe taken up by phytoplankton (Morel et al., 2008). The Fe uptake system of microorganisms can be classified into three categories: (1) transport systems that are specific and particular iron compounds such as Fe-citrate, Fe siderophores or hemes, (2) Fe(II) transporters of various specificities, including divalent metal ion transporters and oxidase-permease complexes that oxidize Fe(II), (3) transport system that includes reductasas able to reduce various Fe(III) species at the cell surface and deliver Fe(II) to option (2).

Furthermore, there are evidences for direct internalization of siderophores by either eukaryotic or prokaryotic phytoplankton. However, both groups appear to be able to acquire iron from organic chelates using reductive mechanisms with different degrees of efficiency (Hopkinson and Morel, 2009). Beside carboxylic acids and catechols, hydroxamates are among the principal functional groups involved in Fe(III) binding in strong chelators like siderophores (Shi et al., 2010).

The transport system of iron based on the siderophore theory occurs predominantly in cyanobacteria (McKnight and Morel, 1979; Boyer et al., 1987; Wilhelm, 1995), although it must be remarked that it is an expensive metabolic mechanism (Völker and Wolf-Gladrow, 1999), so it is usually used in iron-deficient conditions.

Beside the reduction of iron from siderophores, an important reduction has also been studied from diatom cultures, marine as well as fresh (Anderson and Morel, 1980; Maldonado and Price, 2000). This process seems to be affected by more than one transmembrana reductase which is able to reduce Fe(II) organic and inorganic complexes, including Fe-siderophores (Allnutt and Bonner, 1987; Jones et al., 1987; Maldonado and Price, 2000; 2001).

Recent studies (Santana-Casiano et al., 2010) demonstrated that siderophores, where catechol is one of the main functional groups, are capable of reducing Fe(III) to Fe(II), allowing for iron uptake from marine environment. In addition, phytoplanktonic species also excrete important amounts of thiolic groups to the seawater (cysteine and gluthatione), which are strong ligands for metals in the ocean. Cysteine and gluthatione have been measured in natural seawater (van den Berg et al., 1988; Le Gall and van den Berg, 1993). Furthermore, diatoms have been identified as a relevant species due to their capacity to produce higher concentrations of thiol groups than other phytoplankton species (Vasconcelos et al., 2002).

1.3. Fe(II) in seawater

Iron predominantly exists in seawater as Fe(III) because it is the thermodynamically stable form. Fe(III) is very reactive in respect to hydrolysis, adsorption and formation of organic complexes. The fraction of Fe(III) which is complexed with organic ligands is higher than 99% (Gledhill and van den Berg, 1994; Wu and Luther, 1995). However, unusual concentrations of Fe(II) have been measured in different locations around the world. Hong and Kester (1986) found concentrations of Fe(II) above 12 nmol kg^{-1} on the coast of Peru. King et al. (1991) and King (1998) also found Fe(II) concentrations higher than 15 nmol kg^{-1} and 4.2 nmol kg^{-1} in surface seawater (Narragansett Bay, USA). O'Sullivan et al. (1991) reported subnanomolar levels of Fe(II) (0.4 nmol kg^{-1}) in Equatorial Pacific surface waters. In Fuka Bay (Japan), Fe(II) concentrations were related to the spring bloom (Kuma et al., 1992). In addition, during iron fertilization experiments, nanomolar concentrations of Fe(II) were measured after 5 and 8 days of the initial fertilization (Croot et al., 2001; 2005).

Theoretically, once Fe(II) reaches surface waters, it must be oxidized rapidly to Fe(III), with half-life times ($t_{1/2}$) reaching from seconds to several minutes, as a function of the physical-chemistry properties of the media (Millero et al., 1987; Rose and Waite, 2002; Santana-Casiano et al., 2005). In order to be able to measure Fe(II) in oceanic waters, however, some sources of Fe(II) other than external sources as wet deposition or rainwater should exist (Kieber et al., 2001; Hopkinson and Barbeau, 2007; Willey et al., 2009), such as *in situ* production or stabilization of Fe(II), (1) photoreduction of Fe(III) (Waite and Morel, 1984; Wells and Mayer, 1991; Kuma et al., 1992; Miller et al., 1995; Voelker and Sedlak, 1995; Waite et al., 1995), (2) direct or indirect reduction of Fe(II) from marine organisms via enzymatic mechanisms (Anderson and Morel, 1980; Jones et al., 1987; Hutchins et al., 1993; Barbeau et al., 1996; Maldonado and Price, 1999; 2000; Roy et al., 2008). In this case, photoproduction has been considered the most important process to regenerate Fe(II) in surface waters. Therefore, the role of photoproduction must be considered for the increment in the solubility and bioavailability of iron in natural waters (Pehkonen et al., 1992; Johnson et al., 1994; Miller and Kester, 1994; Waite et al., 1995). Another study (Finden et al., 1984) showed clear evidence of photochemical reduction of Fe(III), measuring Fe(II) fixed with bazophenantroline in freshwater. In coastal waters, pH=6.5, Waite and Morel (1984) measured Fe(II) regeneration. In addition, Kuma et al. (1992) demonstrated Fe(II) production induced by light in laboratory experiments for seawater as well as diatom cultures. These studies allow concluding that carboxylic groups (Waite and Morel, 1984; Cunningham, 1988), compounds which contain thiolic groups (Waite and Torikov, 1987), alcohols (Cunningham et al., 1985) and fulvic acids (Waite and Morel, 1984) have an important relation with the photochemical reduction process of Fe(III), from Fe(III) oxides. Specifically, oxalic acids, citric, malic, gliceric, salicilyc, tartaric, gluconic and p-hydroxibenzoic produce an increase in the photoproduction of Fe(II) in solution (Cunningham, 1988; Kuma et al., 1992). Therefore, the

photochemical processes and the presence of specific organic ligands of Fe(II) can explain that Fe(II) will remain in surface waters.

The most accepted mechanism for the photoproduction of Fe(II), in surface waters, is the reduction of complexes of (hydro)oxides of Fe(III) (King et al., 1993),

$$Fe(OH)^{2+} \xrightarrow{h\upsilon} Fe-OH^{*} \rightarrow Fe^{2+} + OH^{-} \quad (1)$$

where only $Fe(OH)^{2+}$ is notably reactive. At pH=8.0, iron is strongly hydrolyzed and forming $Fe(OH)_x$ (x=2-4) (Millero, 1998) and these species must be less reactive. Some kind of reaction of ligand-metal charge transfer (LMTC) must be considered with the organic compounds (Rich and Morel, 1990; King et al., 1993). Consequently, the free Fe(II) will be available for phytoplankton uptake (Hutchins et al., 1999a; Maldonado and Price, 2001; Sunda, 2001). Finally, the organically complexed Fe(II) should be assimilated by organisms via redox and ligand interchange processes (Hutchins et al., 1999a; Maldonado and Price, 2001; Sunda, 2001; Rose et al., 2005).

1.4. Oxidation kinetic of Fe(II) in seawater

An important effort has been made to study the oxidation kinetics of Fe(II) in natural waters, allowing to improve the knowledge of the biogeochemical cycle of Fe(II) in aquatic environments (Stumm and Lee, 1961; Kester et al., 1975; Davison and Seed, 1983; Waite and Morel, 1984; Millero et al., 1987; Millero and Sotolongo, 1989; Liang et al., 1993; King et al., 1995; Rose and Waite, 2002; Santana-Casiano et al., 2004;2005;2006;

González-Dávila et al., 2006; Trapp and Millero, 2007). These studies have looked deeply into the inorganic processes where Fe(II) is involved.

The most accepted mechanism for the oxidation of Fe(II) in natural waters is known as Haber-Weiss mechanism, which includes the molecular oxygen oxidation sequence, superoxide radical, hydrogen peroxide and hydroxyl radical (Haber and Weiss, 1934; Fallab, 1967; Millero and Sotolongo, 1989) :

$$Fe(II) + O_2 \rightarrow Fe(III) + O_2^{\cdot -} \quad (2)$$

$$Fe(II) + O_2^{\cdot -} \xrightarrow{2H+} Fe(III) + H_2O_2 \quad (3)$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{\cdot} + OH^- \quad (4)$$

$$Fe(II) + OH^{\cdot} \rightarrow Fe(III) + OH^- \quad (5)$$

In addition, this mechanism can be completed by considering the reduction of Fe(III), the hydrolysis and formation of colloidal Fe(III), such as the competitive reaction between active oxygen species (O_2^- and H_2O_2) with other species such as copper (Rose and Waite, 2002; Santana-Casiano et al., 2005).

$$Fe(III) + O_2^{\cdot -} \rightarrow Fe(II) + O_2 \quad (6)$$

$$Fe(III) + 3OH^- \rightarrow Fe(OH)_{3(s)} \quad (7)$$

$$Cu(II) + O_2^{\cdot -} \rightarrow Cu(I) + O_2 \quad (8)$$

$$Cu(I) + O_2^{\cdot -} \xrightarrow{2H+} Cu(II) + H_2O_2 \quad (9)$$

The Fe(II) oxidation rate equation with O_2 is given by:

$$\frac{d\,[Fe(II)]}{dt} = -k_{app}[Fe(II)][O_2] \quad (10)$$

Under air saturated conditions, the oxidation rate equation can be considered as a pseudo-first order, where the rate constant $k_{app} = k' / [O_2]$:

$$\frac{d\,[Fe(II)]}{dt} = -k'\,[Fe(II)] \quad (11)$$

Apparent rate constant for Fe(II) oxidation is a function of the weighted sum of oxidation rates for individual Fe(II) species, inorganic and organic (Equation 12).

$$k_{app} = \sum_i \alpha_i k_i + \sum_i \alpha_L k_L \quad (12)$$

where α_i are the molar fractions of each inorganic Fe(II) species and α_L correspond to each Fe(II) organically complexed species.

The oxidation of Fe(II) has been studied in natural waters, at micromolar levels (Kester et al., 1975; Murray and Gill, 1978; Roekens and van Grieken, 1983; Waite and Morel, 1984; Millero et al., 1987; Millero and Izaguirre, 1989) and at nanomolar levels (King et al., 1995; Emennegger et al., 1998; King, 1998; King and Farlow, 2000; Rose and Waite, 2002; González-Davila et al., 2005; Santana-Casiano et al., 2005).

Millero and Izaguirre (1989) and King (1998) have demonstrated that Fe(II) interaction with other major elements of the seawater affects the oxidation rate. King (1998) demonstrated the strong dependence of the

oxidation rate constant with the carbonate concentration, as in equations 13-15.

$$Fe^{2+} + CO_3^{2-} \leftrightarrow FeCO_3 \quad (13)$$

$$Fe^{2+} + 2CO_3^{2-} \leftrightarrow Fe(CO_3)_2^{2-} \quad (14)$$

$$Fe^{2+} + CO_3^{2-} + OH^- \leftrightarrow Fe(CO_3)OH^- \quad (15)$$

The Fe(II) oxidation behaviour showed differences between micromolar concentration and nanomolar levels. At micromolar concentrations, Fe(II) oxidation by oxygen showed a 4:1 stoichiometry (Millero et al., 1987; King, 1998; Santana-Casiano et al., 2000), while the Fe(II) oxidation by H_2O_2 showed a 2:1 stoichiometry (Millero and Sotolongo, 1989; González-Davila et al., 2005).

The Fe(II) oxidation at nanomolar concentration, in air-saturated conditions, has been recently studied (Santana-Casiano et al., 2005), where it must be highlighted that the oxidation rate increased as a function of the HCO_3^- in solution. In addition, as a function of pH, the oxidation rate constant was higher at pH $\leq$ 7.5, although it was lower at pH $\geq$ 7.5 than its value on a micromolar scale. This result is due to the key role of oxidation intermediates, which cannot be observed in micromolar concentrations (Millero et al., 1987; Santana-Casiano et al., 2005).

The Fe(II) oxidation rate has also been studied in a wide range of H_2O_2 concentrations (González-Davila et al., 2005; Santana-Casiano et al., 2006) at nanomolar levels of Fe(II). These studies have a special relevance because they have described an important competition between O_2 (μmol L^{-1}) and H_2O_2 (nmol L^{-1}) for the Fe(II) oxidation. In natural waters, the concentration of H_2O_2 range from 10 to 150 nmol L^{-1}, in oligotrophic waters,

until above 500 nmol L^{-1} in coastal waters (Zika et al., 1985a,b; Moore et al., 1993; Hanson et al., 2001).

In oceans, the photooxidation of organic matter causes the production of reactive oxygen species (Equations 16-18) (Sharma and Millero, 1988a).

$$org \xrightarrow{h\upsilon} org^{+} + e^{-} \quad (16)$$

$$e^{-} + O_2 \rightarrow O_2^{\bullet -} \quad (17)$$

$$2O_2^{\bullet -} + 2H^{+} \rightarrow H_2O_2 + O_2 \quad (18)$$

Under these conditions, Equation 4 is not totally dependant of Equations 2-3. In addition, the oxygen reduction is given by Equations 19-21.

$$O_{2(g)} + 4H^{+} + 4e^{-} \leftrightarrow 2H_2O \quad (19)$$

$$O_{2(g)} + 2H^{+} + 2e^{-} \leftrightarrow H_2O_2 \quad (20)$$

$$H_2O_{2(g)} + 2H^{+} + 2e^{-} \leftrightarrow 2H_2O \quad (21)$$

where the acquisition of the first two electrons from the reaction of O_2 to H_2O_2 is energetically less favourable than the acquisition of the next two electrons from the reaction of H_2O_2 in order to obtain H_2O (Bruland and Rue, 2001; Rose and Waite, 2002). Therefore, the production of H_2O_2 begins from the photooxidation of organic matter, and it will be more relevant in coastal waters (Zika et al., 1985a; Moore et al., 1993).

Under seawater conditions, the speciation of Fe(II) is controlled by Fe^{2+}, $FeCl^{+}$ and $FeSO_4$ for a wide range of pH (6.0-8.2) where the hydrolyzed species of iron, $Fe(OH)_x^{(2-x)-}$ (x=1-2), contribute less to the speciation. At alkaline pH (pH > 8.2), $FeCO_3$ is the dominant species. However, the fractional contribution to the overall rate constant for each Fe(II) species is controlled by the hydrolyzed species of Fe(II), where $Fe(OH)_2$ is the most important one at pH ≥ 8.0, with $Fe(CO_3)_2^{2-}$ the second one. According to the fractional contribution of these species, when oxygen is predominant, the oxidation rate constant showed a second order dependence as a function of pH with the oxygen and a first order dependence with the H_2O_2, compensating the difference in concentrations between O_2 and H_2O_2. Even so, the role of H_2O_2 is important, even at air-saturated levels, although at seawater pH, oxygen is the most relevant oxidative species (González-Dávila et al., 2006).

1.5. The role of organic ligands on the oxidation of Fe(II)

The chemistry of iron is strongly, directly or indirectly, linked to the presence of organic matter in the environment (Figure 1-1). From the first studies on the speciation of Fe(III) in marine environments, the percentage of organically complexed iron was indicated as ≥ 99% (Gledhill and van den Berg, 1994; Wu and Luther, 1995). They showed two types of ligands according to their complexing capacities: L_1 and L_2. The L_1-type of ligands has a huge affinity for iron, and their conditional constant is $K^{cond} \sim 10^{12\text{-}13}$ $Lmol^{-1}$. The technology advances have allowed to identify some of these ligands from marine siderophores, as Aquacheline, Petrobactin, Aerobactin and Desferroxamine B (Macrellis et al., 2001; Barbeau et al., 2002; Gledhill et al., 2004) and it has been possible to account the conditional constants for these siderophores, which are similar to the L_1-type ligands. Generally, this type of ligand (L_1) has been estimated in surface oceanic waters (Barbeau et

al., 2002). The second type of ligand (L_2) has been related with intracellular material, like protoporphyrin, with a conditional constant $K^{cond} \sim 10^{10\text{-}11}$ L mol^{-1} (Rue and Bruland, 1995; 1997; Cullen et al., 2006).

The majority of the studies of the complexation of iron in natural waters have been carried out for Fe(III). Few studies were directed to the effect of natural organic ligands on the oxidation of Fe(II). In this case, not only the key role of the metal complexes with organic ligands must be considered, because these ligands, through photochemical process, also generate important amounts of superoxide radical.

Organic ligands are one of the factors to provoke the stabilization of Fe(II) over time, making possible the presence of Fe(II) levels in natural waters and its utilization by marine organisms for a longer period of time. The organic matter in solution can accelerate, retard or not affect oxidation of Fe(II) in seawater.

Different organic compounds have been taken into account in natural waters. In freshwater, the tannic acid, gallic acid and pirogallol are highlighted because they prevent the oxidation of Fe(II) due to the formation of stronger complexes. Glutamic acid, tartaric acid and glutamine produce the retardation of the Fe(II) oxidation rate. However, citric acid accelerates the same process. Phenol and histidine do not have any effect on the Fe(II) oxidation rate constant (Theis and Singer, 1973; 1974).

In seawater, another organic compounds such as EGTA (ethylene-glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid) can form stronger complexes that inhibit the oxidation of Fe(II). EDTA (ethylenediaminetetraacetic acid) increases the oxidation of Fe(II) because a complex Fe(III)-EDTA is formed which can also produce Fe(II) from photoreduction. Cystine is a relevant organic compound in seawater that is

capable of reducing Fe(III). The role of cysteine is a function of the cysteine-Fe(II) ratio and cysteine-cystine oxidation. Nevertheless, alanine and glutamic acid do not have an effect on the oxidation of Fe(II) in seawater (Santana-Casiano et al., 2000).

Natural organic matter has also been considered in seawater, where the majority of the compounds studied (extracts of sugar cane, acacia, malaleuca and pine (Rose and Waite, 2003a)) accelerated the oxidation kinetics of Fe(II), except three pine extracts and citrate which retarded the oxidation rate. In addition, the effects observed were a function of the concentration for all the studies.

The effect of salicylic acid and phtalic acid on the Fe(II) oxidation has also been studied in seawater (Santana-Casiano et al., 2004). In this case, salicylic acid increased the oxidation rate of Fe(II), while phtalic acid decreased the rate constant. The phtalic acid has been considered as a strong complex reagent for metals in natural waters (Chang and Zylstra, 1999).

Recently, the effect of catechol on Fe(II) regeneration has also been studied (Santana-Casiano et al., 2010). The regeneration process was a function of pH and the major ions in seawater, which described a huge interaction among the semiquinone radical with Mg^{2+} and Ca^{2+} ions. These interactions affected the Fe(II) regeneration in seawater.

Finally, it must be remarked that the organic matter has not only a key role in the oxidation of Fe(II), it is also important for the solubility of iron in marine environment (0.011 nM in NaCl 0.7 M, (Liu and Millero, 2002), allowing for presence of Fe(II) in surface waters for a longer period of time and its availability for phytoplankton uptake.

Therefore, there are research studies that considered individual organic ligands and their interaction with Fe(II), but it is necessary to study, know and characterize the effect of the overall compounds excreted by organisms on the iron chemistry, especially for seawater.

OUTLINE AND AIMS

OUTLINE OF THIS THESIS

The introduction indicates the needs to carry out Fe(II) oxidation experiments in the presence of organic exudates from marine organisms, because the excreted compounds are the main source of specific ligands for iron in seawater. For that, the aim of this PhD Thesis is to characterize the physical chemistry of Fe(II) in the presence of organic exudates excreted by phytoplankton. In addition, due to the competition between different chemical species by the redox oxygen intermediates, in special copper species, it is necessary to characterize the effects of this competition on the chemistry of each metal in natural waters.

The development of the articles that constitutes the present PhD thesis has been carried out for two phytoplankton species, *Phaeodactylum tricornutum* and *Dunaliella tertiolecta*. The first one is the diatom species and the second one is a green flagellate algae. Both species were selected due to great oceanographic dispersion and the knowledge about the organic compounds excreted by them which are of special interest for the metal complexation, as well as the characterization of the most important functional groups on the cell wall that are capable to complex metals in aquatic environments.

The studies developed in this PhD Thesis were carried out in seawater at high nutrient concentrations. These conditions are usually used in f/2 culture media for laboratory experiments. Therefore, the first step should be to characterize the Fe(II) behaviour in the presence of added nutrients in seawater.

Once the Fe(II) behaviour and the interaction between Fe(II) and nutrients are known, the studies about Fe(II) oxidation in the presence of organic exudates from *P. tricornutum* and *D. tertiolecta* were developed.

Finally, interaction studies between Fe(II) and copper were carried out in seawater. Previously, the oxidation of Cu(I) in seawater at nanomolar levels should be studied. Once the Fe(II) and copper oxidation have been described separately, the studies of their interactions were considered.

AIMS OF THIS THESIS

The main aim of the present PhD Thesis was to "*characterize the chemistry of Fe(II) in the presence of organic exudates excreted by phytoplankton species, and in the presence of copper, in seawater*".

This main aim has been structured into five specific aims and five chapters which are included in the PhD Thesis.

- Aim 1: **To characterize the Fe(II) oxidation in natural waters at high nutrient concentrations.**

The two phytoplankton species considered during the PhD Thesis allow us to use the same culture protocol, where seawater was enriched with nutrients that are commonly used in laboratory experiments with f/2 culture media (nitrate, phosphate and silicate). Therefore, the first aim was the study of the interaction between Fe(II) and nutrients in solution and its possible effect on the kinetic oxidation of Fe(II).

In order to reach this aim, a number of studies were carried out about the effect of nutrient concentrations, both all of them together and individual nutrients. This will allow determining what changes are produced on the Fe(II) oxidation as a function of each nutrient, both in seawater and artificial seawater. As the oxidation kinetic depends on the physical-chemistry parameters of the system, this goal implies the study of the effect of pH, temperature and salinity. In this case, it would be possible to obtain the effect that nutrients produce on the oxidation process and to collect experimental data which would help to understand the chemistry of metal at high nutrient concentrations.

The last step to reach this aim was to develop a chemistry kinetic model that described the experimental results obtained previously by considering the interaction between Fe(II) and the nutrients that would be the main responsible factors for the changes in the oxidation rate observed during the experiments.

The development of this aim is presented in Chapter III of this PhD Thesis.

- Aim 2: **To determine the role of organic exudates excreted by *Phaeodactylum tricornutum* on the Fe(II) oxidation rate.**

In order to reach this aim, it would be necessary to characterize the growth of the two species selected in this PhD Thesis under the experimental conditions considered. Once the growth was known, the effect of each growth stage would be considered.

The study of the effect of cell concentrations, for each day of culture, on the Fe(II) oxidation rate constant should be taken into account. Then, if the effect of ligands is a function of the cellular density, it can be determined.

The effect of pH (7.5-8.2), temperature (5-35ºC) and salinity (10-36.720) on the Fe(II) oxidation rate would be considered for each growth stage. These studies would allow to identify the chemical processes in which Fe(II) was involved in the presence of organic exudates from *P. tricornutum*.

Finally, a kinetic model should be developed according to the experimental results obtained previously in order to explain the Fe(II) speciation and the fractional contribution of each Fe(II) species to the overall

oxidation rate constant. In this case, the model should consider a simple ligand model, interacting with Fe(II). The equilibrium constant and rate constant for proposed species would be calculated from the model. This would describe the majority Fe(II) species in a solution enriched with organic exudates and the individual contributions to the overall rate constant in the oxidation process. The development of this aim constitutes Chapter IV.

- Aim 3: **To study the effect of organic exudates excreted by *Dunaliella tertiolecta* on the Fe(II) oxidation rate in seawater.**

The approach indicated in aim 2 was also extended for the effect of *Dunaliella tertiolecta* organic exudates. The growth of the species would be characterized under the experimental conditions considered and the growth stages would be identified. The effect of growth stages should be carried out. Then, the interaction between Fe(II) and the organic exudates from *D. tertiolecta* would be characterized.

The study should be done at different pH (7.5-8.2), temperature (5-35ºC) and salinity (10-36.720) for different cell densities in the presence of organic exudates.

Finally, a kinetic model should be developed which would explain the experimental results collected previously. In this case, two possible ligands would be introduced which are capable to complex Fe(II) and have been reported as majorities in seawater. The kinetic model would allow to account the contribution of each Fe(II) species, including organically complexed, to the overall rate constant. These results are presented in Chapter V.

- Aim 4: **To characterize the oxidation kinetic of Cu(I) in seawater at nanomolar levels.**

The oxidation kinetic of Fe(II) is affect by the presence of species that can react and compete for the redox intermediates produced by oxygen or photooxidation of organic matter in marine environments. Copper is one of these species. In order to study this phenomenon, the oxidation and reduction of copper should be characterized previously in seawater at nanomolar concentrations.

The oxidation kinetic of Cu(I) should be described in seawater as a function of pH (7.17-8.49), temperature (5-35ºC), ionic strength (0.1-0.7 M) and bicarbonate concentrations (0-9 mM). In addition, the reduction of Cu(II) should be studied in order to quantify the amount of Cu(I) which is regenerated under the experimental conditions established. These results are presented in Chapter VI.

- Aim 5: **To determine the competition in the oxidation of Fe(II) in the presence of copper in seawater.**

Competitive oxidation kinetics should be carried out between both metals in marine environments. The effect that can be produced in the process should be studied under different Fe(II) and copper (Cu(I) and Cu(II)) concentrations. These results would allow to identify if there is a relationship between metal concentrations and the Fe(II) oxidation rate constant.

Three different ratios between Fe(II) and Cu(II) concentrations should be selected in order to study the effect of pH (6.0-8.5), H_2O_2 concentration (0-500 nM) and $NaHCO_3$ concentration (2-9 mM). These results are presented in Chapter VII.

CHAPTER II:

Experimental

EXPERIMENTAL

The experimental section has been developed in order to cover the general material and methods used in each chapter. In addition, specific considerations are described in each chapter.

2.1. Chemicals

2.1.1. Seawater solution

The Fe(II) oxidation rates were studied in seawater collected off the coast of Gran Canaria (Spain). The salinity was measured with a Portasal 8410A salinometer, obtaining that salinity was 36.720 (Chapter III-V), 36.691 (Chapter VI) and 36.968 (Chapter VII). The seawater samples were always filtered through 0.1 μm in order to both remove particulate and colloidal matter and to prevent the collapse of the capillary cells.

The ionic strength of the seawater samples was determined from the Equation 22 (Millero, 2001).

$$I_T = 0.0199201 \cdot S \qquad (22)$$

The salinity effect was studied by diluting the samples with Milli-Q water. Due to the strong effect of carbonate species on the Fe(II) oxidation rate, the bicarbonate effect was corrected by considering (Santana-Casiano et al., 2005).

2.1.2. Reagents

The experiments were carried out using a Fe(II) stock solution ($4 \cdot 10^{-4}$ M) prepared using ammonium iron (II) sulphate hexahydrate (Sigma), acidified at a pH of 2 with Suprapur HCl (Sigma) in NaCl 0.7 M.

The Fe(III) stock solutions ($4 \cdot 10^{-4}$ M) were carried out from iron (III) chloride hexahydrate (Sigma) in NaCl 0.7 M.

The nutrients studied in the present Thesis were nitrate (NO_3^-), phosphate (PO_4^{3-}) and silicate (SiO_3^{2-}). The stock solutions of nutrients were prepared by using sodium nitrate (Sigma) (882 mM), potassium hydrogen phosphate (Sigma) (28.8 mM) and sodium silicate (Sigma) (142 mM). Under the different physico-chemical conditions of this work, the concentration of each nutrient was kept at $8.82 \cdot 10^{-4}$ M, $2.93 \cdot 10^{-5}$ M and $1.42 \cdot 10^{-4}$ M for NO_3^-, HPO_4^{2-} and SiO_3^{2-}, respectively.

Stock solutions of Cu(I) ($4 \cdot 10^{-4}$ M) were daily prepared using analytical grade copper (I) chloride (Aldrich). The solutions were acidified at pH 2 with Suprapur HCl and bubbled with N_2 before and after the copper was added. The initial concentration of Cu(I) was kept at 200 nM in the reaction cell except for the studies at different concentrations. Stock solutions of Cu(II) ($4 \cdot 10^{-4}$ M) were prepared using copper (II) chloride (Aldrich) and the initial concentrations were kept at 200 and 400 nM in the reaction cell.

H_2O_2 stock solutions ($4.03 \cdot 10^{-4}$ M) were always prepared in NaCl 0.7 M. The stock solutions were prepared each day and stored under darkness conditions when it was not used.

All solutions were prepared with Milli-Q (18MQ) and the reagents used were trace analytical grade.

2.2. pH measurements

The pH was measured potentiometrically by calibrating the electrode system with Tris-(hydroximethyl) aminomethane (Tris)-artificial seawater buffers (Millero, 1986). The buffers were prepared in 0.005 molkg^{-1} Tris and 0.005 mol kg^{-1} Tris-HCl in artificial seawater. The electrode used in the experiments was a combination electrode, Ross^{TM} Combination, glass body.

The pH can be calculated from Equation 23.

$$pH = pH(s) + \frac{(Es - Ex)F}{2.303RT} = pH_{(s)} + \frac{(Es - Ex)}{1.984 \cdot 10^{-4} T} \quad (23)$$

where the subscript *s* corresponds to Tris and *x* to the sample with unknown pH. Es and Ex, are the potential (mV) for Tris and samples respectively. F, R and T are the Faraday constant (96485.3 C mol^{-1}), the universal gases constant (8.314 J mol^{-1} K^{-1}) and temperature (K).

The pH(s) was calculated by the Equation 24 (Dickson et al., 2007).

$$\begin{aligned} pH(s) = {} & (11911.08 - 18.2499S - 0.039336S^2)\frac{1}{T/K} \\ & - 366.27059 + 0.053993607S + 0.00016329S^2 \\ & + (64.52243 - 0.084041S)\ln(T/K) - 0.11149858(T/K) \end{aligned} \quad (24)$$

Despite the pH measurements in these studies have been done in free-ion scale (pH_F) (Equation 23), in oceanography different scales of pH can be used (Millero et al., 1993), as total pH (pH_T) and seawater pH (pH_{sws}) (Equations 25-27).

$$pH_F = -\log\left[H^+\right]_F \quad (25)$$

$$pH_T = -\log\left[H^+\right]_T \quad (26)$$

$$pH_{sws} = -\log\left[H^+\right]_{sws} \quad (27)$$

where the proton concentration is given by $[H^+]_T = [H^+]_F + [HSO_4^-]$ and $[H^+]_{sws} = [H^+]_F + [HSO_4^-] + [HF]$.

The TRIS-seawater solution was prepared following the Table 2-1 (Millero, 1986). The effects of temperature and salinity on the pK^* of the Tris-buffers were considered in this study. The pH was adjusted to the desired value to ±0.01 with small additions of suprapur HCl 0.1M using an automatic titrator system (Titrino 719S, Methrom).

Table 2-1. Composition of TRIS-seawater buffer solution (Millero, 1986).

Reagent	m	mol kg^{-1}	g kg^{-1}	g dm^{-3} (25ºC)
		m = 0.06 TRIS buffer		
NaCl	0.36664	0.34933	20.416	20.946
Na_2SO_4	0.02926	0.02788	3.960	4.063
KCl	0.01058	0.01008	0.752	0.772
$CaCl_2$	0.01077	0.01026	1.139	1.169
$MgCl_2$	0.05518	0.05258	5.006	5.136
TRIS	0.06	0.05717	6.926	7.106
TRISHCl	0.06	0.05717	9.010	9.244
		g salt	47.209	
		g H_2O	952.791	

2.3. Temperature

The Fe(II) oxidation experiments were carried out as a function of temperature (5-35ºC). The temperature was controlled with a thermostatic bath (AG-2). Temperature affects the oxidation rate and is needed in order to understand its effect on the Fe(II) oxidation process, allowing us to calculate the parameters of both the Arrhenius' equation and the Eyring's equations, the enthalpy and entropy of activation.

2.4. Oxygen concentration

The studies developed in this Thesis were always carried out in air saturated conditions by bubbling pure-air (Carburos Metálicos de Canarias)

throughout the seawater samples during one hour equilibrium time. During experiments, oxygen bubbling was kept on the top of the reaction vessel.

The oxygen equilibrium concentration was determined from the Benson and Krause (1984) expression (Equation 28).

$$\ln[O_2] = -135.29996 + 1.572288 \cdot 10^5 / T - 6.637146 \cdot 10^7 / T^2 + 1.243678 \cdot 10^{10} / T^3 - 8.62106 \cdot 10^{11} / T^4 - S(0.020573 - 12.142 / T + 2363.1 / T^2) + \ln(1 + 10^{-3} S) \quad (28)$$

T is temperature (K) and S corresponds with the salinity.

2.5. Oxidation Experiments

The studies were carried out in a cover water-jacketed glass vessel (250 mL), and controlling the temperature via an AG-2 bath within the range of 5-35ºC (± 0.02 ºC). The samples were stirred at the same rate during all the experiments with a teflon-coated magnetic stirrer. The oxidation kinetics was carried out in air saturated conditions, by bubbling the solution with pure air for 1 hour, previous to and during the experiments. The pH was adjusted to the desired value to ±0.01 with small additions of suprapur HCl 0.1M using an automatic titrator system (Titrino 719S, Methrom). The addition of the Fe(II) or Cu(I) to the sample corresponds to the zero time of reaction.

2.6. Fe(II) measurements

The Fe(II) concentration was determined spectrophotometrically using the ferrozine method (Violler et al., 2000; Santana-Casiano et al., 2005). The ferrozine and Fe(II) form a peak at 562 nm. At selected times, 10 mL of sample were added to a 25 mL glass flask containing ferrozine (50 µL of 0.01 M), acetate buffer (2 mL, pH 5.5) and NaF (50 µL of $7.1 \cdot 10^{-4}$ M). When the NaF was used with an acetate buffer and the ferrozine solution, a stable absorbance reading was observed for over 30 mins (González-Davila, et al, 2005).

A 5 m long waveguide capillary flow cell (World Precision Instruments^{TM}) connected to the UV-Vis detector USB2000 (Ocean Optics^{TM}) was used to measure Fe(II) at nanomolar concentrations. The light used was a halogen light source (HL-2000-FHSA from Mikropack). The capillary flow cell and the UV detector were connected using 400µm optical fiber. The spectra were recorded using the OOIBase32 software by Ocean Optics. The sample was introduced in the column using a peristaltic pump (EXPETEC Perimax 12) with a flux of 1 mL/min.

The apparent oxidation rate constant k_{app} ($M^{-1}min^{-1}$) for the Fe(II) oxidation was defined in Chapter I (Equations 10-12), where the Fe(II) oxidation can be considered as a pseudo-first order equation, under air saturated conditions ($k_{app} = k' / [O_2]$).

The pseudo first-order kinetic rate constant, k', was determined from the linear regression of ln[Fe(II)] vs time for times over half-life time ($t_{1/2}$), where the regressions were permanently $R^2 \geq 0.98$.

2.7. Copper (I) analysis

The Cu(I) concentrations were determined spectrophotometrically using a modified version of the bathocupreine method (Moffett et al., 1985) in order to analyze copper at nanomolar concentrations. At selected times during the experiment, 10 mL of the extracted sample was added to a 25 mL vessel which contained $2 \cdot 10^{-5}$ M bathocupreine (bathocupreinedisulfonic acid, disodium salt hydrate) and 10^{-4} M ethylendiamine reagent. The bathocupreine and the Cu(I) form a complex absorbing at 484 nm. The ethylendiamine forms strong complexes with copper (II) and removes possible interferences in the Cu(I) determination.

The Cu(I) at nanomolar concentration was measured using an UV detector USB2000 (Ocean Optics^{TM}) connected to a 5 m long waveguide capillary cell (LWCC) from World Precision Instruments. A lineal relationship between the absorbance and the concentration of Cu(I) was found for the range 0-336 nM in sodium chloride and seawater solutions, respectively (Equations 29 and 30).

$$Absorbance = 6.59 \cdot 10^{-4} + 2.38 \cdot 10^{-3} \left[Cu(I) \right] \quad (29)$$

$$Absorbance = -1.26 \cdot 10^{-2} + 2.30 \cdot 10^{-3} \left[Cu(I) \right] \quad (30)$$

with a molar absortivity, ε, of 4464 $M^{-1}cm^{-1}$ in seawater and 4767 $M^{-1}\,cm^{-1}$ in NaCl.

2.8. Algae cultures and Organic exudates enrichment

The study of the effect of organic exudates was carried out in natural seawater enriched with organic exudates excreted by two different species, *Phaeodactylum tricornutum* and *Dunaliella tertiolecta.* The experimental method developed in order to obtain the enriched seawater is shown in Figure 2-1.

The cultures were kept in seawater filtered at 0.45 μm and f/2 culture media solution (Guillard, 1975). The pure cells were supplied from the National Algae Bank (BNA) in Gran Canaria.

All cultures were kept in a clean culture chamber (Friocell FC111) at 24ºC under constant light for 24 hours.

The cultures designed to obtain seawater enriched with organic exudates were prepared using only seawater and nutrients from the f/2 media (seawater control) with constant additions of 10^7 cell/L to start the culture until the desired time for the experiment. The nutrient concentrations were $8.82 \cdot 10^{-4}$ M, $2.93 \cdot 10^{-5}$ M and $1.42 \cdot 10^{-4}$ M for NO_3^-, HPO_4^{2-} and SiO_3^{2-}, respectively. The cell density was measured daily using a microscope with a hemacytometer (Microbiotest, Inc.) and measuring the absorbance (640 nm and 670 nm, for *P. tricornutum* and *D. tertiolecta*, respectively) with a spectrophotometer (USB4000).

The growth of species, *P. tricornutum* and *D. tertiolecta* is shown in Figure 2-2A and 2-2B. Different stages were identified in each case. The *P. tricornutum* growth showed an exponential phase ($6.21 \cdot 10^7$ and $2.29 \cdot 10^8$ cell/L) and a stationary phase ($4.98 \cdot 10^8$ cell/L). The *D. tertiolecta* growth was characterized by an exponential phase ($5.52 \cdot 10^7$ and $2.17 \cdot 10^8$ cell/L) and a

stationary phase ($5.04 \cdot 10^8$ cell/L). The maximum cell concentration was reached after eight days.

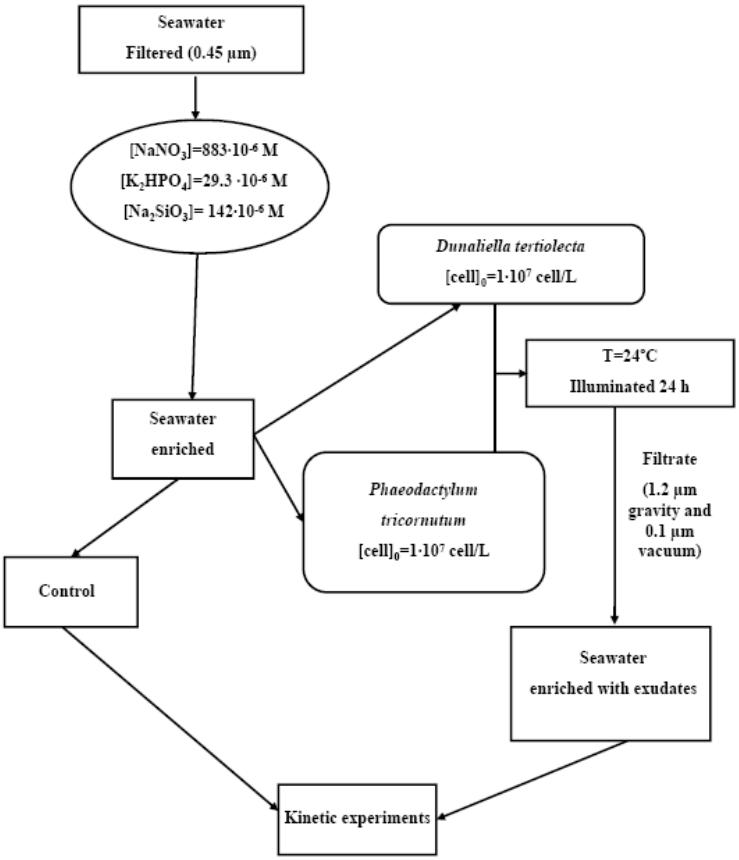

Figure 2-1. Layout of experimental method followed in order to obtain the seawater enriched with organic exudates.

The control seawater was produced using seawater enriched with the same f/2 nutrient concentrations and filtered by 0.1 µm. The cultures were filtered in two steps, first by gravity at 1.2 µm and second by vacuum with 0.1 µm filters. This method was used in order to avoid rupture of cells. The method is summarised in Figure 2-1. All filters used in this work were

previously cleaned with Suprapur HCl solution (24 h at 10% in Milli-Q water) and kept in Milli-Q water.

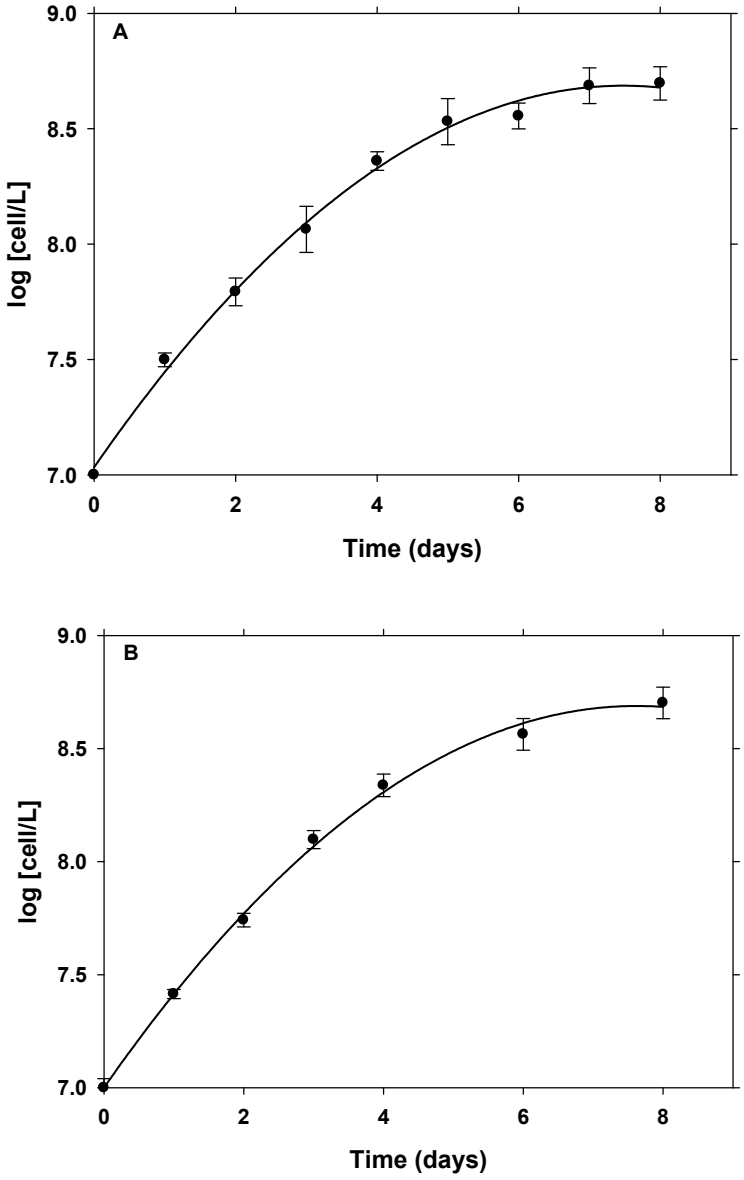

Figure 2-2. (A) The growth curve of the *P. tricornutum* and (B) *D. tertiolecta* under the experimental conditions: f/2 nutrients ($[NO_3^-]$= $8.82 \cdot 10^{-4}$ M, $[HPO_4^{2-}]$= $2.93 \cdot 10^{-5}$ M and $[SiO_3^{2-}]$= $1.42 \cdot 10^{-4}$ M), illuminated for 24 hours and 24 ºC.

2.9. Numerical Model

The Gepasi version 3.30 software was used to simulate the chemical kinetic for all reactants. The overall and the individual rate constants k_i were obtained by adjusting the experimental Fe(II) concentrations/time pair of data to the model output as indicated elsewhere (Santana-Casiano et al., 2004).

Gepasi is a software package for modelling biochemical systems that provides a number of tools to fit model to experimental data. Gepasi allows considering an unlimited number of equations with its parameter estimation. In addition, Gepasi can be also used to find maximum and minima of any variable with a number of adjustable parameters. The most important aspect of Gepasi software is that simplifies the task of model building by assisting the users in translating chemical reactions to mathematic equations (matrices or differential equations). The research also can introduce owner functions in order to estimate a number of parameters.

Table 2-2 shows the reactions considered as a base in the kinetic model developed in this Thesis.

Table 2-2. Stability constants for the formation of Fe(II) and Fe(III) inorganic complexes considered for the kinetic model.

Nº	Species	Log K (0.7 mol L^{-1}, 25ºC)	Ref.
1	$H_2O \leftrightarrow H^+ + OH^-$	-13.69	1
2	$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$	-6.005	1
3	$HCO_3^- \leftrightarrow CO_3^{2-} + H^+$	-9.6	1
4	$Na^+ + HCO_3^- \leftrightarrow NaHCO_3$	-0.53	2
5	$Na^+ + CO_3^{2-} \leftrightarrow NaCO_3^-$	-0.42	2
6	$Ca^{2+} + HCO_3^- \leftrightarrow CaHCO_3^+$	0.33	2
7	$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3$	2.1	2
8	$Mg^{2+} + HCO_3^- \leftrightarrow MgHCO_3^+$	0.28	2
9	$Mg^{2+} + CO_3^{2-} \leftrightarrow MgCO_3$	1.94	2
10	$2Mg^{2+} + CO_3^{2-} \leftrightarrow Mg_2(CO_3)^{2+}$	2.59	2
11	$Mg^{2+} + OH^- \leftrightarrow MgOH^+$	1.70	2
12	$Fe^{2+} + HCO_3^- \leftrightarrow FeHCO_3^+$	0.97	3
13	$Fe^{2+} + CO_3^{2-} \leftrightarrow FeCO_3$	4.33	4
14	$Fe^{2+} + 2CO_3^{2-} \leftrightarrow Fe(CO_3)_2$	6.09	4
15	$Fe^{2+} + CO_3^{2-} + OH^- \leftrightarrow Fe(CO_3)(OH)^-$	8.90	4
16	$Fe^{2+} + H_2O \leftrightarrow Fe(OH)^+ + H^+$	-9.66	5
17	$Fe^{2+} + 2\cdot H_2O \leftrightarrow Fe(OH)_2 + 2H^+$	-20.87	5
18	$Fe^{2+} + Cl^- \leftrightarrow FeCl^+$	-0.12	4
19	$Fe^{2+} + SO_4^{2-} \leftrightarrow FeSO_4$	0.96	4
20	$H^+ + SO_4^{2-} \leftrightarrow HSO_4^-$	-0.10	1
21	$Fe^{3+} + Cl^- \leftrightarrow FeCl^{2+}$	0.57	5
22	$Fe^{3+} + 2Cl^- \leftrightarrow FeCl_2^+$	0.13	5
23	$Fe^{3+} + H_2O \leftrightarrow Fe(OH)^{2+} + H^+$	-2.62	5
24	$Fe^{3+} + 2\cdot H_2O \leftrightarrow Fe(OH)_2^+ + 2H^+$	-6.0	5
25	$Fe^{3+} + 3\cdot H_2O \leftrightarrow Fe(OH)_3 + 3H^+$	-12.5	5
26	$Fe^{3+} + 4\cdot H_2O \leftrightarrow Fe(OH)_4^- + 4H^+$	-21.8	5
27	$Fe(II) + O_2^{\bullet-} + 2H^+ \rightarrow Fe(III) + H_2O_2$	7.00	6
28	$Fe(III) + O_2^{\bullet-} \rightarrow Fe(II) + O_2$	8.18	6
29	$Fe(III) + 3OH^- \rightarrow Fe(OH)_{3(s)}$	4.40	7
30	$Cu(II) + O_2^{\bullet-} \rightarrow Cu(I) + O_2$	8.82	8
31	$Cu(I) + O_2^{\bullet-} + 2H^+ \rightarrow Cu(II) + H_2O_2$	9.30	8

1. Millero, 1995. 2. Millero and Schreiber, 1982. 3. Millero and Hawke, 1992. 4. King, 1998. 5. Millero et al., 1995b. 6. Rush and Bielski, 1985. 7. Rose and Waite, 2002. 8. Zafiriou et al., 1998.

CHAPTER III:

Oxidation of Fe(II) in natural waters at high nutrient concentrations

González, A.G., Santana-Casiano, J.M., Pérez, N., González-Dávila, M. 2010. Oxidation of Fe(II) in natural waters at high nutrient concentrations. Environ. Sci. Technol. 44, 8095-8101.

ABSTRACT

The Fe(II) oxidation kinetic was studied in seawater enriched with nutrients as a function of pH (7.2-8.2), temperature (5-35ºC) and salinity (10-36.720) and compared with that in seawater media. The effect of nitrate (0-$1.77 \cdot 10^{-3}$ M), phosphate (0-$5.80 \cdot 10^{-5}$ M) and silicate (0-$2.84 \cdot 10^{-4}$ M) was studied at pH 8.0 and 25ºC. The experimental results demonstrated that Fe(II) oxidation was faster in high nutrient concentration affecting the lifetime of Fe(II) in nutrient rich waters. Silicate showed the most important effects on the Fe(II) oxidation rate with values similar to those determined in seawater enriched with all nutrients.

A kinetic model was applied to the experimental results in order to account for changes in the speciation and to compute the fractional contribution of each Fe(II) species to the total rate constant as a function of pH. $FeH_3SiO_4^+$ played a key role in the Fe(II) speciation, dominating the process at pH higher than 8.4. At pH 8.0, $FeH_3SiO_4^+$ represented the 18% of total Fe(II) species. Model results show that when the concentration of silicate is $3 \cdot 10^{-5}$ M as in High Nutrient Low Chlorophyll areas, $FeH_3SiO_4^+$ contributed at pH 8.0 with a 4% to the rate, increasing to 11% at $1.4 \cdot 10^{-4}$ M .

