Master's thesis

NTNU
Norwegian University of Science and Technology
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Department of Chemistry

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Effect of iron source on growth and iron uptake in Cyanobacteria *Synechococcus* sp. PCC 7002 wild type and mutants

Master's thesis in Environmental Chemistry
Supervisor: Murat Van Ardelan
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Working on this thesis has been an incredible experience. I had an opportunity to explore different topics, acquire new skills and learn from the best. As I am looking back at this journey, I would like to **thank**:

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За то, что уважаете мой выбор и всегда верите в меня.

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ABSTRACT

Iron is an important trace element that is involved in biological processes and often acts as a limiting nutrient in the ocean. Because of low iron bioavailability in seawater, many organisms have shown ability to adapt to iron-deplete conditions by finding more efficient ways of acquiring iron from the environment (Fraser *et al.*, 2013; González *et al.*, 2018). This work studies response of cyanobacteria species *Synechococcus* sp. PCC 7002 to different iron conditions. This species has shown ability to use such iron acquisition mechanisms as release of siderophores and extracellular iron reduction (Årstøl and Hohmann-Marriott, 2019; Vogel, 2019).

To study the response of *Synechococcus* sp. PCC 7002 as well as role of both iron acquisition pathways in different iron conditions, culturing experiments were conducted. Wild type (WT) and deletion mutant cultures (ΔA2804 and ΔSidOP) were grown on two iron sources ($FeCl_3$ and $FeO(OH)$) in two concentrations (5 μM and 100 nM). During the experiments, optical density of cultures as well as particulate and intercellular iron concentrations were analyzed.

This work is a part of PhD project of Annie Vera Hunnestad at Department of Chemistry which includes several experiments on *Synechococcus* sp. PCC 7002 cultures conducted throughout the years. The project involves additional analyses that are not presented in this thesis.

Based on the results of experiments, in iron-replete conditions all types of organisms showed their ability to sustain growth. Even though a more soluble iron source is generally preferred (in this case, $FeCl_3$), with goethite ($FeO(OH)$) present in high concentrations cultures also showed significant growth. When iron concentrations were lower, iron source and its characteristics became more important together with type of mutation. In lower concentrations of more soluble $FeCl_3$ siderophore-mediated iron uptake pathway may become more important. It can be suggested, that at low concentrations goethite becomes practically unavailable for all cultures. This implies that even wild type culture that can use both iron uptake mechanisms does not have significant advantage on this iron source.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
cFe	Colloidal iron
Chl-a	Chlorophyll a
dFe	Dissolved iron
EDTA	Ethylenediaminetetraacetic acid
HDPE	High density polyethylene
HNLC	High Nutrient - Low Chlorophyll
HR ICP-	High resolution inductively coupled plasma
MS	– mass spectrometry
InFe	Intercellular iron
IsiA	Iron-stress inducible protein A
LDPE	Low density polyethylene
LED	Light emitting diode
LOD	Limit of detection
MQ	Milli-Q
NADP(H)	Nicotinamide Adenine Dinucleotide Phosphate (Hydrogen)
OD	Optical density
PBS	Phycobilisome
PCC	Pasteur Culture collection
PCR	Polymerace chain reaction
PE	Polyethylene
PFe	Particulate iron
PQ	Plastoquinone
PSI(II)	Photosystem I(II)
ROS	Reactive oxygen species
sFe	Soluble iron
sp.	Single species
TFe	Total iron
UP	Ultrapure
WT	Wild type

CHAPTER 1 INTRODUCTION

In most of the ocean iron is present in trace concentrations (down to pico- and nano-molar levels in High nutrient low chlorophyll (HNLC) zones) despite its abundance in the earth's crust (5,6%) (Taylor, 1964; Breitbarth *et al.*, 2010; Longhini, Sá and Neto, 2019). Trace elements in the ocean are on average present in concentrations ≤ 0.1 μM (Morel and Price, 2003), however it is important to note that values can fluctuate locally and seasonally. Trace metals and iron in particular play important roles in biological activities in the ocean and their low bioavailability is an important factor to take into account.

From a biological perspective, the importance of iron in the ocean comes from its crucial role in many biological processes, for example in photosynthesis and nitrogen fixation conducted by microorganisms (Morel and Price, 2003). About 20% of World Ocean are so-called HNLC (high nutrient low chlorophyll) zones, where, despite sufficient supply of macronutrients (like Nitrogen (N), that is often considered a limiting nutrient in the ocean), very low levels of primary production are detected (Pitchford and Brindley, 1999). One of the explanations for existence of HNLC zones was found by detecting a correlation between low productivity and low iron concentrations in seawater (Martin, 1990). Iron has been observed to act as cofactor in many physiological processes and is important in acquisition and utilization of other nutrients. That is due to its flexible redox chemistry which is important in electron transfer reactions. Iron is found within photosynthetic machinery of phytoplankton, indicating importance of the element in photosynthesis (Morel, Rueter and Price, 1991a; Richier *et al.*, 2012; Schoffman *et al.*, 2016).

Some organisms (diazotrophic cyanobacteria) additionally need iron for iron-containing enzymes in charge of nitrogen-fixation apparatus, such as nitrogenase (Shi, Sun and Falkowski, 2007). Iron is also a cofactor in nitrate and nitrite reductase, that are nitrate reducing enzymes. Overall, studies show that iron influences a lot of metabolic pathways and is intertwined with metabolism of other nutrients within cells (Morel, Rueter and Price, 1991a; Schoffman *et al.*, 2016). Since it plays an important role in many physiological processes, iron with its generally low concentrations in seawater can function as a limiting nutrient. However, the main factor that is controlling primary production is not simply low concentration of iron in the solution, but its low bioavailability. In this context bioavailability "may represent the fraction of a chemical accessible to an organism for absorption" (National Research Council, 2003).

Because of low iron bioavailability in seawater many organisms have been shown ability to adapt to iron-deplete conditions by decreasing their iron requirements and/or finding more efficient ways of acquiring iron from the environment (Fraser *et al.*, 2013; González *et al.*, 2018). The latter has been observed in some species of cyanobacteria in the form of using siderophore-mediated and extracellular reductive iron uptake pathways (Lamb *et al.*, 2014; Lis, Kranzler, *et al.*, 2015). Such methods of coping with iron limitation have been observed in particular in *Synechococcus* sp. PCC 7002 (Årstøl and Hohmann-Marriott, 2019; Vogel, 2019).

1.1 Objective and hypothesis

This work studies the response of *Synechococcus* sp. PCC 7002 cultures to different iron conditions. We expect that differences in iron speciation may impact the ability of *Synechococcus* sp. PCC 7002 culture to acquire iron and grow. Theole of each iron uptake mechanism previously observed in this species may also be influenced by different iron conditions.

The objectives of this project are:

- To examine reaction of *Synechococcus* sp. PCC 7002 culture to different iron conditions by using two iron sources ($FeCl_3$ and FeO(OH)) in two concentrations: 5 µM and 100 nM;
- To explore the role of specific genes in *Synechococcus* sp. PCC 7002 in adjusting to different iron conditions by using three types of cultures: wild type and two deletion mutants (ΔA2804 and ΔSidOP).

CHAPTER 2 THEORETICAL BACKGROUND

2.1 Iron speciation in the ocean

Iron bioavailability is a complex topic and is still not fully understood. However, the following has been suggested: in the ocean availability of iron to microorganisms and consequentially ability of said organisms to conduct above-mentioned processes that require iron depends on iron speciation as one of the main factors.

Iron is present in many forms in the ocean. Speciation is a term that is often used to describe the chemical and physical forms in which an element (in this case, iron) is present in the environment. Different species can have different characteristics that dictate their behavior in the environment (Azeez, Prusty and Jagadeesh, 2006). These characteristics can impact bioavailability of an iron species, which is why it is important to understand iron speciation in seawater.

Firstly, in seawater iron is separated into two physical fractions: dissolved (dFe) and particulate (PFe). There are two different operational criteria used to distinguish between the fractions: 0,45 μm (Longhini, Sá and Neto, 2019) or 0,2 μm pore size of a filter (Lough *et al.*, 2019). Dissolved iron fraction is then defined by going through 0,2 or 0,45 μm pore size filter. Particulate, on the other hand, doesn't go through said filter. New technologies have allowed scientists to measure another fraction: soluble (sFe) iron (<0,02 μm pore size) that was later used to determine colloidal (cFe) fraction (from 0,02 to 0,2 (0,45) μm pore size) as difference between dFe and sFe (Wu *et al.*, 2001; von der Heyden and Roychoudhury, 2015). Dissolved (soluble) Fe is the only fraction that is considered readily available to organisms, while cFe is typically the dominant fraction of iron in seawater, for example, in coastal waters and on the surface (Achterberg *et al.*, 2001; Turner and Hunter, 2001).This means that only a small portion of iron pool is easily available to phytoplankton (Morel, Rueter and Price, 1991b; Geider and La Roche, 1994).

As stated above, these criteria are operational, which means they are not representative of all characteristics of iron species. Apart from size differences, it is important to differentiate between organic and inorganic forms, as well as oxidation states.

Mass balance of iron in seawater considering its physical distribution can be described as the following equation (1):

$$Fe_{total} = PFe + cFe + sFe \text{ (Gledhill and Buck, 2012).} \qquad (1)$$

But if a chemical perspective is used, mass balance may be described as (2):

$$Fe_{total} = Fe' + FeL + Fe_{inert} \text{ (Gledhill and Buck, 2012),} \qquad (2)$$

where Fe' stands for labile inorganic iron complexes and free hydrated species, FeL stands for discrete iron organic ligand complexes, and Fe_{inert} – iron fraction bound up in non-labile matrices (Gledhill and Buck, 2012).

As mentioned above, physical fractionation is based on an operational criteria and is should not be expected to include division from a chemical perspective. It is thought that there may be overlapping between physically established fractions when it comes to chemical perspective (Fig.2-1). Thus, sFe is considered to include both inorganic Fe' (free hydrated iron species (Achterberg *et al.*, 2001) and labile inorganic complexes) and organic FeL (e.g. siderophores). The colloidal fraction also includes both inorganic and organic forms, with the latter being represented by weaker ligands (humic substances), metallo-enzymes, viruses, etc. Overall, 99% of dFe is presented in complexes with organic ligands, which means that dissolved unchelated inorganic iron, which is considered to be the most readily available iron source, is the least abundant in seawater (Gledhill and van den Berg, 1995; Wu *et al.*, 2001; Lis, Shaked, *et al.*, 2015). The organic form of PFe is represented by larger biogenic iron, bacteria and detritus. Inorganic forms of cFe and PFe are represented by lithogenic and authigenic particles. Size and density of a particle may define its fate in the environment. Lithogenic PFe tends to sink due to larger size and density, however it was discovered that it may also be converted into biogenic form of different sizes via such processes like consumption by micrograzers, incorporation into larger biogenic particles or dissolution of hydroxides by heterotrophic bacteria (Frew *et al.*, 2006). Biogenic iron can then be made more bioavailable via recycling processes (Frew *et al.*, 2006; von der Heyden and Roychoudhury, 2015).

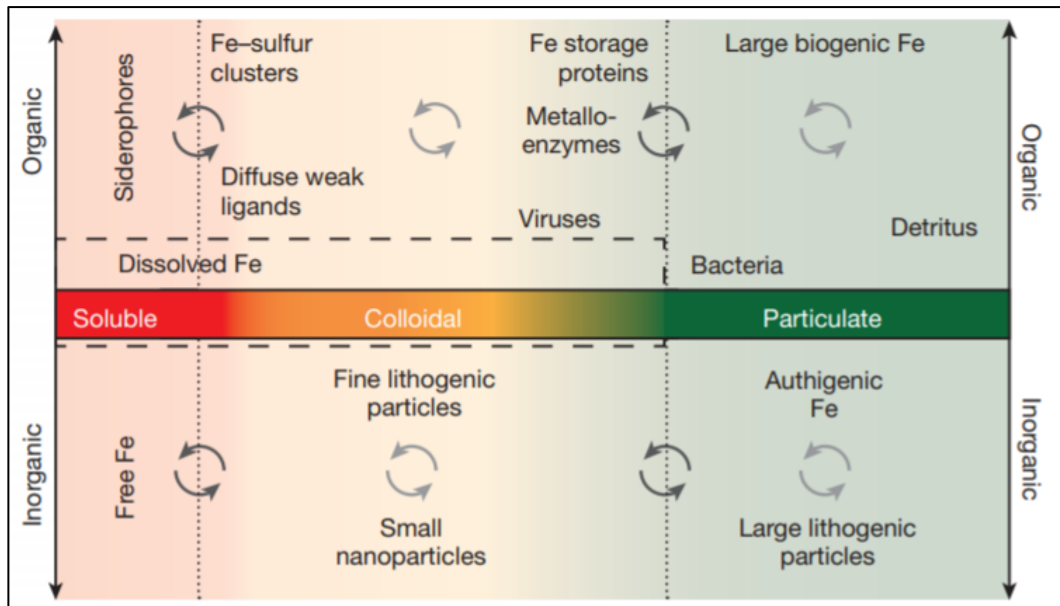


Fig. 2-1 Components of iron pool bound in organic and inorganic complexes divided in size fractions (Tagliabue *et al.*, 2017)

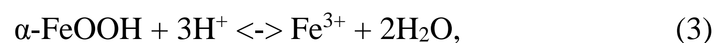
In the ocean iron is present in two environmentally relevant oxidation states: Fe(II) and Fe(III) (ferrous and ferric iron correspondingly). Fe(II) is very soluble, it is commonly found in reducing conditions (sub-oxic zones or in hydrothermal vents), while stable and often insoluble Fe(III) is usually present in oxic conditions in the water column (Schoffman *et al.*, 2016). It has been observed, that ferrous iron Fe(II) is more readily available to phytoplankton than ferric iron because it is more soluble and reactive. In oxic conditions Fe(II) tends to quickly oxidize to Fe(III), that is then easily hydrolyzed to oxides and hydroxides of low solubility (Shaked and Lis, 2012). These are the most common inorganic particulate forms of Fe(III) in seawater (Kraemer, 2004). With time they can become more refractory e.g. being transformed from poorly structured ferrihydrite to more crystalline goethite (Byrne and Kester, 1976) (Raiswell *et al.*, 2008). Apart from oxygen levels in the water column, light also impacts oxidation state of iron present in the solution: in illuminated surface waters Fe(II) contribution to dFe pool can increase due to photochemical reactions (photolysis and photoreduction) (Achterberg *et al.*, 2001; Gledhill and Buck, 2012). Moreover, pH levels influence oxidation rate of Fe(II) to Fe(III) – it tends to be slower in lower pH conditions (Millero *et al.*, 2009). It has been suggested that ocean acidification characterized by lower pH values in the ocean may lead to available iron in the form of Fe(II) increasing in concentrations and becoming more accessible to phytoplankton thus stimulating primary production (Millero *et al.*, 2009). On the other hand, rate of oxidation demonstrates inverse relation to temperature, meaning that increase in

temperature in the ocean may speed up Fe(II) oxidation (Sung and Morgan, 1980; Millero, Sotolongo and Izaguirre, 1987).

Based on the provided information regarding iron speciation, one can say that the most readily available iron (Fe') is barely present in seawater with ferrous iron being quickly oxidized in oxic conditions and particulate and colloidal fractions dominating the iron pool (Shaked and Lis, 2012). This implies that other factors that influence bioavailability of iron should come into play. Such factors include presence of organic ligands, as well as overall physiology of organisms and their Fe uptake mechanisms (Lis, Shaked, *et al.*, 2015). This makes concept of iron bioavailability more complex, with original iron speciation determined by abiotic conditions not being the only factor involved.

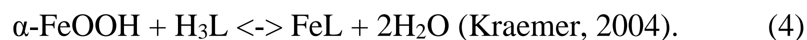
Iron bioavailability is affected by the presence of organic ligands, such as siderophores. Lack of readily available Fe(II) in seawater leads to microorganisms producing organic ligands, e.g. siderophores, that create stable soluble organic complexes with iron, making it more available for acquisition (Kraemer, 2004; Shaked and Lis, 2012). Siderophores are metal chelators with high iron affinity produced by some microorganisms in order to find and complex with iron in the environment. After being released, they can be used by both the producer and other organisms in proximity.

Equation (3) shows dissolution reaction of goethite (as an example) in seawater:



$$\alpha\text{-FeOOH} + 3H^{+} <-> Fe^{3+} + 2H_2O, \qquad (3)$$

while equation (4) shows same reaction in presence of organic ligands (e.g. siderophores) in solution:



$$\alpha\text{-FeOOH} + H_3L <-> FeL + 2H_2O \text{ (Kraemer, 2004).} \qquad (4)$$

It is considered that otherwise free Fe(III) that is quickly hydrolyzed in seawater, in presence of siderophores or other organic ligands can partially stay in solution as part of organic complexes and can be later consumed by organisms. Thus, in upper layers of ocean siderophores are considered to play an important role in solubilization of cFe and PFe (Gledhill and Buck, 2012).

Moreover, biologically mediated reductive processes can take place releasing Fe' from said organic complexes or solid phases. Although it can be quickly hydrolyzed back, as was mentioned before, fresh amorphous hydroxides are a more available iron source compared to aged crystalline phases. In case of reductive dissolution of solid phase, the product of this

reaction can be FeL due to presence of ligands in the solution (Kraemer, 2004; Borer *et al.*, 2005). Biologically mediated reduction can also facilitate transformation of Fe(III) to Fe(II) (Shaked and Lis, 2012).

Bioavailability of different forms and species of iron to phytoplankton should not be seen as black or white, but rather as a spectrum, since many less readily available species can be transformed and utilized by organisms through additional steps (Shaked and Lis, 2012). To summarize, bioavailability of iron doesn't depend solely on concentration or characteristics of iron species, but also on environmental factors (both abiotic and biotic) and physiology of the organism (Lis, Shaked, *et al.*, 2015).

2.2 Iron sources and sinks

Apart from speciation and transformation of iron, it is important to discuss sources of iron in the ocean in order to understand its cycle. The following are considered to be the main sources of iron in the ocean:

- atmospheric deposition,
- fluvial input,
- glacial input,
- interaction with seafloor (hydrothermal vents, submarine groundwater discharge (SGD)),
- circulation/advection,
- death and decay of marine organisms

(Achterberg *et al.*, 2001; Breitbarth *et al.*, 2010; Longhini, Sá and Neto, 2019). Different areas of the ocean may have different dominating sources of iron. It is also important to mention iron input from extraterrestrial materials/dust and volcanic ash, however they are not the focus of this chapter (Breitbarth *et al.*, 2010). Another important source of iron is anthropogenic input e.g. from mining activities and storage of Fe ore. Acute input of iron via disasters on mining facilities (such as collapse of the Fundão tailings dam in Brazil in 2015) can increase iron concentrations in coastal water by several magnitudes (Longhini, Sá and Neto, 2019).

Atmospheric deposition is considered to be the main source of lithogenic iron to the ocean (especially the photic zone). The mechanism behind it is transportation of dust containing iron from land, specifically more arid regions like African deserts that can account for up to 70% of

global supply (Ginoux *et al.*, 2012). Atmospheric input can occur either through dry or wet deposition and is mostly contained within the tropical Atlantic (Longhini, Sá and Neto, 2019). It is important to note that deposited iron can precipitate relatively fast, thus an algal bloom caused by the iron influx can be short-lived. The same has been seen during experiments on artificial iron enrichment, for example EIFEX (European Iron Fertilization Experiment) and so on (Boyd *et al.*, 2000; Yoon *et al.*, 2018).

Fluvial input consists of lithogenic iron input from rivers and estuaries and, consequentially, mainly impacts coastal areas. Estuaries due to their boundary nature between fresh and seawater limit iron input to the ocean. Dissolved iron in fresh water is present in stable colloids that tend to flocculate and precipitate when in contact with seawater leading to removal of estimated 90-95% on the boundary (Johnson, Michael Gordon and Coale, 1997; Longhini, Sá and Neto, 2019). However, due to complexation of iron with low-molecular-weight fulvic acids as well as «ligand exchange reactions with marine iron binding ligands», the overall fluvial input of iron to the ocean increases (Krachler, Jirsa and Ayromlou, 2005).

By glacial input, melting of sea ice, icebergs and glaciers is implied. Sea ice accumulates lithogenic iron from both above (through atmospheric dust) and below (via organic matter on stages of formation and ice algae proliferation) (Breitbarth *et al.*, 2010). For glaciers, meltwaters are the dominant pathway of transporting iron, previously trapped in the glacier, to the ocean (Arrigo *et al.*, 2017). Glacial input, due to its nature, tends to follow seasonal patterns and thus can dictate seasonality of local algal blooms.

When it comes to deep waters, where atmospheric dust is not present in the solution but is precipitated out of the water column, interactions with sediments and hydrothermal vents become the dominant source of iron (Turner and Hunter, 2001). Hydrothermal vents are a source of many trace elements. When hydrothermal plum meets cold and alkaline bottom waters, most of the iron that is found in Fe(II) in the plume (due to low pH and oxygen levels) is oxidized to Fe(III) at around the same rate as it is being released (95%) leaving a very low net input of bioavailable iron (German et al. 1991). In deep water environment it is, however, not as crucial, since due to absence of light, primary producers are not found here. However it was found in areas with high presence of organic ligands, its strong complexation with iron might keep the latter in organic complexes and more available in the water column (Kleint *et al.*, 2016). For example, this can be observed in cases of shallow-water hydrothermal systems, where flux can reach the photic zone where we can expect high concentrations of siderophores

(Kleint, Pichler and Koschinsky, 2017). Some researchers suggest, that hydrothermal activities can be considered iron source on a larger timescale (Tagliabue *et al.*, 2010). Another source of iron in deep water layers is SGD, which implies release of reduced iron Fe(II) from suboxic sediments back into oxic water (Longhini, Sá and Neto, 2019). This release is considered to be caused by such processes as bio-turbation, bio-irrigation, tidal currents etc that disturb the sediment (Klar *et al.*, 2017). Although iron is expected to immediately oxidize to Fe(III) in water column, recent data shows slower rates, implying that there may be a balancing force of complexation of reduced iron Fe(II) by organic ligands present in the water (Klar *et al.*, 2017).

Circulation and advection are overall important in transporting iron from local sources to the open ocean lacking other iron inputs. In shallower areas impact of the continental margin can play an important role. Mixing within the column in proximity of sediments (near land masses and coastal shelf) may resuspend settled iron back into the water column, where it is used by organisms, or, can be transported via advection to downstream areas (Robinson *et al.*, 2016). This source is determined by bottom topography and circulation (currents) in the area (Breitbarth *et al.*, 2010; Robinson *et al.*, 2016) .

Another important source of iron in the water column is death and decay of marine organisms that leads to release of previously consumed iron (Achterberg *et al.*, 2001). On the other hand, the uptake of iron by those organisms is one of the major sinks of iron in the ocean. A second sink is scavenging of dissolved iron by particulate matter via surface adsorption. Extent of importance of these sinks is determined by local factors such as productivity, concentration and size of particles, etc (Achterberg *et al.*, 2001; Turner and Hunter, 2001). The interaction and balance between sinks and sources of iron in marine environment creates the biogeochemical cycle of iron. It is crucial to note importance of sediment – water and marine organisms – water interactions in recycling of iron. As mentioned before, presence of organic ligands and overall biologically mediated processes can increase bioavailability of iron, while excrements and detritus partially release iron back into the solution. At the same time exchange with sediments allows previously settled iron to be reintroduced to the water column (Achterberg *et al.*, 2001). In Fig.2-2 a schematic diagram of biogeochemical iron cycle in the ocean is presented.