CHAPTER III

3.1. Introduction

Natural waters can be ranked as a function of nutrient contents (NO_3^-, HPO_4^{2-} and $Si(OH)_4$) from oligotrophic (low nutrient concentration; $[NO_3^-]$ = 0-6 µM , $[HPO_4^{2-}]$ = 0-0.5 µM, $[Si(OH)_4]$ = 0-10 µM) to eutrophic (high nutrient concentration; $[NO_3^-]$ = 18-25 µM , $[HPO_4^{2-}]$ = 0.8-2 µM, $[Si(OH)_4]$ = 20-60 µM) (Levitus et al., 1993). When the nutrient concentrations increased excessively, eutrophication can take place as it has been described in coastal and estuarine areas (Rabalais et al., 2009) and lagoons (Windom et al., 1999). The enrichment of these nutrients may have significant effects on iron speciation, transformation and transport, depending on local conditions (Öztürk et al., 2003). In culture media as in a f/2 medium, the concentration of nutrients can reach 882 µM, 28.8 µM and 141 µM for NO_3^-, HPO_4^{2-} and $SiOH_4$, respectively (Guillard, 1975), and could affect the Fe speciation and redox chemistry.

The nutrient speciation has been studied in the ocean (Millero, 2006), showing that silicates are usually present as $Si(OH)_4$ (96%) and $Si(OH)_3O^-$ (4%) in aqueous solutions. Nitrates are present as NO_3^- and phosphates mostly as HPO_4^{2-} (79.2%) or PO_4^{3-} (20.4%), interacting the phosphates with Ca^{2+} and Mg^{2+}. Phosphate and Mg^{2+} can be found in the ocean as $MgH_2PO_4^+$ (7%), $MgHPO_4$ (45.8%) and $MgPO_4^-$ (26.6%). In addition, phosphate and Ca^{2+} can be found as $CaH_2PO_4^+$ (0.7%), $CaHPO_4$ (4.9%) and $CaPO_4^-$ (73.2%) (Millero, 2006).

In this chapter, the Fe(II) oxidation was studied in seawater (SW) and seawater enriched with nutrients (SEN). Some studies were done in artificial seawater (ASW) (Millero, 1986; Millero, 2006). Artificial seawater was made following Table 3-1 (Millero, 2006). This solution was also enriched with high nutrient concentrations (ASEN) in order to study the differences with those in seawater.

Table 3-1. Preparation of 1 kg of S=35.00 of Artificial seawater (Millero, 2006).

Salt	g kg^{-1}	mol kg^{-1}	Molecular weight
NaCl	23.9849	0.41040	58.4428
Na_2SO_4	4.0111	0.02824	142.0372
KCl	0.6986	0.00937	74.5550
$NaHCO_3$	0.1722	0.00205	84.0070
$B(OH)_3$	0.1000	0.00084	119.0060
KBr	0.0254	0.00041	61.8322
NaF	0.0029	0.00007	41.9882
$MgCl_2$	5.0290	0.05282	95.211
$CaCl_2$	1.1409	0.01028	110.986
$SrCl_2$	0.0143	0.00009	158.526

This work studied the oxidation of Fe(II) using seawater and seawater enriched with high nutrient concentration (nitrate, phosphate and silicate) (SEN) in air saturated conditions under different pH, temperature and salinity to improve the knowledge of the iron chemistry in natural waters and cultures. A kinetic model (Santana-Casiano et al., 2005) has been applied to the experimental data in order to describe the role played by iron-nutrient species in the oxidation process of Fe(II).

3.2. Results and Discussion

The Fe(II) oxidation was studied as a function of pH (7.2-8.2), temperature (5-35ºC) and salinity (10-36.720). The effect of individual nutrient (nitrate, phosphate and silicate) was also studied. The range of nutrient concentrations, studied individually, varied from 0 to $1.77 \cdot 10^{-3}$ M, $5.80 \cdot 10^{-5}$ M and $2.84 \cdot 10^{-4}$ M for NO_3^-, HPO_4^{2-} and $Si(OH)_4$, respectively.

The Fe(II) oxidation rate constant followed a pseudo-first order behaviour for all studies under the experimental conditions used in the present work. The k_{app} was calculated according to Equations 10-12 (Chapter I) from the pseudo-first order oxidation rate (k'). The apparent rate constant was always higher in the presence of nutrients (Figure 3-1).

3.2.1. Effect of nutrient concentration

The effect of each nutrient on the Fe(II) oxidation rate was determined in seawater enriched with different concentrations of NO_3^- (0-$1.77 \cdot 10^{-3}$ M), HPO_4^{2-} (0-$5.80 \cdot 10^{-5}$ M) and $Si(OH)_4$ (0-$2.84 \cdot 10^{-4}$ M). The selected range of concentrations covered those found in surface ocean oligotrophic waters, poor in nutrients, HNLC waters ($NO_3^- = 29 \cdot 10^{-6}$ M, $HPO_4^{2-} = 1.9 \cdot 10^{-6}$ M and $Si(OH)_4 = 30 \cdot 10^{-6}$ M) (Nolting et al., 1998; Boye et al., 2001), high nutrient-rich waters from eutrophication ($[NO_3^-] \geq 450$ µM, $[HPO_4^{2-}] \geq 80$ µM, $[Si(OH)_4] \geq 100$ µM) (Maier et al., 2009) and waters with f/2 medium prepared for cultures. The nutrient concentrations added to natural seawater in order to prepare the SEN solution is that commonly used in f/2 media when laboratory experiments with phytoplankton are considered (Guillard, 1975; Croot et al., 2000; Shaked et al., 2005). Figure 3-1 showed

the effect of each individual nutrient concentrations on the Fe(II) apparent oxidation rate constant in seawater.

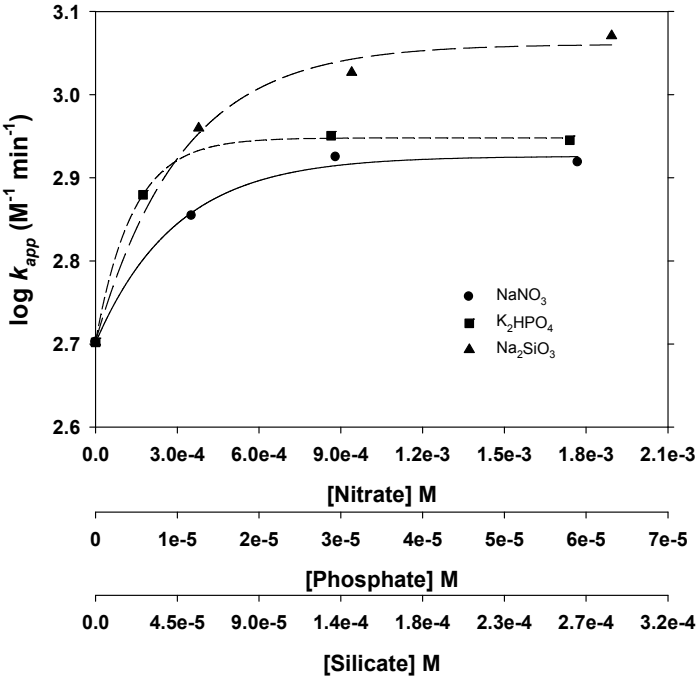

Figure 3-1. Role of each individual nutrient in the rate constant for Fe(II) oxidation process. Temperature and pH were kept at 25ºC and pH 8.0.

The Fe(II) rate constant followed the same behaviour for the three nutrients used. The Fe(II) oxidation rate increased with the nutrient concentration until a maximum. The data have been fitted to an exponential function reaching a maximum (Equations 31-33), where R^2 was 0.995, 0.999 and 0.995 and the standard error of estimation was 0.013, 0.004 and 0.020 for nitrate, phosphate and silicate, respectively.

$$\log k_{app,NIT} = 2.70 + 0.23(1 - e^{(-3391.28[NIT])}) \quad (31)$$

$$\log k_{app,PHP} = 2.70 + 0.25(1 - e^{(-220810.50[PHP])}) \quad (32)$$

$$\log k_{app,SIL} = 2.70 + 0.36(1 - e^{(-21188.17[SIL])}) \quad (33)$$

NIT, PHP and SIL are the total concentrations of nitrate, phosphate and silicate for each condition. Maximum values in log k_{app} were 2.93 ± 0.01, 2.95 ± 0.01 and 3.06 ± 0.01 (M^{-1} min^{-1}) for NO_3^-, HPO_4^{2-} and $Si(OH)_4$, respectively. The changes on the rate constant confirm the effect of nutrient concentrations on the Fe(II) oxidation.

The studies carried out for each nutrient individually (NO_3^- = $8.83 \cdot 10^{-4}$ M, HPO_4^{2-} = $2.93 \cdot 10^{-5}$ M and $Si(OH)_4$ = $1.42 \cdot 10^{-4}$ M), were compared with studies carried out in SW and in SEN, at pH 8.0 and temperature 25ºC (Figure 3-2). The seawater enriched with nitrate, phosphate or silicate was labelled as SNIT, SPHP and SSIL, respectively. The Fe(II) rate constant increased according to SW (log k_{app}= 2.70 ± 0.01 M^{-1} min^{-1}) < SNIT (log k_{app}= 2.93 ± 0.02 M^{-1} min^{-1}) < SPHP (log k_{app}= 2.95 ± 0.05 M^{-1} min^{-1}) < SSIL (log k_{app}=3.03 ± 0.03 M^{-1} min^{-1}) < SEN (log k_{app}= 3.04 ± 0.02 M^{-1} min^{-1}). SSIL and SEN showed similar values in log k_{app} (3.04 and 3.03 M^{-1} min^{-1}) (Figure 3-3). Artificial seawater (ASW) was considered as the reference value in Figure 3-3. This Figure 3-3 depicts various Fe(II) oxidation scenarios as a function of the nutrient contents in environmental waters: oligotrophic waters (SW), theoretical High Nutrient Low Chlorophyll waters (HNLC), seawater enriched with nutrients (SEN) and artificial seawater enriched with nutrients (ASEN), both of them at the same concentration of the f/2 media.

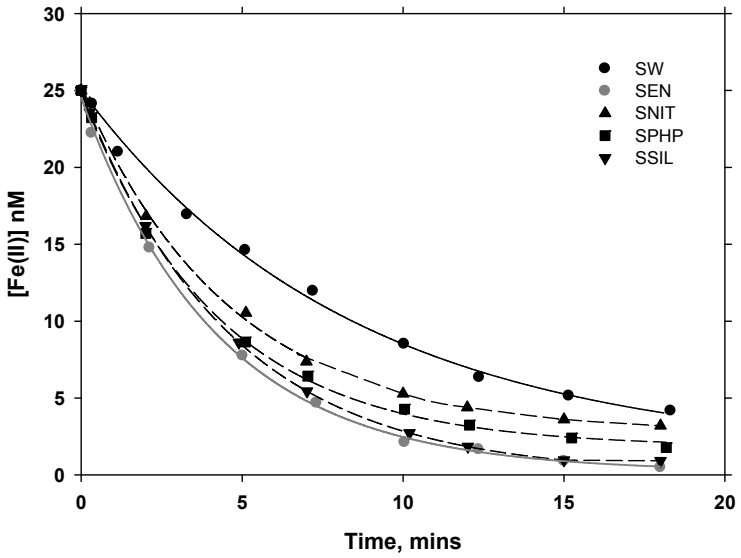

Figure 3-2. Kinetic Fe(II) study for different media, seawater (SW), seawater enriched with high nutrient concentrations (SEN), seawater enriched with nitrate (SNIT) ($8.83 \cdot 10^{-4}$ M), seawater enriched with phosphate (SPHP)($2.93 \cdot 10^{-5}$ M) and seawater enriched with silicate (SSIL)($1.42 \cdot 10^{-4}$ M). Temperature and pH were kept constant for all of them, at 25ºC and 8, respectively.

Seawater with high concentration of each individual nutrient was also considered. The slowest Fe(II) oxidation rate was found in SW and in HNLC seawater, with log k_{app} = 2.70 M^{-1} min^{-1} and 2.75 $M^{-1}min^{-1}$, respectively. The fastest Fe(II) oxidation rate was determined in seawater with high nutrient concentrations, SEN (log k_{app} = 3.04 M^{-1} min^{-1}).

The difference between SW and HNLC was 0.7 mins in half-life time for Fe(II), at pH=8.0 and T=25ºC. The Fe(II) oxidation rate constant for SSIL was similar to the value for ASEN (log k_{app} = 3.03 M^{-1} min^{-1}). This study confirmed that nutrients at high concentrations as in a culture media may play an important role on the Fe(II) rate constant, being the silicate the most active one.

The oxidation of Fe(II) in SW was slightly slower than in ASW ($\Delta \log k_{app}$ = -0.14). The difference observed is explained by the effects of the presence of organic matter in the redox chemistry of iron (Santana-Casiano et al., 2000; 2004). When the water is enriched with nutrient in both SEN and ASEN studies, the effects of the nutrients on the Fe(II) oxidation rate constant exceed those of the organic matter, making them negligible.

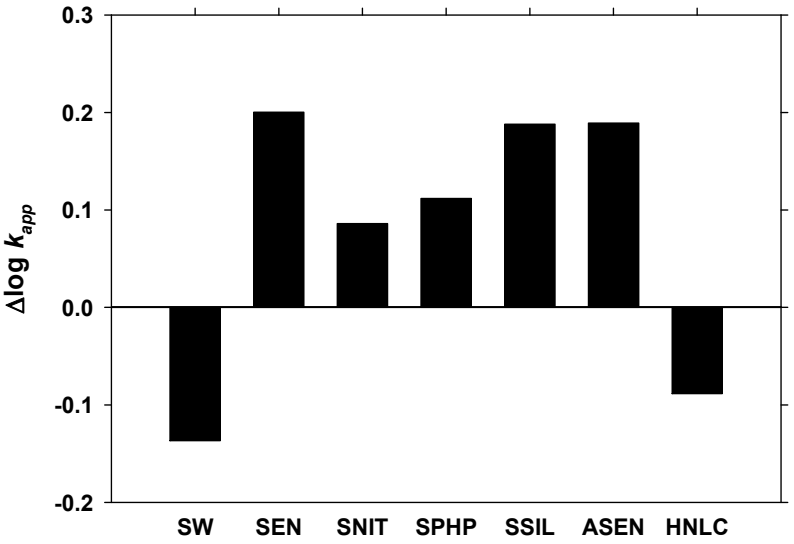

Figure 3-3. Effect of different nutrient concentrations in the rate constant for Fe(II) oxidation and various solutions ($\Delta \log k = \log k_i - \log k_{ASW}$), seawater (SW), seawater enriched with high nutrient concentrations (SEN), seawater enriched with nitrate (SNIT), seawater enriched with phosphate (SPHP), seawater enriched with silicate (SSIL), artificial seawater enriched with nutrients (ASEN) and HNLC simulated waters (HNLC). The rate constant for artificial seawater is considered as the reference value.

3.2.2. pH dependence

The Fe(II) apparent oxidation rate as a function of pH was studied in SW and in SEN. ASW was also considered. The effect of pH was studied in the range 7.2-8.2 (Figure 3-4).

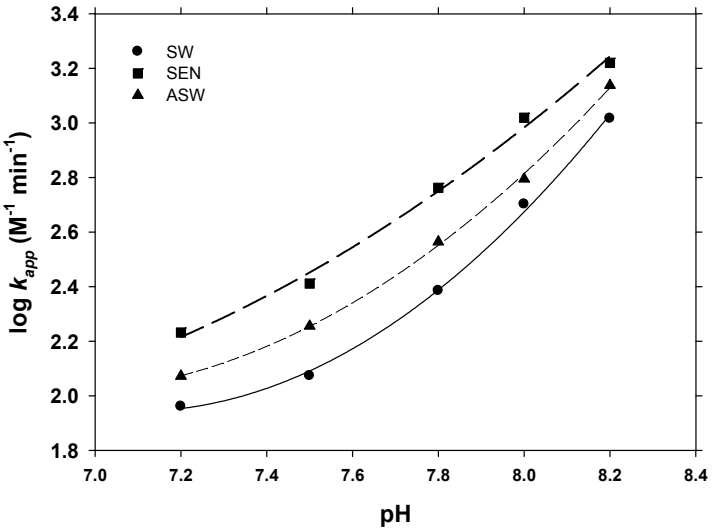

Figure 3-4. Effect of pH on the rate constant for Fe(II) oxidation in seawater (SW), seawater enriched with high nutrient concentrations (SEN) and artificial seawater (ASW). Temperature was 25ºC and pH=8.0.

The rate constants in SEN were always higher than in SW. The pH dependence was fitted to a second order polynomial equation for SW and SEN, respectively (Equations 34-35):

$$\log k_{app,SW} = 46.40 - 12.54\,pH + 0.88\,pH^2 \quad (34)$$

$$\log k_{app,SEN} = 15.09 - 4.26\,pH + 0.34\,pH^2 \quad (35)$$

R^2 was 0.998 and 0.994, where the standard error of estimation was 0.03 and 0.02 for SW and SEN.

The increase observed in the Fe(II) rate constant for seawater at high nutrient concentration (SEN) represented 12% at pH 8.0. As indicated above, the Fe(II) oxidation process increased in the presence of nutrients, indicating the interaction among Fe^{2+} and NO_3^-, HPO_4^{2-} and $Si(OH)_4$ contributed efficiently to the Fe(II) oxidation process. The log k_{app} (2.70 ± 0.01 M^{-1} min^{-1}) for SW at pH=8.0 and T=25ºC was comparable with values in natural Pacific Subarctic waters (log k_{app} = 2.60 M^{-1} min^{-1} at pH=8.0 and 25ºC (Roy et al., 2008)), and with Gulf Stream waters (log k_{app} = 2.99 M^{-1} min^{-1}, at pH=8.0 and 25ºC (Santana-Casiano et al., 2005)). The differences in the Fe(II) rate constant values are related to the original composition of the seawater samples (Millero et al., 1987).

From pH 7.2 to pH 8.2, (Table 3-2) the $t_{1/2}$ was reduced one order of magnitude in both SW (from 37.1 to 3.3 mins) and SEN (from 20 to 2.4 mins). For SW and SEN media, $t_{1/2}$ decreased in 17.2 mins at pH 7.2 and 1.2 mins at pH 8.2 (Table 3-2). At the different pH conditions, the presence of these nutrients decreased the lifetime of Fe(II) in the natural environment. Figure 3-4 also depicts the pH effect on the Fe(II) oxidation rate in ASW. As was pointed out above, the differences are explained by the presence of organic matter in the SW media.

3.2.3. Temperature dependence

The study of the temperature dependence will enable us to know whether the oxidation process of Fe(II) in the presence of high nutrient concentrations differs from the same process in natural seawater. Temperature dependence was studied from 5 to 35ºC, at 10ºC intervals, in SW and SEN (Figure 3-5).

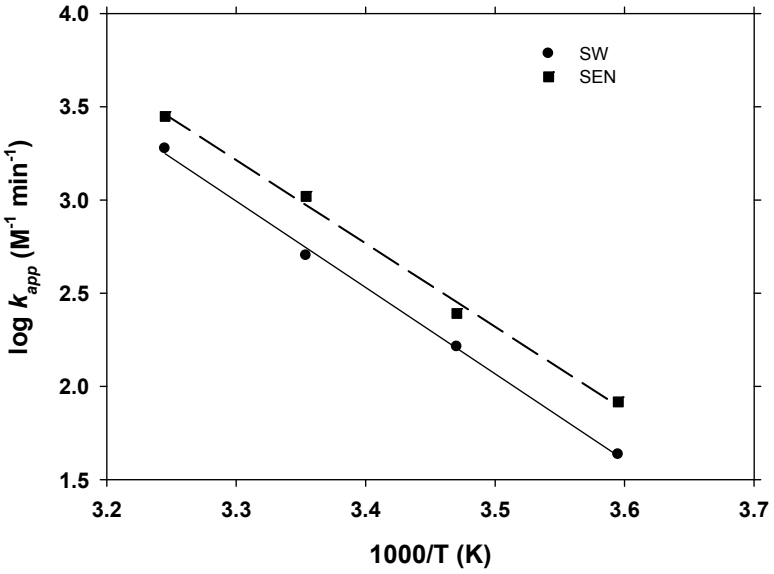

Figure 3-5. Effect of temperature on the rate constant for Fe(II) oxidation in seawater (SW) and seawater with high nutrient concentrations (SEN). pH was kept constant (8.0).

The experimental data were fitted for the SW and SEN solutions, respectively (Equation 36-37).

$$\log k_{app,SW} = 18.29 - 4636 / T \quad (36)$$

$$\log k_{app,SEN} = 17.97 - 4473 / T \quad (37)$$

where T is temperature (K). R^2 was 0.998 and 0.992 with a standard error of estimation of 0.04 and 0.02 for SW and SEN. The rate constants were higher in SEN than in SW at each temperature. However, the plot of log k_{app} versus 1/T gave similar slopes, -4636 K^{-1} in SW and -4473 K^{-1} in the SEN solution.

The Energy of Activation (E_a) was 88 ± 2 kJ mol^{-1} and 86 ± 4 kJ mol^{-1} for SW and SEN respectively. These values were comparable to nanomolar Fe(II) oxidation experiments measured in Gulf Stream seawater (Santana-Casiano et al., 2005) and Subarctic Pacific waters (Roy et al., 2008). These results indicated the mechanism controlling the Fe(II) oxidation in seawater involved the same chemical process as in seawater with high nutrients contents, only accelerated by the presence of these nutrients, especially by silicate. Log k_{app} increased 0.05 units/degree in both solutions. The $t_{1/2}$ values (Table 3-2) were 52.9 mins at 5ºC and 2.1 mins at 35ºC for seawater. The $t_{1/2}$ decreased from 27.6 mins at 5ºC to 1.4 mins at 35ºC in the SEN sample. These differences represented a reduction of 46% in lifetime when the nutrients were present in solution, favouring the disappearance of Fe(II) from the natural waters and increasing its reactivity.

3.2.4. Salinity dependence

The effect of salinity on the Fe(II) rate constant for SW and SEN was carried out by dilution with Milli-Q waters (18 MQ), keeping the nutrient concentrations constant in the SEN solutions. The range of salinities studied was 10 to 36.720 (Figure 3-6).

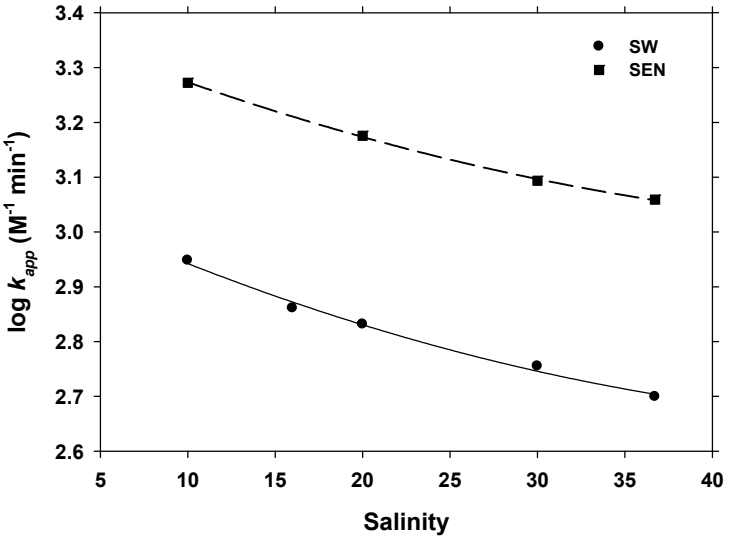

Figure 3-6. Effect of salinity on the rate constant for Fe(II) oxidation in both solutions, seawater (SW) and seawater enriched with high nutrient concentrations (SEN). Temperature and pH were kept constant (25ºC and 8.0).

The effect of HCO_3^- was corrected for both solutions (Santana-Casiano et al., 2005) and fitted to 2 mM. The experimental results for Figure 3-6 were fitted for SW and SEN solutions respectively (Equations 38-39):

$$\log k_{app,SW} = 3.08 - 0.02S + 1.34 \cdot 10^{-4} S^2 \quad (38)$$

$$\log k_{app,SEN} = 3.40 - 0.01S + 1.16 \cdot 10^{-4} S^2 \quad (39)$$

S corresponds to salinity. R^2 was 0.993 and 0.990 for seawater and seawater with nutrients, respectively. The standard error of estimation for the apparent rate constant log k_{app} was 0.02 and 0.01 in each case. These equations included the effect of changes in the carbonate concentrations and salinity.

When the concentration of bicarbonate was corrected and kept constant to 2 mM, log k_{app} increased respect to those without constant bicarbonate concentration. The dependence was fitted for SW (Equation 40) and SEN (Equation 41).

$$\log k_{app,SW} = 3.21 - 0.02S + 1.61 \cdot 10^{-4} S^2 \quad (40)$$

$$\log k_{app,SEN} = 3.51 - 0.02S + 1.16 \cdot 10^{-4} S^2 \quad (41)$$

R^2 was 0.997 and 0.999, respectively; the standard error of estimation for both equations was 0.01. The Fe(II) oxidation process was faster for SEN in all the salinity range. The difference in log k_{app} for both media stayed constant to 0.34 ± 0.02 M^{-1} min^{-1}. The $t_{1/2}$ (Table 3-2) decreased from 6.7 to 2.7 mins when salinity changed from S=36.720 to S=10, in SW, while the $t_{1/2}$ was moved from 3 to 1.3 mins, in SEN.

The apparent rate constant (M^{-1} min^{-1}) can be fitted under the experimental conditions of pH (free scale), temperature (Kelvin) and salinity, in SW (Equation 42) and SEN samples (Equation 43).

$$\log k_{app,SW} = 57.48 - 11.38 pH + 0.81 pH^2 - {}^{4532}/_{T} - 0.02S + 2 \cdot 10^{-4} S^2 \quad (42)$$

$$\log k_{app,SEN} = 28.67 - 3.72 pH + 0.31 pH^2 - {}^{4557}/_{T} - 8.61 \cdot 10^{-3} S - 9.88 \cdot 10^{-5} S^2 \quad (43)$$

R^2 was 0.998 and 0.996 and the standard error of estimation was 0.04 and 0.06, respectively. These equations can be used and applied in the environment, under the experimental conditions used.

3.2.5. Kinetic Model

A kinetic model was applied to the experimental data as a function of pH, in order to account for the speciation of Fe(II) and for the contribution of each individual species on the Fe(II) overall rate constant, in seawater at high nutrient concentration. According to the experimental results, the Fe(II) oxidation rates in the SEN solutions were controlled by the silicate effect. The difference between SW and SEN was constant in the pH-range studied (log k_{SEN}-log k_{SW} = 0.34 ± 0.06 M^{-1} min^{-1}), and the silicate effect can be considered dominant in the entire pH range. Therefore, the base model, with the equilibrium and rate constants for all inorganic species involved in the process for seawater (Santana-Casiano et al., 2005; González-Dávila et al., 2006) was extended by including Equations 44-48 (Reardon, 1979; Smith and Martell, 1991).

$$Si(OH)_4 \leftrightarrow Si(OH)_3O^- \quad (44)$$

$$Mg^{2+} + Si(OH)_3O^- \leftrightarrow MgH_3SiO_4^+ \quad (45)$$

$$Ca^{2+} + Si(OH)_3O^- \leftrightarrow CaH_3SiO_4^+ \quad (46)$$

$$Fe^{3+} + Si(OH)_3O^- \leftrightarrow FeH_3SiO_4^{2+} \quad (47)$$

$$Fe^{2+} + Si(OH)_3O^- \leftrightarrow FeH_3SiO_4^+ \quad \text{(This work)} \quad (48)$$

The Fe(II) apparent rate constant is composed of several individual rate constants for Fe(II) species, which react with oxygen at different rates. The Fe(II) oxidation rate can be determined as a function of the weighted sum of the oxidation rates of the individual Fe(II) species (Equation 49):

$$k = k_{Fe^{2+}}\alpha_{Fe^{2+}} + k_{FeOH^+}\alpha_{FeOH^+} + k_{Fe(OH)_2}\alpha_{Fe(OH)_2} + k_{FeHCO_3^+}\alpha_{FeHCO_3^+} + k_{Fe(CO_3)}\alpha_{Fe(CO_3)} + k_{Fe(CO_3)_2^{2-}}\alpha_{Fe(CO_3)_2^{2-}} + k_{Fe(CO_3)OH^-}\alpha_{Fe(CO_3)OH^-} + k_{FeCl^+}\alpha_{FeCl^+} + k_{FeSO_4}\alpha_{FeSO_4} + k_{FeH_3SiO_4^+}\alpha_{FeH_3SiO_4^+}$$

(49)

where $\alpha_i = [FeX_i] / [Fe(II)]_T$ are the molar fraction of each Fe(II) species in the solution and also a function of the ionic media. k is the apparent overall rate constant (M^{-1} min^{-1}) and k_i are the individual rate constants for the Fe(II) species. Speciation of Fe(II) was included in the kinetic model.

The Fe(II) speciation of seawater enriched with two different silicate concentrations is shown in Figure 3-7. The equilibrium constant for Equation 48 was estimated by the kinetic model as log K = -4.40 and the rate constant for the species $FeH_3SiO_4^+$ fixed to log k = 2.70 (M^{-1} min^{-1}). Considering a silicate concentration of $1.42 \cdot 10^{-4}$ M (Figure 3-7A) as a reference value in eutrophicated waters, the speciation of Fe(II) is dominated by Fe^{2+}, $Fe(CO_3)$, $FeH_3SiO_4^+$, $FeCl^+$, $FeSO_4$, $Fe(CO_3)(OH)^-$. The Fe^{2+} species is the most important one in the Fe(II) speciation from pH 6 (58%) to 8.2 (26%). $Fe(CO_3)$ dominates the speciation at pH between 8.2 (26%) and 8.4 (30%). The role of $FeH_3SiO_4^+$ increased from pH 6, where accounted for the 0.6% of total Fe(II) speciation, becoming the second most important species at pH = 8.4 (30%), reaching at pH 8.5 the 31%. $FeCl^+$ and $FeSO_4$ were also important species from pH 6 (25% and 16%, respectively) to 7.5 (17% and 12%, respectively). $Fe(CO_3)(OH)^-$ became an important contributor at pH higher than 7.6, reaching the 7% at pH=8.5.

At the silicate concentration of $3 \cdot 10^{-5}$ M, typical for surface HNLC regions (Figure 3-7B), $FeH_3SiO_4^+$ was still important, being 5% at pH 8.0 and 9% at pH 8.5. The other Fe(II) species followed a similar distribution. Therefore, $FeH_3SiO_4^+$ should be considered in the Fe(II) speciation in natural

waters, especially in eutrophicated waters, where silicate can be found at high concentrations.

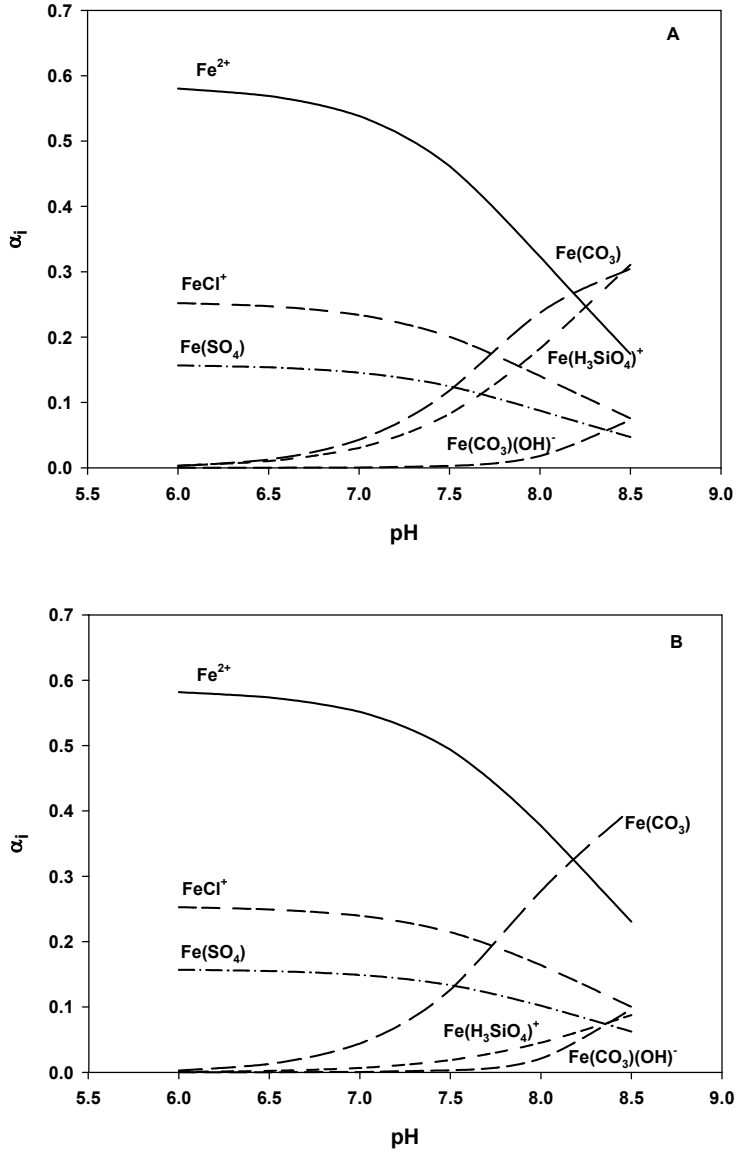

Figure 3-7. Speciation of Fe(II) in seawater enriched with silicate, where the concentration was (A) $1.42 \cdot 10^{-4}$ M and (B) $3 \cdot 10^{-5}$ M, at temperature 25ºC and salinity 36.720.

The individual contribution to the Fe(II) overall kinetic rate was computed from the results of the kinetic model and the speciation of Fe(II) (Equation 49). The results for each fractional contribution are shown as a function of pH in Figure 3-8. The fractional contribution follows a similar distribution for both high silicate concentration ($1.42 \cdot 10^{-4}$ M) and HNLC type silicate concentration ($3 \cdot 10^{-5}$ M) (Figure 3-8A and 3-8B, respectively). The contribution was controlled by Fe^{2+}, $Fe(OH)_2$, $Fe(CO_3)_2^{2-}$, $Fe(OH)^+$, $FeH_3SiO_4^+$, $Fe(CO_3)(OH)^-$ and $Fe(CO_3)$. In SSIL media, the overall contribution was dominated by Fe^{2+} from pH 6 (97%) to 7.7 (22%). At pH higher than 7.7 the dominant species was $Fe(OH)_2$, reaching the 22% at pH 7.7 and 58% at pH 8.5. The contribution of $FeH_3SiO_4^+$ was important in all the pH range. It was 2% at pH 6, 14% at pH 7.5 and 6% at pH 8.5. The contribution of $FeH_3SiO_4^+$ was the fourth most important at pH 8.0 (11% at SEN and 4% at HNLC).

These results confirm that nutrient concentrations, in particular silicate, play an important role in the oxidation of Fe(II) in seawater at high nutrient concentrations, making Fe(II) less available for biological mediated processes in this aquatic media.

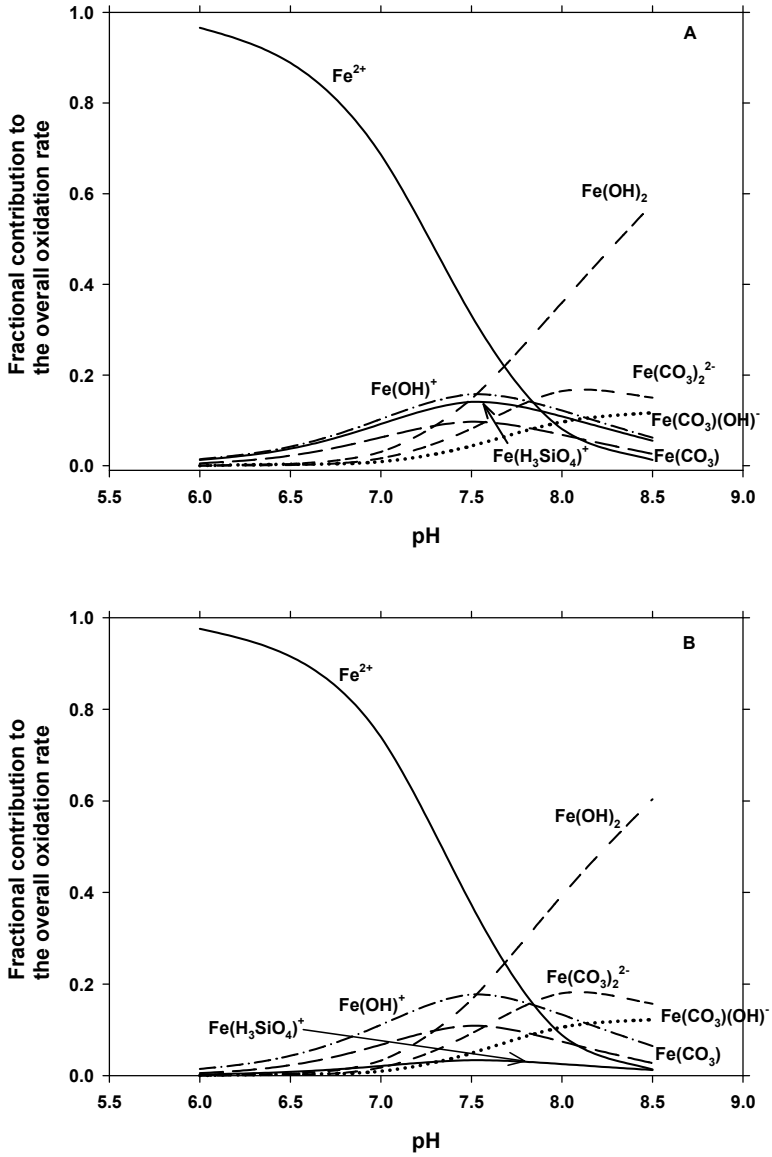

Figure 3-8. Contribution of each individual species on the overall rate constant for Fe(II) oxidation, in seawater enriched with silicate where the concentration was (A) $1.42 \cdot 10^{-4}$ M and (B) $3 \cdot 10^{-5}$ M, at temperature 25ºC and salinity 36.720.

Table 3-2. Half-life time ($t_{1/2}$) for Fe(II) oxidation in seawater (SW) and seawater enriched with nutrients (SEN) (nitrate ($8.83 \cdot 10^{-4}$M), phosphate ($2.93 \cdot 10^{-5}$M) and silicate ($1.42 \cdot 10^{-4}$M).

Media	pH	Temperature (ºC)	Salinity	log k_{app} ($M^{-1} min^{-1}$)	$t_{1/2}$ (min)
Seawater (SW)	7.2	25	36.7	1.96	37.1
	7.5	25	36.7	2.07	28.6
	7.8	25	36.7	2.39	14.0
	8.0	25	36.7	2.70	6.7
	8.2	25	36.7	3.02	3.3
	8.0	5	36.7	1.64	52.9
	8.0	15	36.7	2.21	17.4
	8.0	35	36.7	3.28	2.1
	8.0	25	10.0	3.03	2.7
	8.0	25	16.0	2.92	3.5
	8.0	25	20.0	2.88	4.0
	8.0	25	30.0	2.77	5.5
Seawater enriched with nutrients (SEN)	7.2	25	36.7	2.23	19.9
	7.5	25	36.7	2.41	13.2
	7.8	25	36.7	2.76	5.9
	8.0	25	36.7	3.04	3.0
	8.2	25	36.7	3.22	2.0
	8.0	5	36.7	1.92	27.6
	8.0	15	36.7	2.39	11.5
	8.0	35	36.7	3.45	1.4
	8.0	25	10	3.36	1.3
	8.0	25	20	3.23	1.8
	8.0	25	30	3.12	2.5

3.3. Conclusion

Oxidation of iron (II) has been studied in seawater enriched with high nutrient concentrations. The goal of this work was to know the behaviour of iron in natural waters where the concentration of NO_3^-, HPO_4^{2-} and $Si(OH)_4$, were similar to eutrophicated waters. The oxidation rate was increased due to the presence of nutrients, especially silicate. The Energy of activation (E_a) showed similar values for both solutions (SW and SEN) indicating that the reaction mechanism must be the same. When the salinity increased, the rate constant decreased. The concentration of HCO_3^- was corrected to 2 mM. The rate constant as a function of salinity increased with the effect of bicarbonate. The effects of pH, temperature and salinity were fitted to one equation that describes the oxidation process for seawater and seawater at high nutrient concentration (Equations 42-43).

The rate constant for iron (II) oxidation was function of pH, temperature and salinity. The half-life time for Fe(II), estimated from the pseudo-first order rate constant *k*', decreased seriously in eutrophicated waters. This process changes the speciation of iron and the role of the individual species in the oxidation process of iron (II).

Model revealed that the speciation of iron (II) was controlled by $FeH_3SiO_4^+$ between pH 7.6 and 8.5. $Fe(CO_3)$ was also important in the same range of pH. For lower pH values, the speciation was controlled by $FeCl^+$ and $Fe(SO_4)$. However, the contribution of each species to the overall rate constant was dominated by $Fe(OH)^+$ and $Fe(CO_3)$ from pH 6 to 7.7. $Fe(OH)_2$ showed the most important contribution at pH higher than 8.1. The contribution of $FeH_3SiO_4^+$ was similar to other inorganic species of iron (II). The presence of higher nutrient must be considered, especially in waters with elevated concentration of silicate, like coastal waters or eutrophicated waters, because the oxidation process will be faster. In addition, the speciation of

iron (II), in the range of seawater pH, will be dominated by the Fe(II)-Silicate species.

CHAPTER IV:

The role of the organic exudates of *Phaeodactylum tricornutum* on the Fe(II) oxidation rate constant

González, A.G., Santana-Casiano, J.M., González-Dávila, M., Pérez, N. 2011. The role of the organic exudates of Phaeodactylum tricornutum on the Fe(II) oxidation rate constant. Ciencias Marinas. In press.

ABSTRACT

Fe(II) oxidation kinetics were studied in seawater and in seawater enriched with exudates excreted by *Phaeodactylum tricornutum* as an organic ligand model. The exudates produced after 2, 4 and 8 days of culture at $6.21 \cdot 10^7$ cell/L, $2.29 \cdot 10^8$ cell/L and $4.98 \cdot 10^8$ cell/L were selected. The effects of the pH (7.2-8.2), temperature (5-35ºC) and salinity (10-36.720) on the Fe(II) oxidation rate were studied. All the data were compared with the results for seawater without exudates (seawater control).

The Fe(II) rate constant decreased as a function of the time of culture and cell concentration in the culture at different pH, temperature and salinity. All the experimental data obtained in this study were fitted to a polynomial function in order to quantify the fractional contribution of the organic exudates from the diatoms to the Fe(II) oxidation rate in natural seawater. Experimental results showed that the organic exudates excreted by *Phaeodactylum tricornutum* affect the Fe(II) oxidation, increasing the life time of the Fe(II) in seawater. A kinetic model approach was carried out in order to account for the speciation of each Fe(II) type together with its contribution to the overall rate.

CHAPTER IV

4.1. Introduction

Diatoms are thought to contribute as much as 25% of the global primary production (Scala and Bowler, 2001) and their population is the largest among microalgae in the oceans. Comparing with phytoplankton, diatoms are responsible for about 40% of the marine productivity (Falkowski et al., 1998). Diatoms are unicellular photosynthetic eukaryotes, genus *Chromophytes* and class Bacillariophyceae whose peculiarity is their siliceous cell wall, which can be used as a line of defence against various types of grazers (Ban et al., 1999). Despite their abundance and diversity in nature, few species are used for biotechnological application, for example: (1) silicon production from frustules for technological application in nanotechnology, pollution remediation, aquaculture thanks to the lipid and amino rich algal contents, (2) intracellular metabolites that accumulate in cells (lipids, eicosapentaenoic acid or EPA) for pharmaceutical applications, and (3) extracellular metabolites released into the medium and antibiotics.

In this study, the chosen diatom was *Phaeodactylum tricornutum*, recognised the first time by Bohlin (1897) (Lewin et al., 1958). This species is the only species in the genus *Phaeodactylum* and presents different morphotypes (fusiform, triradiate and oval). *P. tricornutum* is the second diatom for which a whole genome sequence has been generated (Bowler et al., 2008). A notable characteristic of this microalga is its ability to produce EPA in high proportions to the total fatty acid content (Rebolloso-Fuentes et al., 2000).

In natural waters, there is a mixture of organic compounds from microorganisms and various different microalgae. In order to elucidate the influence of the various different organic ligands excreted by these organisms, studies of individual species are required. This chapter studies the effect of the exudates from an individual microalgae, the diatom *P. tricornutum*, on the Fe(II) oxidation rate constant, as a function of the pH (7.2-8.2), temperature (5-35ºC) and salinity (10-36.720). The experiments were carried out at different growth stages. A diatom was selected because of its varied habitat customs and on account of the fact that it is one of the most abundant photosynthetic organisms in marine waters.

4.2. Results and Discussion

4.2.1. Effect of cell concentration

Under an f/2 media with only nutrients added, *P. tricornutum* grew with an exponential phase (2^{nd} day = $6.21 \cdot 10^7$ cell/L, 4^{th} day = $2.29 \cdot 10^8$ cell/L) and a stationary phase (8^{th} day = $4.98 \cdot 10^8$ cell/L), similar to the results obtained in previous work (Vasconcelos et al., 2002; Vasconcelos and Leal, 2008) (Figure 2-2A).

We studied the effect of the organic exudates excreted from the *P. tricornutum* cultures under different growth phases and at different cell concentrations (10^7 - $4.98 \cdot 10^8$ cell/L) (Figure 4-1). The Fe(II) oxidation rate constant decreased in the exudate medium as the cell concentration increased with time of growth (Figure 4-1).

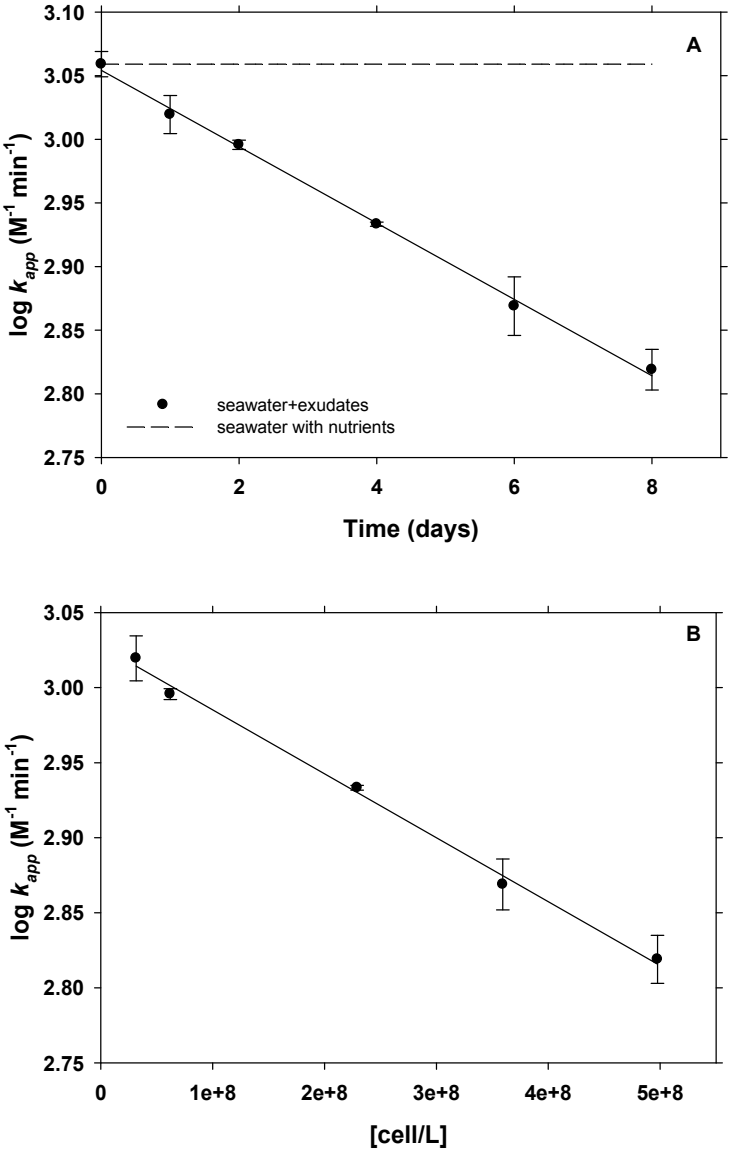

Figure 4-1. The Fe(II) oxidation rate constant in seawater enriched with exudates (A) as a function of the growth stage and (B) as a function of the cell concentration.

The major differences were observed in the case of exudates from $4.98 \cdot 10^8$ cell/L, with the maximum growth obtained in *P. tricornutum* cultures. The log k_{app} at $4.98 \cdot 10^8$ cell/L was 2.82 ± 0.02 (M^{-1} min^{-1}), 0.22 units lower than in seawater enriched with f/2 nutrients (log k_{app} = 3.04 ± 0.01; k_{app} in M^{-1} min^{-1}), a total difference of 436 M^{-1} min^{-1} in k_{app}. Therefore, the Fe(II) rate constant showed that the maximum difference in k_{app} between control seawater and seawater with the extra organic exudates from the *P. tricornutum* cultures was 60% less, at pH=8.0 and T=25ºC. The experimental data were fitted to a polynomial function (Equation 50) in function of a cell concentration ([cell/L]) where the R^2=0.998.

$$\log k_{app,[cell]} = 3.028 - 4.258 \cdot 10^{-10}[cell] \quad (50)$$

The decrease in the Fe(II) oxidation rate in the presence of the organic exudates from *P. tricornutum* resulted in an increase of the half-life time ($t_{1/2}$) from 3.0 mins (seawater) to 3.4 mins ($6.21 \cdot 10^7$ cell/L), 4.0 mins ($2.29 \cdot 10^8$ cell/L) and 5.1 mins ($4.98 \cdot 10^8$ cell/L), at pH 8.0 and T=25ºC (Table 4-1).

This difference indicated that the $t_{1/2}$ was almost double when the seawater was enriched with exudates excreted from $4.98 \cdot 10^8$ cell/L of *P. tricornutum*, allowing the presence of the Fe(II) in solutions for longer periods of time. The linear decrease of log k_{app} as a function of cell concentration in the culture showed that the type of organic ligands that were interacting with Fe(II) either did not change over time or, if changes took place, behaved in a similar way.