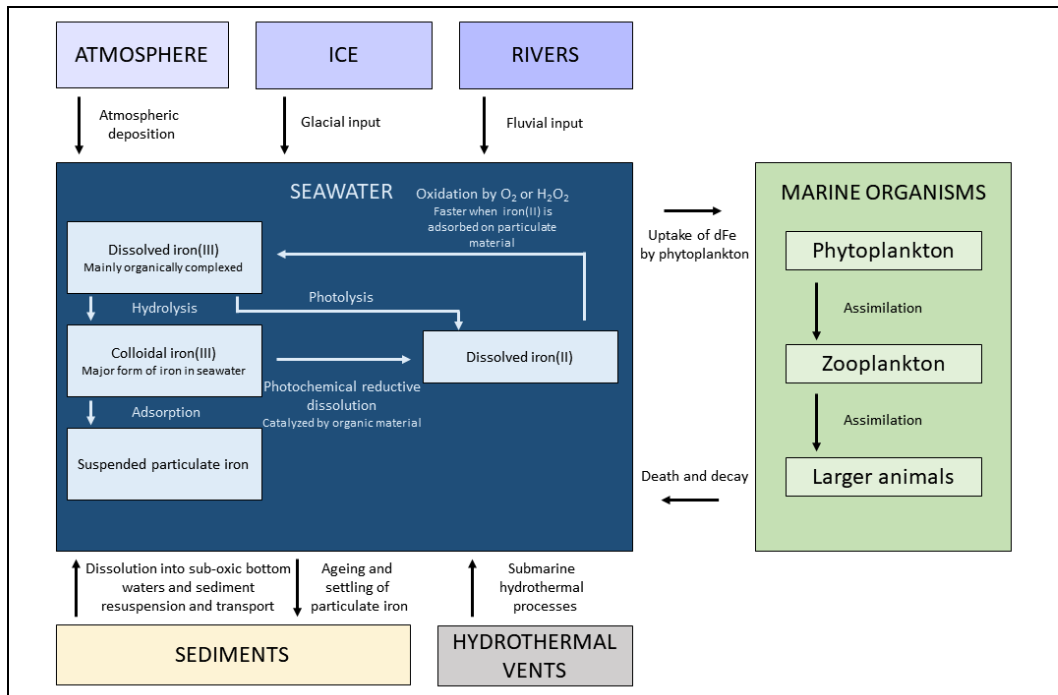


Fig. 2-2 Schematic diagram of biogeochemical iron cycle in the ocean (adapted from (Achterberg *et al.*, 2001))

2.3 Marine cyanobacteria

Cyanobacteria, previously referred to as “blue-green algae” are photosynthetic prokaryotes that can be found in many environments, with seawater being the focus of this study. Cyanobacteria synthesize chlorophyll a as a substrate for harvesting light energy, some species are also nitrogen-fixing, and, as mentioned in the previous chapter, iron is involved in these processes. The defining factor for cyanobacteria presence in an environment is proper light conditions; other important factors are presence of CO_2 and inorganic substances (nutrients) (Mur, Skulberg and Utkilen, 1999; Whitton and Potts, 2012) Different species of cyanobacteria can be found in various aqueous environments with different salinity and temperature where other microalgae species cannot survive (Mur, Skulberg and Utkilen, 1999).

Since cyanobacteria require nutrients, nutrient concentrations can control or even limit their growth. In many ecosystems growth is limited by Phosphorous (P) (Whitton and Potts, 2012). In some cases non-nitrogen-fixing cyanobacteria can be also limited by Nitrogen (N), but some cyanobacteria species (diazotrophic)can fix atmospheric nitrogen which means that their growth is unlikely to be limited by it. However, as was mentioned in the previous chapter, HNLC zones can have high concentrations of N and P, but still show very low productivity.

That is when iron steps in since it is used both in photosynthesis and nitrogen fixation (Morel, Rueter and Price, 1991a) (Shi, Sun and Falkowski, 2007).

2.3.1 Requirements and iron stress

Cyanobacteria are very dependent on iron, that plays an important role in the photosynthetic apparatus. If compared to non-photosynthetic bacteria species, cyanobacteria require approximately 10 times more iron (Singh, McIntyre and Sherman, 2003). Diazotrophic cyanobacteria require even more, since, as mentioned above, they use a lot of iron-containing enzymes for nitrogen fixation (González *et al.*, 2018). Moreover, iron requirements may depend on the environment: coastal microalgae have been observed to have higher minimum cellular iron requirements than oceanic (Marchetti and Maldonado, 2016).

When there is not enough iron present in the environment, it limits cyanobacteria's major physiological processes. Iron starvation can further lead to accumulation of reactive oxygen species (ROS) and cause oxidative stress to cyanobacteria (Latifi *et al.*, 2005; Kranzler *et al.*, 2013a; González *et al.*, 2018). However, it is important to note, that as an essential trace metal, iron has a so-called window of essentiality and in high concentrations can be toxic to an organism (Hopkin, 1993). On the other hand, excess of free iron inside of the cell can also catalyze formation of ROS leading to oxidative stress (Liochev, 1999; Latifi, Ruiz and Zhang, 2009). Fig.2-3 shows, that essential elements can have a negative impact on organisms when concentrations are outside of optimal limits (too low or too high) (Hopkin, 1993; Rainbow and Luoma, 2011). But toxicity of iron is not as common in current oxic ocean conditions and has been seen to be mediated by various mechanisms in cyanobacteria (Shcolnick *et al.*, 2009). Massive iron poisoning is suggested to have occurred in Archaean oceans where oxygen wasn't present in such high concentrations and wasn't able to oxidize most of the available ferrous iron in the bottom waters released through hydrothermal activity (Swanner *et al.*, 2015).

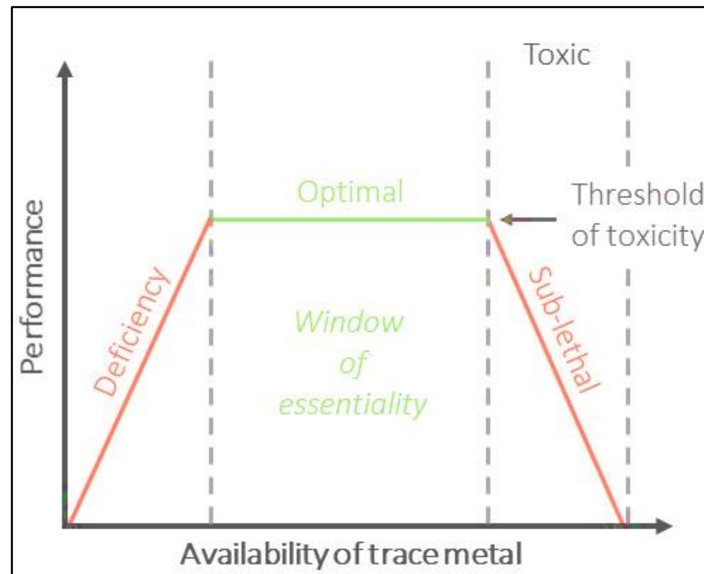


Fig.2-3 Correlation between availability of essential metal and performance of an organism (i.e. physiological processes) (Adapted from (Rainbow and Luoma, 2011))

As mentioned before, cyanobacteria are photosynthetic organisms, meaning that they are able to convert CO_2 and light (as energy source) into cellular energy and O_2. Conversion of solar energy into chemical energy is achieved due to collaborative effort of Photosystems I and II (PSI and PSII) (Shevela, Pishchalnikov and Eichacker, 2013). These are two large pigment-protein reaction center (RC) complexes integrated into the thylakoid membrane within the cytoplasm of the cell. To put it simply, at first, light energy is absorbed by large antenna systems (phycobilisomes or PBSs) of PSII, where it is used to generate electrons from splitting H_2O and production of O_2. After that they are transferred to PSI via the PQ (plastoquinone) pool and another pigment-protein complex: the membrane cytochrome b_6f complex (Shevela, Pishchalnikov and Eichacker, 2013)(Vogel, 2019). From PSI electrons travel to ferredoxin and are used for reduction of NADP+ (Nicotinamide adenine dinucleotide phosphate) to NADPH (Nicotinamide Adenine Dinucleotide Phosphate Hydrogen). The latter acts as cofactor in many reactions within the cell such as lipid and nucleic acid syntheses etc (Ying, 2008). Another outcome of the electron transfer between the systems is synthesis of ATP (adenosine triphosphate) that can be used for storing energy within the cell (Bonora *et al.*, 2012)

Iron is involved in several parts of photosynthetic machinery of cyanobacteria. Overall, under optimal iron conditions a functional photosynthetic apparatus requires around 22-23 iron atoms. Firstly, 3 atoms of Fe are needed for proper functioning of PSII: here iron is mainly used in reaction center proteins and chlorophyll binding proteins. Another 5 are involved in cytochrome b_6f complex; PSI contains another 12 irons; and finally ferredoxin has 2 atoms

(Straus, 1994). In these structures iron is mainly involved in Fe-S (or FeS-X) protein centers, which are electron carriers (Ke, 2005). Based on these numbers PSI has the highest iron requirements per complex in the photosynthetic machinery in cyanobacteria. Under normal circumstances cyanobacteria also has less PSII complexes relative to PSI, and ratio PSI/PSII tends to stay around 3 to 5,8 depending on species (Shevela, Pishchalnikov and Eichacker, 2013).

However, in the ocean easily available iron is often depleted and this can lead to iron stress in cyanobacteria. Response to iron limitation has been studied in different cyanobacteria species. It may lead to lower growth rates and smaller cell size, as well as overall changes in functioning of the cell, especially in the photosynthetic machinery (Straus, 1994; Cunningham and John, 2017; Vogel, 2019). Because of generally iron-deplete conditions in the ocean iron stress can be considered the norm for most cyanobacteria.

PSI has the highest content of iron out of all protein complexes involved in photosynthesis. That is why in Fe-depleted conditions lower amounts of PSI that are synthesized. In *Synechococcus* alteration of the PSI:PSII ratio has been observed changing going down to 1:1. Moreover, PSII synthesis is also often observed to be lower, in order to balance out the systems and limit production of dangerous oxygen radicals. However, under severe iron limitation, the capacity of PBSs to use excess light energy declines thus leading to formation of ROS that causes oxidative stress as mentioned in previous chapter (Facey, Apte and Mitrovic, 2019). Overall genes in charge of assembly of protein complexes involved in photosynthesis are typically down-regulated under iron limitation, while iron acquisition systems are upregulated (Fraser *et al.*, 2013; González *et al.*, 2018). Another observed coping strategy is production of iron-stress inducible protein A (IsiA) that has been described as a chlorophyll binding complex in iron deprived organisms (Sherman and Sherman, 1983; González *et al.*, 2018). In order to partially offset the consequences of fewer PSI complexes, it accumulates around PSI, increasing its size for more efficient light absorption (Ferreira and Straus, 1994; Ryan-Keogh *et al.*, 2012). However, that's not the only potential role of IsiA: it is suggested that it has other functions, such as acting as a chlorophyll storage protein or protecting photosystems from photoinhibition (light-depended decrease in photosynthetic capacity) (Huner *et al.*, 2002; González *et al.*, 2018). Moreover, when the environment is deprived of iron, intercellular iron reserves (within such proteins as ferritin and bacterioferritin) can be used by the cell. In some species of cyanobacteria in iron-deprived environment, some Fe proteins like ferredoxin can

also be replaced with their functional analogs that don't require Fe (in this case – flavodoxin) (Keren, Aurora and Pakrasi, 2004; Baptista and Vasconcelos, 2006; Lodeyro *et al.*, 2012).

In several strains of *Synechococcus* decreases of pigment concentrations have also been observed. Chlorophyll a (Chl-a) is a primary photosynthetic pigment and is an important part of the photosynthetic machinery in the cell. Another important pigment is phycocyanin (PC) that is also involved in photosynthesis. In Fe-depleted environment changes in photosynthetic pigments, e.g. decrease of Chl and PC concentrations may occur (Singh, McIntyre and Sherman, 2003). This can happen due to PBS degradation under nutrient limitation and can subsequently lead to chlorosis. Chlorosis implies change of color to yellow or bleaching of the microorganisms due to photosynthetic apparatus being down-regulated (Collier and Grossman, 1992; Geider and La Roche, 1994).

2.3.2 Iron acquisition mechanisms

Another way of adapting to iron limitation is finding a more efficient way of acquiring iron from an Fe-depleted environment. Siderophore mediated and reductive iron uptake pathways are going to be the focus of this study. It is important to note that this field of research still produces a lot of debate and a lot is yet unknown about iron acquisition strategies of cyanobacteria.

Siderophores are low-molecular-weight metal chelators that some cyanobacteria species and other microorganisms can excrete into the environment. They have high affinity for iron and form strong organic complexes with Fe(III) that are transported into the cell where decomplexation takes place typically in the cytoplasm (Lis, Kranzler, *et al.*, 2015; Årstøl and Hohmann-Marriott, 2019). Different types of siderophores have been observed in different cyanobacteria species. Siderophores are typically classified depending on bidentate ligands incorporated into the siderophore structure, e.g. hydroxamate, catechol etc (Hider and Kong, 2010). For example, *Synechococcus sp. PCC 7002* secretes synechobactins, which are hydroxamate amphiphilic siderophores with citric acid backbone (Hopkinson and Morel, 2009; Kranzler *et al.*, 2013b). It has also been discovered that siderophores can bind with other metals and thus can not only acquire other essential trace metals, but also remove toxic metals outside of the cell. For example, one study showed that with high copper and low iron present in solution, siderophores were primarily used to reduce toxicity of copper, while iron acquisition was conducted by other means (Nicolaisen, Valdebenito and Hahn, 2010). Siderophores play

an important role in iron acquisition not only for the producers, but also for other microorganisms in the environment. Research shows that non-siderophore-producing microorganisms can uptake siderophores of other species in proximity. This phenomenon is called siderophore piracy (D'Onofrio *et al.*, 2010; Traxler *et al.*, 2012; Årstøl and Hohmann-Marriott, 2019). Current genetic analysis shows that most cyanobacteria species are unable to produce siderophores (Toulza *et al.*, 2012).

Inability of certain cyanobacteria species to produce their own siderophores combined with research showing that those species are still able to acquire iron, led scientists to search for another Fe-uptake pathway. Moreover, even species capable of siderophore production showed signs of another siderophore independent pathway. It was proposed that such pathway existed in a form of extracellular ferric Fe reduction mediated by the cell (Lis, Kranzler, *et al.*, 2015). This pathway is thought to be used by organisms on its own or together with the siderophore-mediated uptake described in previous paragraph (Lis, Kranzler, *et al.*, 2015). Despite the evidence of Fe-reductive pathway existence not a lot is known about the process itself (Kranzler *et al.*, 2011). Iron reduction uptake pathway, although not fully understood, is shown to be wide spread among numerous cyanobacteria species in various environments (Lis, Kranzler, *et al.*, 2015).

Recently a theory was suggested that in some cyanobacteria species extracellular reduction is achieved via type IV pili (T4P) donating electrons to iron that is later transported inside the cell. T4P are protein structures or appendages that have been observed on the exterior of some species of bacteria (Schuergers and Wilde, 2015; Vogel, 2019). Research on *Geobacter sulfurreducens* showed that pili might be involved in reduction of ferric iron serving as biological nanowires (Reguera *et al.*, 2005). Another study conducted on deletion mutants of non-siderophore-producing freshwater species *Synechocystis* sp. PCC 6803 showed that in absence of pili-gene PilA1 organisms struggled to survive on iron oxide minerals further proving PilA1 importance in reducing ferric Fe and making it available to the cell (Lamb *et al.*, 2014).

2.3.3 *Synechococcus* sp. strain PCC 7002

The species of cyanobacteria that is the focus of this study is *Synechococcus* sp. PCC 7002 (from this point forward referred to as *Synechococcus* for simplicity). It is a unicellular euryhaline species, which means that it can survive in a wide range of salinities (Ludwig and

Bryant, 2012b). It also shows high tolerance to high-light (UV) irradiation. Another important quality of this strain is its ability to grow very rapidly under optimal conditions. *Synechococcus* is not able to fix nitrogen and thus requires less iron than diazotrophic species.

Synechococcus is a coastal marine strain of cyanobacteria that was originally isolated from a fish pen in Puerto Rico from a collected mud sample. Coastal species tend to have higher iron requirements due to higher iron concentrations in their habitat (Sunda and Huntsman, 2015). Related strains were isolated from different matrices such as sand, seawater and so on all around the world. All these strains were originally growing in coastal areas such as estuaries and tidal zones, where fluctuation of basic factors such as salinity, temperature, light and nutrient concentrations is common. Meaning that in order to survive in these unstable conditions organisms had to learn to adapt to rapid changes (Ludwig and Bryant, 2012b, 2012a).

Most importantly, *Synechococcus* is a model organism for various biotechnology applications (Ludwig and Bryant, 2012b). Firstly, because of its ability to grow fast and flexible growth conditions. Moreover, because its complete genome has been sequenced and made available, and also because it can be genetically transformed quite easily (Ludwig and Bryant, 2011). It allows scientists to conduct experiments in different fields and that is why this strain is used in this study.

In this study wild type and two deletion mutant cultures are used. Wild type (WT) is culture with unmodified organisms. Deletion mutants are organisms missing a specific gene or promoter that in the context of this study is thought to be related to iron acquisition mechanisms. In this study ΔA2804 and ΔSidOP cultures are used.

<u>ΔA2804.</u> *Synechococcus* has been found to express T4P genes that may be involved in reductive iron acquisition pathway (Vogel, 2019). In this study deletion strain of *Synechococcus* (ΔA2804) is used in order to study impact of PilA1 deficit on response of *Synechococcus* to different iron conditions. Mutants were created by replacing targeted gene with kanamycin resistance cassette. Such gene cassettes (small mobile elements of DNA) that confer resistance to an antibiotic (in this case – kanamycin) are common for strain construction purposes in different bacteria (Poteete, Rosadini and St. Pierre, 2006). After that mutants were validated by colony PCR and sequencing. Colony PCR (polymerase chain reaction) is used to verify presence of genetic construct in the organism (Bergkessel and Guthrie, 2013).

<u>ΔSidOP.</u> Second type deletion mutant culture is related to siderophore mediated iron acquisition pathway. It was created by replacing siderophore operon promoter, that controls

cluster of genes related to siderophore production, with spectinomycin resistance cassette. Methodology for creating this type of mutant was originally described in (Vogel, Lale and Hohmann-Marriott, 2017). After that mutants were validated by colony PCR, sequencing and using mass spectrometry (MS) to make sure they do not produce synechobactin. Strain details have been confirmed by Erland Årstøl (E. Årstøl 2020, personal communication, 1 May) and are described in more detail in yet-to-be published work.

2.4 Algal culturing

Algal culturing traces back to 1850s when the first reported attempt of temporarily maintaining microorganisms in laboratory conditions was conducted by Ferdinand Cohn. The methods he used were far from current and algal culturing has come a long way since then (Preisig and Andersen, 2005). The idea of being able to create an indefinitely maintained culture in artificial and fully controlled environment has been studied and developed by many scientists around the world. It is also important to understand, that apart from scientific interest, microalgal culturing has been used for production, e.g. aquaculture (Preisig and Andersen, 2005)

Culturing as a laboratory experiment method for research purposes covers many different aspects of relationship between algae and their environment. Maintaining a culture in controlled conditions has allowed scientists to study importance of different factors and impact of their fluctuations on the microorganisms (Preisig and Andersen, 2005).

There are numerous approaches to culturing and a lot depends on the cultured species and focus of research. Each aspect of created environment can be changed accordingly. Medium can be artificial (AW) and simulate freshwater or seawater environment, or it can be made from filtered natural water (NW). Moreover, different nutrient concentrations can be added depending on species, overall goal of culturing and so on (Watanabe, 2005). In case of this study, culturing is used to extract response of *Synechococcus* to different iron conditions while maintaining other factors relatively constant.

2.4.1 Culturing medium

For experiments with cyanobacteria and trace metals, artificial medium is often used in order to achieve control over its composition as well as metal and ligand concentrations (Harrison and Berges, 2005). In this study Aquil (artificial seawater medium) is used as the medium,

which was first developed by (Morel *et al.*, 1979). Aquil has been used for culturing and for conducting physiological activities on different species of phytoplankton. The recipe used in this study is adapted from (Andersen *et al.*, 2005) and contains salts, nutrient and metal stock and vitamins. Salt composition of Aquil is derived from SOW (synthetic/substitute ocean water) and consists of anhydrous and hydrous salts and filtered deionized water.

Apart from salt composition, another important aspect in making an appropriate medium for culturing is presence of necessary nutrients. Major nutrient, metal and vitamin stocks are prepared separately from Aquil. Major nutrient stock contains phosphorus (P), nitrogen (N) and Silicon (Si); metal stock contains essential trace metals. Metal stocks often include EDTA (ethylenediaminetetraacetic acid) that is a very strong hexadentate chelator. EDTA is used to control metal availability in the solution (Nowack and Sigg, 1997). Without presence of organic ligands in artificial medium iron can precipitate quicker and EDTA is used to prevent that (Sunda, Price and Morel, 2005). As mentioned in previous chapters, presence of organic ligands in natural waters is a very important factor for iron bioavailability.

Artificial seawater media (AW) are typically considered to be better defined compared to NW when it comes to chemical composition because they don't require extensive analysis of the initial seawater. That is what makes Aquil more suitable for trace metal research. However, since preparation of AW involves addition of many different salts, introduction of such impurities as trace metals is still expected (Sunda, Price and Morel, 2005). It is important to acknowledge that and incorporate cleaning techniques into the process of medium preparation. Such cleaning techniques related to trace metal contamination are described in following sections.

Another important step in preparation of culturing medium is sterilization. Sterilization is conducted in order to kill bacteria and other possible life forms in the medium. Presence of bacteria in the solution where microalgae are cultured can influence their growth and outcome of the experiment. Medium can be sterilized by different methods, most common of those being usage of autoclave, microwave and filtration (Kawachi and Noël, 2005).

2.5 Trace metal analysis

It was mentioned in previous chapters, that iron is present in trace amounts in seawater despite being abundant in Earth's crust. However, realistic iron levels in the ocean hadn't been

discovered before 1970s when the issue of sample contamination was addressed (Patterson and Settle, 1976; Fitzwater, Knauer and Martin, 1982; Achterberg *et al.*, 2001). Most of the previous studies were dismissed upon further development of cleaner sampling techniques during that decade. This also lead to more consistent results around the world that would later allow scientists to study distribution patterns of trace metals and explore their cycling in the ocean (Bruland *et al.*, 1979).

Data before the 1970s generally showed higher concentrations of trace metals in seawater and was often inconsistent (Fitzwater, Knauer and Martin, 1982). Several sources of impurities impacting iron levels in seawater samples have been discussed by researchers, such as usage of metal equipment, contact with unfiltered air and so on (Bruland *et al.*, 1979; Fitzwater, Knauer and Martin, 1982; Cutter *et al.*, 2010). It has been shown that presence and usage of metal equipment in proximity can increase metal concentrations in the samples. With air fluxes being an important pathway of iron to the ocean, air is also an important source of contamination to the samples both outdoors and indoors, because of its ability to transfer particles that may contain metals, i.e. dust and rust. Additionally, impurities of equipment and reagents used are considered a possible contamination source (Fitzwater, Knauer and Martin, 1982).

Moreover, another important issue with trace metal contamination of samples that has been observed, is related to phytoplankton and its activity. Contamination of samples with trace metals can lead to, for example, inhibition or alternatively stimulation of growth and primary productivity and thus can skew the results in an unpredictable way (Sanderson *et al.*, 1995). It means, that contamination of samples may impact not only trace metal concentrations, but also metabolic processes of collected microorganisms.

Development of new cleaner techniques for trace metal analysis has been essential in order to provide consistent accurate data (Bruland *et al.*, 1979). Implementation of universally accepted methods and techniques is necessary for deeper understanding of global cycles of said elements. That has been the goal of GEOTRACES program, that was founded in 2006 (SCOR Working Group, 2007). Currently, GEOTRACES guidelines regarding sampling and overall trace metal analysis procedure are commonly used as basis by many researchers in this field (Cutter *et al.*, 2010).

2.5.1 How to minimize contamination

Trace metal analysis guidelines include many recommendations on how to minimize contamination of samples in trace metal analysis. The following measures are common and were used in this project in particular:

Clean laboratory space

Clean laboratory space is a room made specifically to minimize contamination in trace metal analysis. This is achieved mainly by plastic surfaces (or plastic covered surfaces), air filtration system and regular cleaning procedures (EPA, 1996; Cutter *et al.*, 2010).

Laminar flow hood

A laminar flow hood is used to conduct most activities involving samples. Due to laminar flow inside the hood directed towards the user, particles are prevented from getting inside through the open door, when the hood is being used. The air involved in the flow passes through a HEPA (High-efficiency particulate air) filter before reaching the inside of the hood (Cutter *et al.*, 2010). The hood used in trace metal studies ideally should not have metal parts inside.

Plastic film covers

Equipment with metal parts in a clean laboratory can be a source of contamination and is recommended to be replaced. However, in case of that not being a viable option, it is important to cover all metal parts of equipment in polyethylene film or parafilm in advance to ensure no metal surfaces are open in the clean space (EPA, 1996).

Air filtering

Air filtering is another important method to minimize airborne contamination. HEPA filters are implemented in a space where handling of samples takes place, i.e. laminar flow hood, as described previously. Since air is a potential source of contamination, samples should be in contact with filtered air exclusively (either through HEPA or 0,2 um membrane filters) (Cutter *et al.*, 2010). That involves installing aeration systems for cultures similar to the one described in the next chapter.

Acid washing

All plastic equipment that comes in contact with the sample or culture must be previously acid washed. It is especially important for bottles/vials, where samples are stored for a long period of time. The goal of acid washing is lowering pH in order to make metals, that may be adsorbed

on walls of equipment, more soluble and release them into the washing solution. This process consists of several steps: a detergent bath and several baths with decreasing acid concentrations, e.g. starting from 6M and finishing with 0,1M. Equipment is submerged into or filled with the solution for up to several weeks and is rinsed 4 times between each step. Rinsing is started by adding a very small volume of water at first with gradual increase each time. During fourth rinsing the equipment (e.g. bottle, vial, petri dish) is filled up with water allowing it to overflow. Gradual water addition is used in order to slowly increase pH levels from acid wash. That way metals, insoluble at higher pH levels, can be rinsed out before being adsorbed back onto the equipment. LDPE (low density polyethylene) is often used in trace metal analysis because of its ability to withstand different cleaning procedures without being damaged (Dulski, 1999). LDPE is the more favorable compared to HDPE (high density polyethylene) because of higher residual metal content and brittleness of the latter. LDPE equipment is also significantly cheaper compared to Teflon TFE, that is another commonly used material. On the other hand, borosilicate glass – most common labware material, is not recommended for trace metal analysis because it contains significant amounts of metals. Moreover, some acids (e.g. hydrofluoric and phosphoric), as well as caustic conditions may be corrosive with this material and release contamination from it (Dulski, 1999).

Protective gear

While working in the clean laboratory, protective gear is worn, such as clean microporous suits or coats, hairnets and shoe covers. Powder-free nitrile gloves are recommended to be worn at all times while in the laboratory. Once the experiment starts, all equipment entering the space must be rinsed with MQ water and wiped with a dust-free wiper to minimize introduction of new particles to the clean space.

High grade reagents

Another possible source of contamination is impurities in reagents. It is recommended to use the highest available grade of reagents or conduct purification of lower grade chemicals (Cutter *et al.*, 2010).

Chelex resin addition

Chelex resin is a chelating ion exchange resin with high preference for heavy metals, because of which it can act as cleaning agent. Chelex resins (Chelex 20 and Chelex 100) are styrene divinylbenzene copolymers containing paired iminodiacetate ions responsible for binding

polyvalent metal ions (Bio-Rad Laboratories, 2000). Chelex resin can be used for reagent purification (removal of heavy metals) and is commonly used in trace metal analysis.

There are two ways of adding Chelex to the solution: batch and column methods. In this study Chelex 100 resin was added to remove trace metals from medium and nutrient stocks using the batch method. The batch method implies several steps: addition of resin directly to the solution, stirring of the solution for at least 1 hour and removal of resin through filtration. It is recommended to add 5 g of Chelex resin per 100 mL of solution (Bio-Rad Laboratories, 2000). If heavy metal concentrations in the solution are known, required amount of Chelex resin can be calculated according to its wet capacity, which equals 0,4 meq/ml (Bio-Rad Laboratories, 2000).

Sample repetition.

All the above-mentioned measures are used to minimize contamination of samples, however when working with trace metals, it is difficult to avoid it completely. In order to be able to identify contamination in the samples and exclude the outliers, three or more replicates of each sample should be collected and analyzed (Cutter *et al.*, 2010).

2.6 Oxalate wash

In this project measurements of iron concentrations in the culture were conducted. Because of different iron acquisition pathways and survival strategies in phytoplankton, it is important to differentiate between surface-bound and intercellular iron. Intercellular iron measurements show how much iron is truly acquired inside the cell (interior iron pool), while surface-bound iron may be simply attached to the cell on the outside. In order to be able to differentiate between these types of iron without affecting integrity of the cell, scientists have suggested conducting cell washing using reductants (Tovar-Sanchez *et al.*, 2003). Oxalic acid is often used as removing agent of different forms of iron from soils showing good complexing characteristics (Lee *et al.*, 2006). Using oxalate reagent shows high efficiency removal of extracellular iron while also showing low iron contamination risks if appropriate cleaning techniques are implemented (Tovar-Sanchez *et al.*, 2003). In this project, EDTA-oxalate solution recipe was used. It was added to the sample during filtration. Next important step in oxalate wash procedure is NaCl rinse that allows metal-EDTA complexes to be eliminated or

"rinsed" off the filter (Tang and Morel, 2006). A more detailed description of the procedure is provided in the next chapter.

2.7 HR ICP-MS

ICP-MS or Inductively Coupled Plasma – Mass Spectrometry combines high-temperature ICP with a mass spectrometer and is commonly used for elemental analysis in a large variety of samples (Beauchemin, 2016). It is able to determine multiple elements on ultratrace level, which makes it a very important tool in the field of trace element analysis (Thomas, 2013). Liquid (or dissolved solid) sample is delivered to nebulizer where it is converted into an aerosol. Filtered aerosol (2-5% of initial sample) is then introduced to plasma, where it is ionized. After that ions are focused into mass spectrometer via sampler and skimmer: two water-cooled metal interface cones with a small orifice (0,6-1,2 mm) (Beauchemin, 2016). In MS ions of different m/z (mass to charge ratio) values are separated into analyte and nonanalyte ions using quadropole filter (other mass separation devices can also be used depending on the instrument) (Thomas, 2013). High-resolution mass spectrometer is able to reduce or eliminate spectroscopic interferences by using magnetic and electric sectors to separate and focus ions (Skoog *et al.*, 2013). Electric sector disperses ions according to their kinetic energy thus focusing the ion beam, while in mass sector they are dispersed by m/z values (Herbert and Johnstone, 2002; Hoffmann and Stroobant, 2007).

CHAPTER 3 METHODS AND MATERIALS

3.1 Cleaning procedures and implemented measures

The experiment setup was located in a clean laboratory space. Due to external factors, an appropriately equipped facility wasn't available and in case of this study a temporary clean laboratory space was created by cleaning and covering all surfaces with clear polyethylene film thus making an isolated chamber with overlapping plastic screens as the entrance. After that surfaces were additionally cleaned with MQ and wiped with dust free wipers.

All necessary equipment was transferred to the clean space for the laboratory work to be conducted inside. A laminar flow clean hood (AirClean 600) was installed on the bench and was used as the main location for handling and preparing the samples.

Each person operating in the clean space was required to wear protective gear in order to minimize contamination from particles. It consisted of a microporous laminated clean coat (Tyvec©) and single-use shoe and hair covers. Powder-free nitrile gloves were used inside the clean space at all times and had to be regularly changed if working in and out of the clean hood.

Since different cultures were studied during the experiment, cross-contamination was also a possibility and such measures as separate tubing and aeration systems for each culture were implemented.

During this experiment Milli-Q water was used with TOC (total organic carbon) values below 5 ppb (Merck Millipore, 2013). For general cleaning, acids of analytical grade were used. However, for final steps of cleaning, as well as for acidification of samples Ultrapure acids (hydrochloric and nitric of Ultrapure grade) were used.

3.1.1 Acid cleaning

All equipment (such as filtration flasks, bottles, tubing etc) that comes in contact with the sample during the experiment had to be previously acid washed. This process consisted of several steps:

Step 1. 5% detergent bath for 1 week,
Step 2. 3M HCl bath for 3 weeks,
Step 3. 1M HCl bath for 2 weeks,
Step 4. 0,1M Ultrapure HCl bath for 3 weeks.

Equipment was either submerged into a 10L PE box filled with solution or, in case of culturing bottles, was filled up with solution. All equipment was rinsed 4 time between steps, however after Step 1 it was rinsed additionally to make sure detergent was fully removed. After the procedure equipment was rinsed again and stored in a double plastic bag until beginning of the experiment. Culturing bottles were emptied and rinsed when it was time to fill them with sterilized Aquil. For more fragile equipment, such as tubing, that can be broken up by a strong acid, Step 2 was omitted, and Step 3 was cut to 1 week. During cleaning, the inside of tubing was rinsed via acid cleaned syringes to assure that acid was completely washed out.

Equipment had to be cleaned between the experiments as well, with tubing as the only exception, since new acid clean tubing was used for the second experiment. Cleaning procedure between the two experiments consisted of the following steps with quadruple MQ rinsing in between:

Step 5. Ethanol rinse and vigorous shaking,
Step 6. 6M HCl rinse and vigorous shaking,
Step 7. 3M HCl bath for 24 hours,
Step 8. 1M HCl bath for 3 days,
Step 9. 0,1M UP HCl bath for 1 week.

3.2 Medium preparation

Salt composition of Aquil for this study was recalculated from (Andersen, 2005) for larger volumes. Each batch consisted of 20 L, that were stored in acid washed PE collapsible 20L bottles. All salts were dissolved in Milli-Q water (MQ). Anhydrous and hydrous salts (Sigma-Aldrich) were first prepared separately by dissolving in 12L and 6L of MQ correspondingly (18L in total). Two solutions were then combined in acid washed 20L collapsible PE bottle with addition of the final 2L of MQ (Table 3-1).

Table 3-1 Salt composition of Aquil (adapted from (Andersen, 2005))

Salt	Weight per 20 L [g]	Final concentration [M]
	Anhydrous salts	
NaCl	490,80	$4{,}20 \times 10^{-1}$
Na_2SO_4	81,80	$2{,}88 \times 10^{-2}$
KCl	14,00	$9{,}39 \times 10^{-3}$
$NaHCO_3$	4,00	$2{,}38 \times 10^{-3}$
KBr	2,00	$8{,}40 \times 10^{-4}$
H_3BO_3	0,06	$4{,}85 \times 10^{-5}$
NaF	0,06	$7{,}15 \times 10^{-5}$
	Hydrous salts	
MgCl2×H2O	220,00	$5{,}45 \times 10^{-2}$
CaCl2×2H2O	30,80	$1{,}05 \times 10^{-2}$
SrCl2×6H2O	0,34	$6{,}38 \times 10^{-5}$

After combining hydrous and anhydrous salts together, Chelex 100 resin slurry was added to the bottle left on a laboratory shaker for 72 hours. Slurry consisted of 10 g of Chelex mixed with MQ to 20 mL with addition of 10 drops of ammonium acetate buffer. Estimation of required chelex addition was based on previous experiments with Aquil and calculated according to (Bio-Rad Laboratories, 2000) capacity guidelines. After being left on a shaker, Aquil is passed through a chromatography filtration column to remove chelex and collected in a second acid washed PE collapsible 20L bottle.

Sterilization of the medium was done through microwaving as recommended by (Andersen, 2005). Several acid washed 1L PE bottles were used; each portion of Aquil was sterilized for 10 minutes in total. After that Aquil was transferred to acid washed 4L PE culturing vessels, where the cultures were going to be placed.

Three major nutrient stocks: phosphorous (P), nitrogen (N) and silicon (S), were made separately in acid washed PE 1L bottles (Table 3-2). Same as for Aquil, the following cleaning procedures were used for these stocks: chelexing and microwave sterilization.

Additionally, metal and vitamin stocks were made separately (Table 3-2). After all the preparation stocks are stored in a fridge: in darkness and low temperatures (approx. 4C) before it's time to add them to the medium. Right before addition of stocks, they are filter sterilized (0,2 μm). This method is used instead of microwaving in order to avoid potential negative influence of heat exposure.

Table 3-2 Nutrient enrichment of Aquil (adapted from (Andersen, 2005))

Nutrient	**Stock [g/L dH_2O]**	**Quantity per 4L [g]**	**Final concentration [M]**
	Major nutrients		
P ($NaH_2PO_4 \times H_2O$)	1,38	40	1×10^{-3}
N ($NaNO_3$)	8,50	40	1×10^{-2}
Si ($NaSiO_3 \times 9H_2O$)	28,40	4	1×10^{-4}
	Metal/metalloid nutrients		
EDTA	2,920		$1,00\times10^{-5}$
$ZnSO_4 \times 7H_2O$	0,0230		$7,97\times10^{-8}$
$MnCl_2 \times 4H_2O$	0,0240		$1,21\times10^{-7}$
$CoCl_2 \times 6H_2O$	0,0120		$5,03\times10^{-9}$
$Na_2MoO_4 \times 2H_2O$	0,0242		$1,00\times10^{-7}$
	Initial/Final stock [mL/L]		
$CuSO_4 \times 5H_2O$	4,9/1		$1,96\times10^{-8}$
Na_2SeO_3	1,9/1		$1,00\times10^{-8}$
	Vitamins		
Cyanocobalamin (B_{12})	5,5	2	$3,70\times10^{-10}$

The two conducted experiments differed in iron concentrations. In both of the experiments two iron compounds were used as exclusive iron sources: $FeCl_3$ and FeO(OH). These iron sources are chosen for this experiment due to differences in solubility and consequentially bioavailability in seawater environment. Ferric chloride ($FeCl_3$) is considered soluble in water: 74,4 g/100 cc (solvent volume) at 0°C and 535,7 g/100 cc at 100°C (PubChem, 2020). On the other hand, FeO(OH) that is stable in oxic conditions and is considered insoluble in seawater

due to its crystalline structure (Raiswell *et al.*, 2008). Two iron stocks were made, one for each compound. For the second experiment with lower iron concentrations dilution of intermediate stock was achieved by decreasing concentrations from 0,1M to 0,001M levels. For the first experiment intermediate stock was not needed due to higher iron concentrations. Iron stocks were stored in darkness and cold temperatures (approx. 4C) and were kept in a low pH solution (0,01 M HCl) to facilitate solubility. Filter sterilized iron stock were added directly to designated culturing bottles together with other stocks. Added quantities and final concentrations of iron in both experiments are presented below (Table 3-3).

Table 3-3 Iron enrichments of the medium for both experiments

Iron compound	**Final stock concentration [M]**		**Quantity per 4L [mL]**		**Final concentration [M]**	
	Exp. 1	**Exp. 2**	**Exp. 1**	**Exp. 2**	**Exp. 1**	**Exp. 2**
$FeCl_3 \times 6H_2O$	0,1	0,001	0,2	0,4	$0{,}50 \times 10^{-5}$	$1{,}00 \times 10^{-7}$
FeO(OH)	0,1	0,001	0,2	0,4	$0{,}50 \times 10^{-5}$	$1{,}00 \times 10^{-7}$

3.3 Culture addition

All cultures for the experiment were provided by Erland Årstøl from Department of Biotechnology and Food Science at NTNU. Originally cultures were grown at the Department of Biotechnology and Food Science in AA+ medium, which is high in nutrients including iron to sustain rapid growth (Vogel, Lale and Hohmann-Marriott, 2017). Before the experiment cell cultures were centrifuged and washed three times to remove medium and traces of iron from the outside of cells (Vogel, 2019). After that cells were resuspended in previously described Aquil medium. A volume of 40 mL of each culture (with an OD of approximately 1) was added to corresponding culturing vessels.

3.4 Experimental setup

The experiment was conducted in two parts, each 3 weeks long with two different iron concentrations. During the first experiment original iron concentration was 5 µM, during the second – 100 nM. For the experiment $FeCl_3$ and FeO(OH) (goethite) were used as exclusive iron sources, each in WT, ΔA2804 and ΔSidOP cultures (Fig. 3-1). In total, six 4 L bottles were set in the clean laboratory space under constant light (LED lamps, total 70 $\mu Em^{-2}s^{-1}$ measured from inside the bottle) and temperature (22-23 °C) conditions.

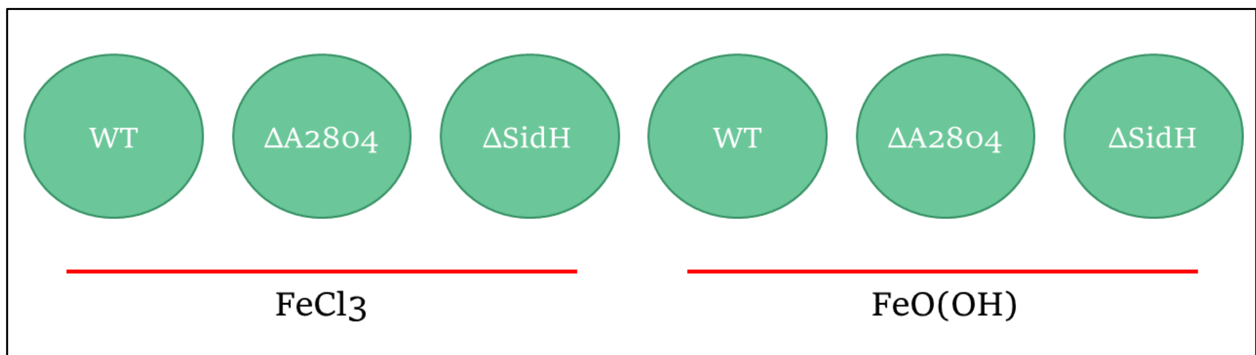


Fig. 3-1 Schematic representation of experimental layout

An air bubbling system was implemented to properly aerate the water while keeping the bottles closed to minimize contamination. As shown in Fig. 3-2, air pump was connected to an acid cleaned 1 L bottle with Milli-Q water through acid cleaned tubing. Additional pieces of tubing were used to connect the 1 L bottle to the main culture bottle through a 0,2 µm polycarbonate membrane filter (Sartorius Stedim) to minimize biological and trace metal contamination. Both bottles had caps with holes for the tubing that were sealed with parafilm after the 'bubbling system' was installed to prevent unfiltered air from entering and possibly impacting iron concentrations. Sampling tubes were installed together with the system. The air pumps were working through the entirety of the experiment to maintain constant levels of oxygen. Each culture bottle was equipped with its own 'bubbling system'. The systems were checked daily to assess the situation and were adjusted if needed.

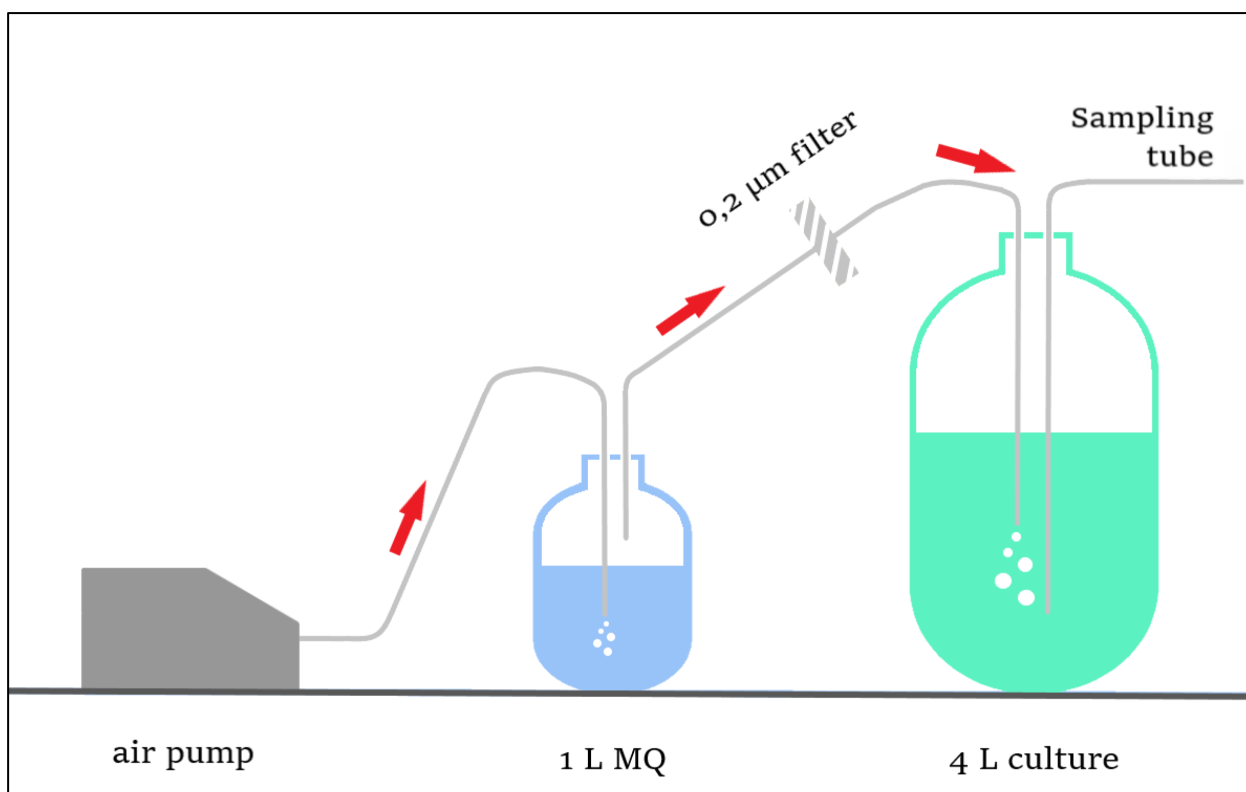


Fig. 3-2 Schematic representation of bubble aeration system used during the experiment. Red arrows show direction of the air flow.

3.5 Sampling procedure

Sampling was conducted in a way that would minimize contamination and cross-contamination of cultures. Each bottle was equipped with a sampling tube, the tip of which was stored in a plastic Ziplock bag when not used. Before each sampling culturing bottles were shaken to homogenize the medium. A peristaltic pump was used for sampling.

Samples were collected in acid-washed 50 mL metal free PE vials. Each culture had 3 designated vials that were rinsed 3 times with MQ before and after each sampling.

3.5.1 Particulate iron (PFe)

Particulate iron samples were collected from each bottle and then prepared by filtration. Nalgene vacuum filter flasks (Thermo Scientific) were used together with 47 mm in diameter 0,2 µm polycarbonate filter (Sartorius Stedim). Filtering system included a peristaltic pump connected to flasks via acid cleaned tubing. A laminar flow clean hood was used to conduct

filtering and store necessary equipment. After each sample vacuum filter flasks were rinsed with MQ.