The exudates produced by the *P. tricornutum* have been identified in seawater enriched with nutrients (Vasconcelos et al., 2002; Vasconcelos and Leal, 2008) under similar growth conditions. It was established that thiolic

compounds were major contributors to the organic ligands released by eukaryotic algae, such as *P. tricornutum*. The cysteine and gluthatione (as thiolic compounds) concentration increased as the cell concentration increased in culture. Cysteine was found to be the most important ligand excreted. Moreover, the Fe(II) regeneration in the presence of cysteine was demonstrated (Santana-Casiano et al., 2000). In addition, free cysteine and compounds identified as glutathione (c-glutamylcysteinylglycine) were found in natural seawater (van den Berg et al., 1988; Le Gall and van den Berg, 1993).

4.2.2. The effect of pH

The effect of pH on the Fe(II) oxidation rate constant was studied from 7.2 to 8.2 (Figure 4-2) in seawater enriched with exudates from *P. tricornutum* cultures, considering the two different growth phases, as seen in Figure 2-2A, an exponential phase (2^{nd} day = $6.21 \cdot 10^7$ cell/L, 4^{th} day = $2.29 \cdot 10^8$ cell/L) and a stationary phase (8^{th} day = $4.98 \cdot 10^8$ cell/L).

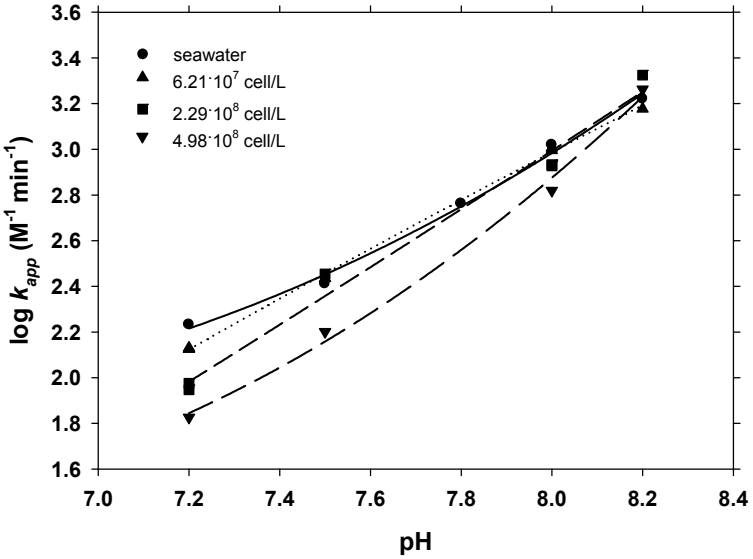

Figure 4-2. The Fe(II) oxidation rate constant as a function of the pH for seawater, and seawater enriched with organic exudates from *P. tricornutum at* T=25ºC. The lines represent the fitting results obtained from Equation 51.

The Fe(II) oxidation rate as a function of the pH (Figure 4-2) was fitted to a second order polynomial function (Equation 51). This second order dependence has been reported in other previous work (Santana-Casiano et al., 2004; 2005; González-Dávila et al., 2006).

$$\log k_{app,pH} = 9.4(\pm 0.1) - 2.94(\pm 0.03)\,pH + 0.27(\pm 0.02)\,pH^2 - 3.7\cdot 10^{-10}(\pm 0.1\cdot 10^{-10})[cell] \quad (51)$$

where R^2 was 0.996 and the standard error of estimation of 0.08 in log k_{app}. In addition, the effect of the organic exudates from *P. tricornutum* was computed by subtracting the pH dependence in seawater control and seawater enriched with exudates (Equation 52), where the R^2 was 0.977 and the standard error of estimation was 0.12 in log k_{app}.

$$\Delta \log k_{app,pH} = -17(\pm 2) + 3.6(\pm 0.5)\,pH - 0.16(\pm 0.03)\,pH^2 + 1.8 \cdot 10^{-9}(\pm 0.2 \cdot 10^{-9})[cell] \quad (52)$$

The log k_{app} decreased with the pH and also as a function of cell concentration in the cultures. The Fe(II) rate constant was slower with exudates collected from higher cell concentration cultures: $4.98 \cdot 10^8$ cell/L < $2.29 \cdot 10^8$ cell/L < $6.21 \cdot 10^7$ cell/L < control seawater (with f/2 nutrients). In addition, the differences between the log k_{app} obtained from seawater enriched with nutrients and the seawater enriched with the organic exudates was higher at lower pH. This difference is more pronounced when the culture is in the stationary phase ($4.98 \cdot 10^8$ cell/L). Here, $\Delta \log k_{app}$ = 0.41 (in M^{-1} min^{-1}) at pH=7.2. In fact, the Fe(II) oxidation rate for pH=7.2 was 60.2% slower in the presence of exudates. In addition, the effect of the organic exudates was more significant at lower pH, being virtually negligible at pH 8.2.

The decrease of the log k_{app} as a function of the pH for the different growth stages, allowed us to show that $t_{1/2}$ increased from 19.9 mins (seawater enriched with nutrients) to 50.6 mins ($4.98 \cdot 10^8$ cell/L) at pH 7.2. Therefore, these studies demonstrated that the organic exudates from the *P. tricornutum* increased the half-life time ($t_{1/2}$) in seawater as a function of the pH.

The effect of the pH on the Fe(II) oxidation is significant because it can control the speciation and the contribution of each species to the overall rate constant for Fe(II) in natural waters (Santana-Casiano et al., 2005), where changes in the pH will modify the speciation of the Fe(II) and the properties of the organic ligands present in solution.

4.2.3. The effect of Temperature

The effect of temperature on the Fe(II) oxidation rate constant was studied between 5-35ºC (Figure 4-3) in the presence of organic exudates. The Fe(II) apparent rate constant increased with temperature and decreased as the cell concentration increased.

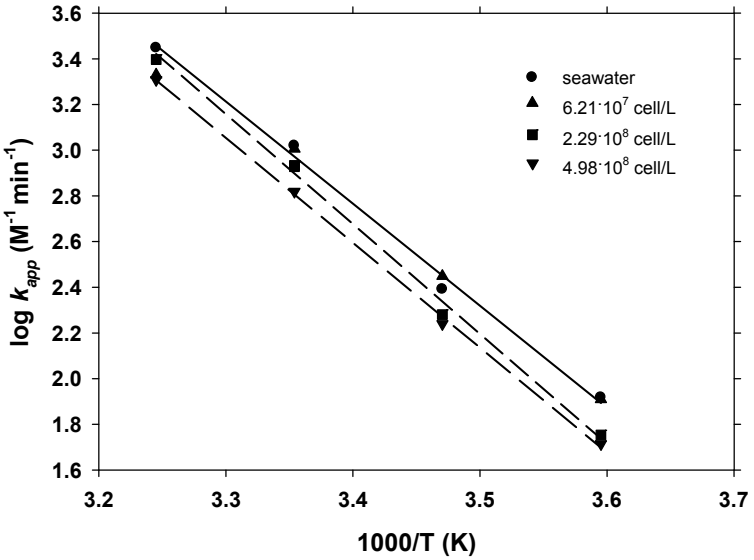

Figure 4-3. The Fe(II) oxidation rate constant as a function of temperature (K) for seawater, and seawater enriched with organic exudates from *P. tricornutum* at pH=8.0. The lines represent the fitting results obtained from Equation 53.

The experimental results were fitted to a linear equation (Equation 53).

$$\log k_{app,T} = 18.1(\pm 0.4) - 4498(\pm 130)/T - 3.5\cdot 10^{-10}(\pm 0.7\cdot 10^{-10})[cell] \quad (53)$$

where R^2 was 0.998 and the standard error of estimation in log k_{app} was 0.06. T was temperature in Kelvin. The effect of the exudates was also computed for temperature dependence, by subtraction from the seawater control (Equation 54), where R^2 was 0.986 and the standard error of estimation was 0.12 in log k_{app}.

$$\Delta \log k_{app,T} = 17.1(\pm 0.9) - 4577(\pm 263)/T + 1.8 \cdot 10^{-9}(\pm 0.2 \cdot 10^{-9})[cell] \quad (54)$$

The Energy of Activation (E_a) was 79 kJ mol^{-1} ($6.21 \cdot 10^7$ cell/L), 92 kJ mol^{-1} ($2.29 \cdot 10^8$ cell/L) and 88 kJ mol^{-1} ($4.98 \cdot 10^8$ cell/L). E_a for seawater, with f/2 nutrients, was 86 kJ mol^{-1}. These values were comparable with previous results in Gulf Stream seawaters (104 kJ mol^{-1}, Santana-Casiano et al., 2005) and Pacific Sub-Arctic waters (97 and 109 kJ mol^{-1}, Roy et al., 2008).

The differences in the log k_{app} between seawater and the seawater enriched with exudates at the highest growth state culture ($4.98 \cdot 10^8$ cell/L) remained constant at 58 ± 6%, in k_{app}, within the temperature range considered. According to the experimental results, the Fe(II) may persist in surface waters between 27.6 mins (seawater) and 44.2 mins ($4.98 \cdot 10^8$ cell/L) at 5ºC (Table 4-1), which could explain the presence of Fe(II) in cold waters (i.e. Southern Ocean, Pacific Sub-Arctic waters) (Roy et al., 2008).

4.2.4. The effect of Salinity

The effect of salinity (10.0 to 36.720) on the Fe(II) oxidation rate constant (Figure 4-4) was studied in the presence of organic exudates from

the *P. tricornutum* cultures, where the bicarbonate concentration was kept constant at 2 mM.

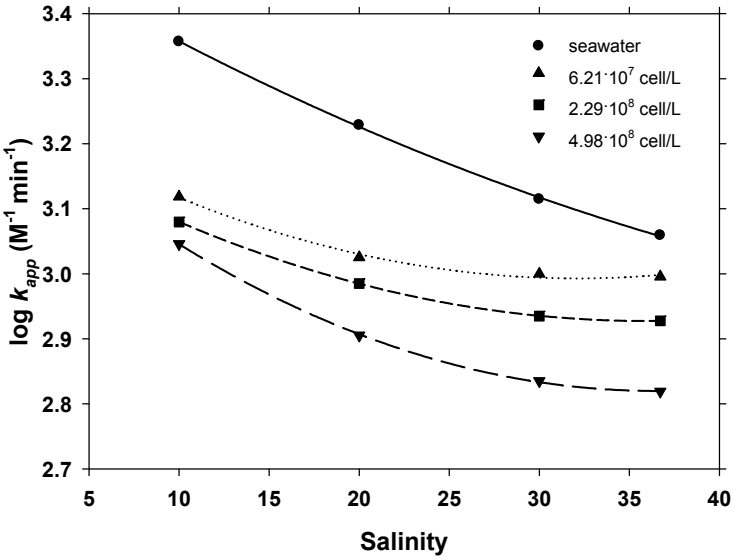

Figure 4-4. The Fe(II) oxidation rate constant as a function of salinity for seawater, and seawater enriched with organic exudates from *P. tricornutum* at pH=8.0. The lines represent the fitting results obtained from Equation 55.

The Fe(II) rate constant decreased as salinity increased, following a second order polynomial function for organic exudates from $6.21 \cdot 10^7$ cell/L, $2.29 \cdot 10^8$ cell/L and $4.98 \cdot 10^8$ cell/L cultures (Equation 55).

$$\log k_{app,S} = 3.39(\pm 0.04) - 0.017(\pm 0.004)S + 1.97 \cdot 10^{-4}(\pm 0.8 \cdot 10^{-4})S^2 - 4.7 \cdot 10^{-10}(\pm 0.4 \cdot 10^{-10})[cell] \quad (55)$$

where R^2 was 0.980 and the standard error of estimation in log k_{app} was 0.02. S corresponded to the salinity. The effect of the exudates as a function of

salinity was also computed by subtracting this effect from the seawater control (Equation 56), where R^2 was 0.963 and the standard error of estimation was 0.13.

$$\Delta \log k_{app,S} = 2.06(\pm 0.02) - 0.015(\pm 0.00)S + 2 \cdot 10^{-4}(\pm 0.5 \cdot 10^{-4})S^2 + 1.8 \cdot 10^{-9}(\pm 0.2 \cdot 10^{-9})[cell] \quad (56)$$

The effect of salinity on the Fe(II) rate constant was less significant in the presence of organic exudates than in their absence (seawater with f/2 nutrients). The difference in the log k_{app} between S=10 and S=36.720 was 0.30 (M^{-1} min^{-1}).

The difference in the log k_{app}, in seawater enriched with *P. tricornutum* exudates, was 0.17 ± 0.05 (M^{-1} min^{-1}). The organic ligands play a key role in the Fe(II) oxidation rate constant stabilization as compared with seawater without ligands, where there is a greater control in the oxidation process by major ionic species. At low salinity values, the oxidation rate in the presence of exudates is slightly faster, which may be related to ionic effects on the complexation capacity and strength.

All the experimental results obtained in the present study were fitted to a polynomial function (Equation 57) (k_{app} in M^{-1} min^{-1}), as a function of the pH (free ion scale), temperature (K), salinity, and cell concentration (cell/L).

$$\log k_{app} = 3.594\,pH - 0.156\,pH^2 - 4577/T - 0.015S + 2.00 \cdot 10^{-4} S^2 - 3.39 \cdot 10^{-10}[cell] \quad (57)$$

R^2 was 0.989 and the standard error of estimation was 0.08.

The effect of the organic ligands from *P. tricornutum* on the Fe(II) oxidation rate can be computed by subtracting the Fe(II) oxidation rate in seawater control (log $k_{app,SWcontrol}$) from the Fe(II) oxidation rate in the presence of organic exudates (log $k_{app,cell}$) (Equation 58-59).

$$\log k_{app} = \log k_{app,SWcontrol} - \log k_{app,cell} \tag{58}$$

$$\log k_{app} = \log k_{app,SWcontrol} + 1.291 - 3.579\,pH + 0.155\,pH^2 + 4577/T + 0.015S - 2\cdot10^{-4}S^2 - 1.833\cdot10^{-9}[cell] \tag{59}$$

where R^2 was 0.999 and the standard error of estimation was 0.001.

These results indicated that the Fe(II) oxidation rate is retarded by the total compounds that form the *P. tricornutum* exudates, where some compounds may accelerate and others delay the Fe(II) oxidation rate. Due to the complex mix of compounds found in the ocean, any extrapolation should be ventured with care. If the behaviour observed in this study for the organic exudates from *P. tricornutum* is assumed to be a model for other diatom cultures, Equation 59 will allow the calculation of the apparent Fe(II) oxidation rate under different experimental conditions. The equation allows us to quantify the equivalent amount of *P. tricornutum* cells that produce a similar effect.

A number of experiments must be carried out in order to improve our knowledge with respect to exudates and their speciation. These studies will allow us to identify what kind of natural organic exudates accelerate or delay the Fe(II) oxidation rate.

4.2.5. Speciation and Fractional contribution to the overall rate constant

In order to compute the effects of the organic ligands produced by *P. tricornutum* (Figure 4-5) and the fractional contribution of each Fe(II) species to the overall rate constant (Figure 4-6), the kinetic model for seawater and seawater at high nutrient concentrations (Santana-Casiano et al., 2005; González-Dávila et al., 2006; González et al., 2010a) was applied. The effect of nutrients was considered because the seawater control was seawater enriched with f/2 nutrients. In addition, the model approach considered the effect of organic exudates in solution by assuming that the Fe(II)-exudates and free Fe(II) are oxidized at different rates. The organic exudates were considered to be one single ligand, ($L + H = LH$; K_a=10^4-10^5, fitting results). The individual contribution for each Fe(II) species to the overall kinetic rate was computed from the results of the kinetic model and the speciation of the Fe(II) (Equation 60).

$$k = k_{Fe^{2+}}\alpha_{Fe^{2+}} + k_{FeOH^+}\alpha_{FeOH^+} + k_{Fe(OH)_2}\alpha_{Fe(OH)_2} + k_{FeHCO_3^+}\alpha_{FeHCO_3^+} + k_{Fe(CO_3)}\alpha_{Fe(CO_3)} + k_{Fe(CO_3)_2^{2-}}\alpha_{Fe(CO_3)_2^{2-}} + k_{Fe(CO_3)OH^-}\alpha_{Fe(CO_3)OH^-} + k_{FeCl^+}\alpha_{FeCl^+} + k_{FeSO_4}\alpha_{FeSO_4} + k_{FeH_3SiO_4^+}\alpha_{FeH_3SiO_4^+} + k_{Fe(II)-L}\alpha_{Fe(II)-L} \quad (60)$$

where $\alpha_i = [FeX_i]/[Fe(II)]_T$ are the molar fractions of each Fe(II) species in the solution. k is the apparent overall rate constant (M^{-1} min^{-1}) and k_i are the individual rate constants for the Fe(II) species. Fe(II)-L represents the Fe(II)-exudate complex.

Organic ligands in natural waters can either accelerate or decelerate Fe(II) oxidation, depending on their structure (Santana-Casiano et al., 2000; Rose and Waite, 2003b). The kinetic model allows us to compute the

concentration of exudates in solution, their equilibrium constants and the oxidation rate for the Fe(II)-exudates. The best fit for all the experiments carried out gave $K_{Fe(II)-L}=10^7$ and log k = 1.08 ± 0.43 (M^{-1} min^{-1}). The total exudate concentration was calculated as 11 ± 1 nM ($6.21 \cdot 10^7$ cell/L), 113 ± 4 nM ($2.29 \cdot 10^8$ cell/L) and 170 ± 10 nM ($4.98 \cdot 10^8$ cell/L). The total ligand concentration was similar to that measured by other authors for the 8th day of culture ($4.98 \cdot 10^8$ cell/L) (Vasconcelos et al., 2002; Vasconcelos and Leal, 2008).

The speciation for all the Fe(II) species involved in these cultures is shown in Figure 4-5. When the exudate concentration increased in the solution, the Fe(II)-L became the most important species. This Fe(II)-L was negligible at $6.21 \cdot 10^7$ cell/L (5 %) and was the first species at $4.98 \cdot 10^8$ cell/L (50%), whereas the Fe^{2+} moved from 58% in seawater reference to 29% in seawater with exudates from $4.98 \cdot 10^8$ cell/L of *P. tricornutum*, at pH under 7.2. At pH 8.5, Fe(II)-L changed from 2% ($6.21 \cdot 10^7$ cell/L) to 20% ($4.98 \cdot 10^8$ cell/L). In addition, the speciation of each Fe(II) species at pH 8.0 adopts the following sequence at under $6.21 \cdot 10^7$ cell/L: $Fe^{2+} > FeCO_3 > Fe(H_3SiO_4)^+ > FeCl^+ > Fe(SO_4) >$ Fe(II)-L $> Fe(CO_3)(OH)^-$. At $4.98 \cdot 10^8$ cell/L, the Fe(II) species followed Fe(II)-L $> Fe^{2+} > FeCO_3 > Fe(H_3SiO_4)^+ > FeCl^+ > Fe(SO_4) > Fe(CO_3)(OH)^-$.

The fractional contribution to the overall rate constant was also computed (Figure 4-6). The model results showed that the Fe(II)-L contribution was important at lower pH. The Fe(II)-L contribution was virtually negligible at the second day of culture ($6.21 \cdot 10^7$ cell/L) (1%) but the second most significant contributor towards the overall oxidation rate in the presence of exudates from $4.98 \cdot 10^8$ cell/L (16%), at pH 6. At pH 8.0, the contribution to the overall oxidation rate of Fe(II)-L was under 0.3% for each case. Whereas the Fe(II) complexes reduced the overall oxidation rate, the

ratio between oxidation rates for other species was practically constant in the seawater reference and for the seawater enriched with exudates at pH ≥ 8.0.

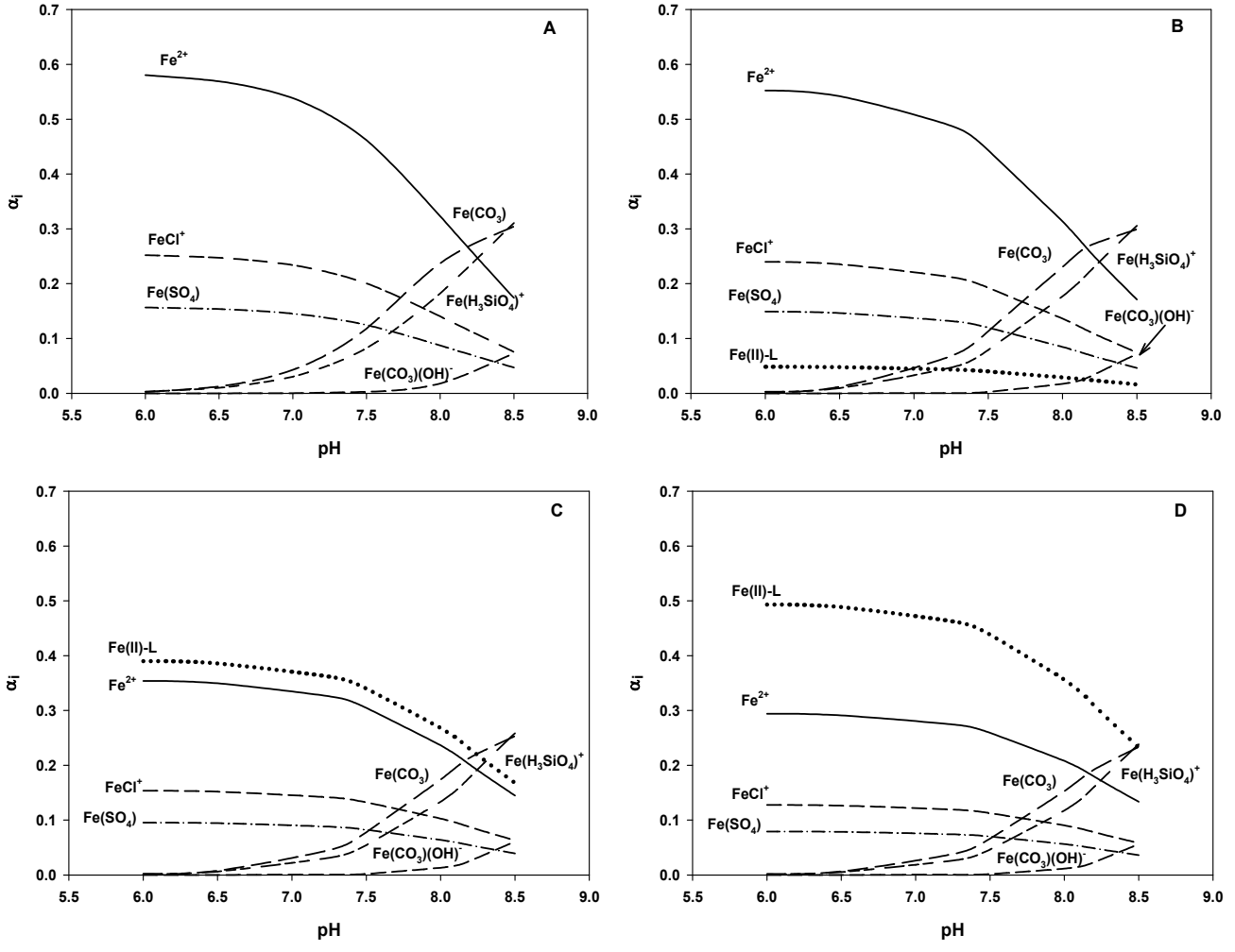

Figure 4-5. The speciation of Fe(II) in different media: (A) control seawater (seawater with f/2 nutrients) (B) seawater enriched with exudates from $6.21 \cdot 10^7$ cell/L (C) seawater enriched with exudates from $2.29 \cdot 10^8$ cell/L (D) seawater enriched with exudates from $4.98 \cdot 10^8$ cell/L.

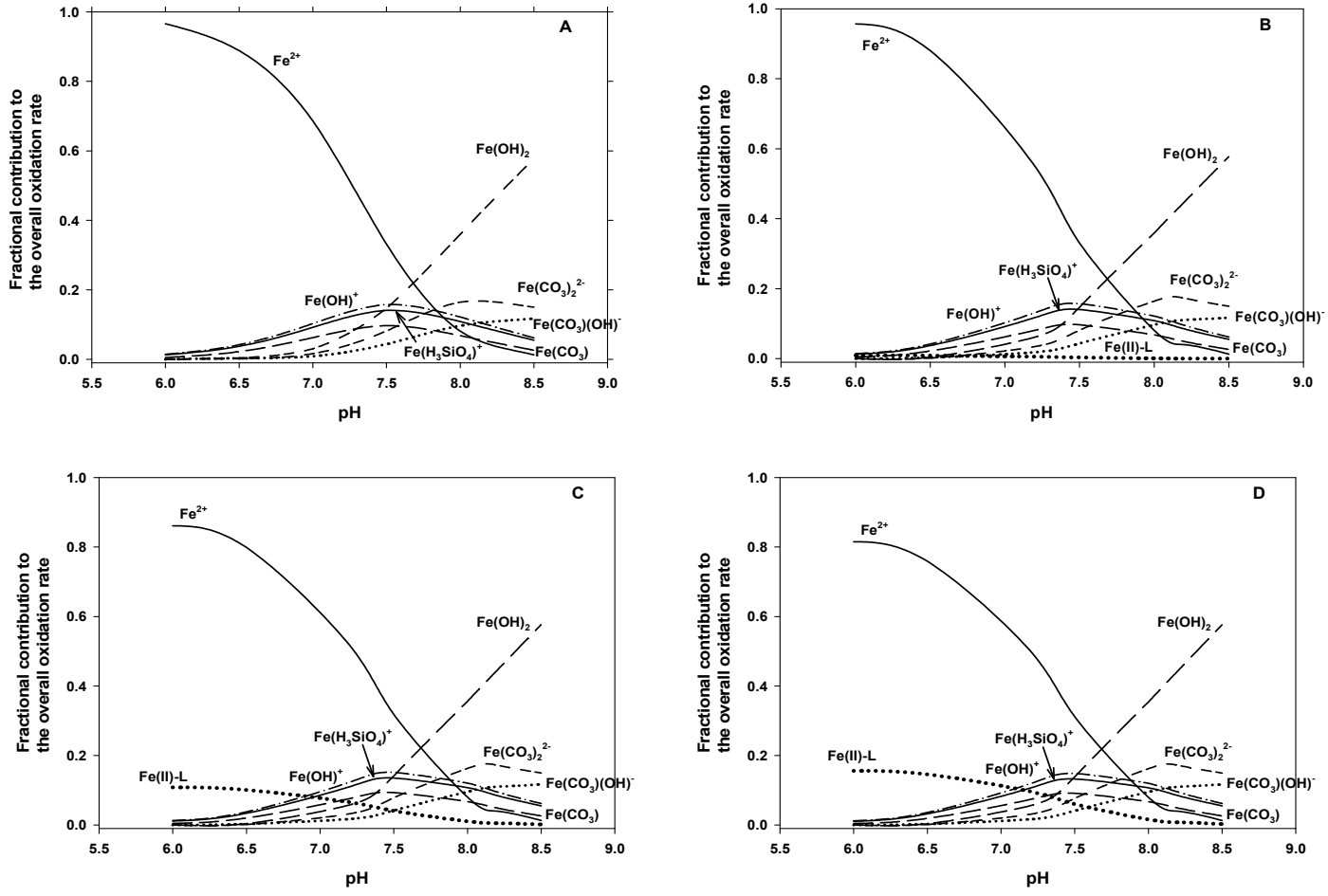

Figure 4-6. The contribution of each Fe(II) species to the overall rate constant for different media: (A) control seawater (seawater with f/2 nutrients) (B) seawater enriched with exudates from $6.21 \cdot 10^7$ cell/L (C) seawater enriched with exudates from $2.29 \cdot 10^8$ cell/L (D) seawater enriched with exudates from $4.98 \cdot 10^8$ cell/L.

Table 4-1. Half-life time accounted from pseudo-first order kinetic rate constant for Fe(II) in the seawater and seawater enriched with organic exudates produced by *P. tricornutum* as a function of pH, temperature and salinity.

Media	pH	Temperature (ºC)	Salinity	log k_{app} (M^{-1} min^{-1})	$t_{1/2}$ (min)
Seawater (reference)	7.2	25	36.72	2.23	19.9
	7.5	25	36.72	2.41	13.2
	7.8	25	36.72	2.76	5.9
	8.0	25	36.72	3.04	3.0
	8.2	25	36.72	3.22	2.0
	8.0	5	36.72	1.92	27.6
	8.0	15	36.72	2.39	11.5
	8.0	35	36.72	3.45	1.4
	8.0	25	10.00	3.36	1.3
	8.0	25	20.00	3.23	1.8
	8.0	25	30.00	3.12	2.5
Seawater enriched with exudates after 2 days culture ($6.21 \cdot 10^7$ cell/L)	7.2	25	36.72	2.13	25.3
	7.5	25	36.72	2.44	12.4
	8.0	25	36.72	3.00	3.4
	8.2	25	36.72	3.18	2.3
	8.0	5	36.72	1.91	28.2
	8.0	15	36.72	2.45	10.1
	8.0	35	36.72	3.33	1.3
	8.0	25	10.00	3.12	2.2
	8.0	25	20.00	3.03	2.9
	8.0	25	30.00	3.00	3.3
Seawater enriched with exudates after 4 days culture ($2.29 \cdot 10^8$ cell/L)	7.2	25	36.72	1.96	37.1
	7.5	25	36.72	2.40	13.6
	8.0	25	36.72	2.93	4.0
	8.2	25	36.72	3.33	1.6
	8.0	5	36.72	1.75	40.3
	8.0	15	36.72	2.28	14.8
	8.0	35	36.72	3.40	1.6
	8.0	25	10.00	3.08	2.4
	8.0	25	20.00	2.99	3.2
	8.0	25	30.00	2.94	3.8
Seawater enriched with exudates after 8 days culture ($4.98 \cdot 10^8$ cell/L)	7.2	25	36.72	1.83	50.6
	7.5	25	36.72	2.20	21.3
	8.0	25	36.72	2.82	5.1
	8.2	25	36.72	3.26	1.9
	8.0	5	36.72	1.71	44.2
	8.0	15	36.72	2.24	16.3
	8.0	35	36.72	3.31	2.0
	8.0	25	10.00	3.05	2.6
	8.0	25	20.00	2.91	3.8
	8.0	25	30.00	2.84	4.7

4.3. Conclusion

The Fe(II) oxidation rate constant is always lower in the presence of the organic exudates from *P. tricornutum* than the level to be found in seawater without ligands. The Fe(II) oxidation rate was linearly related to the cell concentration and time of culture. The rate constant decreased as a function of the growth stage, and the cell concentration as a function of pH, temperature and salinity. The effect of the salinity on log k_{app} in seawater enriched with organic exudates was less important than in seawater, due to the effect of these ligands on the Fe(II) stabilization. The results confirm the longer life-time of Fe(II) in natural surface waters under the effect of phytoplankton exudates. The overall equation (Equation 59) allows us to calculate the fraction of the Fe(II) rate constant that corresponds to the organic exudates from diatoms in natural waters. The kinetic model approach was in line with experimental data and demonstrated that the Fe(II)-exudates from *P. tricornutum* were significant, affecting both the speciation and the contribution to the overall rate of each species of Fe(II) in seawater.

CHAPTER V:

The effect of *Dunaliella tertiolecta* organic exudates on the Fe(II) oxidation kinetic in seawater

González, A.G., Santana-Casiano, J.M., González-Dávila, M., Pérez, N. 2011. The effect of Dunaliella tertiolecta organic exudates on the Fe(II) oxidation kinetic in seawater. Geochim. Cosmochim. Acta. In revision.

ABSTRACT

Fe(II) oxidation was studied in seawater and in seawater enriched with organic exudates collected from *Dunaliella tertiolecta*, in order to demonstrate the role of the ligands excreted by this type of phytoplankton on the Fe(II) oxidation rate. Fe(II) oxidation kinetics were studied as a function of cell density ($1 \cdot 10^7$-$5.04 \cdot 10^8$ cell/L), pH (7.2-8.2), temperature (5-35ºC) and salinity (10-36.720). The effect of the exudates on the apparent Fe(II) rate constant was computed as a function of all the parameters studied, in order to quantify the contribution of the organic ligands excreted by *Dunaliella tertiolecta* to the Fe(II) oxidation rate in seawater. A kinetic modelling approach was used to describe the Fe(II) speciation and the contribution of each Fe(II) species to the overall rate constant. This model considered the presence of two types of ligands: the carboxyl and the amino/phosphoryl groups. Both organic complexes, Fe(II)-LH and Fe(II)-L, play an important role in the Fe(II) speciation, with the carboxylic groups as the most significant contributors to the overall Fe(II) oxidation rate constant at low pH.

CHAPTER V

5.1. Introduction

The green algal genus *Dunaliella* was initially described by (Teodoresco, 1905) and it was named in honour of M.F. Dunal who was the first to recognise the genus at Montpellier (France) (Dunal, 1837). Even since the original observations, the genus *Dunaliella* has been used for numerous studies about physiology, biochemistry, ecology and commercial applications (Avron and Ben-Amotz, 1992; Milledge, 2011). This interesting is a result of several factors including (1) the easy of culturing most of the species, (2) the ability of several species to grow over wide salinity ranges, (3) the accumulation of extremely high levels of β-carotene and (4) the wide tolerance to heavy metals and pesticides by some species (Borowitzka and Siva, 2007). *Dunaliella tertiolecta* has similar features that the rest of *Dunaliella* species, but is characterized because *D. tertiolecta* is always green and radically symmetrical. Cells have dimensions between 5-18 μm in length (mean 9.4-12.4 μm) and 4.5-14 μm wide (mean 7.1-8.2 μm). Generally, flagella are 2-2.5 times the cell length.

In the present chapter, *D. tertiolecta* has been selected as a reference phytoplankton species in order to study the effect of cell exudates on the oxidation of Fe(II) in seawater. In addition, the oxidation of Fe(II) has been considered as a function of the pH (7.2-8.2), temperature (5-25ºC) and salinity (10-36.720), maintaining the air-saturated conditions in the experiments. Finally, a kinetic modelling approach has been carried out in order to compute the individual Fe(II) species and their contribution to the overall rate constant. It must be highlighted that there are few studies on the role of natural exudates on the oxidation process of Fe(II), using

phytoplankton cultures. This study will contribute, therefore, toward the understanding of the bio-geochemical cycle of iron in natural waters.

5.2. Results and Discussion

5.2.1. Cell concentration dependence

The growth of *D. tertiolecta* was shown in the Figure 2-2B indicating that the culture has two different growth stages. The exponential phase (2nd day=$5.52 \cdot 10^7$ cell/L and 4th day=$2.17 \cdot 10^8$ cell/L) and the stationary phase (8th day=$5.04 \cdot 10^8$ cell/L). The effect of cell density on the Fe(II) oxidation rate is shown in Figure 5-1.

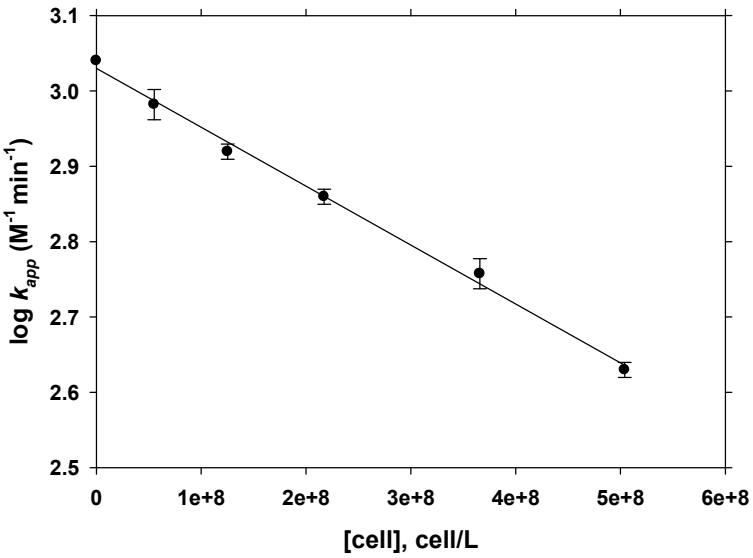

Figure 5-1. The Fe(II) oxidation rate constant in the seawater enriched with the three cell concentrations: $5.52 \cdot 10^7$, $2.17 \cdot 10^8$ and $5.04 \cdot 10^8$ cell/L, at pH=8.0 and T=25ºC.

The log k_{app} decreased linearly with the cell concentrations reaching a maximum difference at $5.04 \cdot 10^8$ cell/L. The $\Delta \log k_{app}$ was 1.39 (M^{-1} min^{-1}). This is equivalent to a reduction of 61% in k_{app}. The experimental data were fitted to a linear equation (Equation 61), with $R^2 = 0.997$ and a standard estimated error of 0.01 in log k_{app}.

$$\log k_{app,cell} = 3.03(\pm 0.01) - 7.8(\pm 0.2) \cdot 10^{-10}[cell] \quad (61)$$

The presence of organic exudates of *D. tertiolecta* affect the oxidation process and the corresponding oxidation rate constant, making the half-life time ($t_{1/2}$) increase from 3.0 mins (seawater control) to 8.0 mins ($5.04 \cdot 10^8$ cell/L). Therefore, the presence of higher concentrations of exudates from *D. tertiolecta* are increasing the number of cells in solution, stabilize Fe(II) in the solution.

The linear relationship between the Fe(II) oxidation rate constants and the cell concentrations showed that the organic exudates are capable of interacting with Fe(II), and they are either the same for all of the growth stages of *D. tertiolecta* or different ligands that behave in a similar way with respect to Fe(II). Therefore, the individual rates for different types of ligands are compensated over time.

The interaction between Fe(II) and organic matter in seawater has been suggested to be an essential process to the permanence of Fe(II) in surface waters. Several substance classes have been reported to be capable of reacting with trace metals: carboxylic groups, amino-acid groups (glutathione-like), humic substances, microbiologically modified organic matter, sulphur-rich substances, transparent exopolymer particles, where phytoplankton exudates represent one of the most important sources of these organic ligands in aqueous environments (Bruland et al., 1991; Aluwihare

and Repeta, 1999; Leal et al., 1999; Quigley et al., 2001; Benner, 2002; Laglera and van den Berg, 2003; Muller et al., 2003; Blake et al., 2004; Bhaskar and Bhosle, 2006; Laglera and van den Berg, 2006; Dryden et al., 2007; Lorenzo et al., 2007; Zhang et al., 2008).

The importance of this complexation process must be highlighted, because Fe(II) oxidation has been identified as an obligatory step before internalisation of Fe(III) (Anderson and Morel, 1982; Maldonado and Price, 2001).

5.2.2. pH dependence

The dependence of log k_{app} as a function of pH was studied in the pH range 7.2 to 8.2 (Figure 5-2) in seawater control and seawater enriched with organic exudates from *D. tertiolecta*. According to the results obtained, the Fe(II) oxidation rate constant decreased as a function of the pH. In addition, the log k_{app} was reduced when the cell concentration was higher in the culture. The Fe(II) oxidation rate constant (log k_{app}) changed from 3.22 ± 0.03 M^{-1} min^{-1} in control seawater to 2.97 ± 0.03 M^{-1} min^{-1} ($5.04 \cdot 10^8$ cell/L), at pH 8.2. At pH 7.2, the log k_{app} changed from 2.23 ± 0.01 M^{-1} min^{-1} (the seawater control) to 2.08 ± 0.03 M^{-1} min^{-1} ($5.52 \cdot 10^7$ cell/L), 2.03 ± 0.02 M^{-1} min^{-1} ($2.17 \cdot 10^8$ cell/L) and 1.86 ± 0.01 M^{-1} min^{-1} ($5.04 \cdot 10^8$ cell/L). The k_{app} was reduced by 43% and 56% at pH 7.2 and 8.2, respectively, when the seawater control was enriched with organic exudates from *D. tertiolecta* ($5.04 \cdot 10^8$ cell/L).

The Fe(II) oxidation rate constant as a function of pH was fitted to a second order equation (Equation 62), where R^2 was 0.998 and the standard estimated error was under 0.04, in log k_{app}.

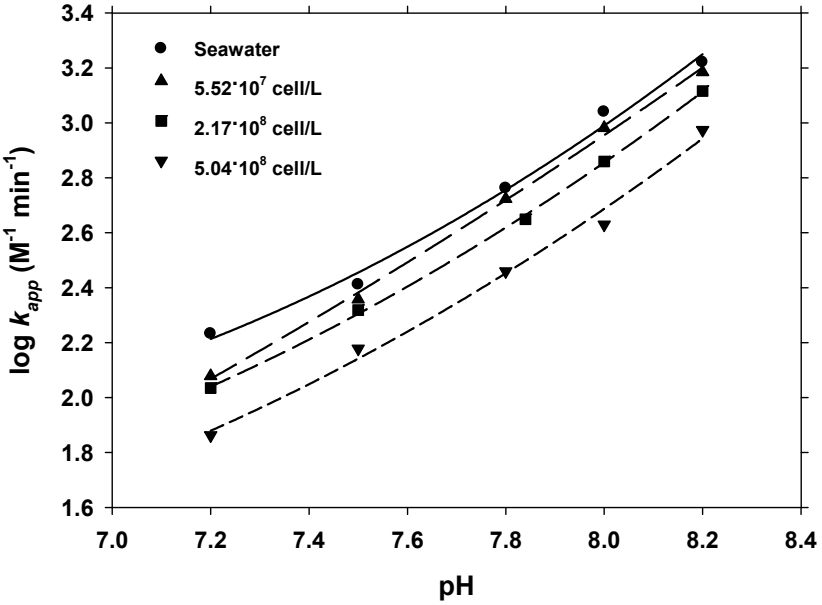

Figure 5-2 The Fe(II) oxidation rate constant as a function of the pH for the seawater and the seawater enriched with organic exudates excreted by the *Dunaliella tertiolecta* for the three different cell densities ($5.52 \cdot 10^7$, $2.17 \cdot 10^8$ and $5.04 \cdot 10^8$ cell/L). The temperature was kept constant (25ºC). The lines represent the fitting results obtained from Equation 62.

The same dependence has been reported in recent studies on Fe(II) oxidation in seawater (Santana-Casiano et al., 2004; González-Dávila et al., 2006; González et al., 2010a).

$$\log k_{app,pH} = 7.1(\pm 0.5) - 2.3(\pm 0.1)\,pH + 0.22(\pm 0.08)\,pH^2 - 5.8(\pm 0.4)\cdot 10^{-10}[cell] \quad (62)$$

The Fe(II) oxidation rate affected only by the cell exudates has been calculated (Equation 63) by subtracting the log k_{app} values in the seawater control and the log k_{app} in the presence of organic exudates for the three cell densities considered in this study. R^2 was 0.997 and the standard estimated

error in log k_{app} was 0.14. The pH dependence has been included in order to show the impact of ocean acidification effect and its impact on the thermodynamics and kinetics of metals in seawater (Millero et al., 2009).

$$\Delta \log k_{app,pH} = -17(\pm 2) + 3.7(\pm 0.7)\,pH - 0.17(\pm 0.04)\,pH^2 + 1.7(\pm 0.2)\cdot 10^{-9}[cell] \quad (63)$$

The half-life time ($t_{1/2}$) is shown in the Table 5-1. The $t_{1/2}$ increased 8.4 mins, 0.5 mins and 0.2 mins, when we compared the result for seawater and seawater enriched with exudates from $5.52\cdot10^7$ cell/L, at pH 7.2, 8.0 and 8.2 respectively. In addition, when exudates from $5.04\cdot10^8$ cell/L of *D. tertiolecta* were present, the $t_{1/2}$ increased by 26.6 mins, 5 mins and 1.6 mins, indicating that Fe(II) can remain longer times in surface seawater as a consequence of the Fe(II)-organic exudate interaction.

5.2.3. Temperature effect

The temperature dependence of the Fe(II) oxidation rate constant was studied in the range 5 to 35ºC at different cell concentrations of *D. tertiolecta* (Figure 5-3A). The experimental data showed a linear dependence of the Fe(II) oxidation rate constants with the temperature. In addition, the log k_{app} values were lower in seawater when the cell concentration was increased. The log k_{app} was 1.92 ± 0.02 (M^{-1} min^{-1}) at 5ºC, in the seawater control, and its value decreased to 1.83 ± 0.01 (M^{-1} min^{-1}), 1.76 ± 0.01 (M^{-1} min^{-1}) and 1.60 ± 0.03 (M^{-1} min^{-1}) for $5.52\cdot10^7$, $2.17\cdot10^8$ and $5.04\cdot10^8$ cell/L, respectively. Therefore, attending to the cell density, the Fe(II) oxidation rate constant can be slowed by 52.14% at 5ºC (k_{app}).

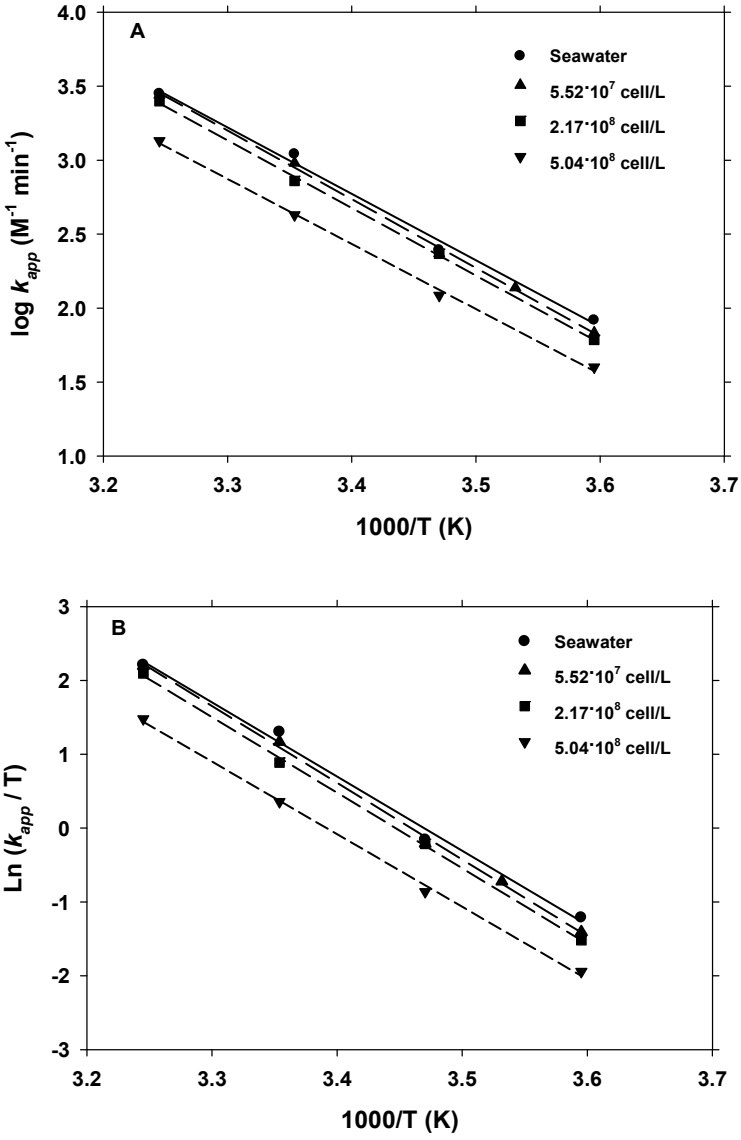

Figure 5-3. The Fe(II) oxidation rate constant as a function of temperature for the seawater and the seawater enriched with the organic exudates excreted by the *Dunaliella tertiolecta* for the three different cell densities ($5.52 \cdot 10^7$, $2.17 \cdot 10^8$ and $5.04 \cdot 10^8$ cell/L). (A) Corresponds to the Arrhenius plot and (B) corresponds to the Eyring plot. The pH was kept constant (8.0). The lines represent the fitting results obtained from Equation 64.

The experimental results were fitted to a linear equation (Equation 64), where R^2 was 0.998 and the standard estimate error was 0.04 in log k_{app} terms.

$$\log k_{app,T} = 18.2(\pm 0.3) - \frac{4534(\pm 80)}{T} - 6.6(\pm 0.5)\cdot 10^{-10}[cell] \qquad (64)$$

The change in the Fe(II) oxidation rate constant due to the presence of organic ligands was also calculated (Equation 65), where R^2 was 0.986 and with a standard estimate error of 0.13.

$$\Delta \log k_{app,T} = 17.2(\pm 0.9) - \frac{4557(\pm 29)}{T} + 1.7(\pm 0.2)\cdot 10^{-9}[cell] \qquad (65)$$

The Arrhenius equation can be used in order to obtain crucial information about collisions and the energy of activation (E_a), according to Equation 66.

$$k = Ae^{-Ea/RT} \qquad (66)$$

where $e^{-Ea/RT}$ provides information about the fraction of collisions with enough energy to react (Atkins, 2008), and, thus k is the rate of successful collisions. These parameters are summarised in Table 5-2. Both the fraction of collisions and the rate of these collisions were cell concentration dependent. The fraction of collisions increased at constant temperature (25ºC) one order of magnitude, when we compared its value in the seawater control and in the seawater enriched with exudates, by $5.04\cdot 10^8$ cell/L.

In addition, the rate of the collisions decreased with the exudate concentration (at 25ºC), more than half, for the lowest and highest cell concentration. This suggests that when the exudate concentration increases, the media becomes more stable and the Fe(II) oxidation rate constant is slowed due to the interaction between the Fe(II) and the exudates in the seawater solutions.

The activation energy was also related to the ligand concentration in the seawater (Table 5-2). The Ea decreased from 89.9 kJ mol^{-1} in the seawater control to 84.2 kJ mol^{-1}, in the seawater enriched with organic exudates from *D. tertiolecta* ($5.04 \cdot 10^8$ cell/L). Comparing the Ea values obtained for the Gulf Stream seawater (Santana-Casiano et al., 2005) and the sub-Arctic Pacific seawaters (Roy et al., 2008), the same behaviour was obtained, with the E_a decreasing for seawater with higher organic matter concentrations. The sub-Arctic Pacific studies were carried out during iron-enrichment experiments and increased phytoplankton biomass, as compared with those in UV-seawater.

The temperature effect on the Fe(II) oxidation rate constant can also be studied from the Eyring representation (Figure 5-3B), in order to compute the enthalpy of activation ($\Delta H^{\ddagger}$) and entropy of activation ($\Delta S^{\ddagger}$). These parameters are shown in Table 5-2. $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ slightly decreased when the seawater was enriched with organic exudates from *D. tertiolecta*. The same effect was shown in the Pacific Sub-Arctic seawater (Roy et al., 2008), with the UV-treated seawater offering higher values for both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ than those in the natural seawater. It is important to remark that the comparison between the results in this study and the results reported for other authors should be carried out with due care, because the seawater controls in this study were prepared by adding f/2 nutrients, and the Fe(II) chemistry is a function of the local conditions (Millero et al., 1987; Öztürk et al., 2003; González et al., 2010a). This study indicates the kinetic parameters for the

Fe(II) oxidation depend on the organic matter concentration in solution. The values of enthalpy and entropy of activation indicate a system that becomes more stable state in the presence of organic exudates, as compared with the seawater control where the organic exudate concentration was lower.

The Fe(II) remained longer in seawater in the presence of the organic exudates from *D. tertiolecta* at the different temperatures considered. At 5ºC, where the Fe(II) oxidation rate is the lowest, the $t_{1/2}$ was 27.6 mins in the control seawater, and became 33.5 mins, 37.5 mins, and 57.3 mins in the seawater enriched with exudates from $5.52 \cdot 10^7$, $2.17 \cdot 10^8$ and $5.04 \cdot 10^8$ cell/L culture, respectively. That is, the Fe(II) can remain in cold waters 30 mins longer in the presence of high exudate concentrations than in the seawater control. This result may explain the measurements of the Fe(II) in cold waters, where high organic matter concentration was present (Croot et al., 2001; 2005; Roy et al., 2008).

5.2.4. Salinity dependence

The salinity dependence on the Fe(II) oxidation rate constant was studied for the range of salinity 10 to 36.720 (Figure 5-4). The Fe(II) oxidation rate showed a higher dependence with salinity in the control seawater than in the presence of organic exudates from *D. tertiolecta*, for both $5.52 \cdot 10^7$, $2.17 \cdot 10^8$ and $5.04 \cdot 10^8$ cell/L.

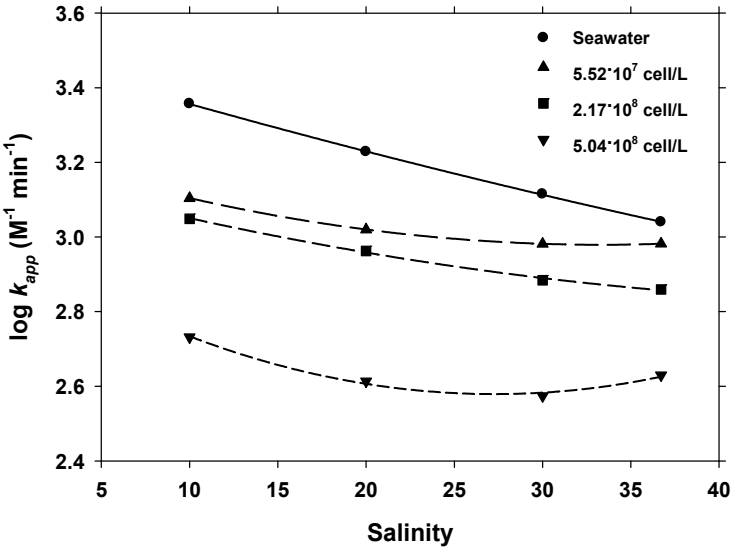

Figure 5-4. The Fe(II) oxidation rate constant as a function of salinity for the seawater and the seawater enriched with the organic exudates excreted by the *Dunaliella tertiolecta* at the three different cell densities ($5.52 \cdot 10^7$, $2.17 \cdot 10^8$ and $5.04 \cdot 10^8$ cell/L). The pH and temperature were kept constant at 8.0 and 25ºC, respectively. The lines represent the fitting results obtained from Equation 67.

The experimental data were fitted to a second order polynomial function (Equation 67), with R^2=0.970 and a standard estimate error of 0.06 in log k_{app}.

$$\log k_{app,S} = 3.4(\pm 0.1) - 0.018(\pm 9 \cdot 10^{-3})S + 2.0(\pm 0.2) \cdot 10^{-4} S^2 - 9.9(\pm 0.8) \cdot 10^{-11}[cell] \quad (67)$$

According to the experimental data, the effect of the organic ligands excreted by *D. tertiolecta* on the Fe(II) oxidation rate constant as compared to that for the control seawater as a function of salinity, was estimated (Equation 68) where R^2=0.943 and a standard estimate error of 0.14 in log k_{app}.

$$\Delta \log k_{app,S} = 2.3(\pm 0.3) - 0.016(\pm 2 \cdot 10^{-3})S + 2.0(\pm 0.5) \cdot 10^{-4} S^2 + 1.7(\pm 0.2) \cdot 10^{-9}[cell] \quad (68)$$

The range of log k_{app} values in the seawater control at the salinities studied was 0.32 ± 0.02 M^{-1} min^{-1} whereas in the seawater enriched with exudates, the range was only 0.14 ± 0.04 M^{-1} min^{-1}, for all the cell densities. This behaviour indicates again organic ligands play a key role in the Fe(II) oxidation rate constant stabilization as compared to seawater without ligands, where there is a greater control over the oxidation process exerted by the interaction with the major ionic species. In the control seawater, the $t_{1/2}$ increased from 1.3 mins at S=10 to 3.0 mins at S=36.720. In the presence of exudates, this effect was slight and the difference in the $t_{1/2}$ between S=10 and S=36.720 was 1.2 mins ($5.52 \cdot 10^7$ cell/L), 2.1 mins ($2.17 \cdot 10^8$ cell/L) and 2.7 mins ($5.04 \cdot 10^8$ cell/L) respectively.

The experimental results for the oxidation of Fe(II) apparent rate constant (log k_{app}, M^{-1} min^{-1}) at different concentrations of cells (cell/L), pH (free ion scale), temperature (K) and salinity were fitted to the polynomial function (Equation 69).

$$\log k_{app} = 3.7(\pm 0.4)pH - 0.17(\pm 0.1)pH^2 - \frac{4557(\pm 80)}{T} - 0.02(\pm 9 \cdot 10^{-3})S + 2(\pm 0.2) \cdot 10^{-4} S^2 - 7.3(\pm 0.3) \cdot 10^{-10}[cell] \quad (69)$$

where R^2 was 0.995 and the standard estimate error was 0.05 in log k_{app}. In addition, the overall effect on the oxidation rate constant due to the presence of cell exudates excreted by *D. tertiolecta*,

$\Delta \log k_{app} = \log k_{app,SW} - \log k_{app,cell}$, is shown in the Equation 70, where R^2 was 0.980 and the standard estimate error was 0.13 in log k_{app}.

$$\begin{aligned}\Delta \log k_{app} = {} & \log k_{app,SW} + 1.1(\pm 0.2) - 3.7(\pm 0.5)\, pH + 0.17(\pm 0.03)\, pH^2 \\ & + \frac{4557(\pm 26)}{T} + 0.016(\pm 2 \cdot 10^{-3})S - 2.0(\pm 0.4) \cdot 10^{-4} S^2 \\ & - 1.7(\pm 0.1) \cdot 10^{-9} [cell]\end{aligned} \tag{70}$$

This equation approach will allow for the calculation of the change in the apparent oxidation rate under different experimental conditions in the presence of ligands excreted by the *D. tertiolecta*. In addition, if the exudates excreted by the *D. tertiolecta* are considered to be a model organic ligand in seawater, this equation may be used to predict the fractional contribution of organic exudates in the Fe(II) oxidation rate constant. These results also suggested that the iron chemistry depends on the local water conditions.

5.2.5. The kinetic modelling approach

The *D. tertiolecta* surface groups were considered in order to identify the functional groups capable of complexing trace metals. Three mean acidity constants were computed corresponding to the carboxylic groups ($K_a=10^{4.9}$), phosphoryl and primary amine groups ($K_a=10^{6.3}$) plus the phenolic and glycine groups ($K_a=10^{10.1}$) (Gonzalez-Davila et al., 1995). The carboxylic groups have been described as the most important group, binding cationic metals in aqueous solutions in numerous aquatic microorganisms (González et al., 2009; 2010b). In order to study the effect played by the organic ligands excreted by the *D. tertiolecta* on the oxidation of the Fe(II), the kinetic model by (Santana-Casiano et al., 2005) was applied but including

new species and contributors. In the model, the ligand speciation was considered as given in Equation 71-72.

$$LH + H \leftrightarrow LH_2 \qquad K_a=10^{4.9} \qquad (71)$$

$$L + H \leftrightarrow LH \qquad K_a=10^{6.3} \qquad (72)$$

The model included the complexation between the Fe(II) and both types of ligands, LH and L (Equation 73-74). The species formed in this fast complexing process can then be oxidized to the Fe(III) at different rates from those computed for the inorganic Fe(II) species (Equation 75-76). The Fe(II) interaction with the ligand with the highest acidity constant was also considered but its role was negligible under the experimental conditions used in this study.

$$Fe(II) + LH \leftrightarrow Fe(II) - LH \qquad K_{Fe(II)-LH} = 9\cdot10^{8}\text{-}1\cdot10^{9} \qquad (73)$$

$$Fe(II) + L \leftrightarrow Fe(II) - L \qquad K_{Fe(II)-L} = 9\cdot10^{6}\text{-}1.5\cdot10^{7} \qquad (74)$$

$$Fe(II) - LH \rightarrow Fe(III) - LH \qquad k_{Fe(II)-LH} = 68\pm9\ M^{-1}\ min^{-1} \qquad (75)$$

$$Fe(II) - L \rightarrow Fe(III) - L \qquad k_{Fe(II)-L} = 54\pm6\ M^{-1}\ min^{-1} \qquad (76)$$

The equilibrium constants (Equations 73-74) and rate constants (Equations 75-76) indicated above were computed using an iterative method via Gepasi software and considering all the experimental conditions discussed above, including the computation of the ligand concentration at the three cell densities used herein. In the first stage, only an individual Fe(II)-complex was accounted, and then, a combined model, including Equations 73 and 74, was performed. The difference between both models, with individual Fe(II) complexes and combined Fe(II)-complexes, was negligible. The total

ligand concentrations were also computed from the model for each culture media considered. The best fit for the experimental data showed that the culture with $5.52 \cdot 10^7$ cell/L had a total ligand concentration of 27.5±2.5 nM, $2.17 \cdot 10^8$ cell/L excreted 70±10 nM, and the culture with $5.04 \cdot 10^8$ cell/L excreted 170±10 nM. These total ligand concentrations excreted by the phytoplankton cultures were in close concordance with results reported by other authors, for *T. weissflogii* (114 nM), *S. Costatum* (106 nM) and *P. tricornutum* (140-228 nM), over the different phases of culture (González-Dávila et al., 2000; Vasconcelos et al., 2002; Vasconcelos and Leal, 2008; Strmečki et al., 2010).

The kinetic modelling approach used in this work allowed us to compute the Fe(II) speciation and the individual species' contribution to the overall rate constant. The Fe(II) speciation is shown in Figure 5-5 for the seawater control (Figure 5-5A), the seawater enriched with organic exudates from $5.52 \cdot 10^7$ cell/L (Figure 5-5B), $2.17 \cdot 10^8$ cell/L (Figure 5-5C) and $5.04 \cdot 10^8$ cell/L (Figure 5-5D). In the seawater control, the Fe(II) speciation was controlled from pH 6 to 8.2 for Fe^{2+}, changing from 58% to 27%. $FeCl^+$ and $Fe(SO_4)$ were also important species from pH 6 to 7.6. At pH=8.0 $Fe(CO_3)$ and $Fe(H_3SiO_4)^+$ played an important role and reached 22% and 19%, respectively. The results for the Fe(II) speciation in seawater with nutrients are in close concordance with previous results (González et al, 2010a). The Fe(II) speciation was radically affected when organic ligands were considered. The speciation was controlled by Fe(II)-LH, from 78% to 98% at pH 6.0 as the number of cells changed from $5.52 \cdot 10^7$ cell/L to $5.04 \cdot 10^8$ cell/L. Fe(II)-LH influence decreased as a function of pH, but was the major species until pH 7.6 when $5.52 \cdot 10^7$ cell/L was present, and until 8.3 at $5.04 \cdot 10^8$ cell/L. The second Fe(II) organic complex considered in this model (Fe(II)-L) became important as the ligand concentration increased. At pH=8.0, Fe(II)-L was 8% ($5.52 \cdot 10^7$ cell/L), 14% ($2.17 \cdot 10^8$ cell/L) and 25% ($5.04 \cdot 10^8$ cell/L). The inorganic species, except Fe^{2+}, were negligible at pH ≤

7.2 in seawater enriched with organic exudates. The model explained the key role played by Fe(II)-organic complexes, such as Fe(II)-LH (carboxyl-like) or Fe(II)-L (phosphoryl or amino-like) in the Fe(II) speciation in natural waters.

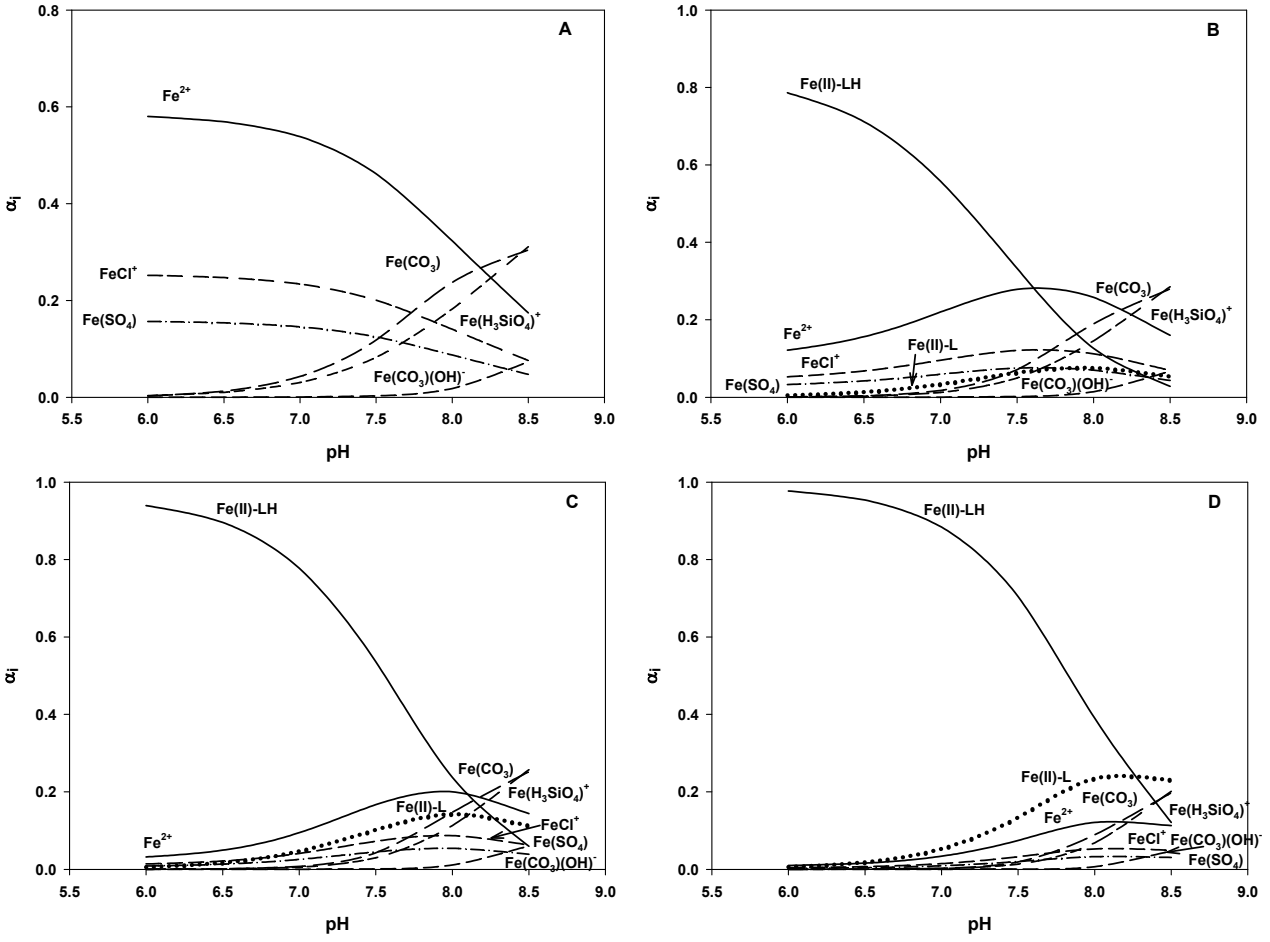

Figure 5-5. The Fe(II) speciation as rendered by the kinetic modelling approach. (A) Seawater control (seawater with f/2 nutrients), (B) seawater enriched with the exudates at $5.52 \cdot 10^7$cell/L, (C) seawater enriched with exudates from $2.17 \cdot 10^8$ cell/L and (D) seawater enriched with exudates from $5.04 \cdot 10^8$ cell/L of *Dunaliella tertiolecta*.

The individual contributions of each Fe(II) species, including Fe(II)-LH and Fe(II)-L species, are shown in Figure 5-6, for the seawater control (Figure 5-6A), seawater enriched with organic exudates excreted by $5.52 \cdot 10^7$ cell/L (Figure 5-6B), $2.17 \cdot 10^8$ cell/L (Figure 5-6C) and $5.04 \cdot 10^8$ cell/L (Figure 5-6D). The individual contribution for each Fe(II) species to the overall kinetic rate was computed from the results of the kinetic model and the speciation of the Fe(II) (Equation 77), where $\alpha_i=[FeX_i]/[Fe(II)]_T$ are the molar fraction of each Fe(II) species in the solution. *k* is the apparent overall rate constant (M^{-1} min^{-1}) and k_i are the individual rate constants for the Fe(II) species.

$$k = k_{Fe^{2+}} + k_{FeOH^+}\alpha_{FeOH^+} + k_{Fe(OH)_2}\alpha_{Fe(OH)_2} + k_{FeHCO_3^+}\alpha_{FeHCO_3^+} + k_{Fe(CO_3)}\alpha_{Fe(CO_3)} + k_{Fe(CO_3)_2^{2-}}\alpha_{Fe(CO_3)_2^{2-}} + k_{Fe(CO_3)OH^-}\alpha_{Fe(CO_3)OH^-} + k_{FeCl^+}\alpha_{FeCl^+} + k_{FeSO_4}\alpha_{FeSO_4} + k_{FeH_3SiO_4^+}\alpha_{FeH_3SiO_4^+} + k_{Fe(II)-LH}\alpha_{Fe(II)-LH} + k_{Fe(II)-L}\alpha_{Fe(II)-L} \quad (77)$$

The oxidation process in seawater control was controlled by the Fe^{2+} from pH 6.0 (96%) to 7.7 (22.5%), where the $Fe(OH)_2$ began to be the most important contributor to the overall rate constant, with 22.5% (pH 7.7) to 55% (pH 8.5). $Fe(OH)^+$ and $Fe(H_3SiO_4)^+$ showed their maximum contribution at pH≈7.6 (15.4% and 13.6%, respectively). $Fe(CO_3)_2^{2-}$ had its maximum values at pH ≥ 8 (16%) followed by $Fe(CO_3)(OH)^-$, with a maximum of 11% at pH=8.5. The results for the seawater control are in close concordance with González et al. (2010a). The presence of organic ligands changed not only the oxidation rate but also the contribution of each individual species to the overall Fe(II) oxidation rate constant. The carboxyl-like complex (Fe(II)-LH) was the most important contributor at pH ≤ 6.8 ($5.52 \cdot 10^7$ cell/L), pH ≤ 7.5 ($2.17 \cdot 10^8$ cell/L) and pH ≤ 7.8 ($5.04 \cdot 10^8$ cell/L). The highest contribution for the phosphoryl- or amino-like complexes (Fe(II)-L) was at pH≈7.4, from 2%

at $5.52 \cdot 10^7$ cell/L to 7% at $5.04 \cdot 10^8$ cell/L. The oxidation process was controlled, at pH ≥ 8.0, by $Fe(OH)_2$, $Fe(CO_3)_2^{2-}$, $Fe(CO_3)(OH)^-$, $Fe(OH)^+$ and $Fe(H_3SiO_4)^+$. The individual contributions to the overall Fe(II) rate constant demonstrated that Fe(II)-LH (carboxyl-like complexes) controlled the Fe(II) oxidation process at low pH, even at low ligand concentrations, and thus must be considered in the kinetic studies of the Fe(II) in natural waters due to its key role in the Fe(II) oxidation.

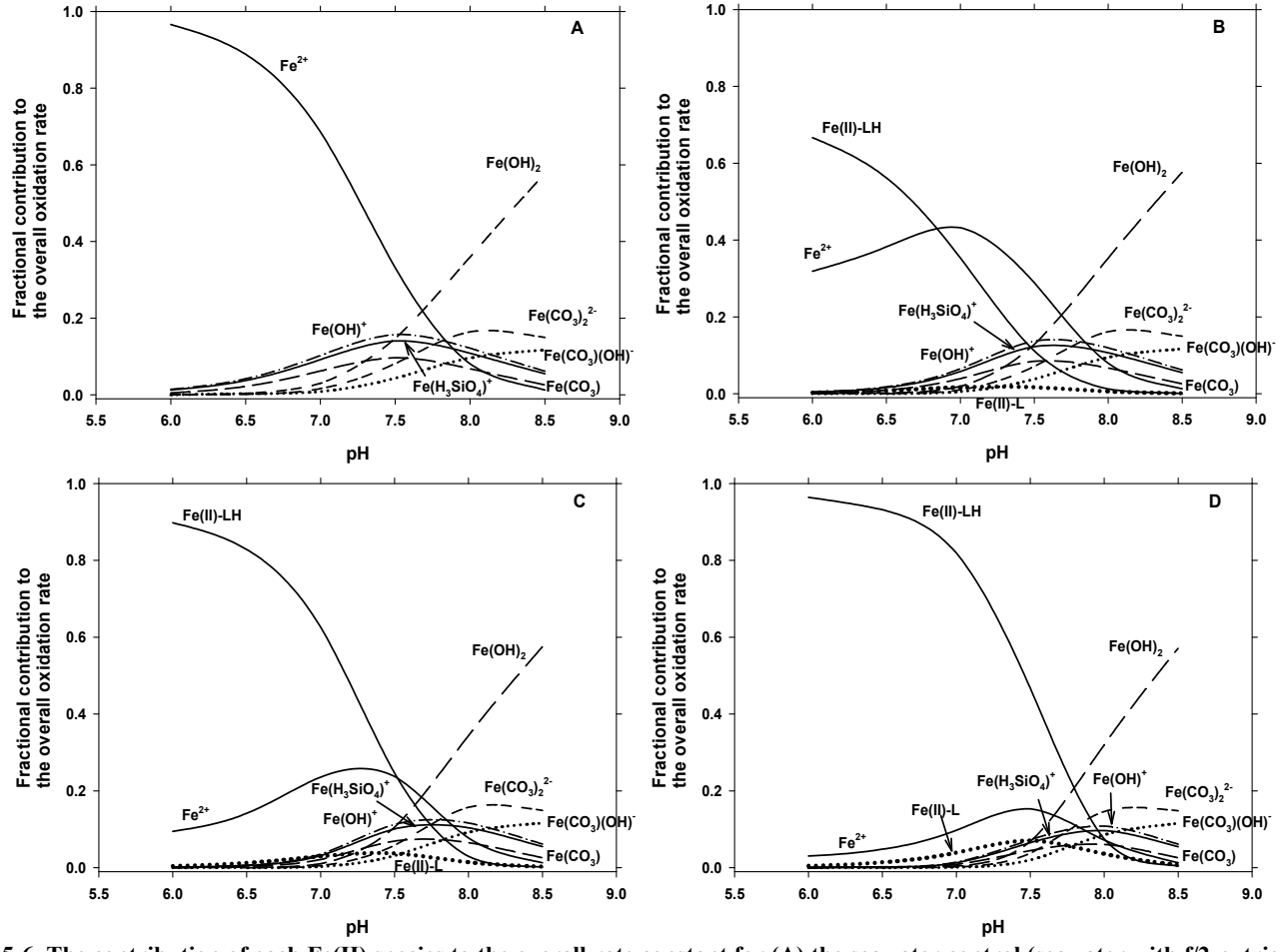

Figure 5-6. The contribution of each Fe(II) species to the overall rate constant for (A) the seawater control (seawater with f/2 nutrients), (B) the seawater enriched with the exudates from $5.52 \cdot 10^7$cell/L, (C) the seawater enriched with the exudates from $2.17 \cdot 10^8$ cell/L and (D) the seawater enriched with the exudates from $5.04 \cdot 10^8$ cell/L of *Dunaliella tertiolecta*.

Table 5-1. The half time ($t_{1/2}$) accounted for the Fe(II) oxidation experiments in the seawater control (by adding f/2 nutrients) and in the seawater enriched with organic exudates excreted by the *D. tertiolecta* as a function of the pH, temperature and salinity.

Media	pH	Temperature (ºC)	Salinity	log k_{app} (M^{-1} min^{-1})	$t_{1/2}$ (min)
Seawater (control)	7.2	25	36.72	2.23	19.9
	7.5	25	36.72	2.41	13.2
	7.8	25	36.72	2.76	5.9
	8.0	25	36.72	3.04	3.0
	8.2	25	36.72	3.22	2.0
	8.0	5	36.72	1.92	27.6
	8.0	15	36.72	2.39	11.5
	8.0	35	36.72	3.45	1.4
	8.0	25	10	3.36	1.3
	8.0	25	20	3.23	1.8
	8.0	25	30	3.12	2.5
Seawater enriched with exudates from $5.52 \cdot 10^7$ cell/L	7.2	25	36.72	2.08	28.3
	7.5	25	36.72	2.36	14.9
	7.8	25	36.72	2.72	6.4
	8.0	25	36.72	2.98	3.5
	8.2	25	36.72	3.19	2.2
	8.0	5	36.72	1.83	33.5
	8.0	10	36.72	2.14	18.6
	8.0	15	36.72	2.37	12.1
	8.0	35	36.72	3.44	1.4
	8.0	25	10	3.10	2.3
	8.0	25	20	3.02	2.9
	8.0	25	30	2.98	3.4
Seawater enriched with exudates from $2.17 \cdot 10^8$ cell/L	7.2	25	36.72	2.03	31.8
	7.5	25	36.72	2.32	16.3
	7.8	25	36.72	2.65	7.6
	8.0	25	36.72	2.86	4.7
	8.2	25	36.72	3.12	2.6
	8.0	5	36.72	1.76	37.5
	8.0	15	36.72	2.37	12.2
	8.0	35	36.72	3.40	1.6
	8.0	25	10	3.05	2.6
	8.0	25	20	2.96	3.3
	8.0	25	30	2.88	4.2
Seawater enriched with exudates from $5.04 \cdot 10^8$ cell/L	7.2	25	36.72	1.86	46.5
	7.5	25	36.72	2.19	22.5
	7.8	25	36.72	2.46	11.8
	8.0	25	36.72	2.63	8.0
	8.2	25	36.72	2.97	3.6
	8.0	5	36.72	1.60	57.3
	8.0	15	36.72	2.09	23.3
	8.0	35	36.72	3.13	2.9
	8.0	25	10	2.73	5.3
	8.0	25	20	2.61	6.8
	8.0	25	30	2.57	8.9

Table 5-2. The thermodynamic parameters computed for the seawater control (with f/2 nutrients) and the seawater enriched with the organic exudates from the *Dunaliella tertiolecta*. The values of the $e^{-Ea/RT}$ and the log *k* were calculated for 25ºC. Pacific Sub-Arctic studies were reported by Roy et al. (2008), and the Gulf Stream seawater values were studied by Santana-Casiano et al. (2005).

Media	A (min^{-1})	$e^{-Ea/RT}$	log *k* (M^{-1} min^{-1})	Ea (kJ mol^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J mol^{-1})
Seawater control	$1.95 \cdot 10^{18}$	$1.76 \cdot 10^{-16}$	2.53	89.9±1.8	87.4±1.3	131±5
Seawater + exudates $5.52 \cdot 10^{7}$ cell/L	$3.20 \cdot 10^{18}$	$2.76 \cdot 10^{-16}$	2.95	88.8±2.2	86.4±2.2	101±6
Seawater + exudates $2.17 \cdot 10^{8}$ cell/L	$1.64 \cdot 10^{18}$	$4.18 \cdot 10^{-16}$	2.89	87.5±1.7	85.1±1.7	96±8
Seawater + exudates $5.04 \cdot 10^{8}$ cell/L	$2.41 \cdot 10^{17}$	$1.78 \cdot 10^{-15}$	2.63	84.2±2.6	81.8±2.5	80±9
Pacific Subarctic UV-seawater	$3.80 \cdot 10^{24}$	$2.57 \cdot 10^{-22}$	2.99	109.6	107.2	135
Pacific Subarctic seawater	$5.37 \cdot 10^{21}$	$6.68 \cdot 10^{-20}$	2.56	95.8	93.4	81
Gulf Stream seawater	$2.75 \cdot 10^{23}$	$4.24 \cdot 10^{-21}$	3.07	102.7	100.2	113

5.3. Conclusion

The aim of this study was to analyze the role of the organic exudates excreted by *Dunaliella tertiolecta* on the Fe(II) oxidation process. The Fe(II) oxidation was analyzed as a function of cell concentration, pH, temperature and salinity, using seawater controls (with f/2 nutrients) and seawater enriched with organic exudates from three cell concentrations, $5.52 \cdot 10^7$, $2.17 \cdot 10^8$ and $5.04 \cdot 10^8$ cell/L. The Fe(II) oxidation rate constant described a linear dependence with the cell concentrations, suggesting that the type of ligands capable of complexing Fe(II) in seawater are either the same along the growing process or have similar properties to bind Fe(II). The pH, temperature and salinity dependence are functions of the cell concentrations in the culture from which the exudates were extracted. All the parameters studied in this work were fitted to a polynomial function in order to compute the log k_{app} in the presence of the organic exudates of *D. tertiolecta* in seawater as a function of pH, temperature and salinity. The kinetic modelling approach describes the presence of ligands, both the carboxyl-like groups and the phosphoryl/amino-like groups on the Fe(II) speciation and on the contribution to the overall rate constant. The results presented in this work may be applied to other natural and artificial media where ligand exudates behave similarly to those excreted by the *D. tertiolecta*.

CHAPTER VI:

Oxidation of Cu(I) at nanomolar levels in seawater

González-Dávila, M., Santana-Casiano, J.M., González, A.G., Pérez, N., Millero, F.J. 2009. Oxidation of copper (I) in seawater at nanomolar levels. Mar.Chem. 115, 118-124.

ABSTRACT

The oxidation and reduction of nanomolar levels of copper in air-saturated seawater and NaCl solutions have been measured as a function of pH (7.17-8.49), temperature (5-35ºC) and ionic strength (0.1-0.7 M). The oxidation rates were fitted to an equation valid at different pH and ionic strength conditions in sodium chloride and seawater solutions:

$$\log k_{(NaCl)} = 5.221 + 0.609\,pH\text{-}1915.433/T\text{-}1.818\sqrt{I} + 0.408I$$
$$\log k_{(sw)} = 5.036 + 0.514\,pH\text{-}1764.915/T\text{-}1.101\sqrt{I} + 0.233I$$

The reduction of Cu(II) was studied in both media for different initial concentrations of copper (II). When the initial Cu(II) concentration was 200 nM, the copper (I) production was 20% and 9% for NaCl and seawater, respectively. The effect of speciation of copper (I) reduced from Cu(II) on the rates was studied. The Cu(I) speciation is dominated by the $CuCl_2^-$ species. On the other hand, the neutral chloride CuCl species dominates the Cu(I) oxidation in the range 0.1 M to 0.7 M chloride concentrations.

CHAPTER VI

6.1. Introduction

Copper redox chemistry controls its speciation in natural waters and its interactions with biological organisms. Copper is an essential redox-active transition metal, which acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses and cell wall metabolism (Yruela, 2005). At elevated concentrations, copper becomes toxic to plants and microorganisms by altering membrane permeability, affecting chromatin structure, protein synthesis, enzyme activities, photosynthesis and respiratory processes (Marschner, 1995; Yruela, 2005). Copper toxicity may result from an intracellular reaction between copper and reduced glutathione, although copper also inhibits the enzyme catalase and reduces the cell defence mechanism against hydrogen peroxide and oxygen free radicals (Sunda, 1988).

The oxidation of Cu(I), at micromolar levels, in seawater and in NaCl solutions has been studied by different authors (Moffett and Zika, 1983; Sharma and Millero, 1988a,b,c). The rate constant k (M^{-1} min^{-1}) for the oxidation of Cu(I) is defined by Equation 78:

$$d[Cu(I)]/dt = -k[Cu(I)][O_2] \quad (78)$$

In the conditions of excess oxygen, the system followed a pseudo-first order kinetics (k') where $k' = k\,[O_2]$. These studies found that the Cu(I) oxidation rate constant depends on the speciation of Cu(I) in the solution

(Millero, 1985). The oxidation of Cu(I) is mainly controlled by the Cl^- concentration. At lower Cl^- concentration, the Cu(I) oxidation is much faster and the back reaction of Cu(II) becomes more important. The most reactive species for the oxidation of Cu(I) are Cu^+ and CuCl (Moffett and Zika, 1983; Millero, 1985; Sharma and Millero, 1988a,b,c).

In seawater, Cu(I) can be formed from Cu(II) due to the photooxidation of dissolved organic matter (Sharma and Millero, 1988a), according to the sequence (Equation 79-81).

$$org \xrightarrow{h\upsilon} org^+ + e^- \quad (79)$$

$$e^- + O_2 \rightarrow O_2^{\cdot -} \quad (80)$$

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (81)$$

Redox cycling between Cu^{2+} and Cu^+ catalyzes production of hydroxyl radicals from superoxide and hydrogen peroxide (Elstner et al., 1988) by the Haber-Weiss reaction and thus enhances the production of reactive oxygen species. These reactions for oxidation and reduction of copper are expressed in the Equation 82-87 (Haber and Weiss, 1934; Barb et al., 1951; Gray, 1969):

$$Cu^+ + OH^{\cdot} \rightarrow Cu^{2+} + OH^- \quad \text{Fast} \quad (82)$$

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^{\cdot} + OH^- \quad \text{Slow} \quad (83)$$

$$H_2O_2 \leftrightarrow H^+ + HO_2^{\cdot -} \quad \text{Fast} \quad (84)$$

$$Cu^{2+} + HO_2^{\cdot -} \rightarrow Cu^+ + HO_2 \quad \text{Slow} \quad (85)$$

$$HO_2 \leftrightarrow H^+ + O_2^{\cdot -} \qquad \text{Fast} \qquad (86)$$

$$Cu^{2+} + O_2^{\cdot -} \rightarrow Cu^+ + O_2 \qquad \text{Fast} \qquad (87)$$

Moreover, copper forms organic complexes with algal exudates and this leads to a large variety of organometallic species which can affect the free ionic copper concentration. Primary photochemical processes, such as the Ligand to Metal Charge Transfer reactions of Cu(II) complexes, could also result in the formation of Cu(I) (Zika, 1981) (Equation 88).

$$\left[Cu(II)L_n\right] + h\upsilon \xrightarrow{LMCT} Cu(I) + (nL)^- \qquad (88)$$

Copper is thought to exist primarily as complexes with organic ligands in seawater (van den Berg, 1984; Moffett and Zika, 1987; Sunda and Hanson, 1987), leaving only around 1% of the total copper as free copper, which is considered to be available or toxic (Allen and Hansen, 1996). Trace metal chemistry is affected by phytoplankton in natural and oceanic waters by surface reactions and metal uptake or production of extracellular organic matter with metal complexity properties (González-Dávila, 1995). The release of extracellular organic compounds appears to be a major source of labile substrate into the dissolved organic matter pool in the open ocean (Duursma, 1961; Wangersky, 1978; Zhou and Wangersky, 1989).

In this work, the oxidation of Cu(I) is studied at nanomolar levels, three orders of magnitude lower than earlier studies (Moffett and Zika, 1983; Sharma and Millero, 1988a,b,c). In addition, we have not added any complexing agent into the reaction vessel in order to enable the formation of Cu(I) from the reduction of Cu(II). The reduction of Cu(II) has been studied because it may be responsible for the decrease in the oxidation rates of Cu(I) in seawater samples. We have studied the oxidation of copper (I) in seawater

and sodium chloride solutions as a function of the initial copper concentration, pH, temperature, sodium bicarbonate and ionic strength. We have also calculated the effect of the various forms of Cu(I) on the kinetic rates. These results allow us to improve our understanding of the biogeochemical cycling of copper in natural waters.

6.2. Results and Discussion

The oxidation of Cu(I) at nanomolar concentration in air saturated solutions followed a pseudo first order kinetics. Table 6-1 gives the half-life times ($t_{1/2}$) for the oxidation kinetics of Cu(I) in 0.7 M NaCl – 2 mM $NaHCO_3$ and seawater solutions at nanomolar and micromolar concentrations. As the pH decreases, an increase in $t_{1/2}$ is obtained in both seawater and NaCl. The $t_{1/2}$ at nanomolar and micromolar Cu(I) concentration in Table 6-1 show differences under similar conditions. Similar differences have also been found for the iron kinetic studies (Santana-Casiano et al., 2005). Values for the Cu(I) oxidation from (Sharma and Millero, 1988c) are also included in Table 6-1. The $t_{1/2}$ obtained by theses authors are lower than those observed in this study. However, Sharma and Millero introduced EDTA in the reaction cell in order to avoid any further Cu(II) reduction. In this study, no complexing agents were introduced in the reaction cell. Ethylendiamine together with bathocupreine were added to the extracted sample before the analysis of Cu(I) to avoid any interference with the Cu(II) when Cu(I) was measured.

Table 6-1. The values of $t_{1/2}$ for the oxidation of Cu(I) in this study in NaCl and seawater as a function of pH and temperatures. Comparisons are also made with the micromolar results from (Sharma and Millero, 1988c) and this study.

Media	pH	Temperature (°C)	$t_{1/2}$ (min)	
NaCl (0.7 M) and 2mM $NaHCO_3$	7.51	25	**27.2**	This study (μM)
	7.76	25	**19.9**	
	8.10	25	**9.5**	
	8.13	25	**8.0**	
	7.17	25	**42.3**	This study (nM)
	7.50	25	**22.3**	
	7.79	25	**16.3**	
	8.05	25	**11.5**	
Seawater (S = 36.691)	7.20	25	**30.8**	
	8.01	25	**12.2**	
	8.11	25	**12.1**	
	7.97	5	**20.1**	
NaCl (1 M)	8.00	25	**1.3**	Sharma and Millero, 1988c
Seawater (S = 35)	8.00	25	**4.0**	
	8.00	5	**15.7**	

6.2.1. Effect of initial Cu(I) concentration

To elucidate the effect of the initial copper (I) concentration on the oxidation of Cu(I) in natural waters and the effect of the Cu(II) back reaction, the experiments were made in 0.7 M NaCl and 2 mM $NaHCO_3$ solutions with

a Cu(I) concentration in the range 50 to 385 nM (Table 6-2). The oxidation rate is not affected by the initial Cu(I) concentration. The average value of log k'_{obs} is -1.31 ± 0.02. The results at an initial concentration of copper (I) of 200 nM can be extrapolated to lower concentrations of 50 nM.

Table 6-2. Effect of initial copper (I) concentrations on the apparent rate constant (min^{-1}) for the oxidation of Cu(I) in NaCl 0.7 M and 2 mM $NaHCO_3$, pH 8.00 and temperature 25ºC.

Media	$[Cu(I)]_0$ (nM)	log k'
NaCl 0.7 M and 2 mM $NaHCO_3$	50	-1.28
	100	-1.33
	200	-1.34
	385	-1.30

6.2.2. Effect of $NaHCO_3$ concentration

The effect of bicarbonate concentration was studied in 0.7 M NaCl and $NaHCO_3$ solutions in the range 0 to 9 mM (Table 6-3). The overall rate constant k (M^{-1} min^{-1}) for seawater and NaCl was computed considering $k = k' / [O_2]$ from Equation 78. The values of $[O_2]$ were determined from solubility equations by Benson and Krause (1984) and Millero et al. (2002). The results were fitted to a second degree polynomial equation (Equation 89), with R^2 of 0.956 and a standard error of estimation of 0.07 in log k.

$$\log k_{(NaHCO_3)} = 2.04(\pm 0.06) + 0.21(\pm 0.03)[NaHCO_3] - 1.54 \cdot 10^{-2}(\pm 0.004)[NaHCO_3]^2 \quad (89)$$

Table 6-3. Effect of $NaHCO_3$ concentration on the rate constant (M^{-1} min^{-1}), in NaCl 0.7 M. pH 8.00 and temperature 25ºC.

Media	$NaHCO_3$ (mM)	log *k* (M^{-1} min^{-1})	Standard deviation
NaCl 0.7 M	0	1.95	0.16
	1	2.30	0.15
	2	2.48	0.02
	4	2.60	0.05
	5	2.70	0.22
	7	2.73	0.04
	9	2.73	0.18

The Cu(I) oxidation rate increased when $NaHCO_3$ was added until 5 mM, being constant from 5 mM to 9 mM. The formation of complexes between the carbonate and Cu(I) (Equations 90 and 91), that can be more reactive than Cu(I) chloride complexes, can account for the observed behaviour.

$$Cu^+ + HCO_3^- \rightarrow CuHCO_3 \quad (90)$$

$$Cu^+ + CO_3^{2-} \rightarrow CuCO_3^- \quad (91)$$

On the other hand, the stabilization of the rate constant at bicarbonate concentration higher than 5 mM can be the result of Cu(II)-carbonate complexation and its reduction to Cu(I). In order to elucidate the effect of this back reaction from Cu(II), the reduction of copper (II) has been studied.

6.2.3. pH effect

The pH effect on the reduction was studied in the pH range 7.17 to 8.49 in NaCl and seawater solutions. The results are shown in Figures 6-1 and 6-2.

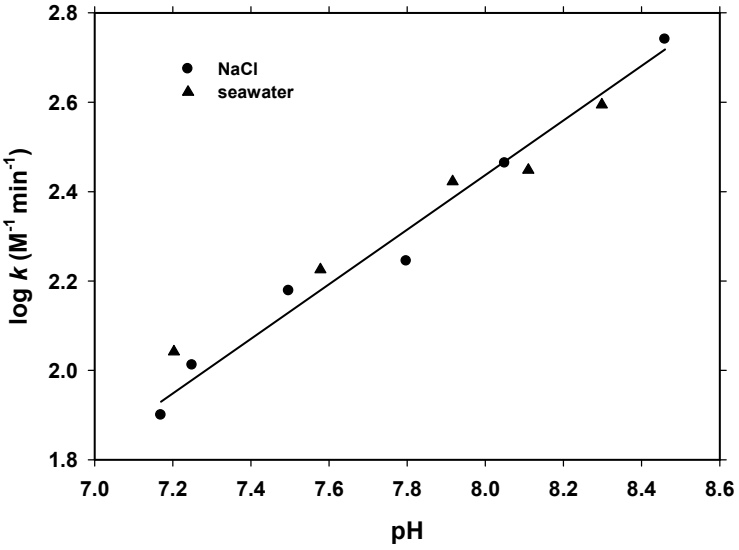

Figure 6-1. Effect of pH on the rate constant (M^{-1} min^{-1}) for the oxidation of Cu(I) in NaCl 0.7 M and seawater (S=36.691) at 25ºC at nanomolar concentrations.

The results were fitted to Equation 92 and 93 for NaCl and seawater at nanomolar levels and Equation 94 for micromolar concentration in sodium chloride solution.

$$\log k_{(NaCl)} = -2.5(\pm 0.3) + 0.61(\pm 0.04)\, pH \qquad (92)$$

$$\log k_{(sw)} = -1.5(\pm 0.3) + 0.49(\pm 0.03)\, pH \qquad (93)$$

$$\log k_{(NaCl,\mu M)} = -4.2(\pm 0.6) + 0.84(\pm 0.07)\, pH \qquad (94)$$

with a standard error of 0.03 and 0.05 in NaCl and seawater at nanomolar levels, with $R^2 = 0.986$ and 0.978, respectively. At micromolar concentration, the standard error of deviation was 0.04 ($R^2 = 0.977$).

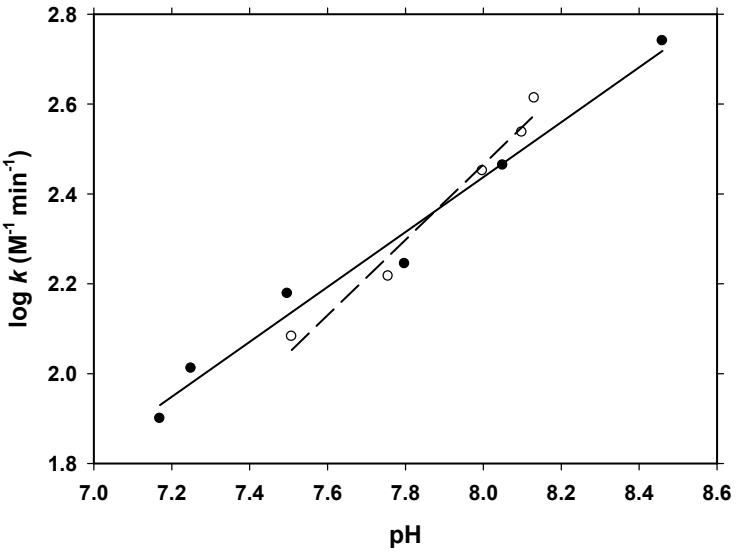

Figure 6-2. Effect of pH on the rate constant (M^{-1} min^{-1}) for the oxidation of Cu(I) in NaCl 0.7 M at 25ºC at micromolar (open circle) and nanomolar (closed circle) concentrations.

The Cu(I) oxidation rate as a function of pH shows a slight effect indicating acidic or basic species (CuOH and $CuClOH^-$) are not strongly involved in the oxidation of copper. In addition, the values are very close in both media indicating that similar complexes are involved and/or the effects due to different ionic interactions in seawater are compensated. Despite the weak dependence with the pH, the slope for micromolar concentration (0.839) is higher than the slope in nanomolar level (0.611), indicating that the

effect of the pH is more important at the micromolar level than at nanomolar. The differences could be due to the presence of intermediate agents such as superoxide or hydrogen peroxide produced in the process, playing a major role in the Cu(II) reduction process at nanomolar concentrations, decreasing the rate constants.

6.2.4. Ionic strength effect

The effect of ionic strength on the oxidation rate is presented in Figure 6-3. The oxidation rate *k* decreased with the increase of ionic strength from 0.1 to 0.7 M. This dependence is in agreement with previous studies (Moffett and Zika, 1983; Millero, 1985; Sharma and Millero, 1988a,b,c).

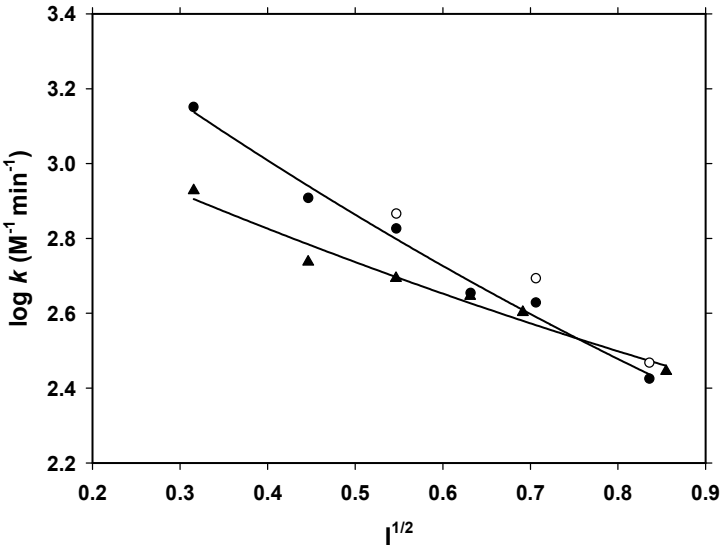

Figure 6-3. Effect of ionic strength on the rate constant (M^{-1} min^{-1}) for the oxidation of Cu(I) in NaCl (closed circle) and seawater (triangle) at pH=8.00 and T=25ºC. The open circles are the results in NaCl with a constant bicarbonate concentration (2 mM).

The observed oxidation rates are fitted to Equation 95 and 96, where R^2 was 0.986 in NaCl and 0.980 in seawater. The standard error of deviation is 0.03 and 0.02, respectively.

$$\log k_{(I,NaCl)} = 3.8(\pm 0.2) - 1.8(\pm 0.7)\sqrt{I} + 0.4(\pm 0.6)I \quad (95)$$

$$\log k_{(I,sw)} = 3.2(\pm 0.2) - 1.1(\pm 0.6)\sqrt{I} + 0.3(\pm 0.5)I \quad (96)$$

The copper chloride complexes formed at high chloride concentrations accounts for the decrease in the rate of oxidation. These ionic strength studies were made by diluting the samples with Milli-Q water that changes the HCO_3^- concentration. When ionic strength was lower than 0.4 M, the values in NaCl were higher than those in seawater ($\Delta \log k = 0.2$), being similar at higher ionic strengths. When the experiments were made at a constant bicarbonate concentration (2 mM) in the NaCl solutions, the rate constants were not affected by the levels of bicarbonate (Figure 6-3). In both media, the main inorganic complexes of Cu(I) are the chloride ions that can be in the forms Cu^+, CuCl, $CuCl_2^-$ and $CuCl_3^{2-}$. As the ionic strength changes, the species distribution and the contribution to the rate will be affected. When the ionic strength is lower the effect of the major ions in seawater can become more important and affect the rate constant. Mg^{2+} and Ca^{2+} may decrease the rate constants in seawater solutions. The concentrations of NaCl are always lower than in NaCl solutions at the same ionic strength and that the back-reaction of Cu(II) is more important at lower Cl^- concentration (Sharma and Millero, 1988c). These processes can account for the differences between NaCl and seawater solutions, at lower ionic strengths.