Filters were put into ca 1 M UP HCL solution 24 hours before sampling. Before usage, each filter was rinsed thoroughly with MQ water from both sides using plastic tweezers and placed onto the filter holder. After the flask was assembled, sample was added to the top and was filtered through using the peristaltic pump. Sample volume varied from 10 to 25 mL depending on the sample density and was always recorded (lower culture density requires higher sample volume to provide enough material for analysis). From each culture samples were collected in triplicates to minimize influence of possible contamination by excluding outliers. Used filters were folded twice and put into acid clean petri dishes that were later covered in parafilm to prevent opening. All petri dishes from each sampling day were stored together in double plastic bags and put into a freezer until further steps. Blank filters were treated the same way as samples, i.e. were stored in the same container for 24 hours before sampling (total 4 filters).

3.5.2 Intercellular iron (InFe)

Intracellular iron sample preparation is similar to particulate in terms of filtration but requires additional steps to separate cells from extracellular iron present in the sample. Sample was not fully filtered with approx. 5 mL left on the filter. At this stage 5 ml of oxalate solution was added. After letting it sit for 5 minutes, filtration was continued until all liquid passed through the filter. The final step was addition of 1,5 mL of NaCl solution 10 times letting the previous portion filter through before adding the next (15 mL in total). Filter was then folded twice and is put into an acid-washed petri dish. Blank filters were treated the same way as samples, i.e. were stored in the same container, washed with oxalate and rinsed with NaCl (total 4 filters).

For oxalate wash EDTA-oxalate solution is made from 100 mM disodium oxalate, 50mM disodium EDTA and 0,3 M NaCl with 0,01 M KCl at pH 7 and stored in 1L PE collapsible bottle inside the clean hood (Tang and Morel, 2006). Afterwards 0,6 M NaCl solution is used for rinsing (Tang and Morel, 2006).

3.5.3 Total iron (TFe)

Samples for total iron were collected in 15 mL metal free tubes. Firstly, tubes were rinsed with sample 3 times. After that the sample could be collected: from each culture 3 samples were collected each time. They were acidified with concentrated Ultrapure HNO_3 to pH <2 and later

analyzed with HR ICP-MS. Acidification is conducted in order to avoid iron adsorption onto the inner walls of the vials and keep it in the solution.

3.6 Optical density (growth)

In order to monitor cultures' growth UV-Vis spectrophotometer (ultraviolet – visible) was used each sampling. It allowed estimation of optical density (OD) of the sample by measuring light scattering and absorbance at wavelength 730 nm (OD_{730}) that is often used in cyanobacteria research (Skubatz and Bendich, 1990; Hu, Westerhoff and Vermaas, 2000; Vogel, Lale and Hohmann-Marriott, 2017). The idea behind spectrophotometry is measurement of optical density of a sample by calculating difference in light intensity before and after it passes through the sample. OD was recorded on a SPECTRONIC 200 E spectrophotometer (Thermo Scientific). A calibration curve for OD_{730} has previously been made by Anne Ilse Maria Vogel (Department of Biotechnology, PhotoSynLab, NTNU, Trondheim) by running a series of cultures with known OD values through a cell counter (hemocytometry), to provide an equation where number of cells can be calculated from measured OD_{730} (Vogel, Lale and Hohmann-Marriott, 2017).

$$Y = 5\times10^{8}X+1\times10^{8}, \qquad (5)$$

Where Y is number of cells, X is OD_{730}.

The final value was calculated as an average of the three replicates for each culture.

3.7 Sample analysis

Before sample analysis, acid digestion of collected filters was conducted using Milestone UltraCLAVE. Acid digestion is used to decompose matrix by adding acid, increasing temperature and pressure (Rohr and Trepp, 1996). It is often used for trace metal analysis. UltraCLAVE is based on high-pressure autoclave design at increasing temperature and pressure that can reach 260C and 2s00 bar correspondingly (Milestone S.r.l.)..

Each filter was placed in a Teflon vial with addition of 5 mL 50% UP HNO_3. Each round consisted of 40 samples, 3 of those were UltraCLAVE blanks and contained only acid. Samples were run in the UltraCLAVE for 150 min. When samples cooled down, previously rinsed Teflon dilution bottle was used to dilute the samples. After sample transfer Teflon bottles were

rinsed twice with MQ, each time they were emptied into the dilution bottle. Dilution weight of each sample was brought to approx. 50 g (calculated based on original acid volume). After closing and carefully shaking the bottle, sample was transferred into a 15 mL ICP-MS vial with the first portion of the sample used for rinsing the vial. Dilution bottle was rinsed 3 times with MQ after each sample. All total, particulate and intercellular iron samples were analyzed by High Resolution ICP-MS.

3.8 Data processing

ICP-MS results were corrected for blanks and calculated back to original sample volumes. Mean values and standard deviation were calculated for each set of samples (triplicates). Outlier elimination was conducted using Grubb's test (Grubbs, 1969):

$$T = |\bar{x} - x|/s, \qquad (6)$$

Where $\bar{x}$ - mean, x – value, s – standard deviation, T – test criterion.

Calculations and results are presented in Appendix B. Additionally, statistical significance was evaluated using two-way ANOVA and Tukey's range tests (Appendix F)

CHAPTER 4 RESULTS

During both experiments on each sampling day photos of cultures were taken in order to track and compare changes in opacity and color (Fig.4-1 – 4-2).

Fig.4-1 Photographs of cultures taken on each sampling day during experiment 1

Fig.4-2 Photographs of cultures taken on each sampling day during experiment 2

4.1 Cell concentrations (OD_{730})

Optical density was measured in triplicates in 1 mL samples collected each sampling day. These values were recalculated using a calibration equation (5), mentioned in previous chapter, providing cell concentration in [№ of cells mL^{-1}]. Because of the formula, $1{,}00\times10^{8}$ is used as

the lower limit and stands for zero concentration of cells. Calculations are presented in Appendix E. All cell concentrations are presented in Table 4-1 in **10^8[№ of cells mL^{-1}]**. For simplicity units will be omitted in the following paragraphs. Results of two-way ANOVA with multiple comparisons (Tukey's tests) analyses are presented in Appendix F.

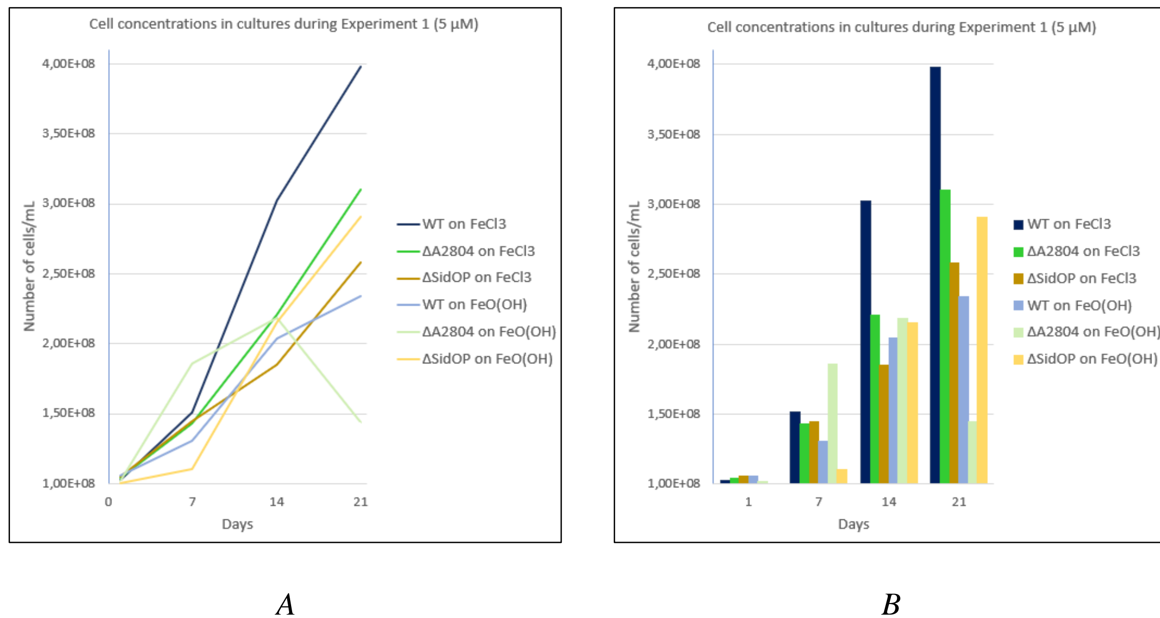
Table 4-1 Mean cell concentrations in cultures during experiments

Day	**Cell concentration, mean±SD 10^8[№ of cells mL^{-1}]**					
	$FeCl_3$			**FeO(OH)**		
	WT	**ΔA2804**	**ΔSidOP**	**WT**	**ΔA2804**	**ΔSidOP**
	Experiment 1 (5 µM)					
1	1,028±0,006	1,042±0,002	1,053±0,010	1,055±0,008	1,020±0,000	1,000±0,000
7	1,512±0,006	1,430±0,012	1,448±0,005	1,305±0,004	1,858±0,019	1,102±0,002
14	3,025±0,005	2,210±0,008	1,847±0,006	2,040±0,007	2,183±0,012	2,155±0,015
21	3,980±0,012	3,100±0,016	2,580±0,008	2,338±0,008	1,442±0,006	2,905±0,004
	Experiment 2 (100 nM)					
1	1,015±0,000	1,028±0,002	1,025±0,000	1,063±0,002	1,047±0,002	1,022±0,002
7	1,158±0,008	1,287±0,002	1,013±0,002	1,025±0,000	1,042±0,002	1,023±0,002
14	1,328±0,002	1,293±0,002	1,025±0,000	1,028±0,005	1,063±0,005	1,000±0,000
21	1,942±0,002	2,000±0,004	1,065±0,000	1,077±0,002	1,080±0,004	1,000±0,000

Graphs showing changes in cell concentrations in different cultures throughout the experiments are presented below (Fig4-3 – 4-4). On the left (A) there are line graphs that show growth for each culture, while on the right (B) there are bar graphs with standard deviation.

4.1.1 Experiment 1 (5 µM)

From day 1 to day 21 all cultures show increase in cell concentration, except for ΔA2804 on goethite, that shows drastic decline from day 14 to 21 (Table 4-1, Fig. 4-3).



A *B*

Fig. 4-3 Graphical representation of mean cell concentrations during experiment 1 (5 µM)

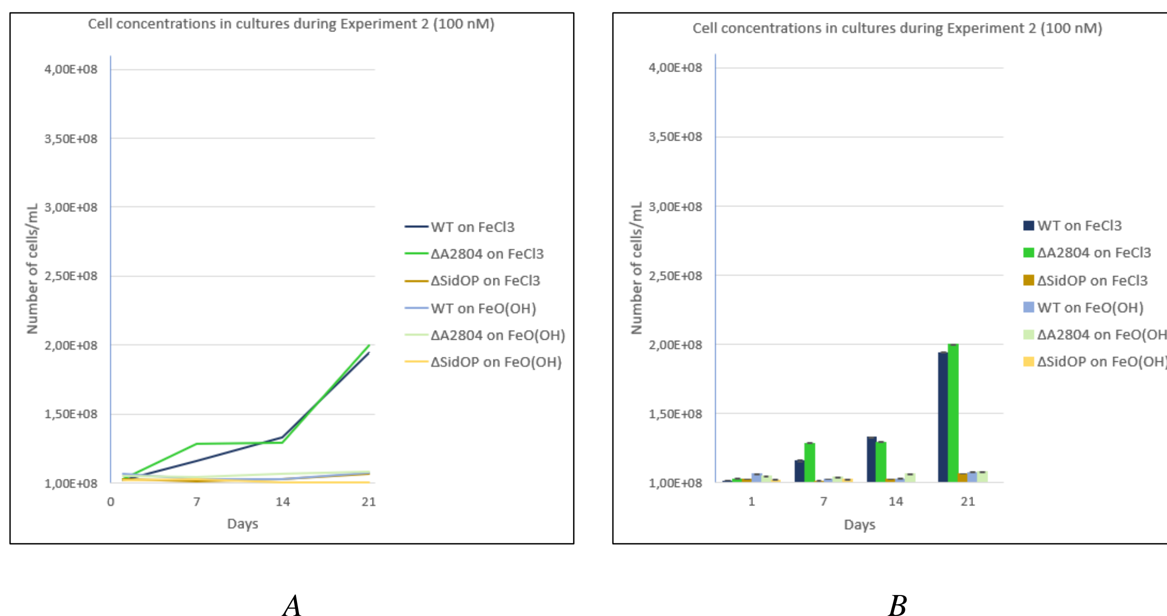
<u>$FeCl_3$.</u> Wild type culture on $FeCl_3$ shows steady increase from day 1 to day 7: from 1,028±0,006 to 1,512±0,006; and continues similar trend towards days 14 and 21, reaching 3,025±0,005 and 3,980±0,012 correspondingly. On days 14 and 21 wild type culture demonstrates maximum mean concentrations for those sampling days. On day 21 WT culture shows absolute maximum among mean cell concentrations, that is observed during both experiments. ΔA2804 culture starts with 1,042±0,002 on day 1 and shows steady increase throughout the experiment: to 1,430±0,012 on day 7 and 2,210±0,008 on day 14. By the end of the experiment this culture shows second highest cell concentration: 3,100±0,016. ΔSidOP culture on $FeCl_3$shows slower increase in concentration, starting with 1,053±0,010 on day 1. By day 7 this culture shows similar trend as ΔA2804 with slightly higher concentrations (1,448±0,005), but by day 14 is slowing down (1,847±0,006) and by the end of the experiment (2,580±0,008) doesn't reach the level of previously described cultures.

<u>FeO(OH).</u> Wild type culture on goethite shows slower growth, compared to the same culture on FeCl3. By day 7 it reaches 1,305±0,004, which is lower than most cultures in this experiment (except for ΔSidOP on goethite). By day 14 it shows a more noticeable increase up to 2,040±0,007 and surpasses ΔA2804 on FeCl3. However by day 21 it slows down and reaches

2,338±0,008. ΔA2804 culture on goethite shows drastic increase and by day 7 reaches 1,858±0,019 which is the maximum mean concentration for that sampling day. After that rapid increase culture slows down to 2,183±0,012 and is surpassed by WT and ΔA2804 on FeCl3. By day 21 this culture shows obvious decline in cell concentration and is the only culture during this experiment to do so. By the end of the experiment concentration drops to 1,442±0,006, which is the minimum for day 21. ΔSidOP culture shows the least growth between days 1 and 7 and only reaches 1,102±0,002. By day 14 it shows a drastic leap up to 2,155±0,015 and catches up with ΔA2804 cultures on both iron sources. By the end of the experiment this culture shows the third highest concentration for day 21: 2,905±0,004, surpassing the rest of the cultures on goethite and ΔSidOP on FeCl3.

4.1.2 Experiment 2 (100 nM)

Two cultures show noticeable growth during the second experiment: wild type and ΔA2804 cultures on FeCl3 (Fig.4-4). ΔSidOP culture on goethite shows zero concentrations in the second half of the experiment.



A *B*

Fig. 4-4 Graphical representation of cell concentrations during experiment 2 (100 nM)

FeCl3. WT culture on $FeCl_3$ shows visible increase throughout the experiment. Between days 1 and 14 concentration slowly grows reaching 1,158±0,008 by day 7 and 1,328±0,002 by day 14. At the end of the experiment it shows a steeper increase up to 1,942±0,002. This culture shows second highest values on days 7 and 21 of this experiment and the highest value on day

14. ΔA2804 culture shows the highest values on days 7 and 21, and is very close to WT type on day 14. At first, it shows a rapid increase to 1,287±0,002 by day 7. Between days 7 and 14 it barely shows any growth and only reaches 1,293±0,002. After that it goes back to a more rapid increase and even slightly surpasses WT by day 21 (2,000±0,004). ΔSidOP doesn't show any significant growth during the experiment. On day 1 and 14 it shows the same cell concentration (1,025±0,000) with a slight decline in between on day 7: 1,013±0,002. On day 21 it shows slight increase and reaches 1,065±0,000. This value is significantly lower than the concentrations observed in the other two cultures throughout the experiment (with exception of day 1).

FeO(OH). All cultures on goethite showed decline of cell concentration at some point of the experiment. All the changes (both negative and positive) in concentrations can be considered insignificant if compared to other cultures (WT and ΔA2804 on FeCl3). Wild type culture shows a decline by day 7, going from 1,063±0,002 to 1,025±0,000. Between days 7 and 14 change in concentration is barely noticeable as it reaches 1,028±0,005. By day 21 it goes up to 1,077±0,002. Cell concentration in ΔA2804 culture between days 1 and 7 is almost constant going from 1,047±0,002 to 1,042±0,002. There is a slight increase after that and it reaches 1,063±0,005 by day 14 and 1,080±0,004 by day 21. The latter is the highest concentration observed in cultures on goethite during the second experiment. ΔSidOP on goethite shows the least growth during the second experiment. It is stable between days 1 and 7 (1,022±0,002 and 1,023±0,002 correspondingly) and after that drops to 0 values until the end of the experiment. It is the only culture to reach absolute 0 values during both of the experiments, however the rest of goethite cultures also show very low concentrations.

4.2 Iron concentrations

PFe and InFe results were corrected for blanks: filter blanks with integrated UltraCLAVE blanks (Table 4-2). Based on the results, filter blanks and oxalate-washed filter blanks showed no significant differences in Fe concentrations, and thus a mean of all blank samples was used as LOD (limit of detection). Values below that were considered 0. After that results were multiplied by final volume of dilution during digestion procedure and divided by original filtered volume. These values [µg/L] were recalculated into [nM] concentrations by dividing them by atomic mass of ^{56}Fe and multiplying by 1000.

Table 4-2 Iron concentrations in blank samples

Blank ID	**^{56}Fe concentration, µg/L**
Blank filter sample 1	0,48
Blank filter sample 2	0,50
Blank filter sample 3	1,25
Blank filter sample 4	1,31
Blank oxalate filter sample 1	0,66
Blank oxalate filter sample 2	1,42
Blank oxalate filter sample 3	0,48
Blank oxalate filter sample 4	0,48
Minimum	*0,48*
Maximum	*1,42*
Standard deviation, %	*51,7*
Mean	***0,82***

Outliers were eliminated on the basis of Grubb's test: for sample size of 3 critical value is T=1,15. Means were recalculated with remaining concentrations.

For the results to be more comparable, iron concentrations in the solution were recalculated into [nM/cell] dividing the value by the number of cells. That way cultures with different growth rates became comparable in terms of iron acquisition. Number of cells in each culture had to be calculated by multiplying cell concentration value by the total culture volume in [mL]. Each sampling day 200 mL of each culture were collected for analyses.

Standard deviation for PFe and InFe of each culture at each sampling day were calculated and plotted together with mean values. Finally, due to very low concentrations, normalization of values had to be conducted for simplicity of representation and comparison. Final unit of measurement for PFe and InFe is **10^{-12}[nM/cell]**. For simplicity units will be omitted in the following paragraphs. Final results are presented in Tables 4-3 and 4-4. Results of two-way ANOVA with multiple comparisons (Tukey's tests) analyses are presented in Appendix F.

Table 4-3 Mean particulate iron concentrations in cultures during two experiments

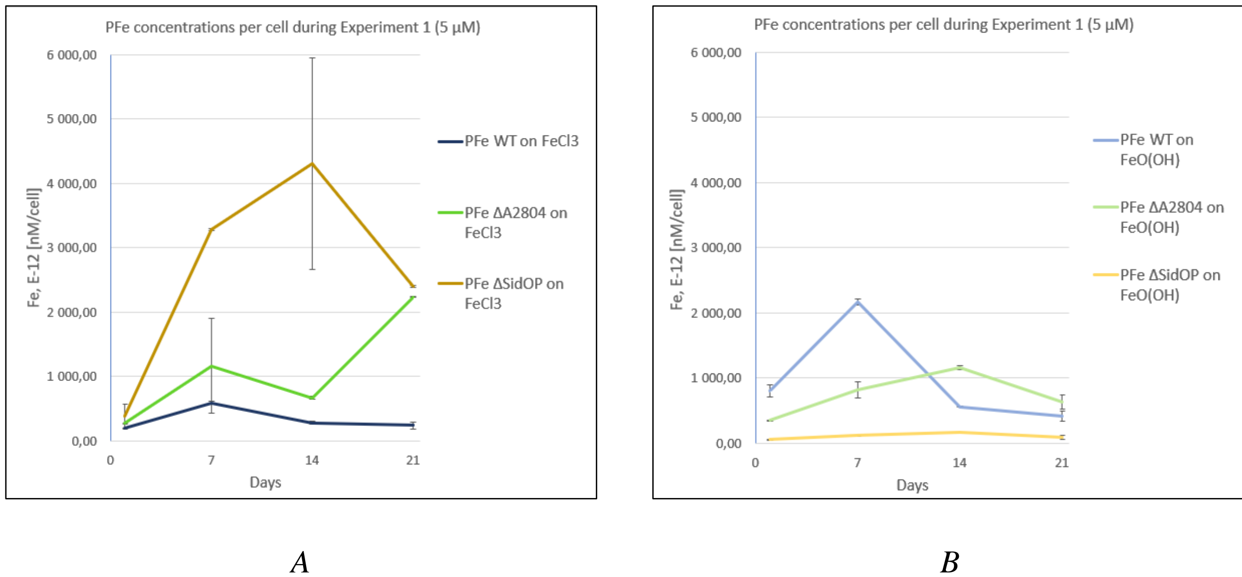
Day	Particulate iron concentration, mean±SD 10^{-12}[nM $cell^{-1}$]					
	$FeCl_3$			FeO(OH)		
	WT	ΔA2804	ΔSidOP	WT	ΔA2804	ΔSidOP
	Experiment 1 (5 µM)					
1	200,1±4,1	283,0±2,9	387,3±186,1	809,3±90,5	355,0±2,5	59,0±0,1
7	593,1±20,1	1169,3±738,1	3289,8±14,7	2165,9±51,4	820,6±131,1	125,1±3,7
14	283,1±26,2	669,8±22,5	4302,4±1643,0	566,1±11,8	1168,63±30,2	169,0±0,4
21	245,5±56,0	2236,4±11,0	2402,1±21,6	420,8±80,3	629,79±109,3	95,1±28,7
	Experiment 2 (100 nM)					
1	149,2±45,8	17,3±3,1	31,3±0,4	38,7±1,8	0,0±0,0	11,1±0,9
7	46,4±21,5	33,3±12,6	25,9±15,8	0,0±0,0	0,0±0,0	0,0±0,0
14	13,8±0,2	17,6±3,3	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0
21	19,9±1,3	38,6±0,6	22,8±17,4	0,0±0,0	0,0±0,0	0,0±0,0

Table 4-4 Mean intercellular iron concentrations in cultures during two experiments

Day	Intercellular iron concentration, mean±SD 10^{-12}[nM $cell^{-1}$]					
	$FeCl_3$			FeO(OH)		
	WT	ΔA2804	ΔSidOP	WT	ΔA2804	ΔSidOP
	Experiment 1 (5 µM)					
1	250,5±139,5	10,2±2,7	53,7±6,0	70,1±21,3	146,4±129,5	51,3±0,9
7	105,3±2,5	155,6±9,3	223,8±18,8	234,2±47,9	325,4±23,5	124,7±3,5
14	217,8±49,1	196,9±15,7	106,2±30,0	198,0±31,3	311,2±45,0	129,2±79,0
21	156,6±0,7	237,7±9,7	121,0±6,4	159,7±1,7	27,3±0,9	65,2±1,1
	Experiment 2 (100 nM)					
1	45,7±25,8	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0
7	19,5±13,3	47,1±6,1	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0
14	3,7±1,0	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0
21	13,2±5,7	16,4±5,3	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0

4.2.1 Experiment 1 (5 μM)

Particulate iron



A *B*

Fig. 4-5 Graphical representation of PFe concentrations during experiment 1 (5 μM) on $FeCl_3$ (A) and FeO(OH) (B) as iron source

FeCl3. Particulate iron concentrations on $FeCl_3$ show growth at some point during the experiment. Wild type culture shows the least growth of PFe with slight peak (593,1±20,1) on day 7 from 200,1±4,1 on day 1. After that this culture shows decline and reaches 283,1±26,2 and 245,5±56,0 by days 14 and 21 correspondingly. ΔA2804 culture shows a more prominent increase throughout the experiment. Starting with 283,0±2,9 on day 1 it reaches its first peak on day 7 (1169,3±738,1). After a decrease to 669,8±22,5 on day 14 this culture shows its maximum on day 21 by reaching 2236,4±11,0. At the end of the experiment it reaches levels similar to ΔSidOP on day 21. ΔA2804 culture on $FeCl_3$ is the only one who shows growth by day 21, the rest of the cultures show some decline by the end of the experiment. ΔSidOP culture shows the highest particulate iron concentrations out of all cultures during the first experiment. It goes from 387,3±186,1 to 3289,8±14,7 between days 1 and 7, showing ten times growth of concentrations in one week. After that it reaches its maximum on day 14: 4302,4±1643,0. By the end of the experiment this culture shows PFe concentration similar to the one showed by ΔA2804 culture: 2402,1±21,6.

FeO(OH). Particulate iron concentrations in cultures grown on goethite are mostly than in cultures on $FeCl_3$ with exception of wild type. WT culture shows more significant increase of PFe concentrations, than the same culture on the other iron source. It starts with higher concentration on day 1 (809,3±90,5) and goes up to 2165,9±51,4 by day 7, which is the maximum mean concentration of PFe on goethite for this experiment. After that it shows a decline and reaches 566,1±11,8 and 420,8±80,3 by days 14 and 21 correspondingly. ΔA2804 culture on goethite shows a steady increase between days 1 and 7: from 355,0±2,5 to 820,6±131,1 and reaches the second highest value out of goethite cultures by day 14 (1168,63±30,2). After that concentrations decline and by day 21 the mean PFe concentration is 629,79±109,3, which is higher than the one observed for the WT culture. ΔSidOP culture on goethite shows the lowest concentrations of PFe throughout the whole experiment. It starts from 59,0±0,1 and then shows steady increase between days 7 and 14: 125,1±3,7 and 169,0±0,4 correspondingly. By day 21 concentration drops to 95,1±28,7.

Intercellular iron

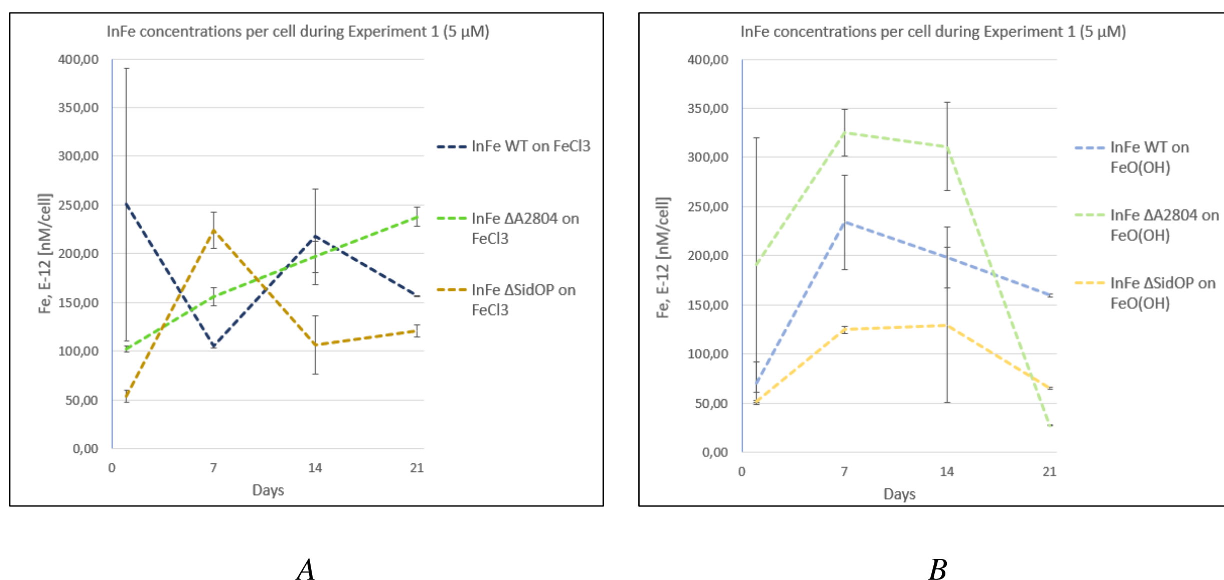


Fig. 4-6 Graphical representation of InFe concentrations during experiment 1 (5 µM) on $FeCl_3$ (A) and FeO(OH) (B) as iron source

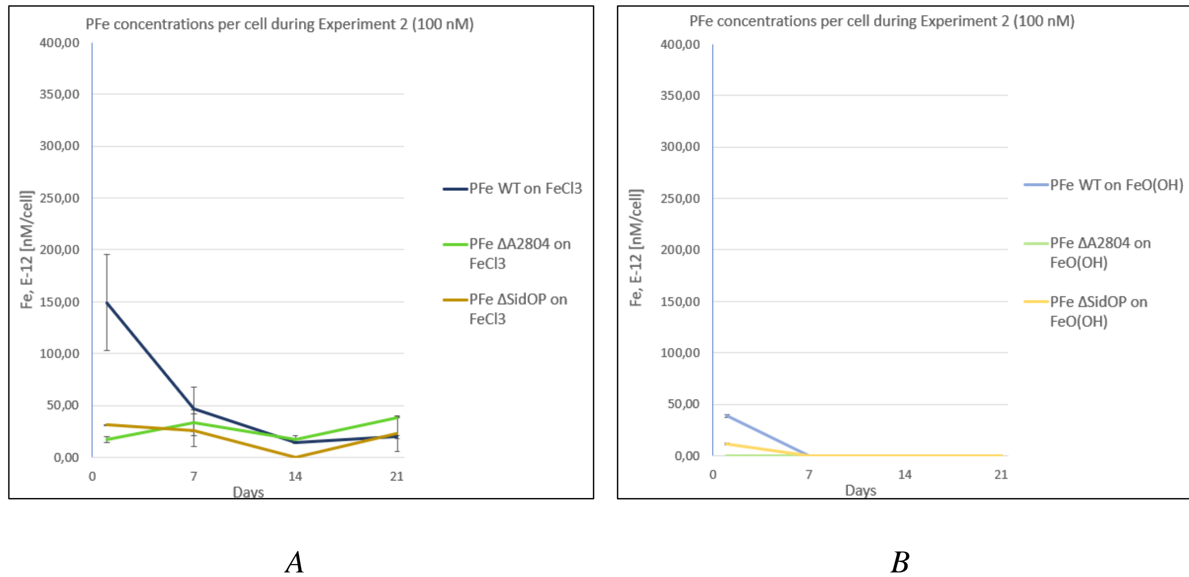
FeCl3. Intercellular iron concentrations in wild type culture show fluctuations throughout the experiment. There is a decrease between days 1 and 7 from 250,5±139,5 to 105,3±2,5. After that by day 14 it almost reaches the same levels as on day 1: 217,8±49,1. By day 21 InFe concentration in WT culture show slight decrease down to 156,6±0,7. In ΔA2804 culture InFe

concentrations show a very steady increase throughout the experiment starting from 10,2±2,7 on day 1, reaching 155,6±9,3 by day 7 and then going up to 196,9±15,7 and 237,7±9,7 by days 14 and 21 correspondingly. This is the highest concentration among the cultures grown on $FeCl_3$ on day 21 and is very close to the highest InFe value in these cultures overall, that was observed in WT culture on day 1. ΔSidOP shows fluctuations of concentrations opposite to the one observed in WT culture with increase between days 1 and 7 from 53,7±6,0 to 223,8±18,8. By day 14 there is a decrease (106,2±30,0) followed by a slight increase up to 121,0±6,4 at the end of the experiment. All of the values for these three cultures are observed in the same range and show peaks on different days: WT – on days 1 and 14, ΔA2804 – on day 21 and ΔSidOP – 7. Because of that it is hard to identify the culture with the highest InFe content. ΔA2804 culture shows the least fluctuations together with the maximum by the end of the experiment, while InFe concentrations in other two cultures fluctuate significantly. There is no common trend shown by cultures on FeCl3.

FeO(OH). Intercellular iron concentrations in wild type culture grown on goethite shows an increase between days 1 and 7 from 70,1±21,3 to 234,2±47,9 and consequent a slight decrease by day 14 and 21: 198,0±31,3 and 159,7±1,7 correspondingly. The latter is the maximum mean concentration observed in these cultures on day 21. In ΔA2804 culture the highest InFe values are observed in cultures on goethite on days 1 to 14. Starting from 146,4±129,5 on day 1 this culture shows increase by day 7 up to 325,4±23,5. On day 14 InFe concentration is 311,2±45,0 which is very close to its previous value, showing no significant change between days 7 and 14. However by the end of the experiment InFe levels drop to 27,3±0,9, which is the lowest value observed throughout this experiment on both iron sources. ΔSidOP is the only culture that doesn't show a maximum on any of the sampling days. It starts from 51,3±0,9 on day 1 and reaches 124,7±3,5 by day 7. Like in case of ΔA2804, InFe concentrations between days 7 and 14 stay somewhat stable in ΔSidOP culture on goethite: on day 14 it is 129,2±79,0. After that a decrease in InFe concentration is observed going down to 65,2±1,1. There is a clear trend in all of the goethite cultures: an increase by day 7, relative stability between days 7 and 14 and a noticeable decline by day 21. The highest InFe concentrations are generally observed in ΔA2804 with exception of the drastic drop day 21. The lowest concentrations are shown by ΔSidOP culture (once again, not including day 21, where ΔA2804 shows the absolute minimum of the entire experiment).

4.2.2 Experiment 2 (100 nM)

Particulate iron



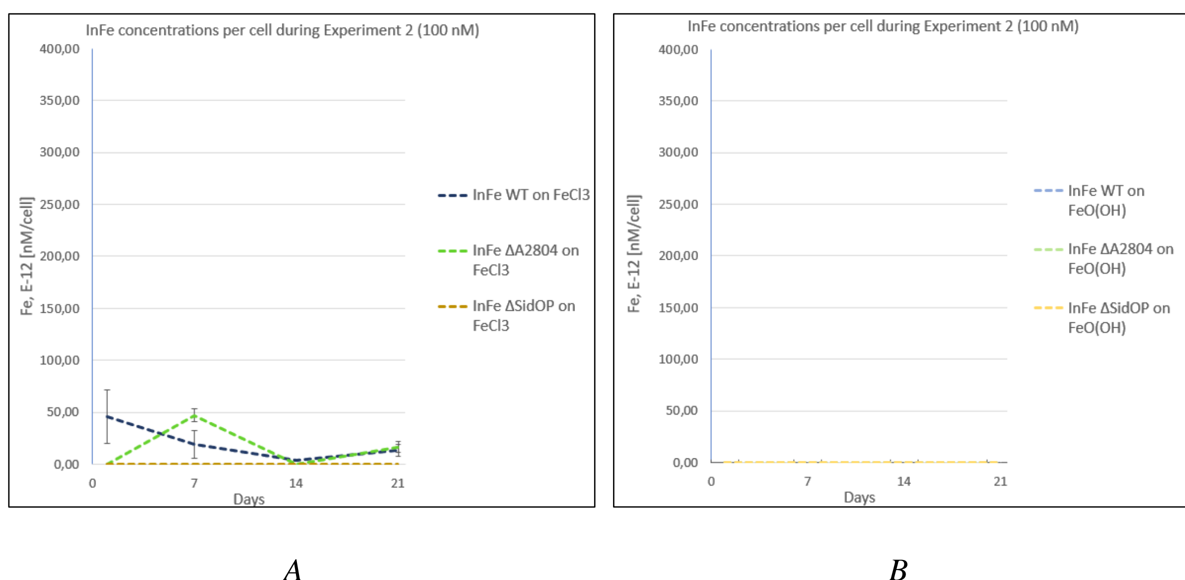
A *B*

Fig. 4-7 Graphical representation of PFe concentrations during experiment 2 (100 nM) on $FeCl_3$ (A) and FeO(OH) (B) as iron source

FeCl3. Wild type culture on $FeCl_3$ as iron source during the second experiment shows a steady decline of PFe concentrations, starting with 149,2±45,8 on day 1 and going down to 46,4±21,5 and 13,8±0,2 on days 7 and 14 correspondingly. It shows a slight increase in PFe levels by the end of the experiment going from 13,8±0,2 to 19,9±1,3 by day 21. At the beginning of the experiment it shows the highest PFe concentration, however on day 21 it reaches the minimum out of cultures grown on FeCl3. ΔA2804 culture shows a different trend with a wave-like profile. It starts with 17,3±3,1 on day 1 and reaches 33,3±12,6 by day 7. After that on day 14 it goes down to the same levels as on day 1 (17,6±3,3) and by day 21 goes up to concentration slightly higher than on day 7: 38,6±0,6. On day 21 it is the highest observed value among cultures on FeCl3. ΔSidOP is the only one out of $FeCl_3$ cultures that reaches concentration below the LOD (i.e. 0 concentration) during this experiment. Between days 1 and 7 it shows slight decline: from 31,3±0,4 to 25,9±15,8. By day 14 it shows a drastic drop to 0 levels. After that it goes back to levels observed on day 7 and reaches 22,8±17,4. With exception of day 14, this culture shows stable PFe concentrations throughout the experiment. WT culture is the only one that shows obvious decline throughout the experiment, while the other two, though demonstrating some fluctuations, still stay within a narrow range.

FeO(OH). Wild type culture on goethite shows drastic decrease of PFe concentrations. After day 1, when PFe concentration is 38,7±1,8, it drops to 0 levels (i.e. below LOD) and stays that way throughout the rest of the experiment (days 7 to 21). In case of ΔA2804 culture, PFe concentrations never go above LOD and stay 0 throughout the experiment. ΔSidOP shows a trend similar to WT culture: detectable concentration on day 1 (11,1±0,9) with 0 levels afterwards. The highest mean concentration of PFe in cultures on goethite in this experiment is the one observed in WT culture on day 1. All of the cultures, even the ones that show initially higher concentrations, show levels below LOD (i.e. 0 levels) throughout the experiment.

Intercellular iron



A *B*

Fig. 4-8 Graphical representation of InFe concentrations during experiment 2 (100 nM) on $FeCl_3$ (A) and FeO(OH) (B) as iron source

FeCl3. Wild type culture is the only one in this experiment with concentrations of InFe above LOD throughout the whole experiment. It starts with 45,7±25,8 on day 1 going down to 19,5±13,3 by day 7. On day 14 it reaches its lowest point (3,7±1,0) and then goes up to 13,2±5,7. Overall it shows a decline from day 1 with the lowest concentration observed on day 14. ΔA2804 is the only other that shows any values above LOD. On day 1 it shows 0 concentration that later increases and reaches 47,1±6,1 by day 7. This is the maximum mean concentration of InFe observed during experiment 2. By day 14 it goes back to 0, showing another increase by the end of the experiment (16,4±5,3). In ΔSidOP culture InFe values do not go above LOD (i.e. 0 level) on any of sampling days. Both WT and ΔA2804 culture show the lowest concentrations

on day 14 with consequent increase by day 21. The difference between these cultures was observed within the first week of the experiment: WT starts with high concentration that goes down by day 7, while ΔA2804 culture starts with levels below LOD and reaches its maximum on day 7.

FeO(OH). All cultures grown on goethite show 0 levels of InFe throughout the second experiment.

CHAPTER 5 DISCUSSION

5.1 Influence of methodology

Due to specifics of trace metal analysis, methods for minimization of contamination were implemented during this experiment. However, because of the nature of said field and the low concentrations measured during the experiments, some discrepancy in data was still observed. For example, during experiment 2 in ΔA2804 culture at one point InFe values were slightly higher than PFe, which can be considered an error, since InFe is a part of the PFe pool. Based on statistical calculations, medial standard deviation between triplicates during both experiments was 8,8%, however values ranged from 0,2% to 88,5%, preventing some of the data from being used for quantitative analysis. Comparison of standard deviation values between two experiments is complicated by presence of Fe concentrations below LOD in experiment 2, reducing the size of dataset. However, presence of high standard deviation between triplicates in both experiments may indicate that contamination was not completely prevented during sample handling and processing. Another reason for observed high standard deviation values can be initial inconsistency in samples. Culturing bottles were thoroughly shaken before sampling; however, inconsistency in samples can be seen as an indicator of uneven distribution of cells within the bottle. Cells could have aggregated in the bottom or on the walls, depending on many factors including iron distribution in the bottle. Total iron samples were analyzed at the beginning and end of experiments in order to observe its dynamics and possibly detect contamination in the cultures. No culture showed increase in total iron concentrations, which would have indicated contamination. On the contrary, each of them showed a decline. The decline in TFe values can be related to precipitation of iron out of solution leading to its aggregation on surfaces, especially in case the crystalline and practically insoluble goethite.

Described possible sources of errors can be improved in future experiments. For example, contamination can be further minimized by conducting experiments in designated stationary clean laboratory space that is intended for this kind of research or by implementing additional measures. Moreover, laboratory shakers can be installed to create constant mixing within culturing bottles in order to prevent aggregation of cells on the surfaces.

Moreover, it is important to note the role of triplicates when it comes to sampling. In this case, triplicate samples (or method triplicates) showed how much iron concentrations can fluctuate in each culture and allowed the elimination of outliers due to discrepancies. A way of improving this system in future experiments would be to add a system of biological triplicates, i.e. having three cultures with similar parameters (mutation, iron source and concentration) instead of one. However, in case when, like in this project, such addition is unavailable, for the sake of trace metal analysis method triplicates are of higher priority. If biological triplicates were used instead of method triplicates, it would have been impossible to differentiate between effects of contamination and differences between biological triplicates. A system should thus consist of both biological and methodological triplicates if practically and financially viable.

5.2 Influence of iron concentration

5.2.1 Experiment 1 (5 μM)

Cultures in experiment 1 had initial 5 μM concentrations of iron. All cultures showed a positive trend in cell concentrations throughout the experiment, with the exception of ΔA2804 culture on goethite as iron source, where a drastic drop in cell concentration was observed between day 14 and day 21. However, in the first half of the experiment this culture still showed significant increase in cell concentrations. This means that all cultures were able to grow on both iron sources. This can be observed in results of statistical analyses, that show significant differences between cell concentrations in cultures on each sampling day. However, with previously observed doubling time of *Synechococcus* culture being ~ 2,6 to 4 hours in optimal conditions, we would expect higher cell concentrations by the end of the three-week-long experiment if all requirements of organisms were met (Ludwig and Bryant, 2011). This may be interpreted as an indicator of the culture experiencing limitation of growth. One of the reasons for that could be insufficient iron supply, that supported growth but at slower rate than under optimal conditions. Another important factor that may have impacted growth rate of cultures, is temperature. In laboratory conditions *Synechococcus* is normally grown in 33-38°C, however in this project temperatures were lower (22-23°C) (Ludwig and Bryant, 2011; Vogel, Lale and Hohmann-Marriott, 2017). This was necessary for conducting temperature-sensitive FIA analyses. Acclimation to lower temperatures may have slowed down the growth rate of cultures (Sakamoto and Bryant, 1997).

PFe concentrations in cultures during experiment 1 show increase from day 1 and peaks during the experiment, something that indicate the cultures' ability to accumulate iron on the outside of the cell. Increase of PFe concentrations at some point during the experiment is observed for both iron sources. Similar trends can be noted for InFe values. Despite showing different trends depending on iron source and mutation all cultures show ability to accumulate iron inside the cell during the experiment on high iron concentration (5 μM).

Another important factor in assessing response of organisms to iron conditions is the color of the cultures. During this experiment a 'healthy' green color was observed in WT cultures on both iron sources, while mutant cultures showed yellow undertones by the end of the experiment. Dependence of color on iron sources and mutations will be discussed in the next sections.

Overall, the iron concentration in experiment 1 can be considered favorable for cultures, since all of the cultures are able to show growth and accumulate InFe. However, even WT culture on bioavailable $FeCl_3$ shows slower increase in cell concentrations than previously observed under optimal conditions. That can be explained by the nature of studied species, that can be found in coastal environment and thus have higher iron requirements. However, there may be other factors slowing down growth, such as temperature conditions. These cultures were originally grown in high-temperature (33-38°C) environment with high iron concentrations (AA+ medium with 14,28 μM) (Vogel, Lale and Hohmann-Marriott, 2017). In this experiment they were put in lower temperature environment with less iron supply. It is difficult to single out the reason for such physiological response, thus we cannot eliminate presence of iron stress to some degree. It is also important to note that temperature was not adjusted during both experiments and thus can be considered constant and excluded as a factor when comparing two experiments. In conclusion, despite signs of limited growth in these conditions all cultures were able to successfully accumulate InFe and show significant increase in cell concentrations on both iron sources.

5.2.2 Experiment 2 (100 nM)

While in experiment 1 all cultures showed significant growth at some point, with most showing a positive trend throughout all three weeks, cultures in experiment 2 with 100 nM iron concentration show a different picture. During this experiment only two cultures demonstrated significant growth: WT and ΔA2804 on $FeCl_3$ as iron source. The rest of the cultures showed

barely any growth with ΔΔSidOP culture on goethite struggling the most, showing zero cell concentrations in the second half of the experiment. That means, that in these iron conditions only $FeCl_3$ was able to support any noticeable growth in cultures. ΔΔA2804 and WT reached very similar cell concentrations by the end of the experiment. That means, that not only iron source is defining the culture's survival, but that also the type of mutation comes into play.

PFe concentrations in cultures on $FeCl_3$ during experiment 2 demonstrate the following patterns: fluctuations with no particular trend in case of ΔSidOP and ΔA2804 cultures and a decrease in concentrations from day 1 as observed in WT culture. Despite Tukey's multiple comparisons test showing no significant changes in PFe concentrations from day 1 to day 21 in either of the $FeCl_3$ cultures, decrease of PFe in WT culture is the most pronounced observed trend that needs to be addressed. There can be several possible explanations for that. Firstly, there may have been higher iron concentrations in the original culture added to Aquil: in form of very strongly surface-bound PFe and as InFe. Another possible explanation can be made, that WT culture was able to initially accumulate PFe and InFe, however limiting iron conditions led to downregulation of genes responsible for photosynthetic machinery causing iron requirements to decrease.

PFe values on day 21 in WT and ΔSidOP cultures on $FeCl_3$ do not show significant differences, however, as was mentioned before, out of these two only in WT noticeable growth was observed. In order to explain this situation, we need to look at InFe values. Out of all $FeCl_3$ cultures ΔSidOP is the only one that has InFe concentrations below LOD throughout the experiment. Similar situation is observed in goethite cultures. That is an indicator of growth dependence on ability of organisms to accumulate iron inside the cell. Despite PFe values comparable to the rest of the $FeCl_3$ cultures, inability of ΔSidOP culture to acquire iron inside the cell prevented its growth. This will be discussed in more detail in the next sections.

Moreover, WT and ΔA2804 on $FeCl_3$ demonstrate no significant differences between their corresponding PFe and InFe values throughout the experiment. That is a sign of organisms internalizing iron right away without storing it on the outside. That can be a sign of these cultures being in fact limited by iron. This can be explained in comparison with experiment 1, where iron species and concentrations of other essential nutrients are the same, however because of higher iron supply cultures are able to grow more, than in experiment 2. This can be attributed to Liebig's law of the minimum that implies limiting nutrient to determine growth of

an organism despite sufficient supplies of other nutrients (de Baar, 1994). In this case comparison between two iron concentrations allows us to suggest iron as the limiting nutrient.