6.2.5. Temperature effect

The effect of temperature in the oxidation kinetic of Cu(I) in NaCl and seawater was studied from 5 to 35ºC and the results are shown in Figure 6-4.

In both media, the rate of oxidation increases with temperature. The plot of log *k* versus 1/T gives slopes very similar, -1884 in NaCl and -1856 in seawater and within the experimental error (Equation 97 and 98).

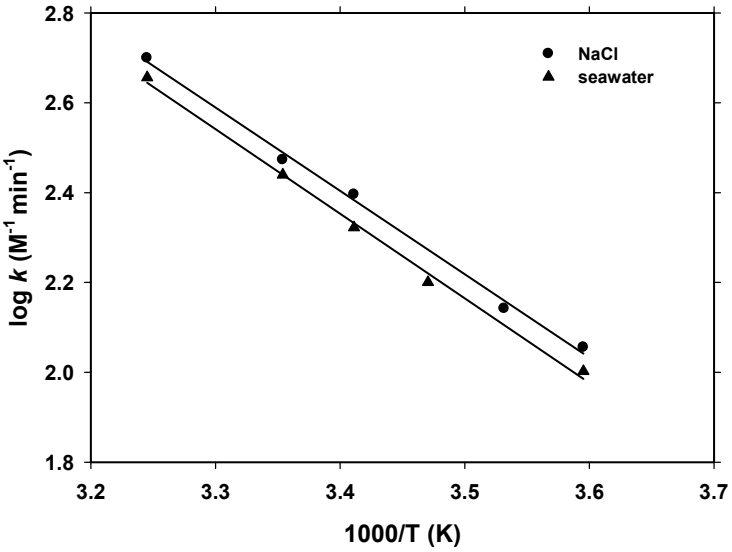

Figure 6-4. Effect of temperature on the rate constant (M^{-1} min^{-1}) for the oxidation of Cu(I) in NaCl 0.7 M and seawater (S=36.691) at pH 8.00.

$$\log k_{(T,NaCl)} = 8.8(\pm 0.2) - 1884(\pm 67)/T \quad (97)$$

$$\log k_{(T,sw)} = 8.7(\pm 0.2) - 1856(\pm 66)/T \quad (98)$$

with a standard error of 0.02 and R^2 0.996 in both media.

The temperature studies allowed us to calculate the Energy of Activation (E_a), the Arrhenius parameter (A), enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) of activation (Atkins, 2008). The values in seawater media were: E_a = 35.54 ± 1.26 kJ mol^{-1}, A = 5.2·10^8 ± 1.7 min^{-1}, $\Delta H^{\ddagger}$ = 33.10 ± 1.25 kJ mol^{-1} and $\Delta S^{\ddagger}$ = -52.17 ± 4.32 J mol^{-1}.

The overall rate constants for the Cu(I) oxidation under different experimental conditions have been fitted to the Equation 99 and 100, considering the combination of all parameters studied at the same time.

$$\log k_{(NaCl)} = 5.221 + 0.609\,pH - 1915.433/T - 1.818\sqrt{I} + 0.408I \qquad (99)$$

$$\log k_{(sw)} = 5.036 + 0.514\,pH - 1764.915/T - 1.101\sqrt{I} + 0.233I \qquad (100)$$

with a standard error of estimation of 0.03 in seawater and NaCl. *k* is the rate constant (M^{-1} min^{-1}), T is temperature (Kelvin) and I is the ionic strength. These equations are valid in the range studied for all these parameters.

6.2.6. Model approach

Some experiments were performed to study the regeneration of Cu(I) from Cu(II) in NaCl and seawater solutions and at oxygen saturated conditions (Figure 6-5). The initial copper (II) concentration was 200 and 400 nM and the Cu(II) reduction was followed over 90 mins. The formation of Cu(I) increased with the initial concentration of Cu(II).

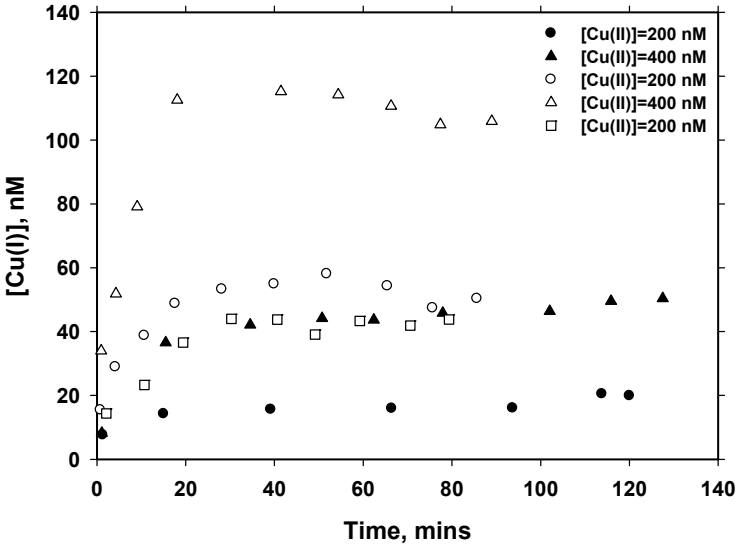

Figure 6-5. The Cu(I) formed by the reduction of Cu(II) in NaCl 0.7 M with $NaHCO_3$ (2 mM) and seawater (S=36.691), using copper (II) concentrations of 200 and 400 nM. The closed symbols are seawater and open symbols are in NaCl. The square symbol corresponds to 200 nM of Cu(II) in NaCl 0.7 M using 9 mM $NaHCO_3$ (T=25ºC and pH=8.00).

At the same initial copper (II) concentration, the concentration of Cu(I) formed in NaCl is higher than in seawater, probably due to the presence of organic compounds that could complex Cu(II) decreasing the reactive Cu(II) species in seawater. At $[Cu(II)]_o$= 200 nM and after 20 mins of reaction, the Cu(I) concentration reaches a constant value of 9% of the initial copper (II) in seawater and 20% in NaCl solutions (Figure 6-5). When the initial copper (II) concentration was 400 nM, the Cu(I) regenerated in NaCl reached 28% and 12% in seawater. When HCO_3^- increased to 9 mM and with 200 nM of initial copper (II) concentration, the maximum concentration of Cu(I) was 16% in NaCl, instead of 20% in 2 mM HCO_3^-. The differences between 2 mM and 9 mM of HCO_3^- must be explained by the interaction between Cu(II) and carbonate (Equation 101).

The reduction of Cu(II) was lower in the presence of higher concentrations of sodium carbonate (Millero et al., 1991; Millero and Hawke, 1992). When Cu(II) and ethylendiamine were initially added to the reaction vessel, the formation of Cu(I) was not observed. The ethylendiamine was preventing the back reaction.

$$CuCO_3 + O_2^{\cdot -} \rightarrow Cu^+ + O_2 + CO_3^{2-} \quad (101)$$

The chemical reduction of Cu(II) to form Cu(I), through reactions 85, 87 and 101, are an important mechanism contributing to the presence of Cu(I) in surface seawater which also decreases the rate constant of oxidation of Cu(I) at nanomolar levels.

The experimental results were used to develop a model in order to calculate the contribution of various species of Cu(I) and its affect on the overall rate constant for the Cu(I) oxidation in NaCl solutions. The overall oxidation rate is given by (Equation 102):

$$k = \alpha_{Cu} k_0 + \alpha_{CuCl} k_1 + \alpha_{CuCl_2} k_2 + \alpha_{CuCl_3} k_3 \quad (102)$$

where α_i are the molar fractions and k_i are the individual rate constants for each species of copper (Millero, 1985). In this model, the Cu(I) formed from the reduction of Cu(II) was included, by considering that after 20 mins the concentration was kept constant at 20% of the initial value. At times lower than 20 mins a linear dependence was applied according to Figure 6-5. The Cu(I) concentration must fulfill the following conditions (Equation 103-106):

$$\left[Cu(I)\right]_{meas} = \left[Cu(I)\right]_0 - \left[Cu(I)\right]_{ox} + \left[Cu(I)\right]_{reg} \quad (103)$$

$$[Cu(I)]_{reg} = [Cu(I)]_{ox} 0.20t/20 \quad (104)$$

$$[Cu(I)]_{ox} = \frac{[Cu(I)]_0 - [Cu(I)]_{meas}}{(1 - 0.20t/20)} \quad (105)$$

$$[Cu(I)]_{real} = [Cu(I)]_0 - [Cu(I)]_{ox} \quad (106)$$

where $[Cu(I)]_0$ is the initial concentration of Cu(I), $[Cu(I)]_{meas}$ is the Cu(I) measured. $[Cu(I)]_{ox}$ is the amount of Cu(I) that is oxidized. $[Cu(I)]_{reg}$ is the copper (I) regenerated. $[Cu(I)]_{reg}$ corrects the percentage of Cu(I) produced in the reduction process. $[Cu(I)]_{real}$ is the copper (I) concentration if only oxidation is taking place. Equation 101 allows us to subtract the Cu(I) regenerated from Cu(II) to the Cu(I) measured. In order to calculate the species contribution to the Cu(I) oxidation in NaCl, the rate constants are calculated as a function of the ionic strength.

The molar fraction α_i for the Cu(I) species (Sharma and Millero, 1988a,b,c) can be determined by (Equation 107-110):

$$\alpha_{Cu} = (1 + \beta_1^* [Cl^-] + \beta_2^* [Cl^-]^2 + \beta_3^* [Cl^-]^3)^{-1} \quad (107)$$

$$\alpha_{CuCl} = \beta_1^* [Cl^-] \alpha_{Cu} \quad (108)$$

$$\alpha_{CuCl_2} = \beta_2^* [Cl^-]^2 \alpha_{Cu} \quad (109)$$

$$\alpha_{CuCl_3} = \beta_3^* [Cl^-]^3 \alpha_{Cu} \quad (110)$$

The stepwise association constants β_i are given by (Equation 111-113):

$$\beta_1 = ([CuCl]/[Cu^+][Cl^-])(\gamma_{CuCl}/\gamma_{Cu}\gamma_{Cl}) \quad (111)$$

$$\beta_2 = ([CuCl_2^-]/[Cu^+][Cl^-]^2)(\gamma_{CuCl_2}/\gamma_{Cu}\gamma_{Cl_2}) \quad (112)$$

$$\beta_3 = ([CuCl_3^{2-}]/[Cu^+][Cl^-]^3)(\gamma_{CuCl_3}/\gamma_{Cu}\gamma_{Cl_3}) \quad (113)$$

where [i] and γ_i are, respectively, the concentrations and activity coefficients for each *i* species. $\beta_i^* = [CuCl_i^{-i+1}]/[Cu^+][Cl^-]$ is the stoichiometric constant.

The equilibrium constants to calculate the speciation were the ones used by (Sharma and Millero, 1988b). The Pitzer parameters (Pitzer and Mayorga, 1973) for the Cu(I) speciation in NaCl are given by (Equation 114-119):

$$Ln\gamma_M = Z_M^2 f^\gamma + 2I \cdot (B_{MCl} + IC_{MCl}) + I^2 (Z_M^2 B'_{NaCl} + Z_M C_{NaCl}) \quad (114)$$

$$Ln\gamma_X = Z_x^2 f^\gamma + 2I \cdot (B_{NaX} + IC_{NaX}) + I^2 (Z_X^2 B'_{NaCl} + Z_M C_{NaCl}) \quad (115)$$

where

$$f^\gamma = -0.392\left[\frac{\sqrt{I}}{1+1.2\sqrt{I}} + 1.67 Ln(1+1.2\sqrt{I})\right] \quad (116)$$

$$B_{MX} = \beta_{MX}^0 + \frac{\beta_{MX}^1}{2I}\left[1-(1+2\sqrt{I})\exp(-2\sqrt{I})\right] \quad (117)$$

$$B'_{MX} = \frac{\beta_{MX}^1}{2I^2}\left[-1+(1+2\sqrt{I}+2I)\exp(-2\sqrt{I})\right] \quad (118)$$

$$C_{MX} = C_{MX}^\phi / (2|Z_M Z_X|^{1/2}) \quad (119)$$

The values of γ_{CuCl} can be estimated from (Equation 120):

$$Ln\gamma_{CuCl} = AI \qquad (120)$$

where A = 0.132 is the salting coefficient for a neutral M^+X^- ion par.

In order to gain an insight into the role played by the different copper chloride species in the oxidation kinetic of Cu(I), the Cu(I) speciation in NaCl has been determined (Figure 6-6). The speciation of Cu(I) is dominated by the $CuCl_2^-$ species, ranging from 92% at 0.1 M to 72% at 0.7 M NaCl, while the $CuCl_3^{2-}$ species achieves a 29% at 0.7 M, following the results at micromolar studies by (Sharma and Millero, 1988a,b,c).

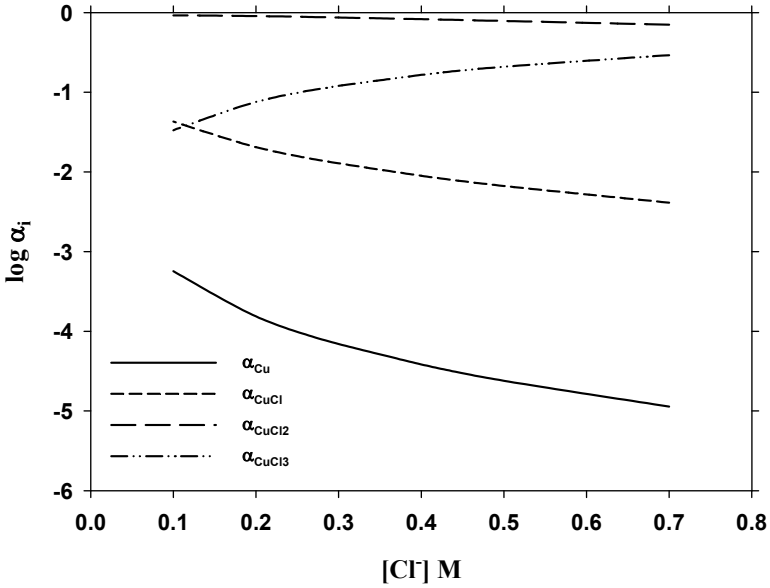

Figure 6-6. Speciation of Cu(I) in NaCl solutions as a function of Cl^- using the molar fractions (α_i) from (Sharma and Millero, 1988c).

The contribution of the different Cu(I) species to the oxidation rate in oxygen saturated condition is given by Equation 102. In our model, the

$CuCl_3^{2-}$ species does not contribute to the overall rate constant. The values of individual rate constants are log $k_0 = 4.47$, log $k_1 = 4.70$ and log $k_2 = 2.38$, for Cu^+, CuCl and $CuCl_2^-$ respectively. The contribution of each species is shown in Figure 6-7. The neutral chloride CuCl species dominates the Cu(I) oxidation in the range studied, 90% at 0.1 M and 54.5% at 0.7 M. $CuCl_2^-$ species increase with the ionic strength, but are less important than CuCl at 0.7 M (45.4%). The contribution of free copper ions is practically negligible; its contribution is 0.1% at 0.7 M and 0.7% at 0.1 M. Differences in the equilibrium constants can lead to both changes in the speciation and in the individual oxidation rates of Cu(I).

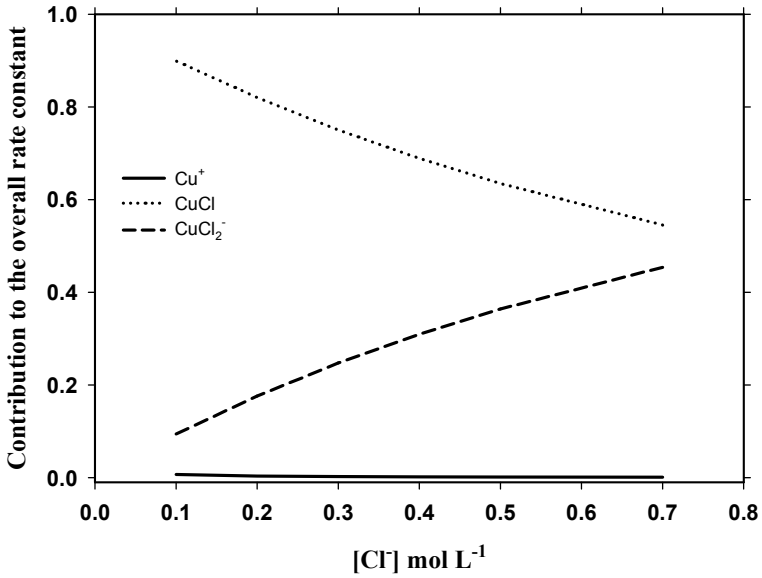

Figure 6-7. Contribution of the various species to the overall rate constant in the range of ionic strength studied.

6.3. Conclusion

The results of this work show that the Cu(I) oxidation rate constants, as a function of pH, are slower at nanomolar levels than at micromolar concentration. The intermediate species generated in the solution, and the Cu(I) regenerated from Cu(II) reduction are more important at nM levels. The effect of pH showed a slight dependence indicating that acid or basic species are not strongly involved in the process. When the temperature increases, the oxidation rate also increases. The copper (I) oxidation rate constant increased with the bicarbonate concentration until 5 mM, but was stabilized between 5-9 mM. The copper (II)-carbonate complexes can account for the effect of the bicarbonate concentration on the oxidation rates. The Cu(I) oxidation rate constant is similar in NaCl and seawater at an ionic strength of 0.7 M. At lower ionic strengths, there are differences between NaCl and seawater. The rate constant is modified by the major role of the interactions with major ions, as Ca^{2+} and Mg^{2+}, and the back-reaction of Cu(II). The $CuCl_2^-$ is the species which dominates the speciation of Cu(I) in sodium chloride. However, neutral CuCl species dominate the Cu(I) contribution to the overall rate constant in the range between 0.1-0.7 M. The results of this work can be extrapolated at low concentrations of copper (50 nM), because there are no measurable effects of the initial copper (I) concentration on the rates.

CHAPTER VII:

Fe(II) and Copper interaction in seawater

ABSTRACT

The competition between Fe(II) and copper species has been studied in seawater at different initial copper concentrations, both Cu(II) and Cu(I) at the range from 0 to 200 nM. In addition, the effect of pH (6.2-8.5), bicarbonate concentration (2- 9 mM) and hydrogen peroxide concentration (0-500 nM) on the Fe(II) apparent rate constant were also studied.

The Cu(II) added in solution was rapidly reduced to Cu(I), at the first 1-2 mins, and it remained in solution after 40 mins. Copper species can react with the natural O_2^- and H_2O_2 present in the seawater. The initial copper additions increase the oxidation rate of Fe(II) under different experimental conditions.

The effect of pH on the Fe(II) apparent oxidation rate with oxygen was a second order dependence for pH over 7.5 and a first order dependence was accounted at lower pH. When Cu(II) was added to the solution, the apparent rate constant was always a first-order pH dependence. These results indicated that $FeOH^+$, $FeCO_3$ and probably $FeHCO_3^+$ are involved in the process.

In addition, the oxidation rate of Fe(II) was function of the initial bicarbonate concentrations, but only when both carbonate and Cu(II) concentrations was over 6 mM and 100 nM, respectively, the oxidation rate increased slightly. The effect of hydrogen peroxide concentrations was also function of the initial Cu(II) additions, but the oxidation rate was equally affected by H_2O_2, thus the observed effect must be only due to the presence of copper species in solution.

CHAPTER VII

7.1. Introduction

The importance of iron in natural waters has been explained in different chapters of this PhD Thesis. In addition, most of the research articles cited have studied the oxidation of Fe(II) in natural waters and its interaction with organic compounds, including Chapters III and V of this manuscript. Nevertheless, the iron redox chemistry can be affected by the presence of other metals in the ocean.

Interactions between iron and first row transition metal species have been described in the literature. Pettine et al. (1998) demonstrated that Fe(II) is an important reductant for Cr(VI). The reduction of hexavalent chromium to Cr(III) by Fe(II) is also a very rapid process (Eary, 1988; Buerge and Hug, 1997). The photochemical production of Fe(II) in the presence of iron-organic colloidal matter was supposed to be responsible for a diurnal cycle of Cr(VI)/Cr(III) ratios in estuarine waters (Kieber and Helz, 1992; Kaczynski and Kieber, 1993). The influence of Cu(II) and other transition metals on the oxidation rates of As(III) with H_2O_2, with important increases in freshwaters and seawater was investigated by Pettine and Millero (2000), and was found to be affected by the presence of competing inorganic and organic ligands that decrease the concentration of uncharged arsenic species that do not react with H_2O_2 and increased charged As reactive species.

Dissolved Fe(II) can also reduce solid Mn(III,IV) (hydro)oxides, and this process has been pointed out as an important secondary reaction to be considered in some natural environments (Postma, 1985; Wehrli, 1990).

According to Chapter VI, copper is an essential redox metal that can act in a number of metabolic processes (Marschner, 1995, Yruela, 2005). The reduction of Cu(II) is also affected by the Fe(II) concentration (Matocha et al., 2005), and it is also known that additions of trace quantities of Cu(II) can catalyze the oxidation of the dissolved Fe(II) by oxygen (Stumm and Lee, 1961; Sayin, 1982). In addition, the presence of Cu(I) higher than 50% of the total copper concentration, in river waters at low oxygen concentration has been attributed to the cycling of Fe(II) and Fe(III) (Glazewski and Morrison, 1996).

The redox mechanism, at air-saturated conditions, in natural waters has been described for iron and copper during this PhD Thesis. Once both processes are known separately, the interaction between both of them can be studied. Reactions that describe the oxidation and reduction mechanism of iron in natural waters were shown in Chapter I.

$$Fe(II) + O_2 \rightarrow Fe(III) + O_2^{\cdot -} \quad (2)$$

$$Fe(II) + O_2^{\cdot -} \xrightarrow{2H^+} Fe(III) + H_2O_2 \quad (3)$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{\cdot} + OH^- \quad (4)$$

$$Fe(II) + OH^{\cdot} \rightarrow Fe(III) + OH^- \quad (5)$$

$$Fe(III) + O_2^{\cdot -} \rightarrow Fe(II) + O_2 \quad (6)$$

$$Fe(III) + 3OH^- \rightarrow Fe(OH)_{3(s)} \quad (7)$$

Reactions that describe the oxidation and reduction of copper in natural waters were presented in Chapter VI.

$$Cu(I) + OH^{\cdot} \rightarrow Cu(II) + OH^{-} \quad (82)$$

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + OH^{\cdot} + OH^{-} \quad (83)$$

$$Cu(II) + HO_2^{\cdot -} \rightarrow Cu(I) + HO_2 \quad (85)$$

$$Cu(II) + O_2^{\cdot -} \rightarrow Cu(I) + O_2 \quad (87)$$

In addition, some reactions must be considered in order to complete the possible species that can affect the oxidation-reduction process for iron and copper (Equations 121-127) (Bielski et al., 1985; Sedlak and Hoigne, 1993; von Piechowski et al., 1993; Millero, 2001).

$$O_2^{-} + HO_2 \xrightarrow{H^+} H_2O_2 + O_2 \quad (121)$$

$$HO_2 \leftrightarrow O_2^{-} + H^+ \quad (122)$$

$$Cu(I) + O_2^{-} \xrightarrow{2H^+} Cu(II) + H_2O_2 \quad (123)$$

$$Cu(I) + HO_2 \xrightarrow{H^+} Cu(II) + H_2O_2 \quad (124)$$

$$Cu(I) + O_2 \rightarrow Cu(II) + O_2^{-} \quad (125)$$

$$Cu(II) + H_2O_2 \rightarrow Cu(I) + HO_2 + H^+ \quad (126)$$

$$Cu(OH)_2 + H_2O_2 \rightarrow Cu(I) + OH^{-} + HO_2 + H_2O \quad (127)$$

The Cu(I) formed in Equation 127 reacts quickly with Cl^- to form chloro complexes (Chapter VI; Millero, 2001; Matocha et al., 2005) (Equations 128-130).

$$Cu(I)_{(aq)} + Cl^- \leftrightarrow CuCl_{(aq)} \quad (128)$$

$$Cu(I)_{(aq)} + 2Cl^- \leftrightarrow CuCl_{2\,(aq)}^- \quad (129)$$

$$Cu(I)_{(aq)} + 3Cl^- \leftrightarrow CuCl_{3\,(aq)}^{2-} \quad (130)$$

The present chapter considers the effect of copper concentration (0-200 nM), Cu(I) and Cu(II), on the Fe(II) oxidation rate constant. By selecting three different Cu(II) concentrations, the effects of pH (6.0-8.5), bicarbonate (2-9 mM) and hydrogen peroxide (0-500 nM) concentrations were also studied. These studies have been carried out in UV-treated seawater, except for the experiments performed to elucidate the effect of bicarbonate concentrations, which were done in NaCl 0.7 M solution.

7.2. Results

7.2.1. Effect of initial copper concentration

The effect of initial copper concentration was studied for Cu(I) and Cu(II) in the range 0 to 200 nM and the initial Fe(II) concentration was always kept constant (100 nM). The experimental results are shown in Figure 7-1 as a function of the ratio, R, between the copper and Fe(II) concentrations added. In both cases, the apparent oxidation rate of Fe(II), log k_{app} = 2.84 ± 0.02 (k_{app} in M^{-1} min^{-1}), increased with the copper concentration. These results were fitted to an equation that rises to a maximum for Cu(II) (Equation 131, with R^2=0.988) and Cu(I) (Equation 132, with R^2=0.996). The standard error of estimation was 0.03 and 0.01, respectively.

$$\log k_{app} = 2.83(\pm 0.02) + 0.41(\pm 0.03) \cdot e^{(-2.6(\pm 0.4) \cdot R_{Cu(II)/Fe(II)})} \quad (131)$$

$$\log k_{app} = 2.85(\pm 0.01) + 0.26(\pm 0.01) \cdot e^{(-4.0(\pm 0.2) \cdot R_{Cu(I)/Fe(II)})} \quad (132)$$

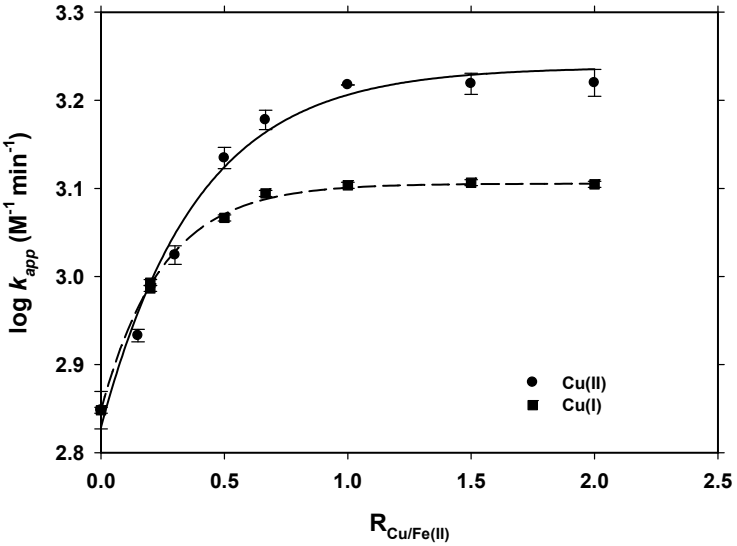

Figure 7-1. Effect of initial copper concentration on the Fe(II) oxidation rate constant at pH=8.0, T=25ºC and $[Fe(II)]_0$=100 nM.

According to the experimental results, both copper species have a similar behaviour when [Cu] is below 0.25 times the [Fe(II)]. The maximum rate constant will be reached with Cu(II) when the ratio Cu(II)/Fe(II) is at least 1. For Cu(I) the maximum is achieved over a ratio Cu(I)/Fe(II) of 0.5. The maximum Fe(II) apparent oxidation rate is obtained in the presence of Cu(II), with a value of log k_{app} = 3.24 ± 0.04, while in the presence of Cu(I) the maximum value was log k_{app} = 3.11 ± 0.02 (k_{app} in M^{-1} min^{-1}). The difference between the rate constants was 0.13 units in log k_{app}. In terms of k_{app}, this difference means 450 M^{-1} min^{-1}.

In order to explain the oxidation of Fe(II) in the presence of copper in solution, the concentration of Cu(I) was followed at pH=8.0, T=25ºC and different Fe(II) concentrations (0-200 nM). The Cu(II) concentrations were kept constant at 100 nM (Figure 7-2). When Cu(II) is added to the solution (Figure 7-2A), Cu(I) is produced even at no Fe(II) added. After addition of 100 nM Cu(II), it is reduced to Cu(I) by O_2^- and H_2O_2 present in the solution at a rate of 4.7 nM min^{-1}. After the formation of Cu(I), it is oxidized and the rate of Cu(I) formation in the solution in strongly reduced, increasing slightly reaching after 40 mins, a concentration of 20 nM. After the initial Cu(I) formation, the final Cu(I) formation rate was 0.2 nM min^{-1}. When Fe(II) is present in solution, the initial formation of Cu(I) is strongly accelerated, reaching a maximum after the first 2-3 mins where the reduction of Cu(II) overcompensates the oxidation of the Cu(I) produced. After this initial stage, the oxidation of Cu(I) dominates, reaching, after 40 mins, a concentration between 30 and 40 nM. The presence of higher Fe(II) concentrations makes the Cu(I) in solution higher but also faster oxidized (Figure 7-2A). The apparent rate constant *k* for Cu(I) moved from 113.19 M^{-1} min^{-1} at 200 nM Fe(II) to 80.17 M^{-1} min^{-1} at 50 nM Fe(II). When Cu(I) instead of Cu(II) was added, (Figure 7-2B), Cu(I) disappeared at a faster rate when no Fe(II) was present. The apparent oxidation rate constant *k* was 471 ± 38 M^{-1} min^{-1}. When Fe(II) is present, Cu(I) can be in solution for a longer time and with higher concentrations directly related to the Fe(II) concentration in solution. At 200 nM Fe(II), the apparent oxidation rate for Cu(I) was k =137 ± 9 M^{-1} min^{-1}, close to the value determined when Cu(II) was initially added at an excess of Fe(II).

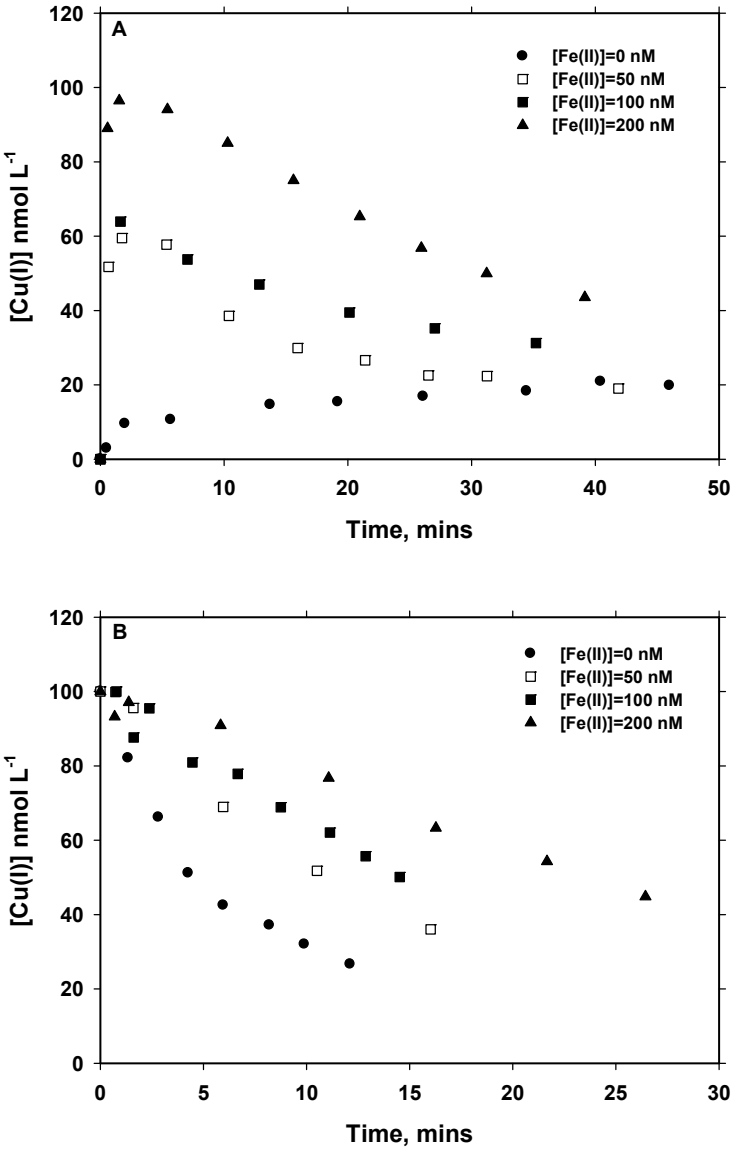

Figure 7-2. Cu(I) concentrations at different Fe(II) initial concentrations. (A) Cu(I) regenerated from Cu(II) and (B) Cu(I) oxidation. The experimental conditions were pH=8.0, T=25ºC and $[Fe(II)]_0$=0, 50, 100 and 200 nM.

7.2.2. Effect of pH

In order to elucidate the effect of pH on the Fe(II) oxidation rate constants in the presence of copper species, the oxidation of Fe(II) was studied at three different Cu(II) initial concentrations (50, 100, 200 nM) and at the pH range 6.0 to 8.5 (in the free-ion scale). The initial Fe(II) concentration was kept constant to 100 nM. Experimental results are shown in Figure 7-3.

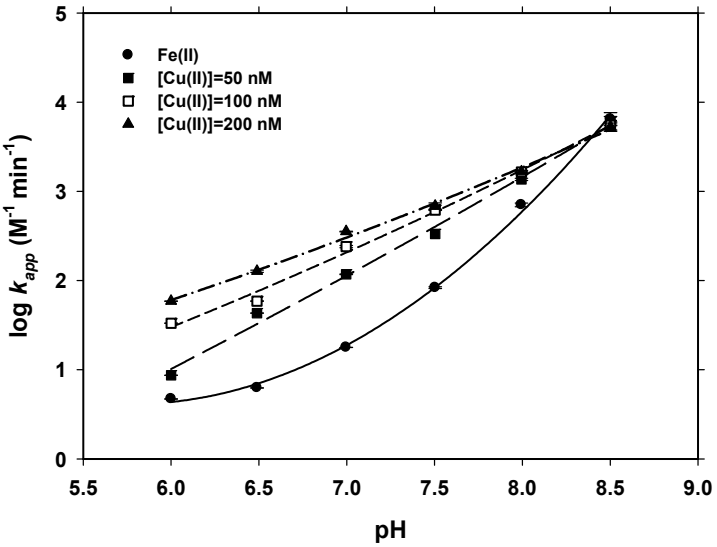

Figure 7-3. Fe(II) oxidation rate as a function of pH for three different initial Cu(II) concentration (50, 100 and 200 nM), at constant temperature (25ºC). $[Fe(II)]_0$ was always 100 nM.

The apparent rate constants (log k_{app}, with k_{app} in M^{-1} min^{-1}) were fitted to second polynomial function for Fe(II) without Cu(II) addition (Equation 133), while the effect of Cu(II) was described by a lineal polynomial equation, for all batches considered as 50 nM (Equation 134), 100 nM (Equation 135) and 200 nM (Equation 136).

$$\log k_{app} = 14.9(\pm 0.3) - 4.96(\pm 0.08)\,pH + 0.43(\pm 0.05)\,pH^2 \quad (133)$$

$$\log k_{app} = -5.6(\pm 0.3) + 1.09(\pm 0.04)\,pH \quad (134)$$

$$\log k_{app} = -4.0(\pm 0.3) + 0.90(\pm 0.04)\,pH \quad (135)$$

$$\log k_{app} = 2.9(\pm 0.2) + 0.77(\pm 0.03)\,pH \quad (136)$$

R^2 was higher than 0.997 for all the equations and the standard error of estimation was always less than 0.09.

The Fe(II) apparent oxidation rate decreased with the pH. In the presence of oxygen (Santana-Casiano et al., 2006) at a pH over 7.5, a slope of 1.89 ± 0.03 was found between log k_{app} and pH, explained by the kinetic controlling effect of $Fe(OH)_2$ and $Fe(CO_3)_2^{2-}$ species. At pH values below 7.5, a slope of 0.84 ± 0.07 is obtained from data in Figure 7-3, due to the higher contribution of $FeOH^+$ and $FeCO_3$ species to the oxidation kinetic rate.

In the presence of Cu(II), the Fe(II) apparent rate constants increased as the initial Cu(II) concentration added increased. The effect of pH in the presence of different Cu(II) levels was more pronounced at pH lowers than 8.0. At pH=6.0, the increment of log k_{app}, referred to the Fe(II) oxidation without Cu(II), was $\Delta\log k_{app}$=0.27, 0.85 and 1.10 (k_{app} in M^{-1} min^{-1}), increasing the initial Cu(II) from 50 nM to 200 nM.

7.2.3. Effect of $NaHCO_3$ concentration

The effect of bicarbonate concentration on the Fe(II) apparent oxidation rate in the presence of Cu(II) was carried out in NaCl 0.7 M solutions with $NaHCO_3$ in the range from 2 to 9 mM. The initial Cu(II)

concentrations were studied in the 50 to 200 nM range. The results are shown in Figure 7-4.

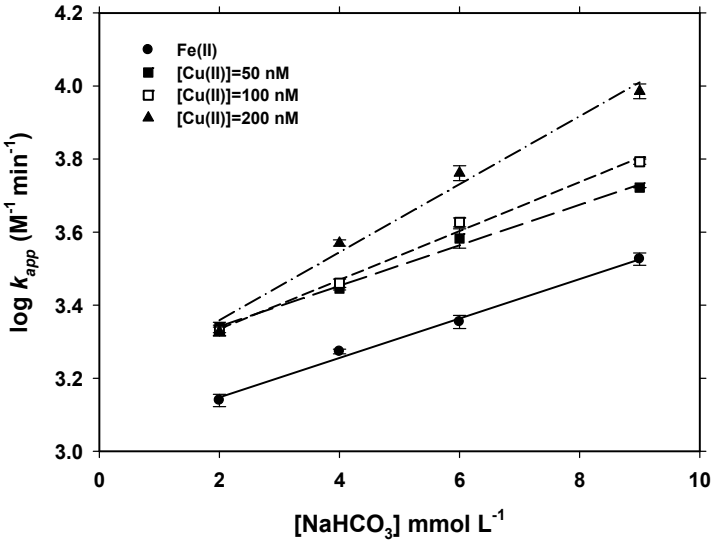

Figure 7-4. Fe(II) oxidation rate as a function of $NaHCO_3$ concentrations at three different initial Cu(II) concentrations (50, 100 and 200 nM). Temperature and pH were kept constant at 25ºC and 8.0 respectively. $[Fe(II)]_0$ was always nM.

The experimental results in the absence of Cu(II) were in concordance with previous studies by Santana-Casiano et al. (2005). The results were fitted to a lineal equation for all the cases: without copper addition (Equation 137) and with Cu(II) additions of 50 nM (Equation 138), 100 nM (Equation 139) and 200 nM (Equation 140). R^2 was always over 0.997 and the standard error of estimation was 0.04 in terms of log k_{app}.

$$\log k_{app} = 3.04(\pm 0.02) + 0.054(\pm 0.003)[NaHCO_3] \quad (137)$$

$$\log k_{app} = 3.23(\pm 0.02) + 0.056(\pm 0.003)[NaHCO_3] \quad (138)$$

$$\log k_{app} = 3.20(\pm 0.02) + 0.067(\pm 0.004)[NaHCO_3] \quad (139)$$

$$\log k_{app} = 3.17(\pm 0.05) + 0.093(\pm 0.009)[NaHCO_3] \quad (140)$$

The Fe(II) apparent oxidation rates increased with the $NaHCO_3$ concentrations from log k_{app} = 3.14 ± 0.02 to 3.53 ± 0.02 (k_{app} in M^{-1} min^{-1}) (King et al., 1995; Santana-Casiano et al., 2005). Under three different Cu(II) concentrations the rate constants kept the observed increase with the $NaHCO_3$ concentration, but the slope was higher as the initial Cu(II) concentration increased over 100 nM. As it was shown before for seawater, (Figure 7-1), the effects in NaCl solutions were close for the different Cu(II) concentrations at 2 mM $NaHCO_3$. At higher $NaHCO_3$ concentrations, the effects due to the presence of Cu(II) that interacts with carbonate ions became more important on the Fe(II) oxidation rates. These slopes were 0.054, for the Fe(II) oxidation rate constants without Cu(II) additions, and increased to 0.056, 0.067 and 0.093 as the Cu(II) levels increased from 50 to 100 and to 200 nM. This made the Fe(II) apparent oxidation rate increased by 0.20, 0.27 and 0.46 in terms of log k_{app} from those at 9 mM $NaHCO_3$ and without Cu(II) added.

7.2.4. Effect of H_2O_2 concentration

The effect of H_2O_2 on the Fe(II) oxidation rate at three Cu(II) initial concentrations (50, 100 and 200 nM) was studied in the range of 50 to 500 nM of H_2O_2. The experimental results are shown in Figure 7-5 together with the effect without Cu(II) addition.

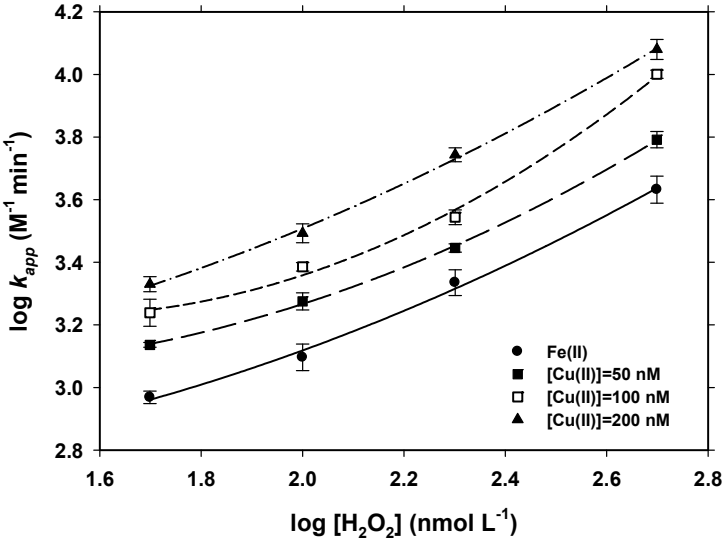

Figure 7-5. Fe(II) oxidation rate as a function of H_2O_2 concentrations at constant pH and Temperature (8.0 and 25ºC, respectively). $[Fe(II)]_0$ was kept constant at 100 nM and the $[Cu(II)]_0$ varied between 50, 100 and 200 nM.

These results clearly indicate that H_2O_2 increased the Fe(II) oxidation rate (González-Davila et al., 2005) and that Cu(II) also increased the oxidation rates. However, the presence of H_2O_2 does not change the observed effects. The additions of Cu(II) were fitted to Equation 141, where $[H_2O_2]$ and [Cu(II)] are in nanomolar levels, with R^2 0.990 and a standard error of estimation of 0.05.

$$\log k_{app} = 3.3(\pm 0.5) - 0.7(\pm 0.5)\log[H_2O_2] + 0.3(\pm 0.1)(\log[H_2O_2])^2 + 2.0(\pm 0.2)\cdot 10^{-3}[Cu(II)] \quad (141)$$

7.3. Discussion

Experimental results collected in the present study demonstrated that the oxidation of Fe(II) is a function of the copper concentration in solution, both Cu(II) and Cu(I). In addition, this process also depends on pH, bicarbonate concentration and hydrogen peroxide concentration.

The non-linear relationship described by the apparent Fe(II) oxidation rate constant at different initial Cu(II) and Cu(I) concentrations (Figure 7-1) in aerated solutions indicated that the presence of copper controlled the Fe(II) oxidation rate. Sayin (1982) suggested that copper ions take part in an oxidation-reduction cycle and act as a catalyst in the oxidation of iron in biotite. Moreover, in anoxic conditions, Abu-Saba et al. (2000) suggested the production of a steady-state Cu(I) concentration after 1-2 mins when Cr(VI) is reduced in the presence of copper. Matocha et al. (2005) also showed that in anoxic conditions, the reduction of Cu(II) to Cu(I) by dissolved Fe(II) was rapid and generally completed in the first 1-2 mins, being strongly affected by the presence of chloride ions.

Our studies in oxic conditions in seawater showed that Cu(I) is produced from Cu(II) in the solution without any Fe(II) addition (Figure 7-2A). Cu(II) can be reduced by the natural H_2O_2 in seawater (Equation 126). This concentration was determined to be between 5-15 nM.

$$Cu(II) + H_2O_2 \rightarrow Cu(I) + HO_2 + H^+ \quad (126)$$

At the seawater pH, HO_2 is present as O_2^-, which can react both with Cu(II) and Cu(I). This process together with the oxidation of Cu(I), oxygen producing Cu(II) and O_2^- (Equation 125) made the Cu(I) concentration reach

a steady state in the solution with a value around 20-30 nM after more than 40 mins.

$$Cu(I) + O_2 \rightarrow Cu(II) + O_2^- \quad (125)$$

When Cu(II) is added to an aerated solution containing Fe(II), Cu(II) is rapidly reduced to Cu(I), reaching a maximum in the first 2-3 mins, while Fe(II) is oxidized to Fe(III). Neither Fe(III) nor Cu(I) are stable under the oxic conditions present in the study. Cu(II) acts as a catalyst, regardless is consumed together with its product, Cu(I), by reactions with oxidant intermediates. Even the catalyst is not totally regenerated, the essential chemical event is catalysis. A schematic depiction proposes in this work that does provide any insight into the redox cycling of iron and copper in oxic seawater is given by Equations 142-145:

$$Fe(OH)_2 + 3H_2O \rightarrow Fe(OH)_3 + O_2^- + 5H^+ + 4e^- \quad (142)$$

$$Cu^{2+} + e^- \rightarrow Cu^+ \quad (143)$$

$$Cu^+ \rightarrow Cu^{2+} + e^- \quad (144)$$

$$4H^+ + O_2^- + 4e^- \rightarrow 2H_2O \quad (145)$$

with the overall reaction (Equation 146):

$$Fe(OH)_2 + O_2 + H_2O \leftrightarrow Fe(OH)_3 + O_2^- + H^+ \quad (146)$$

Copper ions do not appear in the overall reaction, thus acting as a catalyst in the oxidation of iron by oxygen. However, the presence of copper in the oxic seawater also serves as a dominant sink for O_2^- and H_2O_2, and a steady state for Cu(I) is only observed after 30-40 mins with a value that is

independent of the copper initial concentration used in the experiments. The mechanism of interaction between iron and copper has been summarized in Figure 7-6.

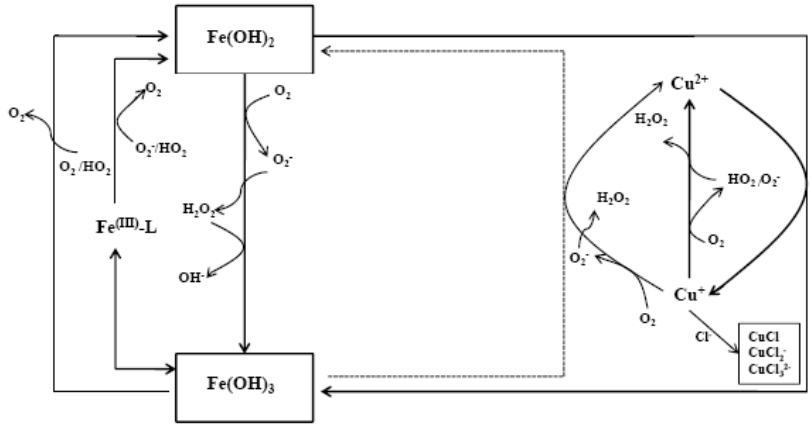

Figure 7-6. Squematic depiction of the redox cycling of iron and copper proposed to seawater under oxic conditions.

As more Fe(II) is present in the solution (Figure 7-2A), more Cu(II) is reduced. At a ratio Fe(II):Cu(II) 2:1, over 95% of the Cu(II) is reduced to Cu(I). Under oxygen saturated conditions, this Cu(I) is oxidized and its concentration decreases until it reaches a steady concentration of around 30 nM of Cu(I), under different Fe(II) concentrations. As a consequence of the production of Cu(I), the amount of Cu(I) in solution increases and [Cu(I)] changes from 31 nM to over 78 nM after 10 mins in solution (Figure 7-2B) without and with 200 nM Fe(II), respectively. Cu(I) remained in solution for a longer time than Fe(II). Considering equimolar concentration ($[Fe(II)]_0$=100 nM and $[Cu(I)]_0$=100 nM), Fe(II) was oxidized in the first 10 mins (at pH=8.0), while Cu(I) concentrations were present in solution after 40 mins.

The decrease in the Cu(I) oxidation rate (Figure 7-2B) at high Fe(II) concentrations is also followed by an increase in the Fe(II) oxidation rate

(Figure 7-1) when copper is present, due to both the reduction of Cu(II) and the production of a higher concentration of intermediates, O_2^- and H_2O_2. At low copper added, a linear increase in the oxidation rate of Fe(II) is observed (Figure 7-1). The Fe(II) oxidation rate increased per nanomolar concentration of Cu(II) added at a rate of 13.6 ± 0.6 M^{-1} min^{-1}. The oxidation of Cu(I) forming Cu(II) under oxic conditions, or the direct addition of Cu(II), oxidized Fe(II) to Fe(III), which presented a similar increase in the oxidation rates. At copper concentrations over 100 nM, the Fe(II) oxidation rates reached a maximum value, in a behaviour similar to that described by a Michaelis Menten catalytic reaction. Cu(II) is also reduced with H_2O_2 producing Cu(I) that will behave as under the addition of Cu(I). Generation of superoxide is also produced that can reacts with Fe(II) increasing the oxidation rate under the presence of Cu(II) with respect to the addition of Cu(I) (Figure 7-1). Moreover, the oxidation of dissolved Cu(I) by Fe(III) can take place (Parker and Espenson, 1969). This process, together with the redox chemistry of both copper and iron species under oxygen saturated conditions are important in lowering the change in the [Fe(II)] to [Cu(I)] ratio. Moreover, as indicated for atmospheric water by Sedlak and Hoigne (1993), in the seawater media, the kinetics of the iron and copper redox cycles are further complicated by the presence of organic matter ligands at concentrations of the same order of magnitude as iron and copper. More than 99% of Fe(III) and 90% of Cu(II) are present in complexed forms in seawater, affecting their redox cycle.

The effect of pH showed that Fe(II) oxidation with oxygen was a second order dependence for pH over 7.5 changing to a pH dependence of order 1 at lower pH values in concordance with values reported by different authors (González-Dávila et al., 2006; Santana-Casiano et al., 2006). When Cu(II) was added to the solution, the apparent rate constant for Fe(II) followed a first order pH dependence for the pH range between 6 and 8.5, indicating that $FeOH^+$, $FeCO_3$, and probably $FeHCO_3^+$, are involved due to

its first-order pH dependence, being also the most affected by the presence of copper. The Fe(II) oxidation rates were similar at pH=8.5 for all the initial Cu(II) concentrations used (Figure 7-3). At low pH values, the oxidation rates increased with the concentration of Cu(II) added, also related to higher predominance of mono carbonate and hydroxyl groups species. Moreover, at high pH, the speciation of Cu(II) is dominated by Cu-carbonate species ($CuCO_3$ and $Cu(CO_3)_2^{2-}$) (Millero et al., 1991) while at low pH values, the free species becomes the most important. The complex formation of Cu(II) with carbonate strongly influences reduction potentials, being free copper a better oxidant. The pH will also affect both the superoxide radical concentration (Equation 122) and the oxidation of Cu(I) and Fe(II), which also contributed to the observed pH dependence (Equations 3 and 123).

$$Fe(II) + O_2^- \xrightarrow{2H^+} Fe(III) + H_2O_2 \quad (3)$$

$$Cu(I) + O_2^- \xrightarrow{2H^+} Cu(II) + H_2O_2 \quad (123)$$

When 50 nM Cu(II) is added to the Fe(II) solution at different carbonate concentrations (Figure 7-4), the Fe(II) apparent oxidation rate slightly increases by 0.15 in log k_{app} from 2 to 9 mM in $NaHCO_3$. Only when both carbonate and Cu(II) concentrations are over 6 mM and 100 nm, respectively, the Fe(II) oxidation rate increases slightly.

The effect of hydrogen peroxide concentration was also function of the initial Cu(II) concentration but, the differences were similar for all the studies in the H_2O_2 range used (0-500 nM). These results suggested that the Fe(II) oxidation rates are equally affected by H_2O_2, and the increase observed is only due to the effect of copper in solution.

In order to explain exactly the Fe(II) and copper interaction in natural waters, further studies are required. Currently, the oxidation of Fe(II)

with Cu(II) additions is studied as a function of temperature and salinity, in particular the key role that can be played by the chloride ions on the resulting Cu(I) ions. In addition, the effects of pH, bicarbonate concentration, hydrogen peroxide concentration, temperature and salinity on the Fe(II) rate constant should also be studied in the presence of Cu(I). Several studies should be carried out under anoxic conditions, in order to determine the role of the reactive oxygen species. Measurements with Cu(I) concentrations are needed because the Fe(II) oxidation mechanism must be characterized by considering Cu(I) speciation. Finally, a kinetic model is being developed in order to explain the variations in the Fe(II) speciation and the contribution of each Fe(II) species to the overall rate constant.

7.4. Conclusion

The results showed in the present chapter demonstrated the fragility of the iron chemistry. The presence of copper species increased the oxidation rate of Fe(II) in seawater under different experimental conditions.

The initial concentration of copper, both Cu(II) and Cu(I), increased the Fe(II) apparent oxidation rate. At the same time, the initial Cu(II) added was rapidly reduced to Cu(I) in the first 1-2 mins, and this Cu(I) undergoes oxidation. The reduction of Cu(II) to Cu(I) also occurred in the absence of Fe(II). The reduction of Cu(II) can be produced due to the reaction with O_2^- and H_2O_2. The oxidation rate of Fe(II) was practically constant when the Cu(II)/Fe(II) ratio was higher than 1 and Cu(I)/Fe(II) was higher than 0.5.

The effect of pH demonstrated that the presence of copper species was important in order to modify the Fe(II) chemistry, respect that in the absence of copper species. The Fe(II) apparent rate constant changed to first-order dependence when Cu(II) was added to the solution, due to the possible

role of $Fe(OH)^{+}$, $FeCO_3$ and $FeHCO_3^{+}$ species. The Fe(II) oxidation rate increased with the bicarbonate concentrations without Cu(II) additions. The most important effect was measured at $NaHCO_3$ and Cu(II) higher than 6 mM and 100 nM respectively. Hydrogen peroxide concentrations increased the oxidation rate of Fe(II), but this increment was similar under different Cu(II) additions, thus the accelerating effect must be only due to the effect of copper species.

In order to elucidate the interaction between iron and copper species, several studies must be developed under different physico-chemical conditions, including experiments in anoxic conditions. These results should support enough information to explain the competitive mechanism between iron and copper species.

CHAPTER VIII:

General Conclusions

GENERAL CONCLUSIONS

The main conclusions that arise from this Thesis are:

1. The oxidation rate of Fe(II) is affected by the local conditions of each natural water samples, according to the nutrient concentrations, organic exudates, temperature, pH, salinity, bicarbonate concentration, hydrogen peroxide concentration and copper concentration.

2. The Fe(II) apparent oxidation rate increased as a function of nutrient concentrations (nitrate, phosphate and silicate), where the interaction with silicate was the most important.

3. The kinetic model developed revealed that the speciation of Fe(II) was controlled by $FeH_3SiO_4^+$ between pH 7.6 and 8.5. In addition, at pH lower than 7.6, the speciation was controlled by $FeCl^+$ and $Fe(SO_4)$. On the other hand, the fractional contribution of $FeH_3SiO_4^+$ to the overall rate constant was similar to other inorganic species of Fe(II) but the oxidation process was controlled by $Fe(OH)^+$, $Fe(CO_3)$ and $Fe(OH)_2$.

4. The presence of higher nutrient concentrations must be considered, especially in coastal waters or eutrophicated waters, because the oxidation of Fe(II) was faster and the half-life time was radically decreased.

5. The presence of organic ligands excreted by *Phaeodactylum tricornutum* remained Fe(II) concentrations in seawater, under different parameters, as cell densities, pH, temperature and salinity.

6. The Fe(II) oxidation rate constant showed a linear dependence with the cell densities or growth time for *P. tricornutum*. These results indicated that the organic ligands are either the same and increase with cell densities, or being different, have a similar mechanism of reaction with Fe(II).

7. The oxidation of Fe(II), as a function of pH, temperature and salinity, was always function of the cell densities, being the oxidation slower respect that in seawater without organic ligands. These results confirmed that Fe(II) can be present for longer time in natural surface waters under the presence of organic exudates excreted by *P. tricornutum*.

8. The kinetic model developed, considering a single ligand model, demonstrated that the organic ligands excreted by *P. tricornutum* affected both the speciation and the fractional contribution of each Fe(II) species, in seawater.

9. The organic exudates from *Dunaliella tertiolecta* showed that the oxidation rate of Fe(II) in seawater, was always function of the cell densities, pH, temperature and salinity. The rate constant was always lower under the presence of organic ligands explaining the presence of Fe(II) concentrations in natural waters.

10. The pH dependence of the Fe(II) rate constant in the presence of organic exudates from *D. tertiolecta*, indicated that these organic ligands interact with Fe(II) modifying the speciation and the fractional contribution of each Fe(II) species.

11. The effect of salinity on the Fe(II) rate constant, was less important in the presence of organic ligands from *D. tertiolecta*, revealing that

there is a great control on the oxidation process the organic ligands over the role played by the major ionic species.

12. The kinetic model approach in order to study the role of the organic ligands from *D. tertiolecta*, on the oxidation of Fe(II) indicated that both ligand models used (carboxyl and amino-like groups) were significant and controlled the oxidation process in a wide range of pH.

13. The oxidation of Fe(II) was dependent on the copper concentration in solution and the Fe(II) rate constant was increased with the copper concentrations.

14. The Fe(II) oxidation rate constant was a second order dependence at pH over 7.5 and a first order dependence at pH over 6. When Cu(II) was added, the pH dependence of the Fe(II) rate was always a first order dependence. Therefore, the $Fe(OH)^{+}$, $FeCO_3$ and $FeHCO_3^{+}$ must be involved in the mechanisms and probably, they are competing with copper species in seawater.

15. The effect of bicarbonate concentrations produced an increasing on the oxidation rate of Fe(II), but only when the concentration of Cu(II) was higher than 100 nM and bicarbonate concentration was over 6 mM, a significant effect was observed.

16. The Fe(II) rate increased with the concentration of hydrogen peroxide concentration in solution. The increment observed was similar for all the conditions. Then, the increasing observed in the presence of Cu(II) was only due to the copper species.

CHAPTER IX:

Future Research

FUTURE RESEARCH

Experimental results collected in the present PhD Thesis demonstrated that the effect of the total organic exudates excreted by *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* retard the oxidation of Fe(II) in seawater under different conditions. The role of nutrients has also been demonstrated, where the Fe(II)-silicate interaction explains the major fraction of the increasing measured to the Fe(II) rate constant. Finally, the competitive mechanism between Fe(II) and copper was described, where the oxidation rate of Fe(II) increases with the copper concentration. These results indicate the fragility of the iron chemistry in natural waters, where the redox process depends on the local conditions.

The results presented in this PhD Thesis have opened a number of doors in order to continue the investigation of Fe(II) oxidation in natural waters.

The organic exudates excreted by different microorganisms must be exhaustively studied, especially the organic exudates that describe redox properties for trace metals in the ocean. The research line must combine the identification of these organic ligands and the laboratory experiments with the more interesting trace metals in order to improve the knowledge about the interactions between metals, as iron or copper, and organic compounds present in natural waters.

On the other hand, the knowledge of the biogeochemical cycles of trace metals requires studies to understand the interactions and the competitive mechanisms between these metals and the rest of chemicals in

solutions, as reactive oxygen species or ionic interactions with major elements of seawater.

Future studies, of both organic and inorganic interactions, must always be carried out for a wide range of physical-chemistry parameters in order to have a large vision of the possible processes that are taking place.

CHAPTER X:

Spanish Summary

Resumen en Español

Capítulo X.1: Introducción

El hierro es un elemento muy importante en el medio natural, debido a que es un micronutriente esencial para la vida de los organismos y que además, juega un papel fundamental en la utilización y producción de gases relacionados con el cambio climático, como pueden ser el CO_2 o el dimetilsulfóxido (Bowie y col., 2002). Existe una fuerte paradoja sobre el hierro en el medio marino, ya que es el cuarto elemento más abundante de la corteza terrestre, pero en cambio, es un metal traza en el océano, donde se encuentra a niveles de concentración subnanomolar. Por ello, se hace crucial conocer los procesos físico-químicos dónde interviene el hierro en el océano, ya que existen aspectos que deben ser investigados con mayor profundidad (Wells y col., 1995), incluyendo su distribución en el océano (de Baar y de Jong, 2001) y su relación con la biota (Geider, 1999). Además, hay que añadir la complejidad de que el hierro está presente en el océano en dos estados de oxidación, Fe(II) y Fe(III) (Sunda, 2001), lo que dificulta aún más el estudio de su ciclo biogeoquímico.

De esta forma, se ha desglosado la introducción en varias secciones que harán posible conocer la parte fundamental de la biogeoquímica del hierro en el medio natural, para luego analizar y discutir los resultados obtenidos en el desarrollo de la presente Tesis Doctoral.

10.1.1. Fuentes de hierro a las aguas oceánicas

La concentración de hierro en el océano está fuertemente ligada a las fuentes continentales, ya que los principales aportes de este metal a las aguas superficiales son los ríos, deposiciones atmosféricas, fuentes hidrotermales y

sedimentos de la plataforma oceánica. Por eso, las concentraciones de hierro van aumentando a medida que nos acercamos a las zonas costeras, donde la influencia continental es más importante (Bowie y col., 2002), por lo que la alta variabilidad geográfica de las fuentes de hierro al océano hacen que la biogeoquímica del hierro dependa de la cuenca y las propiedades de la misma (de Baar y de Jong, 2001). A continuación se detallan cada una de las fuentes de hierro al agua de mar.

- *Aportes a partir de los ríos:*

La entrada de hierro, junto a otros elementos traza, al agua de mar ha sido descrita en detalle por Chester (1990). Los aportes fluviales están distribuidos de forma irregular por la localización de los grandes ríos, sobre todo en el Océano Atlántico, como la descarga del Río Amazonas, Orinoco, Congo y Mississippi.

Los aportes de hierro al agua de mar, a partir de las descargas fluviales, son fundamentalmente de Fe particulado y Fe disuelto. Las cantidades de Fe particulado transportado por los ríos son mucho mayores que las de Fe disuelto, llegando a ser aproximadamente unas 500 veces mayor al Fe disuelto transportado (Chester, 1990). Estas grandes cantidades de Fe particulado que se introducen en el océano, prácticamente son despreciables a nivel oceánico, ya que la mayor parte precipita en los deltas o zonas de descarga, a su llegada a las zonas costeras (de Baar y de Jong, 2001). Dentro de la fracción de Fe disuelto, una gran parte está como pequeñas partículas de Fe coloidal (Fox, 1988), que al llegar al agua de mar sufre un efecto importante de floculación por la diferencia de la fuerza iónica entre ambos medios. La materia orgánica que es transportada por el agua de río, favorecerá la complejación de hierro y la formación de partículas en las zonas de descarga (Fox, 1988). Por lo tanto, el sumidero más importante de Fe particulado, de origen fluvial, es la deposición en los deltas, aunque una

cantidad de Fe particulado puede ser transportado a los fondos oceánicos a través de procesos de transporte y corrientes de turbidez en zonas profundas y cañones submarinos.

- *Deposición atmosférica:*

La deposición atmosférica representa probablemente la fuente dominante de hierro a los océanos (Duce y Tindale, 1991). La entrada de hierro desde la atmósfera sobre las aguas superficiales se produce en dos formas distintas de precipitación, la deposición seca (70%) y deposición húmeda (30%) (Jickells y Spokes, 2001). La deposición seca introduce una importante cantidad de Fe disuelto a las aguas superficiales oceánicas, incluso mayor a la entrada debido al aporte de los ríos en zonas costeras. En este caso, el aporte principal es de Fe(III).

Desde el punto de vista del Fe(II), la contribución más importante se debe a la deposición húmeda, ya que al tener un pH ácido (pH=4-7), cuenta con una alta fracción de Fe(II) disuelto, que se introduce directamente en las aguas superficiales (Millero y col., 1995a). El agua de lluvia, además, es una fuente importante de peróxido de hidrógeno a las aguas superficiales oceánicas (Kieber y col., 2001), participando en los procesos de oxidación de Fe(II) en el agua de mar. Esta agua de lluvia también introduce importantes cantidades de ligandos orgánicos capaces de complejar al Fe(II), incluso tras su deposición en el agua de mar (Willey y col., 2008).

- *Sedimentos marinos:*

Otra fuente importante de Fe disuelto en aguas oceánicas es la movilización de hierro desde los sedimentos marinos. Este hecho ha sido demostrado por el incremento de la concentración de hierro cerca de los márgenes continentales (Chester, 1990; Bowie y col., 2002). Más de la mitad

del hierro presente en sedimentos se encuentra en forma de óxidos de hierro y formando matrices orgánicas. Estas altas concentraciones de hierro medidas en sedimentos se deben principalmente al proceso de diagénesis (Davison y col., 1991; Lovely, 1991; Burdige, 1993; Canfield y col., 1993; Postma, 1993; van Capellen y Wang, 1996).

- *Fuentes hidrotermales:*

La circulación hidrotermal en los fondos oceánicos genera aportes de metales reducidos como Fe y Mn, que al llegar al agua de mar, por la diferencia en temperatura, precipita rápidamente en forma de oxihidróxidos (Campbell, 1991; German y col., 1991), con lo que se forman importantes depósitos ferromanganosos en las zonas cercanas a su emisión. Además, el Fe(II) liberado es rápidamente oxidado, de tal forma que la entrada neta de hierro en las zonas hidrotermales hacia aguas profundas es prácticamente despreciable, comparada con la de otras fuentes como la diagénesis, los ríos o los aportes atmosféricos.

El ciclo biogeoquímico del hierro en el océano se muestra en la Figura 1-1. Las entradas más importantes a las aguas superficiales, como se ha descrito anteriormente, son las entradas fluviales y/o terrestres, junto a la entrada atmosférica. Los sedimentos van a introducir notables cantidades de hierro, que vuelven a su recirculación oceánica, en las zonas profundas.

Como muestra la Figura 1-1, la materia orgánica juega un papel fundamental en la química del hierro, ya sea transportado por los ríos, la lluvia o producida en el propio océano. A través de la radiación solar y/o por fotodegradación de la materia orgánica, se producen intermedios reactivos del oxígeno (O_2^- y H_2O_2) (Zika y col., 1985a; Moore y col., 1993), además de provocar la ruptura de complejos metal-ligando en aguas superficiales, que afectarán al estado en el que se encuentren los cationes metálicos. Los

complejos de hierro liberarán al medio Fe(III), que es altamente insoluble. El Fe(III) se encuentra principalmente en forma coloidal y complejado (99%) con la materia orgánica presente en el agua de mar (Gledhill y van den Berg, 1994; Donat y Bruland, 1995; Wu y Luther, 1995; Bergquist y col., 2006).

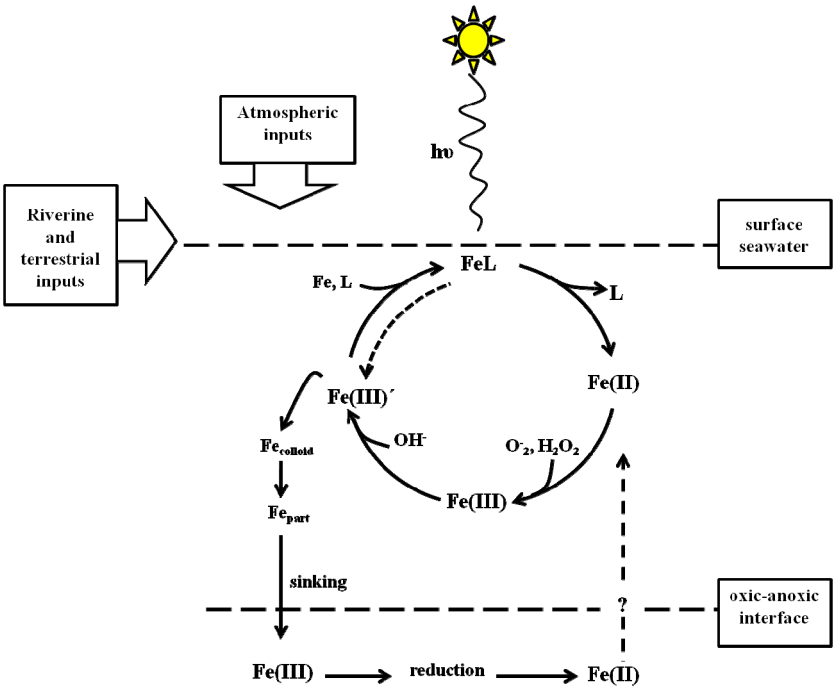

Figura 1-1. Ciclo biogeoquímico del hierro en el océano, modificado de Barbeau (2006).

En cuanto a la distribución vertical (Figura 1-2), el hierro tiene un perfil tipo nutriente en océano abierto, donde las entradas atmosféricas son poco importantes (Johnson y col., 1997), excepto en algunas localizaciones geográficas, dónde la deposición atmosférica introduce grandes cantidades de polvo a la superficie oceánica, como sucede en el Océano Atlántico Noreste (Duce y Tindale, 1991). Además, el hierro también puede estar involucrado

en procesos de adsorción de partículas cambiando su distribución vertical en la columna de agua (Bruland y col., 1994; de Baar y de Jong, 2001).

Un perfil típico de hierro total disuelto en el Océano Atlántico Este (Figura 1-2) muestra que, debido a su relación con los organismos, el hierro en el océano presenta mínimos superficiales, consecuencia de su utilización en la zona fótica por bacterias y fitoplancton, siendo transportado en profundidad. En la zona afótica sufre remineralización profunda vía microbiana (Redfield y col., 1963). El hierro puede presentar, además, un máximo en aguas cercanas a la termoclina, que a veces coincide con máximos de clorofila *a*, lo cual se puede explicar por procesos de complejación orgánica, ya sea por excreción (activa o pasiva) de ligandos específicos de hierro, como son los sideróforos o las porfirinas, o debido al crecimiento de la biota existente (van den Berg, 1995; Rue y Bruland, 1997; Hutchins y col., 1999a). Además, una explicación alternativa puede ser que el máximo de hierro se encuentra en la misma zona del máximo de clorofila debido a la regeneración de hierro a través de la degradación de materia orgánica, o por ingestión de microorganismos, por parte del zooplancton, liberando al medio un contenido importante de hierro (Hutchins y Bruland, 1994; Barbeau y Moffett, 1998). El ciclo se completa con la vuelta del hierro a las zonas superficiales debido a los afloramientos y mezcla turbulenta. El perfil de distribución vertical del hierro está altamente influenciado por las fuentes externas de hierro, principalmente atmosféricas y afloramientos (Bowie y col., 2002). Cabe destacar que el Fe(II) puede mostrar un máximo en profundidades entre 20 y 50 metros, relacionados principalmente con la estabilización con ligandos orgánicos (Bowie y col., 2002; Boye y col., 2006). La concentración de Fe(II) se hace prácticamente despreciable por debajo de los 200 m, aumentando solo en profundidades cercanas al fondo debido a los aportes sedimentarios.

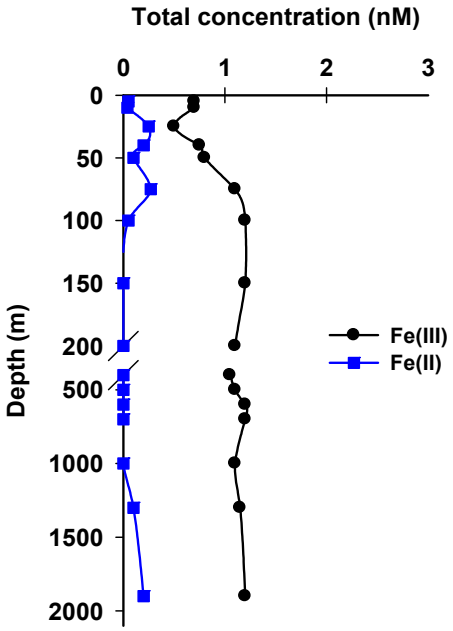

Figura 1-2. Perfil típico de Fe(II) y Fe(III) total disuelto en el Atlántico Este (Boye y col., 2006).

10.1.2. Utilización del hierro por los organismos marinos

Una vez el hierro se encuentra en las aguas superficiales puede ser utilizado por los organismos marinos, ya que es un micronutriente esencial para el crecimiento y el metabolismo. La Tabla 1-1, muestra las enzimas y procesos fundamentales donde se ve involucrado el hierro en el interior de los organismos marinos.

Tabla 1-1. Procesos bioquímicos en los que interviene el Fe dentro de los microorganismos (Stumm y Morgan, 1996).

Metal	Enzima	Funciones
Fe	Citocromo f	Transporte de electrones en la fotosíntesis
	Citocromo b y c	Transporte de electrones en la respiración y en la fotosíntesis
	Ferrodoxina	Transporte de electrones en la fotosíntesis y fijación de nitrógeno
	Proteínas Fe-S	Fotosíntesis y transporte de electrones en la respiración
	Catalasa	Transformación de H_2O_2 a H_2O y O_2
	Peroxidasa	Reducción de H_2O_2 a H_2O
	Quelatasa	Síntesis de Porfirina y Ficobilina
Fe/Mo	Nitrogenasa	Fijación de Nitrógeno
	Nitrato y Nitrito reductasas	Reducción de nitrato a amonio
Fe/Mn	Superóxido dismutasa	Reacción de $O_2^{\cdot-}$ para dar O_2 y H_2O_2

El hierro participa de forma activa y esencial en la fotosíntesis, la respiración y la fijación de nitrógeno. También participa de forma relevante en la síntesis de porfirina y ficobilina, ambos clave en la formación de grupos hemo. El hierro está involucrado en la detoxificación de las especies reactivas del oxígeno (O_2^- y H_2O_2), a través de la catalasa y la peroxidasa. Por lo tanto, estos procesos hacen que el hierro, a pesar de ser un micronutriente, sea de especial interés, sobre todo, en los organismos autótrofos (Sunda y Huntsman, 1997).

Además de las fuentes externas de hierro, existen otras fuentes internas relacionadas a su condición de micronutriente esencial, como son el pastaje y la excreción por el zooplancton, la lisis viral y la remineralización bacteriana. Todas ellas liberan al medio una mezcla importante de compuestos de hierro y ligandos orgánicos, que van a influir en la química del metal (Hutchins y col., 1993).

Algunos estudios han demostrado que el fitoplancton marino es capaz de utilizar el hierro complejado orgánicamente (Glober y col., 1997; Hutchins y col., 1999b; Poorvin y col., 2004; Kustka y col., 2005). Además, estudios de laboratorio, realizados recientemente, soportan la idea de que el Fe(III) no complejado orgánicamente es disponible para la asimilación por los organismos y que además, puede ser una fuente importante de hierro asimilado por el fitoplancton (Morel y col., 2008). El sistema de asimilación de hierro por microorganismos se puede clasificar en tres categorías: (1) sistema de transporte específico o particular para compuestos de hierro como son el Fe-citrato, Fe-sideróforos o grupos hemo, (2) transportadores de Fe(II) de varias características, incluyendo a los transportadores de metales divalentes y los complejos de permeasa-oxidasa, que hacen posible la oxidación de Fe(II), (3) sistema de transporte que incluye a las reductasas para reducir distintas especies de Fe(III) en la superficie celular.

Además, hay evidencias de la internalización directa de los sideróforos, tanto por eucariotas como por procariotas. Sin embargo, ambos grupos de organismos parecen ser capaces de captar hierro a través de procesos de reducción de complejos orgánicos con distintos grados de eficiencia (Hopkinson y Morel, 2009). Los grupos carboxílicos, catecoles e hidroxamatos son los principales grupos funcionales envueltos en la complejación de Fe(III) en ligandos fuertes tipo sideróforos (Shi y col., 2010).

El sistema de transporte de hierro basado en la teoría de los sideróforos ocurre fundamentalmente en cianobacterias (McKnight y Morel, 1979; Boyer y col., 1987; Wilhelm, 1995), aunque hay que destacar que es un sistema metabólicamente muy costoso (Völker y Wolf-Gladrow, 1999), por lo que se produce generalmente en condiciones deficientes de hierro.

Además de reducción de hierro a través de los sideróforos, también se ha observado una importante reducción de Fe(III) en diatomeas, tanto marinas como de agua dulce (Anderson y Morel, 1980; Maldonado y Price, 2000). Este proceso parece estar influenciado por una o más reductasas transmembrana, capaces de reducir complejos de Fe(III) inorgánicos u orgánicos, incluyendo complejos formados por Fe-sideróforos (Allnutt y Bonner, 1987; Jones y col., 1987; Maldonado y Price, 2000; 2001).

Estudios recientes (Santana-Casiano y col., 2010) demuestran que el catecol, uno de los grupos funcionales más representativos de los sideróforos presentes en el medio, a su vez uno de los grupos funcionales más abundantes, es capaces de reducir el Fe(III) a Fe(II), por lo que favorece energéticamente la obtención de hierro del medio marino. Por otra parte, las especies fitoplanctónicas son capaces de excretar importantes cantidades de grupos tiólicos al medio marino (cisteína o glutatión), descritos como fuertes complejantes de metales en el océano. Es importante añadir, que tanto la cisteína como el glutatión han sido determinados en agua de mar (van den Berg y col., 1988; Le Gall y van den Berg, 1993). Incluso, las diatomeas han sido consideradas especies relevantes porque son capaces de producir mayor cantidad de estos compuestos respecto a otras especies fitoplanctónicas (Vasconcelos y col., 2002).

10.1.3. Fe(II) en el agua de mar

El hierro existe en el agua de mar predominantemente como Fe(III), la especie termodinámicamente estable. El Fe(III) es muy reactivo respecto a la hidrólisis, la adsorción y la formación de complejos orgánicos. Realmente, la fracción de Fe(III) que está complejado con compuestos orgánicos es mayor al 99% (Gledhill y van den Berg, 1994; Wu y Luther, 1995). Sin embargo, se han medido concentraciones importantes e inusuales de Fe(II) en

diversos lugares del océano. Hong y Kester (1986) midieron Fe(II) en aguas superficiales superiores a 12 nmol kg^{-1} en las costas de Perú. King y col. (1991) y King (1998) encontraron concentraciones de Fe(II) por encima de 15 nmol kg^{-1} y 4.2 nmol kg^{-1} en agua de mar superficial (Bahía Narragansett, USA). O'Sullivan y col. (1991) publicaron concentraciones de Fe(II) subnanomolares (0.4 nmol kg^{-1}) en aguas superficiales del Pacífico Ecuatorial. En la Bahía de Fuka (Japón), se mostraron altas concentraciones de Fe(II) relacionadas con el bloom de primavera (Kuma y col., 1992). Además, en estudios de fertilización oceánica con hierro, se midieron concentraciones nanomolares de Fe(II) entre 5 y 8 días después de la fertilización (Croot y col., 2001; 2005).

Teóricamente, el Fe(II) una vez presente en agua de mar, debe oxidarse rápidamente a Fe(III), con tiempos de vida media ($t_{1/2}$) desde segundos a varios minutos (Millero y col., 1987; Rose y Waite, 2002; Santana-Casiano y col., 2005), en función de las condiciones físico-químicas del medio. En cambio, como se ha indicado anteriormente, para que sea posible medir Fe(II) en aguas oceánicas, deben existir, además de la fuente principal de Fe(II) como la deposición húmeda o la lluvia (Kieber y col., 2001; Hopkinson y Barbeau, 2007; Willey y col., 2009), otras fuentes de producción o estabilización *in situ* de Fe(II). Estas fuentes pueden ser: (1) la reducción fotoquímica de Fe(III) (Waite y Morel, 1984; Wells y Mayer, 1991; Kuma y col., 1992; Miller y col., 1995; Vöelker y Sedlak, 1995; Waite y col., 1995) (2) la reducción producida directa o indirectamente por organismos marinos o por vía enzimática (Anderson y Morel, 1980; Jones y col., 1987; Hutchins y col., 1993; Barbeau y col., 1996; Maldonado y Price, 1999; 2000; Roy y col., 2008).

El principal proceso de regeneración de Fe(II) en las aguas superficiales es su fotoproducción. Por ello se debe destacar su papel en el incremento de la solubilidad y la biodisponibilidad del hierro en aguas

naturales (Pehkonen y col., 1992; Johnson y col., 1994; Miller y Kester, 1994; Waite y col., 1995). En otro estudio, Finden y col. (1984) mostraron evidencias de la reducción fotoquímica del Fe(III), a través de medidas de Fe(II) fijado con bazofenantrolina en agua dulce. En aguas costeras, a pH=6.5, Waite y Morel, (1984) midieron la regeneración de Fe(II). Kuma y col. (1992), en experimentos de laboratorio, demostraron la producción de Fe(II) inducido por la luz, en agua de mar y en cultivos de diatomeas. En estos estudios se llegó a la conclusión de que los grupos carboxílicos (Waite y Morel, 1984; Cunningham y col., 1988), los compuestos que contienen grupos tiólicos (Waite y Torikov, 1987), alcoholes (Cunningham y col., 1985) y ácidos fúlvicos (Waite y Morel, 1984) tienen una importante relación con los procesos fotoquímicos de reducción de Fe(III), a partir de óxidos de Fe(III). Específicamente, los ácidos oxálico, cítrico, málico, glicérico, salicílico, tartárico, glucónico y p-hidroxibenzóico incrementan la fotoproducción de Fe(II) en disolución (Cunninghan y col., 1988; Kuma y col., 1992). Así, los procesos fotoquímicos y la presencia de ligandos específicos de Fe(II) pueden explicar su presencia en aguas superficiales oceánicas.

Inicialmente, el proceso más aceptado para la foto-producción de Fe(II) en aguas superficiales es la reducción de complejos de (hidro)óxidos de Fe(III) (King y col., 1993).

$$Fe(OH)^{2+} \xrightarrow{h\upsilon} Fe-OH^{*} \rightarrow Fe^{2+} + OH^{-} \qquad (1)$$

donde solo el $Fe(OH)^{2+}$ es notablemente reactivo. A pH=8.0, el hierro está fuertemente hidrolizado formando $Fe(OH)_x$ (x=2-4) (Millero, 1998) y estas especies deben ser menos reactivas. Entonces, se deben considerar también algún tipo de reacción de transferencia de carga metal-ligando (LMTC) con los compuestos orgánicos (Rich y Morel, 1990; King y col., 1993). De esta

forma, el Fe(II) libre quedará disponible para su asimilación por el fitoplancton (Hutchins y col., 1999; Maldonado y Price, 2001; Sunda, 2001). Finalmente, el hierro complejado por ligandos orgánicos debería ser asimilado por los organismos vía procesos redox y procesos de intercambio entre ligandos (Hutchins y col., 1999; Maldonado y Price, 2001; Sunda, 2001; Rose y col., 2005).

10.1.4. Cinética de oxidación de Fe(II) en agua de mar

En los últimos años se ha realizado un enorme esfuerzo en el estudio de la cinética de oxidación de Fe(II) en aguas naturales (Stumm y Lee, 1961; Kester y col., 1975; Davison y Seed, 1983; Waite y Morel, 1984; Millero y col., 1987; Millero e Izaguirre, 1989; Liang y col., 1993; King y col., 1995; Rose y Waite, 2002; Santana-Casiano y col., 2004; 2005; 2006; González-Dávila y col., 2006; Trapp y Millero, 2007) permitiendo un mejor conocimiento del ciclo biogeoquímico del Fe(II) en el medio acuático natural, profundizando en los procesos inorgánicos más importantes donde el Fe(II) se ve involucrado.

El mecanismo más aceptado para la oxidación de Fe(II) en aguas naturales es conocido como el mecanismo de Haber-Weiss, que envuelve la secuencia de oxidación del oxígeno molecular, radical superóxido, peróxido de hidrógeno y radical hidroxilo (Haber y Weiss, 1953; Fallab, 1967; Millero, 1989):

$$Fe(II) + O_2 \rightarrow Fe(III) + O_2^{\cdot -} \quad (2)$$

$$Fe(II) + O_2^{\cdot -} \xrightarrow{2H+} Fe(III) + H_2O_2 \quad (3)$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{\cdot} + OH^{-} \quad (4)$$

$$Fe(II) + OH^{\cdot} \rightarrow Fe(III) + OH^{-} \quad (5)$$

Este mecanismo se debe completar considerando las reacciones de reducción de Fe(III) formado o presente en el medio, la hidrólisis y formación de Fe(III) coloidal, así como con las reacciones de competición con las especies activas del oxígeno ($O_2^{\cdot-}$ y H_2O_2), en las que también participan otras especies activas como el cobre (Rose y Waite, 2002; Santana-Casiano y col., 2005).

$$Fe(III) + O_2^{\cdot-} \rightarrow Fe(II) + O_2 \quad (6)$$

$$Fe(III) + 3OH^{-} \rightarrow Fe(OH)_{3(s)} \quad (7)$$

$$Cu(II) + O_2^{\cdot-} \rightarrow Cu(I) + O_2 \quad (8)$$

$$Cu(I) + O_2^{\cdot-} \xrightarrow{2H^+} Cu(II) + H_2O_2 \quad (9)$$

La ecuación de velocidad para la oxidación de Fe(II) con O_2 viene dada por:

$$\frac{d\,[Fe(II)]}{dt} = -k_{app}[Fe(II)][O_2] \quad (10)$$

En condiciones de saturación de O_2, la ecuación de velocidad puede ser considerada como una ecuación de pseudo-primer orden, donde la constante de velocidad $k_{app} = k' / [O_2]$:

$$\frac{d\,[Fe(II)]}{dt} = -k'\,[Fe(II)] \quad (11)$$

La constante aparente de oxidación de Fe(II) es función de la suma ponderada de las velocidades de oxidación de cada una de las especies individuales de Fe (II), tanto inorgánica como orgánica (Ecuación 12).

$$k_{app} = \sum_i \alpha_i k_i + \sum_L \alpha_L k_L \quad (12)$$

donde α_i es la fracción molar de cada una de las especies de Fe(II) inorgánico y α_L es la fracción molar de las especies de Fe(II) complejado orgánicamente en disolución y que además es función de la fuerza iónica.

La oxidación de Fe(II) ha sido estudiada en aguas naturales, tanto a nivel micromolar (Kester y col., 1975; Murray y Gill, 1978; Roekens y van Grieken, 1983; Waite y Morel, 1984; Millero y col., 1987; Millero e Izaguirre, 1989) como a nivel nanomolar (King y col., 1995; Emennegger y col., 1998; King, 1998; King y Farlow, 2000; Rose y Waite, 2002; González-Dávila y col., 2005; Santana-Casiano y col., 2005).

Millero e Izaguirre (1989) y King (1998) han demostrado que la interacción de Fe(II) con otros iones mayoritarios del agua de mar afecta a la velocidad de oxidación. Así, King (1998) demostró una fuerte dependencia de la velocidad de oxidación de Fe(II) con la concentración de carbonatos, de acuerdo con las Ecuaciones 13-15.

$$Fe^{2+} + CO_3^{2-} \leftrightarrow FeCO_3 \quad (13)$$

$$Fe^{2+} + 2CO_3^{2-} \leftrightarrow Fe(CO_3)_2^{2-} \quad (14)$$

$$Fe^{2+} + CO_3^{2-} + OH^- \leftrightarrow Fe(CO_3)OH^- \quad (15)$$

El comportamiento de la constante de velocidad de oxidación de Fe(II) en agua de mar presenta ciertas diferencias a nivel micromolar respecto a su comportamiento a nivel nanomolar. En concentraciones micromolares, la oxidación de Fe(II) por oxígeno presenta una estequiometria 4:1 (Millero y col., 1987; King, 1998; Santana-Casiano y col., 2000), mientras que la oxidación por peróxido de hidrógeno tiene una estequiometria 2:1 (Millero y Sotolongo, 1989; González-Dávila y col., 2005).

La oxidación de Fe(II) a nivel nanomolar, en condiciones de saturación, ha sido estudiada recientemente (Santana-Casiano y col., 2006), donde cabe destacar que la velocidad de oxidación aumenta en función de la concentración de HCO_3^- en disolución. Pero además, en función del pH, la velocidad de oxidación es mayor a pH $\leq$ 7.5, aunque es menor a pH $\geq$ 7.5 que su valor a escala micromolar. Este comportamiento se debe al papel que juegan los intermedios de oxidación en niveles nanomolares de concentración, que no pueden ser observados a escala micromolar (Millero y col., 1987; Santana-Casiano y col., 2005).

La velocidad de oxidación de Fe(II) también ha sido estudiada para un amplio rango de concentración de H_2O_2 (González-Dávila y col., 2005; Santana-Casiano y col., 2006), a niveles nanomolares de Fe(II). Estos estudios tienen especial relevancia ya que se describió una importante competición entre el O_2 (μmol L^{-1}) y el H_2O_2 (nmol L^{-1}) por la oxidación de Fe(II). En aguas naturales, la concentración de H_2O_2 varía entre 10 y 150 nM, en aguas oligotróficas, hasta alcanzar concentraciones superiores a los 500 nM en aguas costeras (Zika y col., 1985 a, b; Moore y col., 1993; Hanson y col., 2001), por lo que la competición entre H_2O_2 y O_2 como agentes oxidantes es importante.

En el océano, la fotooxidación de la materia orgánica da lugar a la producción de las especies reactivas del oxígeno (Ecuaciones 16-18) (Sharma y Millero, 1988a).

$$org \xrightarrow{h\upsilon} org^{+} + e^{-} \quad (16)$$

$$e^{-} + O_2 \rightarrow O_2^{\bullet -} \quad (17)$$

$$2O_2^{\bullet -} + 2H^{+} \rightarrow H_2O_2 + O_2 \quad (18)$$

En estas condiciones, la Ecuación 4 no es totalmente dependiente de las Ecuaciones 2-3. Además, la reducción del oxígeno viene descrita por (Ecuaciones 19-21).

$$O_{2(g)} + 4H^{+} + 4e^{-} \leftrightarrow 2H_2O \quad (19)$$

$$O_{2(g)} + 2H^{+} + 2e^{-} \leftrightarrow H_2O_2 \quad (20)$$

$$H_2O_{2(g)} + 2H^{+} + 2e^{-} \leftrightarrow 2H_2O \quad (21)$$

donde la adquisición de los primeros dos electrones desde la reacción de O_2 para dar H_2O_2 es energéticamente menos favorable que la adquisición de los siguientes dos electrones desde la reacción de H_2O_2 para obtener H_2O (Bruland y Rue, 2001; Rose y Waite, 2002). Por lo tanto, la producción de peróxido de hidrógeno comienza a partir de la fotooxidación de la materia orgánica, lo cual debe incrementar su importancia cuanto más cerca de la costa, donde la concentración de materia orgánica es mayor y por lo tanto la producción de H_2O_2 debe ser más relevante (Zika y col., 1985a; Moore y col., 1993).

En condiciones de agua de mar, la especiación de Fe(II) está dominada por Fe^{2+}, $FeCl^{+}$ y $FeSO_4$, para un amplio rango de pH (6.0-8.2). En este rango de pH, las especies hidrolizadas de hierro, $Fe(OH)_x^{(2-x)-}$ (x=1-2), contribuyen en menor proporción a su especiación. A pH más alcalinos (pH > 8.2), la especie $FeCO_3$ es las más importante en la especiación. En cambio, la contribución de cada una de las especies de Fe(II) a la constante de velocidad global del proceso está controlada por las especies hidrolizadas de Fe(II), siendo el $Fe(OH)_2$ la especie predominante a pH ≥ 8.0, junto al $Fe(CO_3)_2^{2-}$. Dadas las contribuciones de estas especies, en condiciones donde el oxígeno es predominante, la dependencia de la velocidad de oxidación de Fe(II) es de segundo orden respecto al pH, con el oxígeno, y de orden uno con el H_2O_2, por lo que se compensa la diferencia de concentraciones entre el O_2 y el H_2O_2. Aún así, el papel del H_2O_2 es importante, incluso en niveles de saturación de oxígeno, aunque al pH del agua de mar, el oxígeno es la especie oxidante más relevante (González-Dávila y col., 2006).

10.1.5. El papel de los ligandos orgánicos en la oxidación de Fe(II)

La química del hierro en la naturaleza está fuertemente ligada a la presencia de materia orgánica (Figura 1-1), ya sea de forma directa o de forma indirecta. Desde los primeros estudios sobre la especiación de hierro en el medio marino se mostró que el 99% del Fe disuelto en el océano se encuentra complejado con ligandos orgánicos (Gledhill y van den Berg, 1994; Wu y Luther, 1995), que se pueden clasificar, atendiendo a su capacidad complejante, en dos tipos: L_1 y L_2. Los ligandos tipo L_1 son ligandos de alta afinidad por el hierro, con constantes de complejación de $K^{cond} \sim 10^{12\text{-}13}$ L mol^{-1}. Como resultado de los avances tecnológicos, se han identificado algunos tipos de ligandos específicos de hierro a partir de

sideróforos marinos, como son el Aquacheline, Petrobactin, Aerobactin y Desferroxamine B (Macrellis y col., 2001; Barbeau y col., 2002; Gledhill y col., 2004), estimándose constantes condicionales para estos sideróforos similares a los ligandos del tipo L_1. Generalmente este tipo de ligandos (L_1) se ha determinado en las aguas superficiales oceánicas (Barbeau y col., 2002). El segundo tipo de ligandos (L_2) ha sido relacionado con material intracelular, tipo protoporfirina, con una constante condicional de $K^{cond} \sim 10^{10\text{-}11}$ L mol^{-1} (Rue y Bruland, 1995; 1997; Cullen y col., 2006).

La mayor parte de trabajos que estudian la complejación de hierro en las aguas naturales se han desarrollado para Fe(III), dada la inestabilidad del Fe(II) que hace complicada su caracterización. Sólo algunos estudios han ido dirigidos a estudiar el efecto de ligandos orgánicos naturales en la oxidación de Fe(II). En este caso, además de la importancia que tienen los ligandos orgánicos al complejar al metal, hay que añadir la influencia indirecta de estos ligandos a través de procesos fotoquímicos, dónde se generan importantes cantidades de radical superóxido.

Los ligandos orgánicos son uno de los factores que provocan la estabilización en el tiempo del Fe(II) haciendo posible su presencia en aguas superficiales oceánicas y por lo tanto su utilización por los microorganismos marinos durante un mayor tiempo. La materia orgánica presente en disolución puede provocar una aceleración, un retardo o no tener ningún efecto en la oxidación de Fe(II) en agua de mar. Así se han ido variando los compuestos orgánicos estudiados tanto en agua dulce como en agua de mar, porque hasta la actualidad los estudios han estado dirigidos a conocer el papel de compuestos individuales en la cinética del Fe(II).

En agua dulce, se puede destacar el ácido tánico, el ácido gálico y el pirogalol, que previenen la oxidación de Fe(II), dada la formación de complejos fuertes. El ácido glutámico, el ácido tartárico y la glutamina

provocan una ralentización del proceso de oxidación de Fe(II). En cambio, el ácido cítrico acelera el mismo proceso. El fenol y la histidina no producen ningún efecto en la constante de velocidad de oxidación de Fe(II) en agua dulce (Theis y Singer, 1973; 1974).

Otros compuestos como el EGTA (ácido etilen-glicol-bis(2-aminoetileter)-N,N,N',N'-tetraacético) forman un fuerte complejo que inhibe la oxidación de Fe(II). El EDTA (ácido etilendiaminotetraacético) incrementa la oxidación de Fe(II) formando un complejo fuerte Fe(III)-EDTA que por fotoreducción regenera Fe(II) en el medio. Un compuesto muy interesante en el medio marino es la cisteína, que es capaz de reducir Fe(III). Su papel es función del ratio cisteína-Fe(II) y de la oxidación de cisteína a cistina. En cambio, la alanina y el ácido glutámico no revelan ningún papel en la oxidación de Fe(II) en agua de mar (Santana-Casiano y col., 2000).

En agua de mar se pueden encontrar estudios realizados con materia orgánica natural, dónde la mayor parte de los compuestos utilizados (extractos de materia orgánica procedentes de caña de azúcar, malaleuca, y pino (Rose y Waite, 2003a)) produjeron una aceleración en la cinética de oxidación de Fe(II), excepto tres extractos procedentes de pino y citrato que produjeron una disminución considerable de la velocidad de oxidación. El efecto de estos compuestos en la velocidad de oxidación es proporcional a la concentración utilizada en los estudios.

En agua de mar se ha estudiado el efecto, tanto del ácido salicílico como del ácido ftálico, en la oxidación de Fe(II) en agua de mar (Santana-Casiano y col., 2004). El ácido salicílico produce un incremento en la velocidad de oxidación de Fe(II), mientras que el ácido ftálico actúa disminuyendo la oxidación de Fe(II) en agua de mar. El ácido ftálico ha sido considerado como un fuerte complejante de metales en aguas naturales (Chang y Zylstra, 1999).

Recientemente, también se ha estudiado el efecto que tiene el catecol en la reducción de Fe(III) en agua de mar (Santana-Casiano y col., 2010). En este caso el proceso de regeneración de Fe(II) es función del pH y de los iones del medio, ya que se ha descrito una importante interacción entre el radical semiquinona con los iones Mg^{2+} y Ca^{2+} afectando a la regeneración de Fe(II) en agua de mar.

Es importante destacar que la materia orgánica no sólo tiene un papel fundamental en la cinética de oxidación de Fe(II), sino también en la solubilidad de hierro en el medio marino (0.011 nM en una disolución NaCl 0.7 M; Liu y Millero, 2002), permitiendo que el Fe(II) se mantenga más tiempo en las aguas oceánicas superficiales, aumentando las posibilidades de utilización por el fitoplancton.

Se debe resaltar, que en la bibliografía se ha mostrado el efecto de diversos compuestos individuales en la cinética de oxidación de Fe(II), pero se hace necesario saber, conocer y caracterizar el efecto que tienen el conjunto de compuestos excretados por los organismos en la química del hierro en el medio marino. Un primer paso es considerar los compuestos excretados por tipos concretos de organismos presentes en el medio marino.

Guión Tesis Doctoral

A lo largo de la introducción se ha demostrado la necesidad de hacer estudios de oxidación de Fe(II) en presencia de exudados orgánicos procedentes de organismos marinos, ya que estos compuestos excretados son la principal fuente de ligandos específicos de hierro en el agua de mar. Por esto, el objetivo de la presente Tesis Doctoral es profundizar en la química-física del Fe(II) en presencia de exudados orgánicos procedentes del fitoplancton. Además, dada la competición que existe entre diferentes especies químicas por los distintos intermedios redox de oxígeno, como es el caso de la que se establece entre el Fe(II) y las especies de Cu(I) y Cu(II), se hace necesaria la caracterización de los efectos que pueden tener en la química de cada metal en las aguas naturales.

Para el desarrollo de los trabajos que componen la presente Tesis Doctoral se seleccionaron dos especies de fitoplancton, *Phaeodactylum tricornutum* y *Dunaliella tertiolecta*. La primera una diatomea y la segunda un alga verde flagelada. Ambas especies fueron elegidas tanto por su enorme dispersión oceanográfica como por el conocimiento existente en la bibliografía, tanto de las excreciones de compuestos de interés para la complejación de metales como por la caracterización de los grupos funcionales más importantes en su pared celular, capaces de adsorber metales en el medio.

Los estudios desarrollados en la Tesis Doctoral se llevaron a cabo en agua de mar, con altas concentraciones de nutrientes. Estas concentraciones son propias de medios de cultivo f/2 utilizados comúnmente en estudios de laboratorio. Por lo tanto, el primer paso de este trabajo debía ser caracterizar

el comportamiento del Fe(II) en presencia de los nutrientes añadidos al agua de mar.