As mentioned above, color of culture can also be an indicator of its condition. In experiment 1 only WT culture was green, while others showed a yellow undertone that may indicate chlorosis. It has been mentioned in previous chapters, that chlorosis may be an indicator of nutrient-related stress (in this case, iron limitation), that is expressed through changes in photosynthetic pigment composition (Singh, McIntyre and Sherman, 2003). And although this will be discussed in more detail in following sections, an observation regarding this can be made. During experiment 2 the only two cultures that show color, are the ones that show growth and InFe accumulation: WT and ΔA2804 cultures on $FeCl_3$. However, in both cultures a yellow undertone is observed starting from day 7, when they obtain color. This is not the case in experiment 1, where WT culture stayed green throughout the experiment. Since these cultures are of the same type (wild type) and are grown on the same iron source ($FeCl_3$), the only factor that is different is iron concentration. This means that under lower iron concentrations WT culture is experiences iron stress to a higher degree that is observed through change of color and lower growth. This is not to say that WT on $FeCl_3$ culture in experiment 1 does not experience stress (which was explained above). The question is the degree of said stress.

Overall, experiment 2 showed a more defined iron limitation scenario compared to experiment 1. Lower iron concentration prevented most cultures from showing noticeable growth. Differences between iron sources become more distinct with WT and ΔA2804 on $FeCl_3$ being the only two cultures showing growth throughout the experiment. Cultures on goethite are unable to accumulate InFe and thus do not grow, which implies that in low concentrations goethite is practically unavailable for cultures, even WT. However, that was not the case during experiment 1, where all goethite cultures showed growth. Moreover, ΔSidOP culture did not show significant growth on both iron sources at low iron concentration, which indicates that there are differences in ways each mutation impacts ability of organisms to survive and acquire iron. It can be suggested, that decrease in iron concentration may amplify differences between types of cultures and iron sources. In order to establish these differences, observed influence of these parameters will be discussed below.

5.3 Influence of iron source

As mentioned above, iron source was observed to be one of the factors defining a culture's ability to grow. This effect was more visible under lower iron concentrations.

5.3.1 $FeCl_3$

In higher iron concentrations during experiment 1, the effect of iron source was not as visible as in experiment 2. However, cultures on more available $FeCl_3$ overall showed higher cell concentrations by the end of the experiment 1 with exception of ΔSidOP cultures. In case of ΔSidOP, culture on goethite showed higher concentrations compared to the same culture on $FeCl_3$. Despite these differences, all cultures still showed growth during experiment 1.

By day 21 WT on $FeCl_3$, which showed the highest growth, also showed the least difference between PFe and InFe concentrations per cell, while other cultures on the same iron source had a more statistically significant difference. In case of this culture, the reason behind it is unlikely to be related to iron source, but rather to its type and its behavior on said bioavailable iron source, since other cultures on $FeCl_3$ show a different picture. Moreover, the other two cultures showed quite similar PFe values on day 21, while in WT culture values were significantly lower.

During experiment 1 InFe values for cultures on both iron sources showed values in similar ranges. This means, that even in case of abundance of bioavailable iron source ($FeCl_3$) and a lot of surface-bound iron as observed in ΔA2804 and ΔSidOP cultures, organisms don't internalize more iron. A possible explanation of that can be related to capacity of iron transporters in membrane and overall rate of transportation to inside the cell (Shaked, Kustka and Morel, 2005). Another possible explanation could be fulfillment of iron requirements in $FeCl_3$ cultures that would take away the need for more iron. However, if that was the case, signs of stress, described in previous section, probably would have not been observed.

Another factor that suggests that iron source may play a less important role in environment with high iron concentration is color of cultures on both sources during experiment 1. It is visible, that no matter what source was used, WT cultures showed green color on all sampling days, while mutant cultures demonstrated yellow undertone by the end of the experiment.

During experiment 2 iron source seems to play a more important role in cultures' survival. The only two cultures to show accumulation of InFe and subsequently any significant growth are WT and ΔA2804 cultures on $FeCl_3$. Importance of iron source here comes from $FeCl_3$ being more soluble and its ability to form more available Fe-EDTA complexes that keep iron in solution. That means, that despite low concentrations, iron is still more available.

However, as mentioned before, these cultures, although doing better than the rest, are still experiencing iron stress, which is indicated by lower growth compared to experiment 1 and yellow undertones observed from day 7.

5.3.2 FeO(OH)

Despite goethite being able to sustain growth of all three cultures during experiment 1, differences between same types of cultures on both sources still indicate its lower bioavailability. The case with ΔSidOP cultures, addressed above, is unlikely to be caused solely by iron source, since the other two cultures still grow more on $FeCl_3$.

Cultures on goethite all show no significant difference between PFe and InFe values at the end of experiment 1. Since they are different cultures with iron source as the common factor, it can be suggested that this is due to the characteristics of goethite, that is poorly soluble in oxic conditions and thus is less available (Raiswell *et al.*, 2008). This can be considered a sign of organisms not storing excess iron on the outside but rather internalizing it right away.

During experiment 2 differences between iron sources became more visible. There is no accumulation of PFe and InFe observed for goethite cultures during the experiment with exception of initial PFe values above LOD in WT and ΔSidOP on day 1 that were addressed in previous section. This subsequently leads to no significant growth shown by these cultures.

In experiment 1 the main consequence of changes in iron sources is difference in growth (e.g. cell concentrations by day 21), however, as mentioned above, some exceptions were observed (i.e. ΔSidOP culture). Overall, influence of iron source was not as visible in experiment 1 compared to experiment 2, where goethite cultures were unable to accumulate InFe and show noticeable growth. That can be connected to their inability to acquire a less available iron source when it is present in lower concentrations. There are also differences observed between different types of cultures within these trends. Some of them have been mentioned above. They will be addressed in more detail in the following section.

5.4 Influence of mutation

5.4.1 Wild type culture

Wild type culture consists of genetically unmodified organisms. They show natural response of *Synechococcus* to different iron conditions in laboratory-controlled environment. The culture should be able to utilize both siderophore-mediated and reductive iron uptake mechanisms. In most cases WT culture shows the highest growth by the end of the experiment, compared to other cultures grown on the same iron source. An exception was observed for WT culture on goethite during experiment 1, where its final cell concentration was lower than the one observed in ΔSidOP culture on the same iron source. As mentioned before, PFe values (and difference between PFe and InFe) in WT culture on $FeCl_3$ are the lowest out of all $FeCl_3$ cultures during experiment 1.

WT cultures on both iron sources during experiment 1 are the only cultures that stay green throughout the experiment without showing yellow undertones. However, during experiment 2 with lower iron concentrations WT on $FeCl_3$ showed yellow color from day 7 (when it became opaque). This means, that chlorosis is not only caused by type of mutation, but also by iron concentration. This was discussed in more detail in previous sections.

Yellow undertone and slower growth shown by WT culture on $FeCl_3$ in experiment 2 as compared to the same culture in experiment 1, suggests that it is experiencing a higher degree of iron limitation. It has been studied, that cyanobacteria under iron stress is down-regulating genes responsible for photosynthetic machinery and up-regulating genes in charge of iron acquisition (Fraser *et al.*, 2013)(González *et al.*, 2018). In case of experiment 1, all cultures showed significant growth, no matter the mutation. This leads to a possible conclusion that ability to release siderophores was not a defining factor at higher iron concentration since even cultures that were unable to do that managed to grow on iron in close proximity to the cell. In case of experiment 2, however, siderophores may have become more important. However, since release of siderophores requires a lot of energy and is sometimes referred to as wasteful, a big portion of energy may be lost to that thus limiting growth of cultures (Toulza *et al.*, 2012; Vogel, 2019). Moreover, WT culture on lower concentrations of goethite did not show noticeable growth even though it had an advantage compared to mutant cultures. That may be due to the fact that not only would it have to use siderophores to reach iron scarcely distributed in the environment, but because of low bioavailability of iron source, its reduction also required

a lot of energy. Since energy is a product of photosynthesis, which is heavily dependent on iron conditions, this cycle comes full circle (Shevela, Pishchalnikov and Eichacker, 2013). So, despite having ability to use both iron uptake pathways, WT culture was unable to sustain its growth on goethite. That might explain no growth in PFe and InFe values in WT culture on goethite during experiment 2: iron conditions were probably so unfavorable, that organisms did not have enough resources to use their iron acquisition mechanisms and subsequently sustain growth.

WT culture allowed us to look at responses of unmodified organisms to different iron conditions. The other two types of cultures explored responses of organisms lacking certain genes involved in iron acquisition.

5.4.2 ΔA2804 culture

ΔA2804 is a PilA deletion mutant culture. PilA are suggested to be involved in the reductive iron uptake pathway. These deletion mutants show changes in pili structure: pili are still produced however they are clustered. During experiment 1 ΔA2804 on $FeCl_3$ shows the second highest final cell concentration. However, the same culture on goethite shows a different trend: it starts with high concentrations on day7 and by day 21 drops to the lowest concentration observed that day. This is the only culture that shows a decrease in cell concentration during this experiment. On easily soluble $FeCl_3$ ΔA2804 culture is able to sustain growth throughout the experiment which shows that its potential limited extracellular reductive ability is not a defining factor. However, on goethite this culture becomes transparent by day 21. Bleaching of the culture and significant drop of cell concentration implies that this culture is practically dead by the end of the experiment. It is also supported by drastic decrease of InFe concentrations. It is difficult to explain the exact reason for that. For example, it could have been caused by contamination of culture that led to addition of some toxic substances after day 14. However, a lot of precautions were taken to minimize contamination. Moreover, contamination of the culture could have impacted iron concentrations as well (TFe, PFe and even InFe), however no drastic increase was observed. Another possible reason for such behavior is response to iron conditions. Combination of the unavailable iron source with this type of mutation might have caused a delayed response in the culture. No definite conclusion can be made at this point. This is an example that demonstrates importance of biological triplicates discussed previously, that possibly would have allowed to explain this observation and conclude whether it was a response of the organisms or simply a result of a human error.

During experiment 2 ΔA2804 culture on $FeCl_3$ is one of the only two cultures to show any significant growth. There can be two possible explanations of ΔA2804's success in these iron conditions. Firstly, it can be related to the fact that both ΔA2804 and WT are able to secrete siderophores and acquire iron that is not in close proximity to the cell. Secondly, it can mean that since there is not a lot of iron in the environment, these clustered pili formations may still be somewhat effective in reducing iron that is then acquired inside the cell via iron transporters in the membrane. However, if the latter was the case, the siderophore culture should be able to survive as well, or even better due to presence of unclustered pili. Since that was not observed, it can be suggested that ability to release siderophores is more important in lower concentrations of more a bioavailable iron source. That may be because siderophores allow organisms to reach iron out of their proximity which leads to more iron being accessible. If there is no iron in proximity, extracellular reductive uptake on its own might not be helpful, because there would be no iron to reduce.

As discussed previously, because no culture including WT was able to grow or internalize iron on goethite in low concentrations, it can be considered practically unavailable to organisms at this concentration. In these conditions (i.e. low concentration of less bioavailable iron source) it is impossible to compare efficiency or importance of a specific iron uptake mechanism: all cultures are simply unable to acquire iron and survive.

5.4.3 ΔSidOP culture

ΔSidOP is a deletion mutant culture that is unable to produce siderophores. As mentioned above, usage of siderophores as a way of acquiring iron is often considered too expensive and wasteful in an oceanic environment (Toulza *et al.*, 2012). However, as seen in previous sections, it can be a defining factor of culture's survival at low iron concentrations, as observed during experiment 2 in $FeCl_3$ cultures.

In higher iron concentrations organisms might not use siderophores, since they are generally secreted as a 'last resort' during iron stress (Vogel, 2019). Even though we have observed some iron stress in experiment 1, the fact that siderophore mutants are showing significant growth indicates that organisms are effectively using other uptake mechanisms. That is most likely due to high concentration of iron, which leads to enough iron in close proximity to the cell. It is important to note, that cultures that are still able to release siderophores are showing higher growth in experiment 1 on $FeCl_3$. However, it can be speculated that siderophores are neither

the only nor the defining uptake mechanism used by these cultures in iron-replete conditions. If that was the case, ΔSidOP culture should not be able to survive the conditions in experiment 1.

ΔSidOP culture on goethite shows higher final cell concentration compared to the same culture on $FeCl_3$ and the highest of all goethite cultures. Moreover, ΔSidOP on goethite shows no significant difference between PFe and InFe values throughout the experiment, which can be either a sign of more successful growth (like in case with WT culture on $FeCl_3$) or a sign of adaptation to a less available iron source, since by the end of the experiment 1 all goethite cultures show no significant difference between PFe and InFe. Despite showing high final cell concentration, this culture does not show opaque color before day 21. Compared to other mutant cultures, that go from green to yellow, ΔSidOP on goethite show a yellow undertone right away. These factors can be used to suggest, that this culture was initially adapting to iron conditions (for example, down- and up-regulating specific genes) between days 1 and 7 when it barely shows any growth. After that, since it does not have ability to release siderophores, this energy is not "wasted" and can be used in reductive uptake and growth. That may be the reason why it shows higher cell concentrations compared to other goethite cultures, that may have spent some portion of energy on producing siderophores. It has been mentioned before that in this experiment siderophores should not be considered the defining factor of culture's survival in iron-replete conditions. However, it could have still impacted the rate of growth and final cell concentration in these cultures. It is important to note, however, that definite conclusions regarding that are difficult to make because of the behavior of ΔA2804 culture on goethite discussed previously.

The difference between growth of ΔSidOP cultures on different iron sources during experiment 1 is another interesting observation. The culture grown on the less available iron source showed higher final cell concentration. As was mentioned previously, ΔSidOP on goethite showed no significant difference between PFe and InFe values throughout the experiment, while the same culture on $FeCl_3$ showed the highest observed PFe values and subsequently significant difference between PFe and InFe. It is difficult to find a definite explanation for this phenomenon within the framework of this experiment. However, it may have to do with the nature of goethite as iron source: it might have caused ΔSidOP culture more stress due to its lower bioavailability that made it adapt more efficiently compared to same culture on $FeCl_3$. ΔSidOP culture on $FeCl_3$ shows a lot of extracellular PFe accumulation during the experiment at higher rates than other $FeCl_3$ cultures, however it does not increase its growth or

internalization of iron. That might be related to a previously discussed hypothesis of capacity of iron transporters in membrane acting as a limiting factor. High standard deviation values make it difficult to suggest a definite explanation to observed trends.

During experiment 2 with lower iron concentration none of the ΔSidOP cultures showed any noticeable growth. As discussed previously, in iron-deplete conditions siderophores may be the defining factor as they allow organisms to obtain iron that is not in proximity to the cell. In iron-replete conditions on a less available iron source siderophores may have been a disadvantage, since organisms without ability to release them could potentially use most of their energy on reducing said iron source and showed higher cell concentrations by the end of the experiment (ΔSidOP on goethite in experiment 1). However, in case of lower iron concentrations ability to secrete siderophores may have become a defining factor. That can be observed on $FeCl_3$ as iron source: ΔSidOP culture showed no significant growth. Culture, despite PFe accumulation was unable to internalize it. Based on available data, there is no clear explanation for why this culture was able to accumulate iron on the outside of the cell under low iron concentrations. However, lack of growth in ΔSidOP shows that siderophores were the defining factor for cultures' survival in these iron conditions.

In case of goethite, as mentioned before, it was practically unavailable to all organisms and thus the ability to use different mechanisms did not help. However, it is worth noting that ΔSidOP culture on goethite is the only one that shows zero concentration of cells in the second half of the experiment.

Cultures during conducted experiments showed signs of external and internal iron limitation. External iron limitation is dictated by characteristics of iron source and its concentrations (i.e. poor solubility of goethite), while internal depends on organism's ability to use a certain iron acquisition mechanism. In this case ability to release siderophores have shown to be crucial in iron-deplete conditions.

Based on the collected data, it can be observed, that in experiment 1 only WT cultures managed to sustain green color throughout the experiment. And although it was discussed previously, that in experiment 2 in iron-deplete conditions WT culture also obtained yellow undertone, in case of higher iron concentrations there may be another factor influencing the color. For example, that factor can be overall presence of genetic modifications in mutant cultures, that creates a disadvantage compared to unmodified organisms (Darmon and Leach, 2014).

CONCLUSION

The goal of this project was to study the response of *Synechococcus* sp. PCC 7002 to different iron conditions and explore the roles of iron acquisition mechanisms. This was achieved by conducting experiments that involved culturing of wild type and mutant cultures (ΔSidOP and ΔA2804) on two iron sources ($FeCl_3$ and FeO(OH)) in two concentrations (5 μM and 100 nM).

Higher iron concentrations in experiment 1 can be considered favorable for cultures since all were able to grow and accumulate intercellular iron. Cultures showed some signs of stress (i.e. change of color and/or slower growth compared to optimal conditions), however there is no clear evidence that this is due to iron limitation. Overall, high iron concentrations allowed organisms to absorb iron and grow on both iron sources. On the other hand, during experiment 2 a more defined iron limitation scenario was observed. Lower iron concentrations decreased the availability of goethite as iron source, which allowed growth during experiment 1. This led to no observable intercellular iron accumulation and subsequently no growth in goethite cultures. Only two cultures (both grown on $FeCl_3$) showed significant growth during experiment 2.

During both experiments, cultures on more soluble $FeCl_3$ generally showed higher cell concentrations by day 21. In higher iron concentrations $FeCl_3$ gave cultures an advantage, however cultures on less soluble goethite were also able to show significant growth. When iron concentrations decreased, type of iron source became crucial as it defined culture's ability to grow. Even wild type culture that can use both iron uptake mechanisms, was unable to sustain growth on goethite present in low concentrations, making it practically unavailable to *Synechococcus*.

A similar effect of iron concentrations was observed while comparing different types of cultures. For example, while in higher concentrations significant differences in growth were observed between cultures on $FeCl_3$, iron-deplete conditions amplified said differences. In lower concentrations of $FeCl_3$, siderophore-mediated iron uptake became crucial. That was demonstrated by lack of significant growth and intercellular iron accumulation in ΔSidOP culture on $FeCl_3$. This may be because release of siderophores allowed organisms with this ability to reach iron located further from the cell thus expanding the pool of accessible iron. During experiment 1 this ability may not have been as important due to higher iron concentrations and thus more iron in proximity to each cell.

The results of the experiments showed that differences in iron conditions do impact the ability of *Synechococcus* to acquire iron and grow. They may also dictate the role or importance of certain iron acquisition pathways. Overall, in lower concentrations cultures experienced iron limitation to a higher degree. In these conditions, the importance of iron source and type of mutation becomes more pronounced. Iron limitation experienced by organisms may be presented as a combination of external and internal limitation. External limitation can be defined by iron source characteristics, such a poor solubility of goethite, while internal – by ability of organism to use a certain iron uptake mechanism (i.e. siderophores in iron-deplete conditions). Thus, based on the experimental data, bioavailability of iron to *Synechococcus* was defined by iron concentration, speciation as well as ability of organism to use different iron acquisition pathways.

For future research it is recommended to conduct experiments in a designated stationary clean laboratory to further minimize contamination. Additionally, laboratory shakers should be used for constant mixing of cultures to prevent aggregation of cells on surfaces. If practically and financially viable, biological triplicates should be implemented additional to method triplicates to be able to certainly differentiate between natural response of a culture and effects of contamination.

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APPENDICES

Appendix A ICP-MS results

Table A-1 ICP-MS results (PFe and InFe)

Date				Fe56(MR)	
	Original sample no	Sample info	Original volume filtred in ml	Conc. ng/ml filt	RSD, %
28.10.2019	001	Culture 1A PFe a	25	26,23	2,9
28.10.2019	001	Culture 1A PFe a	25	26,25	3,4
28.10.2019	002	Culture 1A PFe b	25	4,51	2,8
28.10.2019	003	Culture 1A PFe c	25	4,70	1,6
28.10.2019	004	Culture 1A IntraFe a	25	7,44	1,5
28.10.2019	005	Culture 1A IntraFe b	25	8,58	3,2
28.10.2019	006	Culture 1A IntraFe c	25	1,27	3,0
28.10.2019	007	Culture 1B PFe a	25	7,61	3,4
28.10.2019	008	Culture 1B PFe b	25	6,53	3,7
28.10.2019	009	Culture 1B PFe c	25	6,66	3,9
28.10.2019	010	Culture 1B IntraFe a	25	2,38	1,7
28.10.2019	010	Culture 1B IntraFe a	25	2,26	1,8
28.10.2019	011	Culture 1B IntraFe b	25	2,45	2,7
28.10.2019	012	Culture 1B IntraFe c	25	1,22	2,0
28.10.2019	013	Culture 1C PFe a	25	9,17	2,8
28.10.2019	014	Culture 1C PFe b	25	14,52	1,8
28.10.2019	013	Culture 1C PFe a	25	9,01	2,7
28.10.2019	015	Culture 1C PFe c	25	3,77	2,7
28.10.2019	016	Culture 1C IntraFe a	25	1,12	1,0
28.10.2019	017	Culture 1C IntraFe b	25	1,41	2,4
28.10.2019	018	Culture 1C IntraFe c	25	4,16	0,5
28.10.2019	019	Culture 2A PFe a	25	19,48	2,3
28.10.2019	020	Culture 2A PFe b	25	16,32	2,6
28.10.2019	021	Culture 2A PFe c	25	21,51	2,0
28.10.2019	022	Culture 2A IntraFe a	25	2,16	3,3
28.10.2019	023	Culture 2A IntraFe b	25	10,05	2,9
28.10.2019	024	Culture 2A IntraFe c	25	1,15	3,7
28.10.2019	025	Culture 2B PFe a	25	8,05	3,8
28.10.2019	026	Culture 2B PFe b	25	4,96	2,6
28.10.2019	027	Culture 2B PFe c	25	8,16	3,2
28.10.2019	028	Culture 2B IntraFe a	50	1,18	5,5
28.10.2019	030	Culture 2B IntraFe c	25	7,53	2,2
28.10.2019	031	Culture 2C PFe a	25	1,32	0,8
28.10.2019	032	Culture 2C PFe b	25	1,84	2,8
28.10.2019	032	Culture 2C PFe b	25	1,75	1,9
28.10.2019	033	Culture 2C PFe c	25	1,32	1,4
28.10.2019	034	Culture 2C IntraFe a	25	1,13	4,0
28.10.2019	035	Culture 2C IntraFe b	25	1,63	1,1
28.10.2019	036	Culture 2C IntraFe c	25	1,17	2,8
28.10.2019	037	Culture 1A PFe a	25	10,42	1,7