Una vez conocido el comportamiento y las interacciones existentes del Fe(II) con los nutrientes presentes en el agua de mar, se realizaron estudios de oxidación de Fe(II) en presencia de exudados orgánicos, tanto de *P. tricornutum* como de *D. tertiolecta*.

Finalmente, se llevaron a cabo estudios de interacción de Cobre y Fe(II) en agua de mar, para lo que previamente se tuvo que estudiar la oxidación de Cu(I) en agua de mar a escala nanomolar. Una vez conocido el comportamiento del Cobre y el Fe(II) por separado, se procedió al estudio de su interacción.

Objetivos de la Tesis Doctoral

El objetivo principal del trabajo de Tesis era el de "*caracterizar la química del Fe(II) en presencia de compuestos orgánicos excretados por especies de fitoplancton, y en presencia de Cobre, todo ello utilizando como medio el agua de mar*".

Este objetivo principal se ha dividido a su vez, en cinco objetivos específicos que se desarrollan en otros tantos capítulos en los que se ha estructurado la presente Tesis Doctoral.

- Objetivo 1: ***Caracterizar la oxidación de Fe(II) en aguas naturales a altas concentraciones de nutrientes.***

Las dos especies de fitoplancton utilizadas a lo largo de la Tesis Doctoral, han permitido que se utilice un mismo protocolo de cultivo, donde el agua de mar es enriquecida con nutrientes típicamente empleados en medios de cultivo tipo f/2 (nitrato, fosfato y silicato), por lo que el primer objetivo es el estudio de las interacciones entre el Fe(II) y los nutrientes presentes en disolución y su posible efecto en su cinética de oxidación.

Para alcanzar este objetivo, se realizaron estudios del efecto de la concentración de nutrientes, tanto en su conjunto como individualmente. Esto permitirá determinar qué cambios se producen en la cinética de oxidación de Fe(II) en función de cada nutriente, tanto en agua de mar como en agua de mar artificial. Dado que la cinética de oxidación depende de las variables fisicoquímicas del medio, la consecución de este objetivo necesita de la caracterización de la misma en función del efecto del pH, la temperatura y la salinidad. En cada caso, se puede obtener el efecto que los nutrientes están

produciendo en el proceso de oxidación y obtener datos experimentales que ayudaron a comprender cómo es la química del metal en situaciones de altas concentraciones de nutrientes.

Como paso final para alcanzar el objetivo propuesto es necesario la elaboración de un modelo cinético químico que describa los resultados experimentales obtenidos, considerando las reacciones de interacción entre el Fe(II) y los nutrientes que sean responsables mayoritarios de los cambios de la constante de velocidad de oxidación observado en todos los casos de estudio.

El desarrollo de este objetivo constituye el Capítulo III de esta Tesis Doctoral.

- Objetivo 2: ***Determinar el efecto que ejercen los ligandos orgánicos excretados por Phaeodactylum tricornutum en la velocidad de oxidación de Fe(II).***

Para alcanzar este objetivo es necesario caracterizar el crecimiento de la especie en las condiciones establecidas para el desarrollo experimental. Una vez conocido el crecimiento de la especie, se considerarán los efectos producidos en cada uno de los estados de crecimiento.

Otro aspecto a tener en cuenta es el estudio del efecto de la concentración de células en la constante de velocidad de oxidación de Fe(II), para cada día de cultivo. De esta forma se puede determinar si el efecto de los ligandos es función de la densidad celular.

Para cada una de las fases de crecimiento, se considerarán los efectos producidos bajo diferentes rangos de pH (7.5-8.2), temperatura (5-

35ºC) y salinidad (10-36.720) en la oxidación de Fe(II). Estos estudios permitirán conocer los procesos químicos en los que el Fe(II) está envuelto, en presencia de los ligandos orgánicos excretados por *P. tricornutum*.

Finalmente, se desarrollará un modelo cinético, basándonos en los resultados experimentales, para describir la especiación de Fe(II) y la contribución de cada una de las especies de Fe(II) a la constante de velocidad global del proceso. En este caso, se partirá de un modelo de ligando simple, interaccionando con el Fe(II). Con este modelo, se estimarán las constantes de equilibrio y velocidad para la especie propuesta, describiendo las especies mayoritarias de Fe(II) en el medio enriquecido con exudados orgánicos y la contribución individual de cada especie a la velocidad total del proceso de oxidación. El desarrollo de este objetivo constituye el Capítulo IV.

- Objetivo 3: ***Estudiar el efecto que ejercen los exudados orgánicos producidos por el alga Dunaliella tertiolecta en la cinética de oxidación de Fe(II) en agua de mar.***

Los planteamientos indicados en el objetivo 2 se extienden al estudio de los efectos producidos por los exudados orgánicos procedentes de *Dunaliella tertiolecta*. Se caracterizará el crecimiento de la especie en las condiciones de cultivo utilizadas y se identificarán las fases de crecimiento del cultivo para llevar a cabo en cada una de ellas los estudios que permitan determinar el efecto producido bajo diferentes condiciones experimentales. De esta manera, se podrá caracterizar el tipo de interacción que presenta el Fe(II) en presencia de los exudados excretados por *D. tertiolecta*.

El estudio se realizará en función del pH (7.5-8.2), la temperatura (5-35ºC) y la salinidad (10-36.720), para distintas densidades celulares, y en presencia de los exudados producidos.

Finalmente, se desarrollará el modelo cinético que explique los resultados experimentales. En este caso, se introducirán dos posibles tipos de ligandos capaces de complejar Fe(II) en agua de mar, que han sido identificados como mayoritarios en cultivos de *D. tertiolecta*, para estudios anteriores. Con el modelo se estimará la contribución de estas especies a la constante de velocidad total del proceso de oxidación. Los resultados obtenidos constituyen el Capítulo V de la presente Tesis.

- Objetivo 4: ***Caracterización de la cinética de oxidación de Cu(I) a nivel nanomolar en agua de mar.***

La cinética de oxidación del Fe(II) se ve afectada por la presencia de especies que reaccionen y compitan por los intermedios redox generados por el oxígeno o procedentes de la fotooxidación de la materia orgánica presentes en el medio marino. El cobre es una de estas especies interferentes. Para poder estudiar este fenómeno, se debe caracterizar previamente la cinética de oxidación de Cu(I) y la reducción de Cu(II) en agua de mar a nivel nanomolar.

Se describirá la cinética de oxidación de Cu(I) en agua de mar, en función del pH (7.17-8.49), la temperatura (5-35ºC), la fuerza iónica (0.1-0.7 M) y la concentración de bicarbonato (0-9 mM). Se realizarán además estudios de reducción de Cu(II), para cuantificar la cantidad de Cu(I) regenerado en las condiciones de trabajo. Los resultados obtenidos conforman el Capítulo VI.

- Objetivo 5: ***Determinar la competición que se establece en la cinética de oxidación de Fe(II) bajo la presencia de cobre en agua de mar.***

Se realizarán estudios cinéticos de oxidación competitiva entre ambos metales en el medio marino. Se considerarán los efectos producidos trabajando tanto a concentraciones constantes o variables de Fe(II), Cu(I) o Cu(II). Esto nos permitirá conocer si existe una relación entre la concentración de ambos metales y la constante de velocidad del Fe(II).

Se seleccionarán tres proporciones distintas entre Fe(II) y Cu(II) para estudiar el efecto del pH (6.0-8.5) y la concentración de H_2O_2 (0-500 nM) y la concentración de $NaHCO_3$ (2-9 mM). Los resultados encontrados serán mostrados en el Capítulo VII de esta Tesis Doctoral.

Capítulo X.2: Material y Métodos

La sección experimental ha sido desarrollada englobando los aspectos generales del material y métodos empleados en cada capítulo de esta Tesis Doctoral. Además, las consideraciones particulares, para estudios concretos de cada capítulo, serán caracterizadas y detalladas en el inicio del mismo capítulo.

10.2.1. Reactivos

10.2.1.1. Agua de mar

La velocidad de oxidación de Fe(II) ha sido estudiada en agua de mar procedente del Noreste de Gran Canaria (España). La salinidad de las muestras de agua de mar fue medida con un salinómetro Portasal 8410 A, obteniendo un valor de salinidad de 36.720 (para el agua de mar utilizada en los estudios de los Capítulos III-V), 36.691 (para el agua de mar utilizada en los estudios realizados en el Capítulo VI) y 36.968 (estudios del Capítulo VII). El agua de mar fue siempre filtrada por 0.1 μm, para prevenir el colapso de la columna capilar de largo paso de luz.

La fuerza iónica (I) de las muestras de agua de mar se determinó a través de la Ecuación (22) (Millero, 2001).

$$I_T = 0.0199201 \cdot S \qquad (22)$$

El efecto de la salinidad, en la constante de velocidad de oxidación de Fe(II), se llevó a cabo diluyendo las muestras originales con agua Milli-Q, corrigiendo el efecto de la concentración de bicarbonato (Santana-Casiano y col., 2005).

10.2.1.2. Disoluciones

Los experimentos fueron desarrollados utilizando una disolución stock de Fe(II) ($4 \cdot 10^{-4}$ M), acidificada a pH 2 con HCl suprapuro en NaCl 0.7 M.

La disolución stock de Fe(III) se preparó a $4 \cdot 10^{-4}$ M, también en NaCl 0.7 M.

Los nutrientes utilizados en la presente Tesis Doctoral, para realizar estudios de oxidación de Fe(II) fueron: nitrato (NO_3^-), fosfato (PO_4^{3-}) y silicato (SiO_3^{2-}). Las disoluciones stock de cada uno de ellos fue preparada a 882 mM, 28.8 mM y 142 mM, para nitrato, fosfato y silicato respectivamente. Bajo las condiciones experimentales estudiadas en este trabajo, la concentración de nutrientes utilizada en los distintos estudios fue de $8.83 \cdot 10^{-4}$ M, $2.93 \cdot 10^{-5}$ M y $1.42 \cdot 10^{-4}$ M para NO_3^-, HPO_4^{2-} y SiO_3^{2-}, respectivamente.

Las disoluciones stock de Cu(I) ($4 \cdot 10^{-4}$M) se prepararon diariamente en NaCl 0.7 M y acidificadas a pH 2 con HCl suprapuro. El stock de Cu(I) se burbujeó con N_2 de alta pureza, una hora antes de añadir el Cu(I), y se mantuvo el burbujeo hasta finalizar los estudios.

Los estudios de Cu(II) se realizaron utilizando un stock de Cu(II) ($4 \cdot 10^{-4}$M), preparado en NaCl 0.7 M.

El peróxido de hidrógeno (H_2O_2) se preparó cada día en NaCl 0.7 M. El stock de H_2O_2 fue preparado a $4.03 \cdot 10^{-4}$M y almacenado en oscuridad mientras no era utilizado.

Todas las disoluciones preparadas en este trabajo se hicieron a partir de reactivos de grado analítico de metales traza y con agua Milli-Q (18MQ).

10.2.2. Medidas de pH

El pH se medió potenciométricamente, calibrando el electrodo con las disoluciones tamponadoras (Tris-agua de mar) (Millero, 1986). Estas disoluciones tampón, se prepararon con 0.005 mol kg^{-1} de Tris y Tris-HCl en agua de mar artificial. Se utilizó un electrodo combinado (Ross) de vidrio. El pH se calculó a partir de la Ecuación 23.

$$pH = pH(s) + \frac{(Es - Ex)F}{2.303RT} = pH(s) + \frac{(Es - Ex)}{1.984 \cdot 10^{-4} T} \qquad (23)$$

donde s corresponde al Tris y x corresponde a la muestra, cuyo pH es desconocido. Es y Ex, son valores de potencial (mV) para el Tris y para la muestra, respectivamente. F, R y T representan la constante de Faraday (96485.3 C mol^{-1}), la constante universal de los gases (8.314 J mol^{-1} K^{-1}) y la temperatura (K), respectivamente.

El pH(s) se calcula a partir de la ecuación de Dickson y col. (2007) (Ecuación 24).

$$pH(s) = (11911.08 - 18.2499S - 0.039336S^2)\frac{1}{T/K} - 366.27059 + 0.053993607S + 0.00016329S^2 + (64.52243 - 0.084041S)\ln(T/K) - 0.11149858(T/K) \quad (24)$$

Las medidas de pH en esta Tesis Doctoral han sido representadas como pH en escala de ión libre (pH_F), aunque cabe destacar que en oceanografía se pueden utilizar varias escalas de pH (Millero y col., 1993), el pH en escala total (pH_T) y pH en escala de agua de mar (pH_{sws}).

10.2.3. Temperatura

El efecto de la temperatura en la velocidad de oxidación de Fe(II) fue estudiado en un rango entre 5-35ºC. La temperatura fue controlada con un baño termostático AG-2 durante todo el estudio. El efecto de la temperatura es importante ya que permite realizar las estimaciones termodinámicas necesarias, para comprender el proceso de oxidación de Fe(II) que se produce en el medio natural.

10.2.4. Concentración de oxígeno

Los estudios desarrollados en esta Tesis Doctoral fueron siempre realizados en condiciones de sobresaturación de oxígeno, burbujeando las muestras durante una hora con aire de alta pureza (Carburos Metálicos de Canarias).

La concentración de equilibrio de oxígeno de las muestras, en cada caso, fue estimada a través de la ecuación de Benson y Krause (1984) (Ecuación 28).

$$\ln[O_2] = -135.29996 + 1.572288 \cdot 10^5 / T - 6.637146 \cdot 10^7 / T^2 + 1.243678 \cdot 10^{10} / T^3 - 8.62106 \cdot 10^{11} / T^4 - S(0.020573 - 12.142 / T + 2363.1 / T^2) + \ln(1 + 10^{-3} S) \quad (28)$$

T es la temperatura (K) y S es la salinidad.

10.2.5. Experimentos de oxidación

Los estudios de oxidación de Fe(II) se realizaron en un matraz de reacción termostático (250 mL), controlando la temperatura con un baño termostático en el rango de 5-35ºC. Las muestras se agitaron durante todo el estudio con un agitador de teflón. La cinética de oxidación de Fe(II) se estudió en agua de mar en condiciones de sobresaturación de oxígeno en todos los casos, burbujeando la muestra durante 1 hora con aire de alta pureza. El pH se ajustó al valor deseado, añadiendo alícuotas de HCl 0.1 M a través de un valorador automático (Titrino 719S, Methrom). La adición de Fe(II) a la muestra corresponde con el tiempo cero de reacción.

10.2.6. Medidas de Fe(II)

La concentración de Fe(II) se determinó espectrofotométricamente utilizando el método de la ferrozina (Violler y col., 2000; Santana-Casiano y

col., 2005). La ferrozina y el Fe(II) forman un complejo 1:3 con una banda de absorción a 562 nm. A los tiempos establecidos, se extraen 10 mL de muestra y se introducen en matraces de 25 mL, a los que previamente se han añadido ferrozina, disolución tampón de acetato y NaF.

Las medidas de Fe(II) a concentraciones nanomolares se obtienen utilizando una columna capilar de 5 metros de paso de luz (World Precision Instruments^{TM}) conectado a un detector UV-vis (USB-2000, de Ocean Optics^{TM}). Todo el sistema está conectado a través de fibra óptica. Las muestras se introducen en el sistema de análisis con una bomba peristáltica (EXPECTEC Perimax 12) con un flujo de 1 mL/min.

La constante de velocidad aparente de Fe(II) (k_{app}; M^{-1} min^{-1}) se determinó a través de las Ecuaciones 10-12 presentadas en el Capítulo I, donde en condiciones sobresaturadas de oxígeno la cinética se puede estudiar como un sistema de pseudo-primer orden y $k_{app} = k' / [O_2]$.

La constante de velocidad de pseudo-primer orden, *k'*, se determinó a través de la regresión lineal de la representación de ln[Fe(II)] vs tiempo (min).

10.2.7. Análisis de Cu(I)

La concentración de Cu(I) se midió espectrofotométricamente con el método de la bazocupreina (Moffett y col., 1985) para concentraciones nanomolares. A tiempos establecidos, se extraen alícuotas de la disolución experimental para introducirlos en matraces en los que previamente se añaden $2 \cdot 10^{-5}$ M de bazocupreina y 10^{-4} M de etilendiamina. La bazocupreina y el Cu(I) forman un complejo que absorbe luz a 484 nm. Además, la etilendiamina se utiliza porque evita la interferencia del Cu(II) en los estudios.

La determinación de Cu(I) se realiza con un sistema idéntico al utilizado para la determinación de Fe(II).

10.2.8. Cultivo de algas y Enriquecimiento de exudados orgánicos

El efecto de los exudados orgánicos en la oxidación de Fe(II), para las aguas naturales, se realizó teniendo en cuenta dos especies, *Phaeodactylum tricornutum* y *Dunaliella tertiolecta*. El método experimental diseñado para la obtención del agua de mar enriquecida con los exudados de estas especies se mostró en la Figura 2-1.

Los cultivos puros fueron proporcionados por el Banco Nacional de Algas (Taliarte, Gran Canaria) y mantenidos en un medio f/2 (Guillard, 1975).

Todos los cultivos utilizados para la obtención de agua de mar enriquecida con exudados orgánicos fueron elaborados con agua de mar y solamente los nutrientes del medio f/2 (agua de mar control). Una vez obtenida la muestra enriquecida, se realizaron los estudios cinéticos previamente considerados.

La densidad celular se midió diariamente a través de contajes a microscopio, haciendo uso de un hemacitómetro (Microbiotests, Inc.) y con medidas espectrofotométricas (USB4000).

El crecimiento de las dos especies (*P. tricornutum* y *D. tertiolecta*) se mostró en la Figura 2-2A y 2-2B.

Los cultivos se filtraron en dos etapas, una primera etapa en gravedad, para evitar la rotura celular y por lo tanto la introducción de material intracelular rico en compuestos de hierro al medio de estudio, y la segunda etapa empleando vacío.

Todos los filtros utilizados en este caso fueron limpiados previamente con HCl al 10% en agua Milli-Q.

10.2.9. Modelo numérico

Para el desarrollo de los distintos modelos cinéticos mostrados en la Tesis Doctoral, se utilizó el programa informático Gepasi 3.30, para simular la cinética de todos los reactivos introducidos en el mismo. La constante global e individual de cada uno de ellos, k_i, se obtuvo ajustando los valores resultantes del programa a los valores experimentales en cada caso. Estos valores se ajustaron a las concentración de Fe(II) y tiempo de reacción utilizados (Santana-Casiano y col., 2004). Las ecuaciones utilizadas como base para el desarrollo de los modelos cinéticos presentados en esta Tesis Doctoral se muestran en la Tabla 2-2.

Capítulo X.3: Oxidación de Fe(II) en aguas naturales a altas concentraciones de nutrientes

La aguas naturales pueden clasificarse en función de la concentración de nutrientes, principalmente NO_3^-, HPO_4^{2-} y $Si(OH)_4$. De esta forma existen aguas oligotróficas (bajo contenido en nutrientes) y eutróficas (alto contenido en nutrientes) (Levitus y col., 1993). En cambio, cuando la concentración de nutrientes aumenta excesivamente, se llega a un estado de eutrofización, el cuál ha sido descrito tanto en aguas costeras como en zonas estuarinas (Rabalais y col., 2009) y en lagunas (Windom y col, 1999). Estas concentraciones elevadas de nutrientes van a producir cambios en las propiedades químicas del agua, afectando a la especiación de hierro (Özturk y col., 2003). Además, en la mayoría de los estudios de laboratorio, donde se utilizan medios de cultivo como el f/2 medio (Guillard, 1975), se emplean concentraciones importantes de nutrientes, por lo que podrían afectar a la especiación y a la química redox del hierro.

En este trabajo se estudió la cinética de oxidación de Fe(II) en agua de mar (SW) y en agua de mar enriquecida con nutrientes (SEN). Se estudió el efecto del pH (7.2-8.2), la temperatura (5-35ºC) y la salinidad (10-36.720). Algunos estudios fueron realizados en agua de mar artificial (ASW) y agua de mar artificial enriquecida con nutrientes (ASEN). Finalmente, se desarrolló un modelo cinético que describe el papel que juegan los nutrientes en la cinética del Fe(II).

10.3.1. Efecto de la concentración de nutrientes

Se consideró el efecto de cada nutriente individual en la velocidad de oxidación de Fe(II). Este efecto se determinó en un amplio rango de concentraciones (NO_3^- = 0-1.77·10^{-3} M, HPO_4^{2-} = 0-5.80·10^{-5} M y $Si(OH)_4$ = 0-2.84·10^{-4} M). Este rango engloba tanto los valores presentes en aguas oligotróficas, áreas ricas en nutrientes e incluso, zonas afectadas por eutrofización (Nolting y col., 1998; Boye y col., 2001; Maier y col., 2009). El efecto que cada nutriente ejerce sobre la cinética de oxidación de Fe(II) se mostró en la Figura 3-1. En todos los casos, la cinética de oxidación de Fe(II) siguió un mismo patrón, aumentando hasta un valor máximo. Por esto, los resultados fueron ajustados a una ecuación exponencial (Ecuaciones 31-33).

Los estudios realizados con distintas concentraciones de nutrientes en agua de mar se compararon con otros medios, como son agua de mar enriquecida, agua de mar artificial y agua de mar artificial enriquecida con nutrientes. La constante de velocidad aparente (k_{app}) aumentó siguiendo la siguiente estructura: SW (log k_{app}=2.70 ± 0.01 M^{-1} min^{-1}) < SNIT (log k_{app}=2.93 ± 0.02 M^{-1} min^{-1}) < SPHP (log k_{app}=2.95 ± 0.05 M^{-1} min^{-1}) < SSIL (log k_{app}=3.03 ± 0.03 M^{-1} min^{-1}) < SEN (log k_{app}=3.04 ± 0.02 M^{-1} min^{-1}). SSIL y SEN mostraron valores similares de k_{app} (3.04 y 3.03 M^{-1} min^{-1}) (Figura 3-3).

Este estudio demuestra que los nutrientes, a altas concentraciones, juegan un papel importante en la oxidación de Fe(II), donde el nutriente más activo para el proceso de oxidación es el silicato.

10.3.2. Dependencia del pH

El efecto del pH en la oxidación de Fe(II) fue estudiado tanto en agua de mar como en agua de mar enriquecida con nutrientes (Figura 3-4). Log k_{app} fue siempre mayor en SEN que en SW. La dependencia del pH fue ajustada a una ecuación polinómica de segundo grado en ambos casos (Ecuación 34-35).

El incremento en la velocidad de oxidación de Fe(II) debida a la presencia de nutrientes, a pH 8.0, supone que el tiempo de vida medio disminuye 1.4 min (Tabla 3-2).

10.3.3. Dependencia de la temperatura

El estudio de la dependencia de la cinética de oxidación de Fe(II) con la temperatura, en presencia de altas concentraciones de nutrientes, permite conocer si el proceso de oxidación de Fe(II) sigue el mismo proceso cinético que en agua de mar. Los resultados experimentales del efecto de la temperatura en la velocidad de oxidación de Fe(II) se mostraron en la Figura 3-5. Estos resultados fueron fijados a una ecuación lineal, tanto para agua de mar como para agua de mar enriquecida con nutrientes (Ecuación 36-37).

La energía de activación (E_a) estimada en este estudio fue similar a los valores presentados por Santana-Casiano y col. (2005), en aguas de la corriente del Golfo, y Roy y col. (2008), en aguas de Pacífico Sub-Ártico.

Según los resultados obtenidos en este estudio, el mecanismo de reacción que controla la oxidación de Fe(II) en agua de mar es el mismo que en presencia de altas concentraciones de nutrientes, solamente acelerado por la presencia de dichos nutrientes, especialmente por los silicatos.

10.3.4. Dependencia de la salinidad

El efecto de la salinidad en la oxidación de Fe(II) se mostró en la Figura 3-6. Los resultados experimentales fueron ajustados a una ecuación polinómica de segundo grado (Ecuación 38-41).

La constante aparente de velocidad aumenta a medida que disminuye la salinidad, pero siempre es mayor cuando el agua de mar contiene altas concentraciones de nutrientes (SEN). Así el tiempo de vida medio disminuyó desde 6.7 mins a 3 mins en valores de S=10.

Todos los resultados experimentales fueron englobados en una ecuación polinómica, en función del pH (en escala libre), la temperatura (K) y salinidad (Ecuación 42-43). Estas ecuaciones pueden ser utilizadas bajo las condiciones experimentales consideradas en este trabajo.

Finalmente, se desarrolló un modelo cinético, considerando como modelo base el propuesto por Santana-Casiano y col. (2005) y González-Dávila y col. (2006), introduciendo nuevos equilibrios que contemplan la especiación del silicato, que es el nutriente que principalmente afecta a la aceleración de la oxidación de Fe(II), y la interacción entre silicato y Fe(II), en agua de mar (Ecuación 44-48).

El modelo cinético indica que a altas concentraciones de $Si(OH)_4$ ($1.42 \cdot 10^{-4}$ M), la especie $FeH_3SiO_4^+$ incrementó su fracción molar desde un 0.6% a pH 6.0 hasta un 31% a pH 8.4 (Figura 3-7A) Cuando la concentración de $Si(OH)_4$ considerada es de $3 \cdot 10^{-5}$ M (Figura 3-7B), la especie $FeH_3SiO_4^+$ llega a alcanzar un 9% a pH 8.5. Por lo que, $FeH_3SiO_4^+$ debería ser considerada en la especiación de Fe(II) en aguas naturales, especialmente en aguas eutrofizadas donde las concentración de silicatos es especialmente alta.

En términos de contribución a la velocidad global del proceso de oxidación, el modelo indica que las especies que controlan el proceso de oxidación siguen el siguiente orden: Fe^{2+}, $Fe(OH)_2$, $Fe(CO3)_2^{2-}$, $Fe(OH)^+$, $FeH_3SiO_4^+$, $Fe(CO_3)(OH)^-$ y $Fe(CO_3)$. La contribución de $FeH_3SiO_4^+$ es la cuarta más importante a pH 8.0. Por lo tanto, los resultados muestran nuevamente como la presencia de nutrientes, principalmente silicatos, juega un papel importante en la oxidación de Fe(II) en agua de mar, provocando que el Fe(II) permanezca menos tiempo en el medio marino, y por lo tanto, haciéndolo menos disponible para los procesos biológicos en medio acuático.

Capítulo X.4: El papel de los exudados orgánicos de Phaeodactylum tricornutum en la velocidad de oxidación de Fe(II)

En el medio marino existe una cantidad importante de compuestos orgánicos generados por los microorganismos y diversos tipos de microalgas. Este capítulo se centra en determinar el efecto que tienen los exudados orgánicos producidos por una diatomea, *Phaeodactylum tricornutum*, en la oxidación de Fe(II). Se estudió el efecto de la densidad celular, el efecto del pH (7.2-8.2), la temperatura (5-35ºC) y la salinidad (10-36.720).

10.4.1. El efecto de la concentración celular

El cultivo de *P. tricornutum*, en agua de mar y nutrientes del f/2 (Guillard, 1975), mostró un crecimiento exponencial. Se caracterizó por una fase exponencial entre 2 y 4 días, donde la concentración de células fue de $6.21 \cdot 10^7$ cel/L y $2.29 \cdot 10^8$ cel/L, respectivamente. En el octavo día de cultivo, se alcanza la fase estacionaria ($4.98 \cdot 10^8$ cel/L), siendo este el máximo crecimiento celular encontrado en las condiciones de estudio. El crecimiento de *P. tricornutum* fue similar al encontrado en trabajos previos (Vasconcelos y col., 2002; Vasconcelos y Leal, 2008) (Figura 2-2A).

El efecto de los exudados orgánicos, excretados por *P. tricornutum* en la oxidación de Fe(II), fue estudiado para cada día de cultivo, desde 10^7 - $4.98 \cdot 10^8$ cel/L (Figura 4-1). La constante de velocidad aparente del Fe(II) fue siempre disminuyendo linealmente cuando la concentración celular aumentó. Los resultados experimentales fueron ajustados a una ecuación polinomial (Ecuación 50).

Por lo tanto, de acuerdo a los resultados obtenidos, la disminución lineal de la velocidad de oxidación de Fe(II) en presencia de exudados orgánicos de *P. tricornutum*, sugiere que el tipo de ligandos orgánicos que interaccionan con Fe(II) o bien no cambian en el tiempo o, aún cambiando, su comportamiento es similar.

10.4.2. El efecto del pH

El efecto del pH en la oxidación de Fe(II) se estudió en un amplio rango de pH (7.2-8.2) (Figura 4-2). k_{app} disminuyó con el pH y con la concentración de células presentes en el cultivo. Además, a medida que disminuyó el pH, la diferencia en log k_{app} aumentó. Todos los resultados experimentales fueron ajustados a la Ecuación 51, en función del pH y la densidad celular. Con el objeto de obtener una ecuación que describa el efecto de los exudados, se determinó la Ecuación 52.

10.4.3. El efecto de la temperatura

El efecto de la temperatura en la oxidación de Fe(II) se mostró en la Figura 4-3. Los resultados experimentales obtenidos se fijaron en la Ecuación 53. Además, el efecto de los exudados orgánicos excretados por *P. tricornutum*, en función de la temperatura y la densidad celular fueron ajustados a la Ecuación 54.

En este caso, se determinó la energía de activación, que fue de 79 kJ mol^{-1} ($6.21 \cdot 10^7$ cel/L), 92 kJ mol^{-1} ($2.29 \cdot 10^8$ cel/L) y 88 kJ mol^{-1} ($4.98 \cdot 10^8$

cel/L). La E_a para agua de mar, con los nutrientes del f/2 medio fue de 86 kJ mol^{-1}.

De acuerdo con los resultados obtenidos, la presencia de exudados orgánicos provoca un mayor tiempo de permanencia del Fe(II) en agua de mar.

10.4.4. El efecto de la salinidad

El efecto de la salinidad en la oxidación de Fe(II) se mostró en la Figura 4-4. Todos los resultados fueron introducidos en la Ecuación 55, y la Ecuación 56 engloba el efecto real de los exudados excretados.

La constante de velocidad aparente aumenta a medida que disminuye la salinidad, pero este efecto se hizo menos importante en presencia de compuestos orgánicos. Por lo que dichos exudados juegan un estricto control en la estabilización del Fe(II) en agua de mar.

Todos los resultados obtenidos en este trabajo han sido considerados en una ecuación polinómica (Ecuación 57) en función de la densidad celular (cel/L), el pH (escala libre), temperatura (K) y salinidad. Además, el efecto específico de los ligandos excretados por *P. tricornutum* fueron fijados a la Ecuación 58-59.

10.4.5. Especiación y contribución parcial a la constante de velocidad global

Finalmente, un modelo cinético fue diseñado a partir del modelo cinético establecido por Santana-Casiano y col. (2005), González-Dávila y col. (2006) y González y col. (2010a). En este modelo se tienen en cuenta todas las posibles reacciones de Fe(II) en el medio marino, más la interacción con los silicatos, que presentó la mayor actividad en la oxidación de Fe(II). A partir de los resultados del modelo, en comparación con los resultados experimentales, se puede estimar la constante de equilibrio y de velocidad para la interacción entre los ligandos orgánicos excretados por la diatomea (L) y el Fe(II). De esta forma, $K_{Fe(II)-L}=10^7$ y log k = 1.08 ± 0.43 (M^{-1} min^{-1}). La concentración de ligandos fue estimada en 11 ± 1 nM ($6.21 \cdot 10^7$ cel/L), 113 ± 4 nM ($2.29 \cdot 10^8$ cel/L) y 170 ± 10 nM ($4.98 \cdot 10^8$ cel/L). Estos resultados están en concordancia con los obtenidos por otros autores (Vasconcelos y col., 2002; Vasconcelos y Leal, 2008).

La especiación de todas las especies de Fe(II) se mostraron en la Figura 4-5. A medida que aumenta la concentración de ligandos, la especie Fe(II)-L va incrementando su importancia, hasta llegar a ser la especie dominante entre pH 6.2 y 8.5.

La contribución a la velocidad total del proceso de oxidación de Fe(II) se mostró en la Figura 4-6. La especie Fe(II)-L fue más importante a pH inferiores a 8, llegando a ser la segunda especie que más contribuye al proceso a pH 6 en la última fase de crecimiento.

Capítulo X.5: El efecto de los exudados orgánicos de Dunaliella tertiolecta en la cinética de oxidación de Fe(II) en agua de mar

En este capítulo se estudia el efecto de los exudados orgánicos, excretados por *Dunaliella tertiolecta*, en función de la densidad celular, el pH (7.2-8.2), la temperatura (5-35ºC) y la salinidad (10-36.720). Este trabajo contribuye a mejorar el conocimiento del ciclo biogeoquímico del hierro en las aguas naturales en presencia de ligandos orgánicos.

10.5.1. Dependencia de la concentración de células

El crecimiento de *D. tertiolecta* se mostró en la Figura 2-2B. El efecto de la densidad celular, en la velocidad de oxidación de Fe(II) se mostró en la Figura 5-1, donde todos los resultados obtenidos se fijaron a la Ecuación 61.

La velocidad de oxidación de Fe(II) disminuyó linealmente con la concentración celular. La diferencia entre el valor mínimo y máximo de la constante de velocidad, se traduce en un 61% de reducción de la velocidad en términos de k_{app}. Esto significa que el tiempo de vida medio aumentó en 5 min, a pH 8.0 y T=25ºC, solo por la presencia de exudados orgánicos producidos por $5.04 \cdot 10^8$ cel/L.

10.5.2. Dependencia del pH

La dependencia de la velocidad de oxidación de Fe(II) con el pH en el rango entre 7.2 y 8.2, se representó en la Figura 5-2. Según los resultados obtenidos, log k_{app} disminuyó con el pH. Estos resultados fueron fijados a la Ecuación 62. La velocidad de oxidación fue siempre menor a medida que aumentaba la concentración de ligandos o de células en el cultivo experimental. De tal forma que el tiempo de vida medio aumentó 26.6 min, 5 min y 1.6 min, para pH 7.2, 8 y 8.2, respectivamente. El efecto ejercido solo por los exudados orgánicos se calculó mediante la diferencia de los resultados obtenidos para cada concentración celular y el valor obtenido en agua de mar control (Ecuación 63).

10.5.3. El efecto de la temperatura

La dependencia de la velocidad de oxidación de Fe(II) en presencia de exudados orgánicos de *D. tertiolecta*, se mostró en la Figura 5-3. En este caso se usaron dos tipos de representaciones, la de Arrhenius (Figura 5-3A) y la de Eyring (Figura 5-3B). Los resultados experimentales fueron fijados a la Ecuación 64. El efecto debido únicamente a la presencia de exudados orgánicos se muestra en la Ecuación 65.

La fracción de colisiones satisfactorias, las colisiones con suficiente energía para provocar reacción, la velocidad de dichas colisiones, la energía de activación y el factor de Arrhenius, la entalpía y la entropía de activación se mostraron en la Tabla 5-2. Tanto la fracción de colisiones como la velocidad de las mismas, son dependientes de la concentración de células, de la cantidad de ligandos presentes en el medio. En este caso, tanto $\Delta H^{\ddagger}$ como $\Delta S^{\ddagger}$, disminuyeron ligeramente con la presencia de ligandos orgánicos en el

medio. Esto indica que el sistema tiende a un estado más estable en presencia de ligandos orgánicos excretados por el fitoplancton.

El tiempo de vida media, indica una estabilización del Fe(II) en agua de mar en función de los ligandos orgánicos presentes. A 5ºC, el $t_{1/2}$ aumenta desde 27.6 min a 57.3 min ($5.04 \cdot 10^8$ cel/L). Esto puede explicar la presencia de Fe(II) en aguas superficiales de zonas frías donde la cantidad de materia orgánica también puede ser elevada (Croot y col., 2001; 2005; Roy y col., 2008).

10.5.4. La dependencia de la Salinidad

El efecto de la salinidad en la oxidación de Fe(II) se mostró en la Figura 5-4. Los resultados se ajustaron a la Ecuación 67. Además, el efecto debido a los ligandos orgánicos excretados por *D. tertiolecta*, en función de la salinidad, se mostraron en la Ecuación 68.

La velocidad de oxidación disminuyó en función del número de células presente en el cultivo experimental. El valor de la constante de velocidad aumenta cuando la salinidad disminuye, pero este efecto es menos importante en presencia de exudados orgánicos, ya que estos provocan una estabilización del Fe(II) y por lo tanto ejerce un mayor control, en el proceso de oxidación, que los iones mayoritarios del agua de mar.

10.5.5. Modelo cinético

Los grupos funcionales presentes en la superficie celular de *D. tertiolecta* han sido estudiados (González-Dávila y col., 1995). Estos grupos

presentan una constante de acidez de $K_a=10^{4.9}$, $K_a=10^{6.3}$ y $K_a=10^{10.1}$. El primer valor corresponde a grupos carboxílicos, el segundo a grupos amino y el último a grupos fenólicos. De estos tres grupos funcionales, los carboxílicos han sido destacados por su gran capacidad de complejar metales en microorganismos (González y col., 2009; González y col., 2010b).

Teniendo en cuenta los tipos de ligandos caracterizados, se amplió el modelo cinético diseñado por Santana-Casiano y col. (2005) y González y col. (2010a), incluyendo la Ecuaciones de disociación de los ligandos y su interacción con Fe(II) (Ecuación 71-76).

Las constantes de equilibrio y las constantes de velocidad fueron estimadas a través de un método iterativo, usando el software Gepasi y considerando todos los resultados experimentales obtenidos.

Para optimizar el modelo cinético se realizaron tres tipos de modelos cinéticos. Dos modelos, que consideraban los grupos carboxílicos y amino por separado, y un tercero con ambos en conjunto. No se ha considerado a los grupos fenólicos, ya que al introducirlos su participación en el proceso era despreciable.

La concentración de ligandos se determinó en cada caso, y a través del mejor ajuste se determinó que la concentración de ligandos era de 27.5 ± 2.5 nM ($5.52\cdot10^7$ cel/L), 70 ± 10 nM ($2.17\cdot10^8$ cel/L) y 170 ± 10 nM ($5.04\cdot10^8$ cel/L). Estos valores están en concordancia con los encontrados por otros autores para distintos grupos de microorganismos (González-Dávila y col., 2000; Vasconcelos y col., 2002; Vasconcelos y Leal., 2008; Strmecki y col., 2010).

El resultado del modelo cinético (Figura 5-5) indica que la especiación de Fe(II) está controlada por el complejo Fe(II)-LH a pH

inferiores a 8.3 ($5.04 \cdot 10^8$ cel/L), alcanzando un 98% a pH 6.0. El complejo Fe(II)-L llega a alcanzar un 25% del Fe(II) total para exudados provenientes de cultivos con $5.04 \cdot 10^8$ cel/L.

En cuanto a la contribución de cada especie a la constante global de velocidad de oxidación, el modelo indica (Figura 5-6) que la especie Fe(II)-LH es la especie más importante para pH inferiores a 7.8 ($5.04 \cdot 10^8$ cel/L), 7.5 ($2.17 \cdot 10^8$ cel/L) y 6.8 ($5.52 \cdot 10^7$ cel/L). La especie Fe(II)-L solo llegó a alcanzar un 7% de la velocidad total a pH 7.4, cuando la concentración de células fue $5.04 \cdot 10^8$ cel/L.

Por lo tanto, el modelo confirma la importancia que tienen ambos tipos de ligandos en la oxidación de Fe(II) en aguas naturales. Además, la contribución del Fe(II)-LH (grupos carboxílicos) es la más importante a bajos pH.

Capítulo X.6: Oxidación de Cu(I) en agua de mar a niveles nanomolares

La química redox del cobre controla su especiación en aguas naturales y su interacción con los organismos marinos. A elevadas concentraciones, el cobre llega a ser tóxico para los organismos, alterando la permeabilidad de la membrana, afectando a diversos procesos metabólicos (Marschner, 1995; Yruela, 2005).

La oxidación de Cu(I) ha sido estudiada a niveles micromolares, tanto en agua de mar como en NaCl (Moffett y Sharma, 1983; Sharma y Millero, 1988a,b,c). En estos estudios se encontró que la especiación de Cu(I) estaba controlada fundamentalmente por los iones cloruro.

En este trabajo se determinó la oxidación de Cu(I) a escala nanomolar, en función de la concentración inicial de Cu(I), el pH, la temperatura y la salinidad. Además se determinó la fracción de Cu(I) reducido a partir del Cu(II) en condiciones saturadas de oxígeno. En este trabajo, además, no se introdujeron reactivos que complejarán al Cu(II) en la disolución experimental, por lo que se permite que el proceso siga su curso, lo más similar al medio natural.

10.6.1. Efecto de la concentración inicial de Cu(I)

La constante de velocidad de oxidación de Cu(I) no es dependiente de la concentración inicial de Cu(I) (Tabla 6-2), para un rango de concentraciones entre 50 y 385 nM, por lo que los estudios se desarrollaron a 200 nM de Cu(I) inicial, pudiendo extrapolarse su efecto a concentraciones inferiores.

10.6.2. Efecto de la concentración de $NaHCO_3$

El efecto del bicarbonato fue estudiado en NaCl 0.7 M en un rango de $NaHCO_3$ entre 0 y 9 mM (Tabla 6-3). Los resultados experimentales obtenidos fueron fijados a una ecuación polinomica de segundo orden (Ecuación 89). La constante de velocidad de oxidación de Cu(I) aumentó hasta 5 mM de $NaHCO_3$, y permaneció constante entre 5 y 9 mM. Esto puede ser explicado por los complejos formados entre Cu(I) y carbonatos, que son más reactivos que los complejos Cu(I) y cloruros. A concentraciones altas de bicarbonato, el proceso de oxidación de Cu(I) es prácticamente constante, por la presencia de complejos Cu(II)-carbonato y su reducción a Cu(I).

10.6.3. Efecto del pH

El efecto del pH se estudió en agua de mar y en NaCl 0.7 M, en un rango entre 7.17 y 8.49. Los resultados se muestran en la Figura 6-1 y 6-2. Además los resultados fueron fijados a sendas ecuaciones lineales (Ecuaciones 92-94).

La oxidación de Cu(I), en función del pH, mostró una débil dependencia, indicando que las especies ácidas o básicas no tienen una gran importancia en el proceso de oxidación de Cu(I). Los valores en agua de mar y en NaCl son muy similares, por lo que los complejos son similares o el efecto de los distintos iones mayoritarios del agua de mar se compensan en el proceso. En este caso, también existen diferencias entre la escala nanomolar y la escala micromolar, que puede ser explicado por la presencia de especies intermedias cuyos efectos no son apreciables a niveles de concentración micromolar.

10.6.4. Efecto de la fuerza iónica

La fuerza iónica se estudió realizando diluciones de la muestra original para un rango entre 0.1 y 0.7 M. La dependencia de segundo orden mostrada en los resultados experimentales (Figura 6-3 y Ecuaciones 95-96) están en concordancia con los mostrados por otros autores (Moffett y Zika, 1983; Millero, 1985; Sharma y Millero, 1988a,b,c). Los complejos Cu(I)-cloruros formados a altas fuerzas iónicas provocan una disminución en la constante de velocidad. Cuando la fuerza iónica disminuye, la diferencia entre agua de mar y NaCl se explica debido a la interacción del Cu(I) con otros iones mayoritarios presentes en el agua de mar, como son el Mg^{2+} y el Ca^{2+}.

10.6.5. Efecto de la temperatura

El efecto de la temperatura se estudió en agua de mar y NaCl 0.7 M en un rango entre 5-35ºC. En ambos casos la velocidad de oxidación aumentó con la temperatura (Figura 6-4 y Ecuaciones 97-98).

Los estudios de la dependencia de la constante de velocidad de oxidación con la temperatura ha permitido estimar la Energía de Activación (Ea), el parámetro de Arrhenius (A), Entalpía ($\Delta H^{\ddagger}$) y Entropía ($\Delta S^{\ddagger}$) de activación (Atkins, 2008). Los valores para agua de mar fueron: $E_a = 35.54 \pm 1.26$ kJ mol^{-1}, $A = 5.2 \cdot 10^8 \pm 1.7$ min^{-1}, $\Delta H^{\ddagger} = 33.10 \pm 1.25$ kJ mol^{-1} y $\Delta S^{\ddagger} = -52.17 \pm 4.32$ J mol^{-1}.

10.6.6. Reducción de Cu(II)

En este trabajo se determinó la cantidad de Cu(I) que se produce a partir de Cu(II) en distintas condiciones. Se determinó que la concentración de Cu(I) es función de la concentración inicial de Cu(II) y de la concentración de bicarbonato presente en la disolución. El Cu(I) generado en los primeros 20 mins, era aproximadamente un 20% en NaCl y un 11% en agua de mar.

10.6.7. Modelo cinético

Los resultados experimentales obtenidos en el trabajo se han utilizado para diseñar un modelo cinético para las especies de Cu(I) (Millero, 1985). En este modelo se tuvo en cuenta la regeneración de Cu(I) a partir del Cu(II) (Ecuaciones 102-106). Este modelo fue creado a partir de las ecuaciones del modelo de Pitzer (Pitzer y Mayorga, 1973).

El modelo indica que la especiación de Cu(I) está controlada por las especies $CuCl_2^-$, la cual abarca un 92% a fuerza iónica 0.1 M y 72% a 0.7 M. La segunda especie más importante es el $CuCl_3^{2-}$ (Figura 6-6). En cuanto a la contribución a la velocidad final del proceso, la especie $CuCl_3^{2-}$ no contribuye al proceso de oxidación de Cu(I) (Figura 6-7). La especie CuCl domina la contribución a la constante de velocidad, desde un 90% a fuerza iónica 0.1 M y un 45.4% a 0.7 M. Además, la contribución de las especies libres de Cu(I) es prácticamente despreciable.

Capítulo X.7: Interacción entre Fe(II) y Cobre en agua de mar

La relevancia del hierro en aguas naturales ha sido explicada en distintos capítulos de la presente Tesis Doctoral. En estos capítulos se ha detallado la interacción del hierro con los compuestos orgánicos, pero el equilibrio redox del hierro puede estar afectado por otros metales en el océano. Así, la interacción entre el hierro y otros metales de transición ha sido estudiada por varios autores. Pettine y col. (1998) demostraron que el Fe(II) es un importante reductor de Cr(VI), que además es un proceso muy rápido (Eary y Rai, 1988; Buerge y Hug, 1997). El Fe(II) también ha sido descrito como un reductor de (hidro)óxidos de Mn(III, IV) (Postma, 1985; Wehrli, 1990).

En este capítulo se deben considerar todas las reacciones posibles de oxidación y reducción tanto de hierro como de cobre en aguas naturales, descritas en los Capítulos I y VI. Pero también deben ser incluidas otras posibles ecuaciones que completarán el proceso de oxidación-reducción de cobre en aguas naturales (Ecuaciones 121-127).

En este capítulo se ha estudiado el efecto de la concentración de cobre (0-200 nM), tanto Cu(II) como Cu(I), en la velocidad de oxidación de Fe(II). También, seleccionando tres concentraciones de Cu(II) (50, 100 y 200 nM) se estudió el efecto del pH (6.0-8.2), la concentración de bicarbonato (2-9 mM) y la concentración de peróxido de hidrógeno (0-500 nM) en la constante de velocidad aparente de Fe(II).

10.7.1. El efecto de la concentración inicial de cobre

El efecto de la concentración inicial de Cu(II) en la velocidad de oxidación de Fe(II) fue estudiado manteniendo constante la concentración de Fe(II), a pH = 8.0 y T = 25ºC. Los resultados obtenidos se presentaron en la Figura 7-1, en función del ratio, R, entre la concentración de cobre y de Fe(II). Estos resultados fueron fijados a las Ecuaciones 131-132. En ambos casos, con Cu(I) y Cu(II), la velocidad de oxidación de Fe(II) aumentó con la concentración inicial de cobre. Los resultados fueron similares para R inferior a 0.25. En el caso donde se añadió Cu(II), la velocidad aparente de oxidación de Fe(II) alcanzó su máximo a R próximo a 1, mientras que para Cu(I) ese máximo fue alcanzado para R cercanos a 0.5.

En varios estudios se midió la concentración de Cu(I), añadiendo concentraciones de Fe(II) entre 0 y 200 nM. Estos estudios se hicieron añadiendo Cu(II) o Cu(I) en cada caso. Los resultados muestran que en estudios donde se añaden Cu(II) y Fe(II) se produce una importante reducción de Cu(II) a Cu(I), de tal forma que en los primeros 1-2 mins se llega a alcanzar el 97%, en presencia de 200 nM de Fe(II). Este Cu(I), una vez formado, sufre oxidación. La velocidad de oxidación de Cu(I) fue más lenta que cuando se añadía inicialmente Cu(I) y Fe(II).

10.7.2. El efecto del pH

El efecto del pH, en la velocidad de oxidación de Fe(II), se estudió en el rango de 6.0-8.5, para tres concentraciones de Cu(II) (50, 100 y 200 nM). Los resultados fueron mostrados en la Figura 7-3 y fijados a las Ecuaciones 133-136.

La constante de velocidad aparente disminuye con el pH. En ausencia de Cu(II) mostró una dependencia de segundo orden para pH superiores a 7.5 y de primer orden para pH inferiores a 7.5. En cambio, cuando el Cu(II) era añadido al estudio, el efecto del pH describió una dependencia de primer orden en todo el rango de pH estudiado. Esto es indicativo de que probablemente las especies $FeOH^+$, $FeCO_3$ y $FeHCO_3^+$ están tomando mayor importancia en el proceso de oxidación. La formación de complejos Cu-carbonato pueden influir de forma notable en los potenciales de reducción, siendo el Cu(I) mejor oxidante. Además, al variar el pH, también se modifica la concentración de radical superóxido en el medio, lo que conlleva un cambio en la oxidación de Cu(I) y Fe(II).

10.7.3. El efecto de la concentración de $NaHCO_3$

La concentración de bicarbonato se varió entre 2-9 mM en NaCl 0.7 M, manteniendo constante la concentración de Fe(II) (100 nM) y para tres concentraciones de Cu(II) (50, 100 y 200 nM). Los resultados se mostraron en la Figura 7-4 y fueron fijados en las Ecuaciones 137-140.

Para las distintas concentraciones iniciales de Cu(II) añadido, la constante aparente de velocidad de Fe(II) a 2 mM de bicarbonato fue similar. Solamente cuando la concentración de Cu(II) fue superior a 100 nM y la concentración de bicarbonato fue superior a 6 mM, se observa un incremento importante en la velocidad oxidación de Fe (II).

10.7.4. El efecto de la concentración de H_2O_2

El efecto de la concentración de peróxido de hidrógeno, en la velocidad de oxidación de Fe(II), fue estudiado en el rango de 0 a 500 nM de peróxido de hidrógeno. Estos resultados se mostraron en la Figura 7-5 y fueron fijados a la Ecuación 141, donde Cu(II) y H_2O_2 corresponden a valores nanomolares.

El efecto del H_2O_2 mostró un incremento similar en todos los casos, con ausencia y presencia de Cu(II). Por lo tanto, el incremento observado a distintas concentraciones de Cu(II) añadido, es debido a la presencia de especies de cobre.

Finalmente, para conocer y describir con exactitud el mecanismo competitivo existente entre hierro y cobre en aguas naturales se deben desarrollar más estudios. Incluso, estudios en condiciones anóxicas que aporte mayor información del papel que juegan los intermedios en el proceso redox de ambos metales.

Conclusiones Generales

Las conclusiones generales a las que se han llegado en este trabajo son:

1. La velocidad de oxidación de Fe(II) se ve afectada por las condiciones locales de cada agua natural, de acuerdo a la concentración de nutrientes, ligandos orgánicos, temperatura, pH, salinidad, concentración de bicarbonato, concentración de peróxido de hidrógeno y la concentración de cobre.

2. La velocidad de oxidación de Fe(II) aumenta en función de la concentración de nutrientes (nitrato, fosfato y silicato), donde la interacción entre Fe(II) y silicato es la más importante.

3. El modelo cinético revela que la especiación de Fe(II) está controlada por $FeH_3SiO_4^+$ para pH entre 7.6 y 8.5. Además, a pH más bajos, inferiores a 7.6, la especiación está controlada por $FeCl^+$ y $Fe(SO_4)$. Por otro lado, la contribución de cada especie a la velocidad total del proceso de oxidación de Fe(II) está controlada por $Fe(OH)^+$, $Fe(CO_3)$ y $Fe(OH)_2$.

4. La presencia de altas concentraciones de nutrientes debe ser considerada, especialmente en aguas costeras o aguas eutrofizadas, ya que la constante de velocidad aumenta y por lo tanto disminuye el tiempo de vida medio del Fe(II).

5. La presencia de compuestos orgánicos excretados por *Phaeodactylum tricornutum* hacen posible la existencia de

concentraciones de Fe(II) en agua de mar, en diferentes condiciones de densidad celular, pH, temperatura y salinidad.

6. La constante de velocidad de Fe(II) muestra una dependencia linear con la densidad celular o tiempo de cultivo de *P. tricornutum*, lo que nos indica que los ligandos orgánicos excretados o bien son los mismos en el tiempo o siendo distintos, tienen un mismo mecanismo de reacción con el Fe(II).

7. La velocidad de oxidación de Fe(II) en función del pH, temperatura y salinidad, es función de la densidad de células presentes en el cultivo original, siendo la velocidad de oxidación más lenta respecto a sus valores en ausencia de exudados orgánicos.

8. El modelo cinético desarrollado, considerando un modelo de ligando simple, demuestra que los exudados orgánicos de *P. tricornutum* se deben tener en cuenta, tanto debido a su efecto en la especiación como en la contribución a la velocidad de cada especie de Fe(II).

9. Los exudados orgánicos de *Dunaliella tertiolecta* muestran que la velocidad de oxidación de Fe(II) en agua de mar es función de la densidad celular, el pH, la temperatura y la salinidad. Esta velocidad de oxidación disminuye cuanto mayor era el número de células en el cultivo original.

10. La dependencia de la velocidad de oxidación de Fe(II) con el pH, en presencia de exudados de *D. tertiolecta*, indican que estos ligandos orgánicos interactúan con Fe(II) y modifican la especiación y la contribución de las distintas especies de Fe(II) al proceso global.

11. El efecto de la salinidad, en la velocidad de oxidación es menos importante en presencia de ligandos orgánicos que en su ausencia. Esto se debe a que hay un mayor control del proceso cinético por los ligandos orgánicos que por los iones mayoritarios del agua de mar.

12. El modelo cinético desarrollado para estudiar el papel que juegan los exudados de *D. tertiolecta* en la oxidación de Fe(II) indica que ambos ligandos considerados (grupos carboxílicos y aminos) son significativos y controlan el proceso en un amplio rango de pH.

13. La velocidad de oxidación de Fe(II) depende de la concentración de cobre en agua de mar. Además, dicha velocidad de oxidación aumenta con la concentración de cobre.

14. La constante de velocidad de Fe(II) describe una dependencia de segundo orden a pH mayores a 7.5 y de primer orden a pH superiores a 6. Cuando se añade Cu(II) al agua de mar, la dependencia de la constante de velocidad de Fe(II) con el pH fue siempre de primer orden. Esto se debe a la participación de especies como $Fe(OH)^+$, $FeCO_3$ o $FeHCO_3^+$, que además deben estar compitiendo con las especies de cobre en disolución.

15. El efecto de la concentración de bicarbonato produce un incremento en la velocidad de oxidación de Fe(II), pero solo se observa un efecto verdaderamente significativo para concentraciones de Cu(II) superiores a 100 nM y de bicarbonato superiores a 6 mM.

16. La concentración de peróxido de hidrógeno provoca un incremento en la velocidad de oxidación de Fe(II). El

incremento medido para cada caso, incluso con Cu(II) añadido, es similar, por lo que la diferencia observada en todos los estudios se debe simplemente a la presencia de Cu(II) en el medio.

REFERENCES

REFERENCES / REFERENCIAS

Abu-Saba K. E., Sedlak D. L. and Flegal A. R. (2000) Indirect reduction of hexavalent chromium by copper in the presence of superoxide. *Mar. Chem.* **69,** 33-41.

Allen H. E. and Hansen D. L. (1996) The importance of trace metal speciation to water quality criteria. *Water Environ. Res.* **68,** 42-54.

Allnutt F. C. T. and Bonner W. D. J. (1987) Characterization of iron uptake from ferrioxamine B by *Chlorella vulgaris*. *Plant Physiol.* **85,** 746-750.

Aluwihare L. I. and Repeta D. J. (1999) A comparison of the chemical characteristics of oceanic DOM and extracelular DOM produced by marine algae. *Mar. Ecol. Prog. Ser.* **186,** 105-117.

Anderson M. A. and Morel F. M. M. (1980) Uptake of Fe(II) by a diatom in oxic culture medium. *Mar. Biol. Lett.* **1,** 263-268.

Anderson M. A. and Morel F. M. M. (1982) The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* **27,** 789-813.

Atkins P. W. (2008) *Quimica Física.* Médica Panamericana, cop., Buenos Aires.

Avron M. and Ben-Amotz A. (1992) *Dunaliella: Physiology, biochemistry and biotechnology.* CRC Press, Boca Raton.

Ban S., Burns G., Castel J., Chaudron Y., Christou R., Escribano R., Umani S. F., Gasparini S., Ruiz F. G., Hoffmeyer M., Lanora A., Kang H. K., Laabir M., Lacoste A., Miralto A., Ning X., Poulet S. and Smetacek V. (1999) Diatoms and the ocean carbon cycle. *Protist* **150,** 25-32.

Barb W. G., Baxendale J. H., George P. and Hargrave K. R. (1951) Reactions of ferrous and ferric ions with hydrogen peroxide. 1. The ferrous ion reaction. *Transactions of the Faraday Society* **47,** 462-500.

Barbeau K. (2006) Photochemistry of organic iron(III) complexing ligands in oceanic systems. *Photochem. Photobiol.* **82,** 1505-1516.

Barbeau K. A. and Moffett J. W. (1998) Dissolution of iron oxides by phagotrophic protists: Using a novel method to quantify reaction rates. *Environmental Science and Technology* **32,** 2969-2975.

Barbeau K., Moffett J. W., Caron D. A., Croot P. L. and Erdner D. L. (1996) Role of protozoan grazing in relieving iron limitation of phytoplankton. *Nature* **380,** 61-64.

Barbeau K., Zhang G. P., Live D. H. and Butler A. (2002) Petrobactin, a photoreactive siderophore produced by the oil-degrading marine bacterium Marinobacter hydrocarbonoclasticus. *J. Am. Chem. Soc.* **124,** 378-379.

Benner R. (2002) Chemical composition and reactivity. In *Biogeochemistry of dissolved organic matter* (eds. D. A. Hansell and C. Carlson). Academic Press, New York. pp. 59-90.

Benson B. B. and Krause D. J. (1984) The concentration and isotopic fractionation of oxygen dissolved in fresh water and seawater in equilibrium with the atmosphere. *Limnol. Oceanogr.* **29,** 620-632.

Bergquist B. A., Wu J. and Boyle E. A. (2007) Variability in oceanic dissolved iron is dominated by the colloidal fraction. *Geochim. Cosmochim. Acta* **71,** 2960-2974.

Bhaskar P. V. and Bhosle N. B. (2006) Bacterial extracellular polymeric substance (EPS): a carrier of heavy metals in the marine food-chain. *Environ. Int.* **32,** 191-198.

Bielski H. J., Cabelli D. E., Ravindra L. A. and Ross A. B. (1985) Reactivity of HO_2/O_2^- radicals in aqueous solution. *J. Phys. Chem. Ref. Data* **14,** 1041-1100.

Blake A. C., Chadwick D. B., Zirino A. and Rivera-Duarte I. (2004) Spatial and temporal variations in copper speciation in San Diego Bay. *Estuaries* **27,** 437-447.

Borowitzka M. A. and Siva C. J. (2007) The taxonomy of the genus *Dunaliella* (Chlorophyta, Dunaliellales) with emphasis on the marine and halophilic species. *J. Appl. Phycol.* **19,** 567-590.

Bowie A. R., Whitworth D. J., Achterberg E. P., Mantoura R. F. C. and Worsfold P. J. (2002) Biogeochemistry of Fe and other trace elements (Al, Co, Ni) in the upper Atlantic Ocean. *Deep-Sea Res. Part. I.* **49,** 605-636.

Bowler C., Allen A. E., Badger J. H., Grimwood J., Jabbari K., Kuo A., Maheswari U., Martens C., Maumus F., Otillar R. P., Rayko E., Salamov A., Vandepoele K., Beszteri B., Gruber A., Heijde M., Katinka M., Mock T., Valentin K., Verret F., Berges J. A., Brownlee C., Cadoret J. -., Chiovitti A., Choi C. J., Coesel S., De Martino A., Detter J. C., Durkin C., Falciatore A., Fournet J., Haruta M., Huysman M. J. J., Jenkins B. D., Jiroutova K., Jorgensen R. E., Joubert Y., Kaplan A., Kröger N., Kroth P. G., La Roche J., Lindquist E., Lommer M., Martin-Jézéquel V., Lopez P. J., Lucas S., Mangogna M., McGinnis K., Medlin L. K., Montsant A., Secq M. -. O. -., Napoli C., Obornik M., Parker M. S., Petit J. -., Porcel B. M., Poulsen N., Robison M., Rychlewski L., Rynearson T. A., Schmutz J., Shapiro H., Siaut M., Stanley M., Sussman M. R., Taylor A. R., Vardi A., Von Dassow P., Vyverman W., Willis A., Wyrwicz L. S., Rokhsar D. S., Weissenbach J., Armbrust E. V., Green B. R., van de Peer Y. and Grigoriev I. V. (2008) The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* **456,** 239-244.

Boye M., Aldrich A., van den Berg C. M. G., de Jong J. T. M., Nirmaier H., Veldhuis M., Timmermans K. R. and de Baar H. J. W. (2006) The chemical speciation of iron in the north-east Atlantic Ocean. *Deep-Sea Res. Part. I* **53,** 667-683.

Boye M., van den Berg C. M. G., de Jong J. T. M., Leach H., Croot P. and de Baar H. J. W. (2001) Organic complexation of iron in the Southern Ocean. *Deep-Sea Res. Part I.* **48,** 1477-1497.

Boyer G. L., Gillam A. H. and Trick C. G. (1987) Iron chelation and uptake. In *The Cyanobacteria* (eds. P. Fay and C. van Baalen). Elsevier, New York. pp. 415-436.

Bruland K. W. and Rue E. L. (2001) Analytical methods for the determination of concentrations and speciation of iron. In *The Biogeochemistry of iron in seawater* (eds. D. R. Turner and K. A. Hunter). Willey, England. pp. 255-289.

Bruland K. W., Donat J. R. and Hutchins D. A. (1991) Interactive influence of bioactive trace metals on biological production in oceanic waters. *Limnol. Oceanogr.* **36,** 1555-1577.

Bruland K. W., Orians K. J. and Cowen J. P. (1994) Reactive trace metals in the stratified central North Pacific. *Geochim. Cosmochim. Acta* **58,** 3171-3182.

Buerge I. J. and Hug S. J. (1997) Kinetics and pH dependence of chromium(VI) reduction by iron(II). *Environ. Sci. Technol.* **31,** 1426-1432.

Burdige D. J. (1993) The biogeochemistry of manganese and iron reduction in marine sediments. *Earth Science Reviews* **35,** 249-284.

Campbell A. C. (1991) Mineralogy and chemistry of marine particles by synchrotron x-ray spectroscopy, Mösssbauer spectroscopy, and plasma-mass spectrosmetry. In *Marine particles: Analysis and characterization* (eds. D. C. Hurd and D. W. Spencer). American Geophysical Union, Washington, DC. pp. 375-390.

Canfield D. E., Thamdrup B. and Hansen J. W. (1993) The anaerobic degradation of organic matter in Danish coastal sediments: Iron reduction, manganese reduction, and sulfate reduction. *Geochim. Cosmochim. Acta* **57,** 3867-3883.

Chang H. and Zylstra G. J. (1999) Characterization of the phthalate permease OphD from *Burkholderia cepacia* ATCC 17616. *J. Bacteriol.* **181,** 6197-6199.

Chester R. (1990) *Marine geochemistry.* Unwin Hyman, London.

Croot P. L., Bowie A. R., Frew R. D., Maldonado M. T., Hall J. A., Safi K. A., La Roche J., Boyd P. W. and Law C. S. (2001) Retention of dissolved iron and FeII in an iron induced Soutern Ocean phytoplankton bloom. *Geophys. Res. Lett.* **28,** 3425-3428.

Croot P. L., Laan P., Nishioka J., Strass V., Cisewski B., Boye M., Timmermans K. R., Bellerby R. G., Goldson L., Nightingale P. and de Baar H. J. W. (2005) Spatial and temporal distribution of Fe(II) and H_2O_2 during EisenEx, and open ocean mesoscale iron enrichment. *Mar. Chem.* **95,** 65-88.

Croot P. L., Moffett J. W. and Brand L. E. (2000) Production of extracellular Cu complexing ligands by eucaryotic phytoplankton in response to Cu stress. *Limnol. Oceanogr.* **45,** 619-627.

Cullen J. T., Bergquist B. A. and Moffett J. W. (2006) Thermodynamic characterization of the partitioning of iron between soluble and colloidal species in the Atlantic Ocean. *Mar. Chem.* **98,** 295-303.

Cunningham K. M. (1988) Mechanisms for aqueous photolysis of adsorbed benzoate, oxalate, and succinate on iron oxyhydroxide (goethite) surfaces. *Environ. Sci. Technol.* **22,** 1090-1097.

Cunningham K. M., Goldberg M. C. and Weiner E. R. (1985) Photodissolution of iron oxides in the presence of adsorbed alcohols. *Photochem. Photobiol.* **41,** 409-416.

Davison W. and Seed G. (1983) The kinetics of the oxidation of ferrous iron in synthetic and natural waters. *Geochim. Cosmochim. Acta* **47,** 67-79.

Davison W., Grime G. W., Morgan J. A. W. and Clarke K. (1991) Distribution of dissolved iron in sediment pore waters at submillimetre resolution. *Nature* **352,** 323-325.

de Baar H. J. W. and de Jong J. T. M. (2001) Distributions, sources and sinks of iron in seawater. In *The biogeochemistry of iron in seawater* (eds. D. R. Turner and K. A. Hunter). John Wiley & Sons Ltd., The Netherlands. pp. 123-254.

Dickson A. G., Sabine C. L. and Christian J. R. (2007) Guide to best practices for ocean CO_2 measurements. *PICES Special Publication* **3,** 191.

Donat J. R. and Bruland K. W. (1995) Trace elements in the oceans. In *Trace Elements in Natural waters* (eds. B. Slbu and E. Steinnes). CRC Press, Boca Raton. pp. 247-292.

Dryden C. L., Gordon A. S. and Donat J. R. (2007) Seasonal survey of copper-complexing ligands and thiol compounds in a heavily utilized, urban estuary: Elizabeth River, Virginia. *Mar. Chem.* **103,** 276-288.

Duce R. A. and Tindale N. W. (1991) Atmospheric transport of iron and its deposition in the ocean. *Limnol.Oceanogr.* **36,** 1715-1726.

Dunal M. F. (1837) Note sur les algues qui colourent en rouge certaines eaux des marais salants méditerranéens. *Compt. Rend. Acad. Sci. Ser III-Sciences De La Vie* **15,** 585-587.

Duursma E. K. (1961) Dissolved organic carbon, nitrogen and phosphorus in the sea. *Neth. J. Sea. Res.* **1,** 1-148.

Eary L. E. (1988) Chromate removal from aqueous wastes by reduction with ferrous ion. *Environ. Sci. Technol.* **22,** 972-977.

Elstner E. F., Wagner G. A. and Schütz W. (1988) Activated oxygen in green plants in relation to stress situation. *Curr. Top. Plant. Biochem. Physiol.* **7,** 159-187.

Emennegger L., King D. W., Sigg L. and Sulzberger B. (1998) Oxidation kinetics of Fe(II) in a eutrophic Swiss lake. *Environ. Sci. Technol.* **32,** 2990-2996.

Falkowski P. G., Barber R. T. and Smetacek V. (1998) Biogeochemical controls and feedbacks on ocean primary production. *Science* **281,** 200-206.

Fallab S. (1967) Reactions with molecular oxygen. *Angew. Chem.* **6,** 496-507.

Finden D. A. S., Tipping E., Jaworski G. H. M. and Reynolds C. S. (1984) Light-induced reduction of natural iron(III) oxide and its relevance to phytoplankton. *Nature* **309,** 783-784.

Fox L. E. (1988) The solubility of colloidal ferric hydroxide and its relevance to iron concentrations in river water. *Geochim. Cosmochim. Acta* **52,** 771-777.

Geider R. J. (1999) Complex lessons of iron uptake. *Nature* **400,** 815-816.

German C. R., Campbell A. C. and Edmond J. M. (1991) Hydrothermal scavenging at the Mid-Atlantic Ridge: Modification of trace element dissolved fluxes. *Earth Planet. Sci. Lett.* **107,** 101-114.

Glazewski R. and Morrison G. M. (1996) Copper(I)/copper(II) reactions in an urban river. *Sci. Total Environ.* **189-190,** 327-333.

Gledhill M., McCormack P., Ussher S., Achterberg E. P., Mantoura R. F. C. and Worsfold P. J. (2004) Production of siderophore type chelates by mixed bacterioplankton populations in nutrient enriched seawater incubations. *Mar. Chem.* **88,** 75-83.

Gledhill M. and van den Berg C. M. G. (1994) Determination of complexation of iron(III) with natural organic complexing ligands in sea water using cathodic stripping voltammetry. *Mar. Chem.* **47,** 41-54.

Glober C. J., Hutchins D. A., Fisher N. S., Cosper E. M. and Sañudo-Wilhelmy S. A. (1997) Release and bioavailability of C, N and Fe following virial lysis of a marine chrysophyte. *Limnol. Oceanogr.* **42,** 1492-1504.

González A. G., Pokrovsky O. S., Shirikova L. S., Pokrovski G. S., Jimenez-Villacorta F., González-Dávila M., Santana-Casiano J. M. and Emnova E. E. (2009) Speciation of Fe adsorbed on and incorporated into soil and aquatic bacteria: XAS structural study. *Geochim. Cosmochim. Acta.* **73,** A453-A453.

Gonzalez A. G., Santana-Casiano J. M., Perez N. and Gonzalez-Davila M. (2010a) Oxidation of Fe(II) in natural waters at high nutrient concentrations. *Environ. Sci. Technol.* **44,** 8095-8101.

González A. G., Shirokova L. S., Pokrovsky O. S., Emnova E. E., Martínez R. E., Santana-Casiano J. M., González-Dávila M. and Pokrovski G. S. (2010b) Adsorption of copper on *Pseudomonas aureofaciens*: Protective role of surface exopolysaccharides. *J. Colloid Interface Sci.* **350,** 305-314.

González-Davila M. (1995) The role of phytoplankton cells on the control of heavy metal concentration in seawater. *Mar. Chem.* **48,** 215-236.

González-Dávila M., Santana-Casiano J. M. and Laglera L. M. (2000) Copper adsorption in diatom cultures. *Mar. Chem.* **70,** 161-170.

González-Davila M., Santana-Casiano J. M. and Millero F. J. (2005) Oxidation of iron (II) nanomolar with H_2O_2 in seawater. *Geochim. Cosmochim. Acta* **69,** 83-93.

González-Dávila M., Santana-Casiano J. M. and Millero F. J. (2006) Competition between O_2 and H_2O_2 in the oxidation of Fe(II) in natural waters. *J. Sol. Chem.* **35,** 95-111.

Gonzalez-Davila M., Santana-Casiano J., Perez-Pena J. and Millero F. J. (1995) Binding of Cu(ll) to the surface and exudates of the alga *Dunaliella tertiolecta* in seawater. *Environ. Sci. Technol.* **29,** 289-301.

Gray R. D. (1969) Kinectics of oxidation of copper(I) by molecular oxygen in perchloric acid-acetonitrile solutions. *JACS* **91,** 56.

Guillard R. R. L. (1975) Culture of phytoplankton for feeding marine invertebrates. 29-60.

Haber F. and Weiss J. (1934) The catalytic decomposition of hydrogen peroxide by iron salts. *Proc. R. Soc. Lond. A*.332-351.

Hanson A. K., Tindale N. W. and Abdel-Moati M. A. R. (2001) An equatorial Pacific rain event: Influence on the distribution of iron and hydrogen peroxide in surface waters. *Mar. Chem.* **75,** 69-88.

Hong H. and Kester D. R. (1986) Redox state of iron in the offshore waters of Peru. *Limnol. Oceanogr.* **31,** 512-524.

Hopkinson B. M. and Barbeau K. A. (2007) Organic and redox speciation of iron in the eastern tropical North Pacific suboxic zone. *Mar. Chem.* **106,** 2-17.

Hopkinson B. M. and Morel F. M. M. (2009) The role of siderophores in iron acquisition by photosynthetic marine microorganisms. *Biometals* **22,** 659-669.

Hutchins D. A. and Bruland K. W. (1994) Grazer-mediated regeneration and assimilation of Fe, Zn and Mn from planktonic prey. *Mar. Ecol. Prog. Ser.* **110,** 259-269.

Hutchins D. A., DiTrullio G. R. and Bruland K. W. (1993) Iron and regenerated production-evidence for biological iron recycling in 2 marine environments. *Limnol. Oceanogr.* **38,** 1242-1255.

Hutchins D. A., Witter A. E., Butler A. and Luther G. W. (1999a) Competition among marine phytoplankton for different chelated iron species. *Nature* **400,** 858-861.

Hutchins D. A., Franck V. M., Brzezinski M. A. and Bruland K. W. (1999b) Inducing phytoplankton iron limitation in iron-replete coastal waters with a strong chelating ligand. *Limnol. Oceanogr.* **44,** 1009-1018.

Jickells T. D. and Spokes L. J. (2001) Atmospheric Iron Inputs to the Oceans. In *The Biogeochemistry of Iron in Seawater* (eds. D. R. Turner and K. A. Hunter). John Wiley & Sons, LTD, The Netherlands. pp. 85-121.

Johnson K. S., Coale K. H., Elrod V. A. and Tindale N. W. (1994) Iron photochemistry in seawater from equatorial Pacific. *Mar. Chem.* **46,** 319-334.

Johnson K. S., Michael Gordon R. and Coale K. H. (1997) What controls dissolved iron concentrations in the world ocean? *Mar. Chem.* **57,** 137-161.

Jones G. J., Palenik B. P. and Morel F. M. M. (1987) Trace-metal reduction by phytoplankton. The role of plasmalemma redox enzymes. *J. Phycol.* **23,** 237-244.

Kaczynski S. E. and Kieber R. J. (1993) Aqueous trivalent chromium photoproduction in natural waters. *Environ. Sci. Technol.* **27,** 1572-1576.

Kester D. R., Byrne R. H.,Jr. and Liang Y. (1975) Redox reactions and solution complexes of iron in marine systems. In *Marine Chemistry of the Coastal Environment* (ed. I. M. Chuch). ACS Symposium Series 18; American Chemical Society, Washington, DC. pp. 56-79.

Kieber R. J. and Helz G. R. (1992) Indirect photoreduction of aqueous chromium(VI). *Environ. Sci.Technol.* **26,** 307-312.

Kieber R. J., Williams K., Willey J. D., Skrabal S. and Avery G. B. (2001) Iron speciation in coastal rainwater: Concentration and deposition to seawater. *Mar. Chem.* **73,** 83-95.

King D. W. (1998) Role of carbonate speciation on the oxidation rate of Fe(II) in aquatic systems. *Environ. Sci. Technol.* **32,** 2997-3003.

King D. W., Aldrich R. A. and Charnecki S. E. (1993) Photochemical redox cycling of iron in NaCl solutions. *Mar. Chem.* **44,** 105-120.

King D. W. and Farlow R. (2000) Role of carbonate speciation on the oxidation of Fe(II) by H_2O_2. *Mar. Chem.* **70,** 201-209.

King D. W., Lin J. and Kester D. R. (1991) Determination of Fe(II) in seawater at nanomolar concentrations. *Anal. Chim. Acta.* **247,** 125-132.

King D. W., Lounsbury H. A. and Millero F. J. (1995) Rates and mechanism of Fe(II) oxidation at nanomolar total iron concentration. *Environ. Sci. Technol.* **29,** 818-824.

Kuma K., Nakabayashi S., Suzuki Y. and Matsunaga K. (1992) Dissolution rate and solubility of colloidal hydrous ferric oxide in seawater. *Mar. Chem.* **38,** 133-143.

Kustka A. B., Shaked Y., Milligan A. J., King D. W. and Morel F. M. M. (2005) Extracellular production of superoxide by marine diatoms: Contrasting effects on iron redox chemistry and bioavailability. *Limnol. Oceanogr.* **50,** 1172-1180.

Laglera L. M. and van den Berg C. M. G. (2003) Copper complexation by thiol compounds in estuarine waters. *Mar. Chem.* **82,** 71-89.

Laglera L. M. and van den Berg C. M. G. (2006) Photochemical oxidations of thiols and copper complexing ligands in estuarine waters. *Mar. Chem.* **101,** 130-140.

Le Gall A. and van den Berg C. M. G. (1993) Cathodic stripping voltammetry of glutathione in natural waters. *Analyst.* **118,** 1411-1415.

Leal M. F. C., Vasconcelos M. T. S. D. and van den Berg C. M. G. (1999) Copper-induced release of complexing ligands similar to thiols by *Emiliana huxleyi* in seawater cultures. *Limnol. Oceanogr.* **47,** 1750-1762.

Levitus S., Conkright M. E., Reid J. L., Najjar R. G. and Mantyla A. (1993) Distribution of nitrate, phosphate and silicate in the world oceans. *Prog. Oceanogr.* **31,** 245-273.

Lewin J. C., Lewin R. A. and Philiplott D. E. (1958) Observations on *Phaeodactylum tricornutum. J. Gen. Microbiol.* **18,** 418-426.

Liang L., McNabb J. A., Paulk J. M., Gu B. and McCarthy J. F. (1993) Kinetics of Fe(II) oxygenation at low partial pressure of oxygen in the presence of natural organic matter. *Environ. Sci. Technol.* **27,** 1864-1870.

Liu X. and Millero F. J. (2002) The solubility of iron in seawater. *Mar. Chem.* **77,** 43-54.

Lorenzo J. I., Nieto-Cid M., Alvarez-Salgado X. A., Perez P. and Beiras R. (2007) Contrasting complexing capacity of dissolved organic matter produced during the onset, development and decay of a simulated bloom of the marine diatom *Skeletonema costatum*. *Mar. Chem.* **103,** 61-75.

Lovely D. R. (1991) Dissimilatory Fe(III) and Mn(IV) reduction. *Microb. Rev.* **55,** 259-287.

Macrellis H. M., Trick C. G., Rue E. L., Smith G. and Bruland K. W. (2001) Collection and detection of natural iron-binding ligands from seawater. *Mar. Chem.* **76,** 175-187.

Maier G., Glegg G. A., Tappin A. D. and Worsfold P. J. (2009) The use of monitoring data for identifying factors influencing phytoplankton bloom dynamics in the eutrophic Taw Estuary, SW England. *Mar. Pollut. Bull.* **58,** 1007-1015.

Maldonado M. T. and Price N. M. (1999) Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean. *Deep-Sea Res. Part II.* **46,** 2447-2473.

Maldonado M. T. and Price N. M. (2000) Nitrate regulation of Fe reduction and transport by Fe-limited *Thalassiosira oceanica*. *Limnol. Oceanogr.* **45,** 814-826.

Maldonado M. T. and Price N. M. (2001) Reduction and transport of organically bound iron by *Thalassiosira oceanica* (Bacillariophyceae). *J. Phycol.* **37,** 298-309.

Marschner H. (1995) *Mineral nutrition in higher plants.* Academic press, London.

Matocha C. J., Karathanasis A. D., Rakshit S. and Wagner K. M. (2005) Reduction of copper(II) by iron(II). *J. Environ. Qual.* **34,** 1539-1546.

McKnight D. M. and Morel F. M. M. (1979) Release of weak an strong copper-complexing agents by algae. *Limnol. Oceanogr.* **24,** 823-837.

Milledge J. J. (2011) Commercial application of microalgae other than as biofuels: A brief review. *Reviews in Environmental Science and Biotechnology* **10,** 31-41.

Miller W. L. and Kester D. (1994) Photochemical iron reduction and iron bioavailability in seawater. *J. Mar. Res.* **52,** 325-343.

Miller W. L., D.W. King, Jie Lin and Kester D.R. (1995) Photochemical redox cycling of iron in coastal seawater. *Mar. Chem.* **50,** 63-77.

Millero F. J. (1985) The effect of ionic interactions on the oxidation of metals in natural-waters. *Geochim. Cosmochim. Acta* **49,** 547-553.

Millero F. J. (1986) The pH of estuarine waters. *Limnol. Oceanogr.* **31,** 839-847.

Millero F. J. (1989) Effect of ionic interactions on the oxidation of Fe(II) and Cu(I) in natural waters. *Mar. Chem.* **28,** 1-18.

Millero F. J. (1995) Thermodynamics of the carbon dioxide system in the oceans. *Geochim. Cosmochim. Acta* **59,** 661-677.

Millero F. J. (1998) Solubility of Fe(III) in seawater. *Earth Planet. Sci. Lett.* **154,** 323-329.

Millero F. J. (2001) *The physical chemistry of natural waters.* Wiley-Interscience. Miami, FL.

Millero F. J. (2006) *Chemical Oceanography.* Taylor and Francis Group, Boca Raton, FL.

Millero F. J. and Hawke D. J. (1992) Ionic interactions of divalent metals in natural waters. *Mar. Chem.* **40,** 19-48.

Millero F. J. and Izaguirre M. (1989) Effect of ionic strength and ionic interactions on the oxidation of Fe(II). *J. Solution Chem.* **18,** 585-599.

Millero F. J. and Schreiber D. R. (1982) Use of the ion pairing model to estimate activity coefficients of the ionic components of natural waters. *Am. J. Sci.* **282,** 1508-1540.

Millero F. J. and Sotolongo S. (1989) The oxidation of Fe(II) with H_2O_2 in seawater. *Geochim. Cosmochim. Acta* **53,** 1867-1873.

Millero F. J., Gonzalez-Davila M. and Santana-Casiano J. M. (1995a) Reduction of Fe(III) with sulfite in natural waters. *J. Geophys. Res.* **100,** 7235-7244.

Millero F. J., Huang F. and Laferiere A. L. (2002) Solubility of oxygen in the major sea salts as a function of concentration and temperature. *Mar. Chem.* **78,** 217-230.

Millero F. J., Zhang J.Z., Fiol S., Sotolongo S., Roy R.N., Lee K. and Mane S. (1993) The use of buffers to measure the pH of seawater. *Mar. Chem.* **51,** 707-711.

Millero F. J., Sharma V. K. and Karn B. (1991) The rate of reduction of Cu(II) with hydrogen peroxide in seawater. *Mar. Chem.* **36,** 71-83.

Millero F. J., Sotolongo S. and Izaguirre M. (1987) The oxidation kinetics of Fe(II) in seawater. *Geochim. Cosmochim. Acta* **51,** 793-801.

Millero F. J., Woosley R., DiTrolio B. and Waters J. (2009) Effect of ocean acidification on the speciation of metals in seawater. *Oceanography* **22,** 72-85.

Millero F.J., Yao W., Aicher J. (1995b) The speciation of Fe(II) and Fe(III) in natural waters. *Mar. Chem.* **50,** 21-39.

Moffett J. W. and Zika R. G. (1983) Oxidation-Kinetcis of Cu(I) in seawater-Implications for its existence in the marine-environment. *Mar. Chem.* **13,** 239-251.

Moffett J. W. and Zika R. G. (1987) Solvent-Extraction of copper acetylacetonate in studies of copper(II) speciation in seawater. *Mar. Chem.* **21,** 301-313.

Moffett J. W., Zika R. G. and Petasne R. G. (1985) Evaluation of bathocuproine for the spectrophotometric determiantion of copper(I) in copper redox studies with applications in studies of natural-waters. *Anal. Chim. Acta* **175,** 171-179.

Moore C. A., Farmer C. T. and Zika R. G. (1993) Influence of the Orinoco River on hydrogen peroxide distribution and production in the eastern Caribbean. *J.Geophys. Res.* **98,** 2289-2298.

Morel F. M. M., Kustka A. B. and Shaked Y. (2008) The role of unchelated Fe in the iron nutrition of phytoplankton. *Limnol. Oceanogr.* **53,** 400-404.

Muller F. L. L., Jacquet S. and Wilson W. H. (2003) Biological factors regulating the chemical speciation of Cu, Zn, and Mn under different nutrient regimes in a marine mesocosm experiment. *Limnol. Oceanogr.* **48,** 2289-2302.

Murray J. W. and Gill G. (1978) The geochemistry of iron in Puget Sound. *Geochim. Cosmochim. Acta* **42,** 9-19.

Nolting R. F., Gerringa L. J. A., Swagerman M. J. W., Timmermans K. R. and De Baar H. J. W. (1998) Fe (III) speciation in the high nutrient, low chlorophyll Pacific region of the Southern Ocean. *Mar. Chem.* **62,** 335-352.

O'Sullivan D. W., Hanson A. K., Miller W. L. and Kester D. R. (1991) Measurement of Fe(II) in surface water of the equatorial Pacific. *Limnol. Oceanogr.* **36,** 1727-1741.

Öztürk M., Bizsel N. and Steinnes E. (2003) Iron speciation in eutrophic and oligotrophic Mediterranean coastal waters; impact of phytoplankton and protozoan blooms on iron distribution. *Mar. Chem.* **81,** 19-36.

Parker O. J. and Espenson J. H. (1969) Reactions involving copper(I) in perchlorate solution. A kinetic study of the reduction of iron(III) by copper(I). *Inorg. Chem.* **8,** 1523-1526.

Pehkonen S. O., Erel Y. and Hoffmann M. R. (1992) Simultaneous spectrophotometric measurement of Fe(II) and Fe(III) in atmospheric water. *Environ. Sci. Technol.* **26,** 1731-1736.

Pettine M. and Millero F. J. (2000) Effect of metals on the oxidation of As(III) with H_2O_2. *Mar. Chem.* **70,** 223-234.

Pettine M., D'Ottone L., Campanella L., Millero F. J. and Passino R. (1998) The reduction of chromium (VI) by iron (II) in aqueous solutions. *Geochim. Cosmochim. Acta* **62,** 1509-1519.

Pitzer K. S. and Mayorga G. (1973) Thermodynamics of electrolytes. II. Activity and osmotic coefficients for strong electrolytes with one or both ions univalent. *J. Phys. Chem.* **77,** 2300-2308.

Poorvin L., Rinta-Kanto J. M., Hutchins D. A. and Wilhelm S. W. (2004) Viral release of iron and its bioavailability to marine plankton. *Limnol. Oceanogr.* **49,** 1734-1741.

Postma D. (1985) Concentration of Mn and separation from Fe in sediments-I. Kinetics and stoichiometry of the reaction between birnessite and dissolved Fe(II) at 10°C. *Geochim. Cosmochim. Acta* **49,** 1023-1033.

Postma D. (1993) The reactivity of iron oxides in sediments: A kinetic approach. *Geochim. Cosmochim. Acta* **57,** 5027-5034.

Quigley M. S., Santschi P. H., Guo L. and Honeyman B. D. (2001) Sorption irreversibility and coagulation behavior of ^{234}Th with marine organic matter. *Mar. Chem.* **76,** 27-45.

Rabalais N. N., Turner R. E., Díaz R. J. and Justić D. (2009) Global change and eutrophication of coastal waters. *ICES J. Mar. Sci.* **66,** 1528-1537.

Reardon E. J. (1979) Complexing of silica by iron(III) in natural waters. *Chem. Geol.* **25,** 339-345.

Rebolloso-Fuentes M. M., Acién-Fernández G. G., Sánchez-Pérez J. A. and Guil-Guerrero J. L. (2000) Biomass nutrient profiles of the microalga *Porphyridium cruentum*. *Food Chem.* **70,** 345-353.

Redfield A. C., Ketchum B. H. and Richards F. A. (1963) The influence of organisms on the compostion of seawater. In *The sea* (ed. M. N. hill). John Wiley & Sons, New York. pp. 26-77.

Rich H. W. and Morel F. M. M. (1990) Availability of well-defined iron colloids to the marine diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* **35,** 652-662.

Roekens E. J. and van Grieken R. (1983) Kinetics of iron(II) oxidation in seawater of various pH. *Mar. Chem.* **13,** 195-202.

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

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

Rose A. L. and T.D. Waite (2003b) Kinetics of iron complexation by dissolved natural organic matter in coastal waters. *Mar. Chem.* **84,** 85-103.

Rose A. L., Salmon T. P., Lukondeh T., Neilan B. A. and Waite T. D. (2005) Use of superoxide as an electron shuttle for iron acquasition by the marine cyanobacterium Lyngbya majuscula. *Environ. Sci. Technol.* **39,** 3708-3715.

Roy E. G., Wells M. L. and King W. D. (2008) Persistence of iron(II) in surface waters of the western subarctic Pacific. *Limnol. Oceanogr.* **53,** 89-98.

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

Rue E. L. and Bruland K. W. (1997) The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. *Limnol. Oceanogr.* **42,** 901-910.

Rush J. D. and Bielski B. H. J. (1985) Pulse radiolytic studies of the reactions of HO_2/O_2 - with Fe(II)/Fe(III) ions. The reactivity of HO_2/O_2 - with ferric ions and its implication on the occurrence of the Haber-Weiss reaction. *J. Phys. Chem.* **89,** 5062-5066.

Santana-Casiano J. M., González-Dávila M. and Millero F. J. (2004) The oxidation of Fe(II) in NaCl-HCO_3 - and seawater solutions in the presence of phthalate and salicylate ions: A kinetic model. *Mar. Chem.* **85,** 27-40.

Santana-Casiano J. M., González-Dávila M. and Millero F. J. (2005) Oxidation of nanomolar level of Fe(II) with oxygen in natural waters. *Environ. Sci. Technol.* **39,** 2073-2079.

Santana-Casiano J. M., González-Dávila M. and Millero F. J. (2006) The role of Fe(II) species on the oxidation of Fe(II) in natural waters in the presence of O_2 and H_2O_2. *Mar. Chem.* **99,** 70-82.

Santana-Casiano J. M., González-Dávila M., González A. G. and Millero F. J. (2010) Fe(III) reduction in the presence of Catechol in seawater. *Aquat. Geochem.* **16,** 467-482.

Santana-Casiano J. M., González-Dávila M., Rodríguez M. J. and Millero F. J. (2000) The effect of organic compounds in the oxidation kinetics of Fe(II). *Mar. Chem.* **70,** 211-222.

Sayin M. (1982) Catalytic action of copper on the oxidation of structural iron in vermiculitized biotite. *Clays & Clay Minerals* **30,** 287-290.

Scala S. and Bowler C. (2001) Molecular insights into the novel aspects of diatom biology. *Cellular and Molecular Life Sciences* **58,** 1666-1673.

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

Shaked Y., Kustka A. B. and Morel F. M. M. (2005) A general kinetic model for iron acquisition by eukaryotic phytoplankton. *Limnol. Oceanogr.* **50,** 872-882.

Sharma V. K. and Millero F. J. (1988a) Oxidation of Cu (I) in seawater. *Environ. Sci. Technol.* **22 (7),** 768-771.

Sharma V. K. and Millero F. J. (1988b) The oxidation of Cu(I) in Electrolyte Solutions. *J. Solution Chem.* **17 (6),** 581-599.

Sharma V. K. and Millero F. J. (1988c) Effect of ionic interactions on the rates of oxidation of Cu (I) with O_2 in natural waters. *Mar. Chem.* **25,** 141-161.

Shi D., Xu Y., Hopkinson B. M. and Morel F. M. M. (2010) Effect of ocean acidification on iron availability to marine phytoplankton. *Science* **327,** 676-679.

Smith R. M. and Martell A. E. (1991) *Critical stability constants. Vol.4: Inorganic Complexes*. Plennum Press, New York.

Strmečki S., Plavšićc M., Steigenberger S. and Passow U. (2010) Characterization of phytoplankton exudates and carbohydrates in relation to their complexation of copper, cadmium and iron. *Mar. Ecol. Prog. Ser.* **408,** 33-46.

Stumm W. and Lee G. F. (1961) Kinetic product of ferrous iron. *Ind. Eng. Chem.* **53,** 143-146.

Stumm W. and Morgan J. J. (1996) *Aquatic chemistry: Chemical Equilibria and Rates in Natural Waters (3rd Edition)*. John Wiley & Sons, INC., New York.

Sunda W. G. (1988) Trace metals interactions with marine phytoplankton. *Biol. Oceanogr.* **4,** 411-441.

Sunda W. G. (2001) Bioavailability an Bioaccumulation of Iron in the Sea. In *The Biogeochemistry of Iron in Seawater* (eds. D. R. Turner and K. A. Hunter). John Wiley & Sons, LTD., The Netherlands. pp. 41-78.

Sunda W. G. and Hanson A. K. (1987) Measurement of free cupric ion concentration in seawater by a ligand competition technique involving copper sorption onto C18 SEP-PAK cartridges. *Limnol. Oceanogr.* **32,** 537-551.

Sunda W. G. and Huntsman S. A. (1997) Interrelated influence of iron, light and cell size on marine phytoplankton growth. *Nature* **390,** 389-392.

Teodoresco E. C. (1905) Observations morphologiques et biologiques sur le genre *Dunaliella*. *Rev. Gen. Bot.* **118,** 353-371.

Theis T. L. and Singer P. C. (1973) The stabilization of ferrous iron by organic compounds in natural waters. In *Trace-Metals and metal-organics interactions in natural waters* (ed. P. C. Singer). Ann Arbor Sci. Publ., Ann Arbor, MI.

Theis T. L. and Singer P. C. (1974) Complexation of iron(II) by organic matter and its effect on iron(II) oxygenation. *Environ. Sci. Technol.* **8,** 569-573.

Trapp J. M. and Millero F. J. (2007) The oxidation of iron(II) with oxygen in NaCl brines. *J. Solution Chem.* **36,** 1479-1493.

van Capellen Y. and Wang Y. (1996) Cycling of iron and manganese in surface sediments: a general theory for the coupled transport and reaction of carbon, oxygen, nitrogen, sulfur, iron and manganese. *Am. J. Sci.* **296,** 197-243.

van den Berg C. M. G. (1984) Determining the complexing capacity and conditional stability constant of complexes of copper (II) in seawater by cathodic stripping voltammetry of copper-catechol complex ions. *Mar. Chem.* **15,** 1-18.

van den Berg C. M. G. (1995) Evidence for organic complexation of iron in seawater. *Mar. Chem.* **50,** 139-157.

van den Berg C. M. G., Househam B. C. and Riley J. P. (1988) Determination of cystine and cysteine in seawater using cathodic stripping voltammetry in the presence of Cu(II). *J Electroanal Chem* **239,** 137-148.

Vasconcelos M. T. S. D. and Leal M. F. C. (2008) Exudates of different marine algae promote growth and mediate trace metal binding in *Phaeodactylum tricornutum*. *Mar. Environ. Res.* **66,** 499-507.

Vasconcelos M. T. S. D., Leal M. F. C. and van den Berg C. M. G. (2002) Influence of the nature of the exudates released by different marine algae on the growth, trace metal uptake and exudation of *Emiliania huxleyi* in natural seawater. *Mar. Chem.* **77,** 187-210.

Violler E., Inglett P. W., Hunter K., Roychuodhury A. N. and Cappellen P. (2000) The ferrozine method revisited: Fe(II)/ Fe(III) determination in natural waters. *App. Geochem.* **15,** 785-790.

Voelker B. M. and Sedlak D. L. (1995) Iron reduction by photoproduced superoxide in seawater. *Mar. Chem.* **50(1-4),** 93-102.

Völker C. and Wolf-Gladrow D. A. (1999) Physical limits on iron uptake mediated by siderophores or surface reductases. *Mar. Chem.* **65,** 227-244.

von Piechowski M., Nauser T., Hoigné J. and Bühler R. E. (1993) O_2^- decay catalyzed by Cu^{2+} and Cu^+ ions in aqueous solutions: a pulse radiolysis study for atmospheric chemistry. *Ber. Bunsenges. Phys. Chem.* **97,** 762-771.

Waite T. D. and Morel F. M. M. (1984) Photoreductive dissolution of colloidal iron oxide: Effect of citrate. *J. Colloid Interface Sci.* **102,** 121-137.

Waite T. D., Szymczak R., Espey Q. I. and Furnas M. J. (1995) Diel variations in iron speciation in northern Australian shelf waters. *Mar. Chem.* **50,** 79-91.

Waite T.D. and Torikov A. (1987) Photo-assisted dissolution of colloidal iron oxides by thiol-containing compounds. 2. Comparison of lepidocrocite (γ,-FeOOH) and hematite (α-Fe_2O_3) dissolution. *J. Colloid Interface Sci.* **119,** 228-235.

Wangersky P. J. (1978) Production of dissolved organic matter. In *Marine Ecology, Dynamics* (ed. O. Kinne). Wiley, New York. pp. 115-220.

Wehrli B. (1990) Redox reactions of metal ions at mineral surfaces. In *Aquatic chemical kinetics* (ed. W. Stumm). John Wiley & Sons, New York. pp. 331-336.

Wells M. L. and Mayer L. M. (1991) The photoconversion of colloidal iron oxyhydroxides in seawater. *Deep Sea Research Part A, Oceanographic Research Papers* **38,** 1379-1395.

Wells M. L., Price N. M. and Bruland K. W. (1995) Iron chemistry in seawater and its relationship to phytoplankton: A workshop report. *Mar. Chem.* **48,** 157-182.

Wilhelm S. W. (1995) Ecology of iron-limited cyanobacteria: A review of physiological responses and implications for aquatic systems. *Aquat. Microb. Ecol.* **9,** 295-303.

Willey J. D., Kieber R. J. and Yavari J. R. (2009) Fe(II) in coastal rainwater: Changing stability and concentrations. *Aquat. Sci.* **71,** 144-150.

Willey J. D., Kieber R. J., Seaton P. J. and Miller C. (2008) Rainwater as a source of Fe(II)-stabilizing ligands to seawater. *Limnol. Oceanogr.* **53,** 1678-1684.

Windom H. L., Niencheski L. F. and Smith Jr. R. G. (1999) Biogeochemistry of nutrients and trace metals in the estuarine region of the patos lagoon (Brazil). *Estuar. Coast. Shelf Sci.* **48,** 113-123.

Wu J. F. and Luther G.W. (1995) Complexation of Fe(III) by natural organic-ligands in the Northwest Atlantic-Ocean by a competitive ligand equilibration method and kinetic approach. *Mar. Chem.* **50,** 159-177.

Yruela I. (2005) Copper in plants. *Brazilian Journal of Plant Physiology* **17,** 145-156.

Zafiriou O. C., Voelker B. M. and Sedlak D. L. (1998) Chemistry of the superoxide radical (O_2^-) in seawater: Reactions with inorganic copper complexes. *J. Phys. Chem. A* **102,** 5693-5700.

Zhang S., Xu C. and Santschi P. H. (2008) Chemical composition and 234Th (IV) binding of extracellular polymeric substances (EPS) produced by the marine diatom Amphora sp. *Mar. Chem.* **112,** 81-92.

Zhou X. and Wangersky P. J. (1989) Study of copper-complexing organic ligands: isolation by a Sep-Pak C18 column extraction technique and characterization by chromarod thin-layer chromatography. *Mar. Chem.* **26,** 21-41.

Zika R. G. (1981) Marine Organic Photochemistry. In *Marine Organic Chemistry.* (eds. E. K. Duursma and R. Dawson). Elsevier, Amsterdam. pp. 299-325.

Zika R. G., Moffett J. W., Petasne R. G., Cooper W. J. and Saltzman E. S. (1985a) Spatial and temporal variations of hydrogen peroxide in Gulf of Mexico waters. *Geochim. Cosmochim. Acta* **49,** 1173-1184.

Zika R. G., Saltzman E. S. and Cooper W. J. (1985b) Hydrogen peroxide concentrations in the Peru upwelling area. *Mar. Chem.* **17,** 265-275.

"The function of education is to teach one to think intensively and to think critically... Intelligence plus character – that is the goal of true education".

Martin Luther King, Jr. quotes (American Baptist Minister and Civil-Rights Leader. 1929-1968)