Date				Fe56(MR)	
	Original sample no	Sample info	Original volume filtred in ml	Conc. ng/ml filt	RSD, %
28.10.2019	038	Culture 1A PFe b	25	19,70	2,1
29.10.2019	039	Culture 1A PFe c	25	18,41	2,1
29.10.2019	040	Culture 1A IntraFe a	15	3,46	2,9
29.10.2019	041	Culture 1A IntraFe b	15	3,31	0,2
29.10.2019	042	Culture 1A IntraFe c	15	5,08	1,7
29.10.2019	043	Culture 1B PFe a	15	66,01	2,1
29.10.2019	044	Culture 1B PFe b	15	12,65	1,1
29.10.2019	045	Culture 1B PFe c	15	27,96	0,5
29.10.2019	046	Culture 1B IntraFe a	15	4,35	1,4
29.10.2019	047	Culture 1B IntraFe b	15	5,02	2,2
29.10.2019	048	Culture 1B IntraFe c	15	4,83	2,8
29.10.2019	049	Culture 1C PFe a	25	100,82	1,1
29.10.2019	050	Culture 1C PFe b	25	74,92	0,8
29.10.2019	051	Culture 1C PFe c	25	101,94	1,5
29.10.2019	051	Culture 1C PFe c	25	101,51	1,0
29.10.2019	052	Culture 1C IntraFe a	15	7,40	2,8
29.10.2019	053	Culture 1C IntraFe b	15	6,08	1,5
29.10.2019	054	Culture 1C IntraFe c	15	7,19	1,1
29.10.2019	055	Culture 2A PFe a	15	58,65	0,4
29.10.2019	056	Culture 2A PFe b	15	61,50	1,6
29.10.2019	057	Culture 2A PFe c	15	87,08	1,8
29.10.2019	058	Culture 2A IntraFe a	15	6,69	2,4
29.10.2019	059	Culture 2A IntraFe b	15	8,02	1,3
29.10.2019	060	Culture 2A IntraFe c	15	4,78	0,9
29.10.2019	061	Culture 2B PFe a	15	27,70	1,1
29.10.2019	062	Culture 2B PFe b	15	39,63	2,1
29.10.2019	063	Culture 2B PFe c	15	29,91	1,9
29.10.2019	064	Culture 2B IntraFe a	15	13,68	2,9
29.10.2019	065	Culture 2B IntraFe b	15	11,56	2,5
29.10.2019	066	Culture 2B IntraFe c	15	13,32	2,9
29.10.2019	067	Culture 2C PFe a	15	2,84	3,8
29.10.2019	068	Culture 2C PFe b	15	3,02	2,8
29.10.2019	069	Culture 2C PFe c	15	4,16	4,8
29.10.2019	070	Culture 2C IntraFe a	15	6,77	2,1
29.10.2019	071	Culture 2C IntraFe b	15	3,00	2,9
29.10.2019	072	Culture 2C IntraFe c	15	2,84	1,6
29.10.2019	073	Culture 1A PFe a	10	18,77	2,8
29.10.2019	074	Culture 1A PFe b	10	15,04	1,5
29.10.2019	075	Culture 1A PFe c	10	17,95	1,6
29.10.2019	076	Culture 1A IntraFe a	10	14,64	2,4
30.10.2019	077	Culture 1A IntraFe b	10	16,05	0,6
30.10.2019	078	Culture 1A IntraFe c	10	9,13	0,7
30.10.2019	079	Culture 1B PFe a	10	28,80	2,8
30.10.2019	080	Culture 1B PFe b	10	30,81	2,2
30.10.2019	081	Culture 1B PFe c	10	50,04	1,7
30.10.2019	082	Culture 1B IntraFe a	10	9,74	3,2
30.10.2019	083	Culture 1B IntraFe b	10	8,15	4,0
30.10.2019	084	Culture 1B IntraFe c	10	8,39	3,4

Date	Original sample no	Sample info	Original volume filtred in ml	Fe56(MR)	
				Conc. ng/ml filt	RSD, %
30.10.2019	085	Culture 1C PFe a	10	152,51	0,6
30.10.2019	086	Culture 1C PFe b	10	238,27	0,9
30.10.2019	087	Culture 1C PFe c	10	89,18	1,0
30.10.2019	088	Culture 1C IntraFe a	10	5,19	2,0
30.10.2019	089	Culture 1C IntraFe b	10	4,16	1,0
30.10.2019	090	Culture 1C IntraFe c	10	2,49	1,8
30.10.2019	091	Culture 2A PFe a	10	43,05	2,8
30.10.2019	092	Culture 2A PFe b	10	23,74	2,8
30.10.2019	093	Culture 2A PFe c	10	22,77	2,4
30.10.2019	094	Culture 2A IntraFe a	10	6,44	0,2
30.10.2019	095	Culture 2A IntraFe b	10	9,56	2,3
30.10.2019	096	Culture 2A IntraFe c	10	8,40	2,0
30.10.2019	097	Culture 2B PFe a	10	50,05	2,2
30.10.2019	098	Culture 2B PFe b	10	74,33	3,0
30.10.2019	099	Culture 2B PFe c	10	52,70	2,0
30.10.2019	100	Culture 2B IntraFe a	10	16,34	0,1
30.10.2019	101	Culture 2B IntraFe b	10	13,11	2,6
30.10.2019	102	Culture 2B IntraFe c	10	11,60	2,1
30.10.2019	103	Culture 2C PFe a	10	7,32	4,5
30.10.2019	104	Culture 2C PFe b	10	7,35	1,9
30.10.2019	105	Culture 2C PFe c	10	3,88	3,7
30.10.2019	106	Culture 2C IntraFe a	10	10,30	4,4
30.10.2019	107	Culture 2C IntraFe b	10	4,33	0,7
30.10.2019	108	Culture 2C IntraFe c	10	2,20	0,4
30.10.2019	109	Culture 1A PFe a	10	12,76	2,9
30.10.2019	110	Culture 1A PFe b	10	22,75	2,9
30.10.2019	111	Culture 1A PFe c	10	20,22	3,5
30.10.2019	112	Culture 1A IntraFe a	10	11,90	1,0
30.10.2019	113	Culture 1A IntraFe b	10	9,19	1,7
30.10.2019	114	Culture 1A IntraFe c	10	11,80	0,6
31.10.2019	115	Culture 1B PFe a	10	131,20	1,8
31.10.2019	116	Culture 1B PFe b	10	132,50	1,3
31.10.2019	117	Culture 1B PFe c	10	98,01	2,6
31.10.2019	118	Culture 1B IntraFe a	10	14,72	4,4
31.10.2019	119	Culture 1B IntraFe b	10	14,02	1,2
31.10.2019	120	Culture 1B IntraFe c	10	13,31	2,4
31.10.2019	121	Culture 1C PFe a	10	99,85	1,1
31.10.2019	122	Culture 1C PFe b	10	118,92	2,4
31.10.2019	123	Culture 1C PFe c	10	116,80	1,7
31.10.2019	124	Culture 1C IntraFe a	10	6,24	5,4
31.10.2019	125	Culture 1C IntraFe b	10	6,06	0,3
31.10.2019	126	Culture 1C IntraFe c	10	5,51	2,2
31.10.2019	127	Culture 2A PFe a	10	22,44	2,5
31.10.2019	128	Culture 2A PFe b	10	13,90	2,4
31.10.2019	129	Culture 2A PFe c	10	19,80	3,2
31.10.2019	130	Culture 2A IntraFe a	10	7,03	3,0
31.10.2019	131	Culture 2A IntraFe b	10	9,32	3,7
31.10.2019	132	Culture 2A IntraFe c	10	7,17	1,6

Date				Fe56(MR)	
	Original sample no	Sample info	Original volume filtred in ml	Conc. ng/ml filt	RSD, %
31.10.2019	133	Culture 2B PFe a	10	16,81	2,1
31.10.2019	134	Culture 2B PFe b	10	13,84	1,9
31.10.2019	135	Culture 2B PFe c	10	21,14	1,7
31.10.2019	136	Culture 2B IntraFe a	10	0,72	2,7
31.10.2019	137	Culture 2B IntraFe b	10	0,77	2,4
31.10.2019	138	Culture 2B IntraFe c	10	1,35	1,6
31.10.2019	139	Culture 2C PFe a	10	7,37	3,1
31.10.2019	140	Culture 2C PFe b	10	3,56	2,1
31.10.2019	141	Culture 2C PFe c	10	4,82	2,3
31.10.2019	142	Culture 2C IntraFe a	10	1,75	1,9
31.10.2019	143	Culture 2C IntraFe b	10	3,54	3,3
31.10.2019	144	Culture 2C IntraFe c	10	3,66	1,2
27.11.2019	153	Culture 1A PFe a	20	3,07	2,5
27.11.2019	153	Culture 1A PFe a	20	2,99	2,7
27.11.2019	154	Culture 1A PFe b	20	4,80	3,1
27.11.2019	155	Culture 1A PFe c	20	2,33	3,3
27.11.2019	156	Culture 1A IntraFe a	20	-0,06	1,3
27.11.2019	157	Culture 1A IntraFe b	20	0,45	3,3
27.11.2019	158	Culture 1A IntraFe c	20	1,62	1,3
27.11.2019	159	Culture 1B PFe a	20	0,33	3,3
27.11.2019	160	Culture 1B PFe b	20	-0,19	3,4
27.11.2019	161	Culture 1B PFe c	20	0,45	4,0
27.11.2019	161	Culture 1B PFe c	20	0,50	3,4
27.11.2019	162	Culture 1B IntraFe a	20	-0,35	1,4
27.11.2019	163	Culture 1B IntraFe b	20	0,09	4,8
27.11.2019	164	Culture 1B IntraFe c	20	-0,23	3,2
27.11.2019	165	Culture 1C PFe a	20	0,96	1,8
27.11.2019	166	Culture 1C PFe b	20	0,70	3,5
27.11.2019	167	Culture 1C PFe c	20	0,72	3,3
27.11.2019	168	Culture 1C IntraFe a	20	0,24	5,1
27.11.2019	169	Culture 1C IntraFe b	20	-0,21	5,7
27.11.2019	170	Culture 1C IntraFe c	20	-0,20	4,2
27.11.2019	171	Culture 2A PFe a	20	0,88	1,6
27.11.2019	172	Culture 2A PFe b	20	2,29	4,0
27.11.2019	173	Culture 2A PFe c	20	0,96	3,6
27.11.2019	174	Culture 2A IntraFe a	20	-0,24	3,9
27.11.2019	175	Culture 2A IntraFe b	20	-0,08	2,2
27.11.2019	176	Culture 2A IntraFe c	20	0,66	4,1
27.11.2019	177	Culture 2B PFe a	20	-0,21	4,8
27.11.2019	178	Culture 2B PFe b	20	-0,52	5,9
27.11.2019	179	Culture 2B PFe c	20	-0,37	4,5
27.11.2019	180	Culture 2B IntraFe a	20	-0,29	5,0
27.11.2019	181	Culture 2B IntraFe b	20	-0,17	0,6
27.11.2019	182	Culture 2B IntraFe c	20	-0,34	2,7
27.11.2019	183	Culture 2C PFe a	20	0,27	3,6
27.11.2019	184	Culture 2C PFe b	20	0,23	3,1
27.11.2019	185	Culture 2C PFe c	20	0,68	1,7
27.11.2019	186	Culture 2C IntraFe a	20	-0,06	3,9

Date				Fe56(MR)	
	Original sample no	Sample info	Original volume filtred in ml	Conc. ng/ml filt	RSD, %
27.11.2019	187	Culture 2C IntraFe b	20	-0,15	0,8
27.11.2019	188	Culture 2C IntraFe c	20	-0,26	1,9
27.11.2019	189	Culture 1A PFe a	20	0,54	3,0
28.11.2019	190	Culture 1A PFe b	20	1,83	4,3
28.11.2019	191	Culture 1A PFe c	20	1,05	3,6
28.11.2019	192	Culture 1A IntraFe a	20	0,37	1,8
28.11.2019	193	Culture 1A IntraFe b	20	0,15	5,2
28.11.2019	194	Culture 1A IntraFe c	20	0,92	1,7
28.11.2019	195	Culture 1B PFe a	20	1,26	2,5
28.11.2019	196	Culture 1B PFe b	20	1,03	2,5
28.11.2019	197	Culture 1B PFe c	20	0,44	2,9
28.11.2019	198	Culture 1B IntraFe a	20	1,23	2,1
28.11.2019	199	Culture 1B IntraFe b	20	1,12	1,8
28.11.2019	200	Culture 1B IntraFe c	20	1,52	2,3
28.11.2019	201	Culture 1C PFe a	20	0,65	3,0
28.11.2019	201	Culture 1C PFe a	20	0,61	2,7
28.11.2019	202	Culture 1C PFe b	20	0,12	6,0
28.11.2019	202	Culture 1C PFe b	20	0,10	1,3
28.11.2019	203	Culture 1C PFe c	20	0,93	1,0
28.11.2019	204	Culture 1C IntraFe a	20	-0,58	2,4
28.11.2019	205	Culture 1C IntraFe b	20	-0,50	2,8
28.11.2019	206	Culture 1C IntraFe c	20	0,28	3,0
28.11.2019	207	Culture 2A PFe a	20	-0,21	3,2
28.11.2019	208	Culture 2A PFe b	20	-0,47	3,3
28.11.2019	209	Culture 2A PFe c	20	-0,65	3,7
28.11.2019	210	Culture 2A IntraFe a	20	-0,74	2,6
28.11.2019	211	Culture 2A IntraFe b	20	-0,61	2,7
28.11.2019	212	Culture 2A IntraFe c	20	-0,77	5,0
28.11.2019	213	Culture 2B PFe a	20	-0,63	4,8
28.11.2019	214	Culture 2B PFe b	20	-0,80	0,4
28.11.2019	215	Culture 2B PFe c	20	-0,66	0,9
28.11.2019	216	Culture 2B IntraFe a	20	-0,66	4,0
28.11.2019	217	Culture 2B IntraFe b	20	-0,80	2,3
28.11.2019	218	Culture 2B IntraFe c	20	-0,96	5,3
28.11.2019	219	Culture 2C PFe a	20	-0,79	2,1
28.11.2019	220	Culture 2C PFe b	20	-0,64	3,4
28.11.2019	221	Culture 2C PFe c	20	-0,56	4,9
28.11.2019	222	Culture 2C IntraFe a	20	-0,58	4,8
28.11.2019	223	Culture 2C IntraFe b	20	-0,96	5,9
28.11.2019	224	Culture 2C IntraFe c	20	-0,91	4,0
28.11.2019	225	Culture 1A PFe a	20	0,74	2,5
28.11.2019	226	Culture 1A PFe b	20	0,37	4,5
28.11.2019	227	Culture 1A PFe c	20	0,37	2,4
29.11.2019	228	Culture 1A IntraFe a	20	0,13	1,1
29.11.2019	229	Culture 1A IntraFe b	20	1,27	3,9
29.11.2019	230	Culture 1A IntraFe c	20	0,07	2,6
29.11.2019	231	Culture 1B PFe a	20	0,34	3,2
29.11.2019	232	Culture 1B PFe b	20	0,55	3,2

Date				Fe56(MR)	
	Original sample no	Sample info	Original volume filtred in ml	Conc. ng/ml filt	RSD, %
29.11.2019	233	Culture 1B PFe c	20	0,49	1,1
29.11.2019	234	Culture 1B IntraFe a	20	0,05	1,2
29.11.2019	235	Culture 1B IntraFe b	20	-0,17	4,3
29.11.2019	236	Culture 1B IntraFe c	20	-0,26	6,1
29.11.2019	237	Culture 1C PFe a	20	1,11	1,7
29.11.2019	238	Culture 1C PFe b	20	-0,18	6,1
29.11.2019	239	Culture 1C PFe c	20	-0,15	2,8
29.11.2019	240	Culture 1C IntraFe a	20	-0,19	2,3
29.11.2019	241	Culture 1C IntraFe b	20	-0,68	0,8
29.11.2019	242	Culture 1C IntraFe c	20	-0,45	1,2
29.11.2019	243	Culture 2A PFe a	20	-0,94	3,1
29.11.2019	244	Culture 2A PFe b	20	-0,35	5,1
29.11.2019	245	Culture 2A PFe c	20	-0,93	3,3
29.11.2019	246	Culture 2A IntraFe a	20	-0,89	4,8
29.11.2019	247	Culture 2A IntraFe b	20	-0,91	4,5
29.11.2019	248	Culture 2A IntraFe c	20	-0,85	3,7
29.11.2019	249	Culture 2B PFe a	20	0,15	0,6
29.11.2019	250	Culture 2B PFe b	20	-0,58	3,2
29.11.2019	251	Culture 2B PFe c	20	-0,09	2,5
29.11.2019	252	Culture 2B IntraFe a	20	-0,88	4,0
29.11.2019	253	Culture 2B IntraFe b	20	-0,86	5,0
29.11.2019	254	Culture 2B IntraFe c	20	-0,58	3,8
29.11.2019	255	Culture 2C PFe a	20	-1,18	8,9
29.11.2019	256	Culture 2C PFe b	20	-0,88	6,4
29.11.2019	257	Culture 2C PFe c	20	-0,81	1,6
29.11.2019	258	Culture 2C IntraFe a	20	-0,79	5,5
29.11.2019	259	Culture 2C IntraFe b	20	-0,84	4,0
29.11.2019	260	Culture 2C IntraFe c	20	-1,03	6,0
29.11.2019	261	Culture 1A PFe a	20	0,80	4,8
29.11.2019	262	Culture 1A PFe b	20	0,68	1,7
29.11.2019	263	Culture 1A PFe c	20	0,72	1,4
29.11.2019	264	Culture 1A IntraFe a	20	0,39	1,4
29.11.2019	265	Culture 1A IntraFe b	20	0,30	6,2
01.12.2019	266	Culture 1A IntraFe c	20	0,78	1,6
01.12.2019	267	Culture 1B PFe a	20	1,45	4,6
01.12.2019	268	Culture 1B PFe b	20	1,49	2,9
01.12.2019	269	Culture 1B PFe c	20	1,15	1,6
01.12.2019	270	Culture 1B IntraFe a	20	0,36	4,4
01.12.2019	271	Culture 1B IntraFe b	20	0,66	4,3
01.12.2019	272	Culture 1B IntraFe c	20	0,85	2,7
01.12.2019	273	Culture 1C PFe a	20	0,95	2,1
01.12.2019	274	Culture 1C PFe b	20	0,13	5,0
01.12.2019	275	Culture 1C PFe c	20	0,31	2,4
01.12.2019	276	Culture 1C IntraFe a	20	-0,90	7,7
01.12.2019	277	Culture 1C IntraFe b	20	-0,64	1,9
01.12.2019	278	Culture 1C IntraFe c	20	-0,57	2,8
01.12.2019	279	Culture 2A PFe a	20	-0,68	4,2
01.12.2019	280	Culture 2A PFe b	20	-0,45	4,5

Date				Fe56(MR)	
	Original sample no	Sample info	Original volume filtred in ml	Conc. ng/ml filt	RSD, %
01.12.2019	281	Culture 2A PFe c	20	-0,79	2,5
01.12.2019	282	Culture 2A IntraFe a	20	-0,30	7,7
01.12.2019	283	Culture 2A IntraFe b	20	-0,84	5,0
01.12.2019	284	Culture 2A IntraFe c	20	-0,95	1,5
01.12.2019	285	Culture 2B PFe a	20	-0,88	2,3
01.12.2019	286	Culture 2B PFe b	20	-0,81	2,3
01.12.2019	287	Culture 2B PFe c	20	-0,98	1,3
01.12.2019	288	Culture 2B IntraFe a	20	-0,97	3,4
01.12.2019	289	Culture 2B IntraFe b	20	-0,87	3,8
01.12.2019	290	Culture 2B IntraFe c	20	-0,91	2,4
01.12.2019	291	Culture 2C PFe a	20	-0,96	3,4
01.12.2019	292	Culture 2C PFe b	20	-1,14	2,9
01.12.2019	293	Culture 2C PFe c	20	-1,10	6,8
01.12.2019	294	Culture 2C IntraFe a	20	-1,02	2,9
01.12.2019	295	Culture 2C IntraFe b	20	-1,07	2,5
01.12.2019	296	Culture 2C IntraFe c	20	-1,02	3,2
01.12.2019	296	Culture 2C IntraFe c	20	-1,00	6,3

Table A-2 ICP-MS results (TFe)

Date of delivery			Fe56(MR)	
	Original sample no	Sample info	Conc. µg/L	RSD, %
08.01.2020	D001	Culture 1A 03.10.19 a	99,05	1,4
08.01.2020	D002	Culture 1A 03.10.19 b	95,04	2,8
08.01.2020	D003	Culture 1A 03.10.19 c	94,52	1,7
08.01.2020	D004	Culture 1B 03.10.19 a	281,43	1,7
08.01.2020	D005	Culture 1B 03.10.19 b	291,07	2,2
08.01.2020	D006	Culture 1B 03.10.19 c	282,85	1,7
08.01.2020	D007	Culture 1C 03.10.19 a	284,77	2,9
08.01.2020	D008	Culture 1C 03.10.19 b	276,91	0,5
08.01.2020	D009	Culture 1C 03.10.19 c	283,51	1,4
08.01.2020	D010	Culture 2A 03.10.19 a	273,83	1,3
08.01.2020	D011	Culture 2A 03.10.19 b	260,75	0,3
08.01.2020	D012	Culture 2A 03.10.19 c	270,12	0,5
08.01.2020	D013	Culture 2B 03.10.19 a	129,05	2,0
08.01.2020	D014	Culture 2B 03.10.19 b	131,38	2,0
08.01.2020	D015	Culture 2B 03.10.19 c	133,31	1,6
08.01.2020	D016	Culture 2C 03.10.19 a	20,42	1,7
08.01.2020	D017	Culture 2C 03.10.19 b	20,61	0,6
08.01.2020	D018	Culture 2C 03.10.19 c	21,05	1,1
08.01.2020	D019	Culture 1A 24.10.19 a	27,22	2,1
08.01.2020	D020	Culture 1A 24.10.19 b	26,29	2,4
08.01.2020	D021	Culture 1A 24.10.19 c	26,10	0,6
08.01.2020	D022	Culture 1B 24.10.19 a	103,19	1,5
08.01.2020	D023	Culture 1B 24.10.19 b	104,60	1,1
08.01.2020	D024	Culture 1B 24.10.19 c	99,93	2,2

Date of delivery			Fe56(MR)	
	Original sample no	**Sample info**	**Conc. µg/L**	**RSD, %**
08.01.2020	D025	Culture 1C 24.10.19 a	13,90	1,8
08.01.2020	D026	Culture 1C 24.10.19 b	13,64	0,5
08.01.2020	D027	Culture 1C 24.10.19 c	13,42	1,4
08.01.2020	D028	Culture 2A 24.10.19 a	58,14	2,3
08.01.2020	D029	Culture 2A 24.10.19 b	58,14	1,0
08.01.2020	D030	Culture 2A 24.10.19 c	58,83	2,4
08.01.2020	D031	Culture 2B 24.10.19 a	9,98	2,2
08.01.2020	D032	Culture 2B 24.10.19 b	10,29	0,5
08.01.2020	D033	Culture 2B 24.10.19 c	10,11	4,0
08.01.2020	D034	Culture 2C 24.10.19 a	9,12	1,6
08.01.2020	D035	Culture 2C 24.10.19 b	9,19	1,3
08.01.2020	D036	Culture 2C 24.10.19 c	8,93	2,2
08.01.2020	D037	Culture 1A 07.11.19 a	5,32	4,2
08.01.2020	D038	Culture 1A 07.11.19 b	5,26	2,4
08.01.2020	D039	Culture 1A 07.11.19 c	5,21	3,3
08.01.2020	D040	Culture 1B 07.11.19 a	5,71	1,9
08.01.2020	D041	Culture 1B 07.11.19 b	5,83	1,9
08.01.2020	D042	Culture 1B 07.11.19 c	5,90	0,3
08.01.2020	D043	Culture 1C 07.11.19 a	6,64	1,3
08.01.2020	D044	Culture 1C 07.11.19 b	6,38	2,6
08.01.2020	D045	Culture 1C 07.11.19 c	6,75	1,0
08.01.2020	D046	Culture 2A 07.11.19 a	1,87	1,4
08.01.2020	D047	Culture 2A 07.11.19 b	2,00	2,5
08.01.2020	D048	Culture 2A 07.11.19 c	2,04	2,6
08.01.2020	D049	Culture 2B 07.11.19 a	1,30	3,0
08.01.2020	D050	Culture 2B 07.11.19 b	1,34	2,1
08.01.2020	D051	Culture 2B 07.11.19 c	1,36	1,4
08.01.2020	D052	Culture 2C 07.11.19 a	2,13	2,5
08.01.2020	D053	Culture 2C 07.11.19 b	2,08	3,4
08.01.2020	D054	Culture 2C 07.11.19 c	2,20	2,5
08.01.2020	D055	Culture 1A 27.11.19 a	2,43	2,9
08.01.2020	D056	Culture 1A 27.11.19 b	2,37	4,5
08.01.2020	D057	Culture 1A 27.11.19 c	2,49	1,2
08.01.2020	D058	Culture 1B 27.11.19 a	2,44	4,1
08.01.2020	D059	Culture 1B 27.11.19 b	2,94	0,9
08.01.2020	D060	Culture 1B 27.11.19 c	2,92	3,3
08.01.2020	D061	Culture 1C 27.11.19 a	3,07	2,7
08.01.2020	D062	Culture 1C 27.11.19 b	3,18	0,6
08.01.2020	D063	Culture 1C 27.11.19 c	3,14	1,8
08.01.2020	D064	Culture 2A 27.11.19 a	0,37	3,2
08.01.2020	D065	Culture 2A 27.11.19 b	0,25	5,2
08.01.2020	D066	Culture 2A 27.11.19 c	0,38	3,0
08.01.2020	D067	Culture 2B 27.11.19 a	0,29	1,3
08.01.2020	D068	Culture 2B 27.11.19 b	0,41	3,4
08.01.2020	D069	Culture 2B 27.11.19 c	0,41	1,4
08.01.2020	D070	Culture 2C 27.11.19 a	0,29	4,1
08.01.2020	D071	Culture 2C 27.11.19 b	0,29	3,0
08.01.2020	D072	Culture 2C 27.11.19 c	0,20	3,7

Appendix B ICP-MS data processing

Table B-1 PFe and InFe ICP-MS data processing

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
BEGINNING OF THE EXPERIMENT				FIRST STAGE OF THE EXPERIMENT, 20 uM										
1A	PFe	WT	1	469,148	211,27	223,34	105,72	1,15		82,324	4,11333E+11		200,1	4,1
			2	80,620	211,27	223,34		0,58	80,620		4,11333E+11	1,95997E-10		0,0
			3	84,028	211,27	223,34		0,57	84,028		4,11333E+11	2,04281E-10		0,0
1A	InFe	WT	4	132,967	103,04	70,29	68,215	0,43	132,967	103,039	4,11333E+11	3,23259E-10	250,5	139,5
			5	153,410	103,04	70,29		0,72	153,410		4,11333E+11	3,72957E-10		0,0
			6	22,740	103,04	70,29		1,14	22,740		4,11333E+11	5,52831E-11		0,0
1B	PFe	PilA	7	136,046	123,95	10,54	8,5067	1,15		117,906	4,16667E+11		283,0	2,9
			8	116,684	123,95	10,54		0,69	116,684		4,16667E+11	2,80042E-10		0,0
			9	119,127	123,95	10,54		0,46	119,127		4,16667E+11	2,85906E-10		0,0
1B	InFe	PilA	10	41,482	35,66	12,08	33,882	0,48	41,482	42,601	4,16667E+11	9,95572E-11	102,2	2,7
			11	43,719	35,66	12,08		0,67	43,719		4,16667E+11	1,04926E-10		0,0
			12	21,766	35,66	12,08		1,15			4,16667E+11			0,0
1C	PFe	Sid	13	162,500	163,16	96,05	58,865	0,01	162,500	163,164	4,21333E+11	3,85679E-10	387,3	186,1
			14	259,541	163,16	96,05		1,00	259,541		4,21333E+11	6,15999E-10		0,0
			15	67,452	163,16	96,05		1,00	67,452		4,21333E+11	1,60092E-10		0,0
1C	InFe	Sid	16	20,100	39,91	30,02	75,211	0,66	20,100	22,645	4,21333E+11	4,7706E-11	53,7	6,0

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
			17	25,190	39,91	30,02		0,49	25,190		4,21333E+11	5,97856E-11		0,0
			18	74,454	39,91	30,02		1,15			4,21333E+11			0,0
2A	PFe	WT	19	348,232	341,51	46,78	13,697	0,14	348,232	341,512	4,22E+11	8,25195E-10	809,3	90,5
			20	291,739	341,51	46,78		1,06	291,739		4,22E+11	6,91324E-10		0,0
			21	384,565	341,51	46,78		0,92	384,565		4,22E+11	9,11291E-10		0,0
2A	InFe	WT	22	38,566	79,62	87,14	109,45	0,47	38,566	29,578	4,22E+11	9,1389E-11	70,1	21,3
			23	179,704	79,62	87,14		1,15			4,22E+11			0,0
			24	20,589	79,62	87,14		0,68	20,589		4,22E+11	4,87897E-11		0,0
2B	PFe	PilA	25	143,836	126,10	32,48	25,761	0,55	143,836	144,840	4,08E+11	3,52538E-10	355,0	2,5
			26	88,605	126,10	32,48		1,15			4,08E+11			0,0
			27	145,845	126,10	32,48		0,61	145,845		4,08E+11	3,57463E-10		0,0
2B	InFe	PilA	28	21,028	59,72	80,29	134,46	0,48	21,028	59,716	4,08E+11	5,15396E-11	190,7	129,5
			30	134,580	59,72	80,29		0,93	134,580		4,08E+11	3,29854E-10		0,0
2C	PFe	Sid	31	23,540	26,43	4,93	18,662	0,59	23,540	23,583	4E+11	5,88493E-11	59,0	0,1
			32	32,126	26,43	4,93		1,15			4E+11			0,0
			33	23,626	26,43	4,93		0,57	23,626		4E+11	5,90657E-11		0,0
2C	InFe	Sid	34	20,167	23,43	5,02	21,409	0,65	20,167	20,539	4E+11	5,04168E-11	51,3	0,9
			35	29,202	23,43	5,02		1,15			4E+11			0,0
			36	20,912	23,43	5,02		0,50	20,912		4E+11	5,22796E-11		0,0
1A	PFe	WT	37	186,328	289,24	89,87	31,072	1,15		340,703	5,74433E+11		593,1	20,1
			38	352,259	289,24	89,87		0,70	352,259		5,74433E+11	6,13229E-10		0,0
			39	329,146	289,24	89,87		0,44	329,146		5,74433E+11	5,72993E-10		0,0
1A	InFe	WT	40	61,935	70,61	17,55	24,851	0,49	61,935	60,511	5,74433E+11	1,07819E-10	105,3	2,5
			41	59,087	70,61	17,55		0,66	59,087		5,74433E+11	1,02862E-10		0,0
			42	90,803	70,61	17,55		1,15			5,74433E+11			0,0

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
1B	PFe	PilA	43	1180,132	635,39	491,22	77,31	1,11	1180,132	635,388	5,434E+11	2,17176E-09	1169,3	738,1
			44	226,129	635,39	491,22		0,83	226,129		5,434E+11	4,16138E-10		0,0
			45	499,903	635,39	491,22		0,28	499,903		5,434E+11	9,19954E-10		0,0
1B	InFe	PilA	46	77,707	84,57	6,18	7,3072	1,11	77,707	84,572	5,434E+11	1,43002E-10	155,6	9,3
			47	89,691	84,57	6,18		0,83	89,691		5,434E+11	1,65056E-10		0,0
			48	86,318	84,57	6,18		0,28	86,318		5,434E+11	1,58848E-10		0,0
1C	PFe	Sid	49	1802,493	1653,53	272,15	16,459	0,55	1802,493	1810,585	5,50367E+11	3,27508E-09	3289,8	14,7
			50	1339,411	1653,53	272,15		1,15			5,50367E+11			0,0
			51	1818,678	1653,53	272,15		0,61	1818,678		5,50367E+11	3,30448E-09		0,0
1C	InFe	Sid	52	132,323	123,19	12,67	10,287	0,72	132,323	123,193	5,50367E+11	2,40427E-10	223,8	18,8
			53	108,724	123,19	12,67		1,14	108,724		5,50367E+11	1,97549E-10		0,0
			54	128,530	123,19	12,67		0,42	128,530		5,50367E+11	2,33536E-10		0,0
2A	PFe	WT	55	1048,614	1235,02	279,91	22,665	0,67	1048,614	1074,082	4,959E+11	2,11457E-09	2165,9	51,4
			56	1099,551	1235,02	279,91		0,48	1099,551		4,959E+11	2,21728E-09		0,0
			57	1556,893	1235,02	279,91		1,15			4,959E+11			0,0
2A	InFe	WT	58	119,577	116,13	29,07	25,035	0,12	119,577	116,126	4,959E+11	2,41132E-10	234,2	47,9
			59	143,319	116,13	29,07		0,94	143,319		4,959E+11	2,89007E-10		0,0
			60	85,483	116,13	29,07		1,05	85,483		4,959E+11	1,7238E-10		0,0
2B	PFe	PilA	61	495,269	579,50	113,42	19,572	0,74	495,269	579,497	7,06167E+11	7,01349E-10	820,6	131,1
			62	708,460	579,50	113,42		1,14	708,460		7,06167E+11	1,00325E-09		0,0
			63	534,762	579,50	113,42		0,39	534,762		7,06167E+11	7,57275E-10		0,0
2B	InFe	PilA	64	244,629	229,76	20,33	8,8473	0,73	244,629	229,758	7,06167E+11	3,46418E-10	325,4	23,5

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
			65	206,596	229,76	20,33		1,14	206,596		7,06167E+11	2,92559E-10		0,0
			66	238,050	229,76	20,33		0,41	238,050		7,06167E+11	3,37102E-10		0,0
2C	PFe	Sid	67	50,802	59,68	12,77	21,405	0,69	50,802	52,357	4,18633E+11	1,21353E-10	125,1	3,7
			68	53,912	59,68	12,77		0,45	53,912		4,18633E+11	1,2878E-10		0,0
			69	74,317	59,68	12,77		1,15			4,18633E+11			0,0
2C	InFe	Sid	70	120,957	75,11	39,73	52,893	1,15		52,190	4,18633E+11		124,7	3,5
			71	53,658	75,11	39,73		0,54	53,658		4,18633E+11	1,28173E-10		0,0
			72	50,723	75,11	39,73		0,61	50,723		4,18633E+11	1,21163E-10		0,0
1A	PFe	WT	73	335,560	308,45	35,00	11,348	0,77	335,560	308,453	1,0896E+12	3,07966E-10	283,1	26,2
			74	268,934	308,45	35,00		1,13	268,934		1,0896E+12	2,46819E-10		0,0
			75	320,864	308,45	35,00		0,35	320,864		1,0896E+12	2,94478E-10		0,0
1A	InFe	WT	76	261,754	237,30	65,46	27,583	0,37	261,754	237,305	1,0896E+12	2,4023E-10	217,8	49,1
			77	287,017	237,30	65,46		0,76	287,017		1,0896E+12	2,63415E-10		0,0
			78	163,143	237,30	65,46		1,13	163,143		1,0896E+12	1,49727E-10		0,0
1B	PFe	PilA	79	514,963	653,44	209,57	32,072	0,66	514,963	532,890	7,956E+11	6,47263E-10	669,8	22,5
			80	550,818	653,44	209,57		0,49	550,818		7,956E+11	6,9233E-10		0,0
			81	894,546	653,44	209,57		1,15			7,956E+11			0,0
1B	InFe	PilA	82	174,106	156,62	15,30	9,7659	1,14	174,106	156,620	7,956E+11	2,18836E-10	196,9	15,7
			83	145,726	156,62	15,30		0,71	145,726		7,956E+11	1,83165E-10		0,0
			84	150,029	156,62	15,30		0,43	150,029		7,956E+11	1,88573E-10		0,0
1C	PFe	Sid	85	2726,641	2860,23	1337,71	46,769	0,10	2726,641	2860,230	6,648E+11	4,10145E-09	4302,4	1643,0
			86	4259,726	2860,23	1337,71		1,05	4259,726		6,648E+11	6,40753E-09		0,0
			87	1594,323	2860,23	1337,71		0,95	1594,323		6,648E+11	2,3982E-09		0,0

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
1C	InFe	Sid	88	92,805	70,57	24,40	34,571	0,91	92,805	70,574	6,648E+11	1,39598E-10	106,2	30,0
			89	74,446	70,57	24,40		0,16	74,446		6,648E+11	1,11982E-10		0,0
			90	44,471	70,57	24,40		1,07	44,471		6,648E+11	6,6894E-11		0,0
2A	PFe	WT	91	769,611	533,69	204,50	38,318	1,15		415,725	7,344E+11		566,1	11,8
			92	424,410	533,69	204,50		0,53	424,410		7,344E+11	5,77901E-10		0,0
			93	407,040	533,69	204,50		0,62	407,040		7,344E+11	5,54249E-10		0,0
2A	InFe	WT	94	115,210	145,43	28,12	19,337	1,07	115,210	145,434	7,344E+11	1,56877E-10	198,0	31,3
			95	170,830	145,43	28,12		0,90	170,830		7,344E+11	2,32611E-10		0,0
			96	150,263	145,43	28,12		0,17	150,263		7,344E+11	2,04607E-10		0,0
2B	PFe	PilA	97	894,837	1055,34	238,12	22,563	0,67	894,837	918,544	7,86E+11	1,13847E-09	1168,6	30,2
			98	1328,928	1055,34	238,12		1,15			7,86E+11			0,0
			99	942,252	1055,34	238,12		0,47	942,252		7,86E+11	1,19879E-09		0,0
2B	InFe	PilA	100	292,067	244,59	43,28	17,696	1,10	292,067	244,591	7,86E+11	3,71587E-10	311,2	45,0
			101	234,381	244,59	43,28		0,24	234,381		7,86E+11	2,98195E-10		0,0
			102	207,326	244,59	43,28		0,86	207,326		7,86E+11	2,63773E-10		0,0
2C	PFe	Sid	103	130,796	110,56	35,64	32,233	0,57	130,796	131,133	7,758E+11	1,68596E-10	169,0	0,4
			104	131,470	110,56	35,64		0,59	131,470		7,758E+11	1,69463E-10		0,0
			105	69,411	110,56	35,64		1,15			7,758E+11			0,0
2C	InFe	Sid	106	184,127	100,27	75,09	74,886	1,12	184,127	100,268	7,758E+11	2,37338E-10	129,2	79,0
			107	77,413	100,27	75,09		0,30	77,413		7,758E+11	9,97847E-11		0,0
			108	39,265	100,27	75,09		0,81	39,265		7,758E+11	5,06123E-11		0,0
1A	PFe	WT	109	228,150	332,15	92,86	27,958	1,12	228,150	332,149	1,3532E+12	1,686E-10	245,5	56,0
			110	406,771	332,15	92,86		0,80	406,771		1,3532E+12	3,00599E-10		0,0

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
			111	361,527	332,15	92,86		0,32	361,527		1,3532E+12	2,67164E-10		0,0
1A	InFe	WT	112	212,809	196,01	27,51	14,033	0,61	212,809	211,885	1,3532E+12	1,57263E-10	156,6	0,7
			113	164,271	196,01	27,51		1,15			1,3532E+12			0,0
			114	210,962	196,01	27,51		0,54	210,962		1,3532E+12	1,55898E-10		0,0
1B	PFe	PilA	115	2345,587	2155,52	349,46	16,213	0,54	2345,587	2357,173	1,054E+12	2,22541E-09	2236,4	11,0
			116	2368,759	2155,52	349,46		0,61	2368,759		1,054E+12	2,2474E-09		0,0
			117	1752,215	2155,52	349,46		1,15			1,054E+12			0,0
1B	InFe	PilA	118	263,075	250,55	12,55	5,0085	1,00	263,075	250,548	1,054E+12	2,49597E-10	237,7	9,7
			119	250,592	250,55	12,55		0,00	250,592		1,054E+12	2,37753E-10		0,0
			120	237,978	250,55	12,55		1,00	237,978		1,054E+12	2,25785E-10		0,0
1C	PFe	Sid	121	1785,021	1999,78	186,95	9,3483	1,15		2107,153	8,772E+11		2402,1	21,6
			122	2126,102	1999,78	186,95		0,68	2126,102		8,772E+11	2,42374E-09		0,0
			123	2088,203	1999,78	186,95		0,47	2088,203		8,772E+11	2,38053E-09		0,0
1C	InFe	Sid	124	111,555	106,15	6,82	6,4285	0,79	111,555	106,147	8,772E+11	1,27171E-10	121,0	6,4
			125	108,405	106,15	6,82		0,33	108,405		8,772E+11	1,23581E-10		0,0
			126	98,480	106,15	6,82		1,12	98,480		8,772E+11	1,12266E-10		0,0
2A	PFe	WT	127	401,152	334,52	78,16	23,366	0,85	401,152	334,519	7,95033E+11	5,04573E-10	420,8	80,3
			128	248,481	334,52	78,16		1,10	248,481		7,95033E+11	3,12542E-10		0,0
			129	353,923	334,52	78,16		0,25	353,923		7,95033E+11	4,45167E-10		0,0
2A	InFe	WT	130	125,611	140,14	22,91	16,351	0,63	125,611	126,932	7,95033E+11	1,57995E-10	159,7	1,7
			131	166,555	140,14	22,91		1,15			7,95033E+11			0,0
			132	128,254	140,14	22,91		0,52	128,254		7,95033E+11	1,61318E-10		0,0

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
2B	PFe	PilA	133	300,585	308,70	65,63	21,259	0,12	300,585	308,700	4,90167E+11	6,13231E-10	629,8	109,3
			134	247,510	308,70	65,63		0,93	247,510		4,90167E+11	5,04951E-10		0,0
			135	378,006	308,70	65,63		1,06	378,006		4,90167E+11	7,71179E-10		0,0
2B	InFe	PilA	136	12,943	16,98	6,28	36,993	0,64	12,943	13,362	4,90167E+11	2,64063E-11	27,3	0,9
			137	13,780	16,98	6,28		0,51	13,780		4,90167E+11	2,81132E-11		0,0
			138	24,218	16,98	6,28		1,15			4,90167E+11			0,0
2C	PFe	Sid	139	131,837	93,90	34,74	36,995	1,09	131,837	93,901	9,877E+11	1,33479E-10	95,1	28,7
			140	63,644	93,90	34,74		0,87	63,644		9,877E+11	6,44366E-11		0,0
			141	86,223	93,90	34,74		0,22	86,223		9,877E+11	8,72971E-11		0,0
2C	InFe	Sid	142	31,263	53,32	19,13	35,881	1,15		64,349	9,877E+11		65,2	1,1
			143	63,277	53,32	19,13		0,52	63,277		9,877E+11	6,40653E-11		0,0
			144	65,421	53,32	19,13		0,63	65,421		9,877E+11	6,62354E-11		0,0
		Filter blanks	145											
			146											
			147											
			148	SECOND STAGE OF THE EXPERIMENT, 100 nM										
		Oxalate filter blanks	149											
			150											
			151											
			152											
1A	PFe	WT	153	54,17002	60,58	22,79	37,621	0,28	54,17001877	60,581269	4,06E+11	1,33424E-10	149,2	45,8
			154	85,89167	60,58	22,79		1,11	85,89166509		4,06E+11	2,11556E-10		0,0
			155	41,68212	60,58	22,79		0,83	41,6821239		4,06E+11	1,02665E-10		0,0
1A	InFe	WT	156	0	12,37	14,99	121,22	0,82		18,551896	4,06E+11		45,7	25,8

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
			157	8,062153	12,37	14,99		0,29	8,062152609		4,06E+11	1,98575E-11		0,0
			158	29,04164	12,37	14,99		1,11	29,04163953		4,06E+11	7,15311E-11		0,0
1B	PFe	PilA	159	5,837566	4,75	4,31	90,72	0,25	5,837565817	7,120088	4,11333E+11	1,41918E-11	17,3	3,1
			160	0	4,75	4,31		1,10			4,11333E+11			0,0
			161	8,40261	4,75	4,31		0,85	8,402610173		4,11333E+11	2,04277E-11		0,0
1B	InFe	PilA	162	0	0,00				0	0	4,11333E+11		0,0	0,0
			163	0					0		4,11333E+11	0		0,0
			164	0					0		4,11333E+11	0		0,0
1C	PFe	Sid	165	17,10731	14,20	2,52	17,765	1,15		12,745771	4,1E+11		31,1	0,4
			166	12,59524	14,20	2,52		0,64	12,59523937		4,1E+11	3,07201E-11		0,0
			167	12,8963	14,20	2,52		0,52	12,89630192		4,1E+11	3,14544E-11		0,0
1C	InFe	Sid	168	0	0,00				0	0	4,1E+11	0	0,0	0,0
			169	0					0		4,1E+11	0		0,0
			170	0					0		4,1E+11	0		0,0
2A	PFe	WT	171	15,67802	24,61	14,16	57,537	0,63	15,67801944	16,446961	4,25333E+11	3,68605E-11	38,7	1,8
			172	40,93677	24,61	14,16		1,15			4,25333E+11			0,0
			173	17,2159	24,61	14,16		0,52	17,21590233		4,25333E+11	4,04763E-11		0,0
2A	InFe	WT	174		0,00				0	0	4,25333E+11	0	0,0	0,0
			175						0		4,25333E+11	0		0,0
			176	0					0		4,25333E+11	0		0,0
2B	PFe	PilA	177	0	0,00				0	0	4,18667E+11	0	0,0	0,0
			178	0					0		4,18667E+11	0		0,0
			179	0					0		4,18667E+11	0		0,0

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
2B	InFe	PilA	180	0	0,00				0	0	4,18667E+11	0	0,0	0,0
			181	0					0		4,18667E+11	0		0,0
			182	0					0		4,18667E+11	0		0,0
2C	PFe	Sid	183	4,891062	7,07	4,40	62,147	0,50	4,891061642	4,5425813	4,08667E+11	1,19683E-11	11,1	0,9
			184	4,194101	7,07	4,40		0,65	4,194101023		4,08667E+11	1,02629E-11		0,0
			185	12,13115	7,07	4,40		1,15			4,08667E+11			0,0
2C	InFe	Sid	186	0	0,00				0	0	4,08667E+11	0	0,0	0,0
			187	0					0		4,08667E+11	0		0,0
			188	0					0		4,08667E+11	0		0,0
1A	PFe	WT	189	9,738374	20,43	11,61	56,83	0,92	9,73837438	20,42811	4,40167E+11	2,21243E-11	46,4	21,5
			190	32,77819	20,43	11,61		1,06	32,7781886		4,40167E+11	7,44677E-11		0,0
			191	18,76777	20,43	11,61		0,14	18,76776589		4,40167E+11	4,26379E-11		0,0
1A	InFe	WT	192	6,591431	8,58	7,17	83,639	0,28	6,59143072	8,5754898	4,40167E+11	1,49749E-11	19,5	13,3
			193	2,603931	8,58	7,17		0,83	2,603931427		4,40167E+11	5,91579E-12		0,0
			194	16,53111	8,58	7,17		1,11	16,53110714		4,40167E+11	3,75565E-11		0,0
1B	PFe	PilA	195	22,46974	16,28	7,53	46,24	0,82	22,46973754	16,277622	4,88933E+11	4,59566E-11	33,3	12,6
			196	18,46326	16,28	7,53		0,29	18,46326402		4,88933E+11	3,77623E-11		0,0
			197	7,899863	16,28	7,53		1,11	7,899863234		4,88933E+11	1,61573E-11		0,0
1B	InFe	PilA	198	21,99184	23,05	3,63	15,749	0,29	21,9918433	23,048677	4,88933E+11	4,49792E-11	47,1	6,1
			199	20,06441	23,05	3,63		0,82	20,0644142		4,88933E+11	4,10371E-11		0,0

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
			200	27,08977	23,05	3,63		1,11	27,08977323		4,88933E+11	5,54059E-11		0,0
1C	PFe	Sid	201	11,26307	9,96	7,43	74,601	0,17	11,26307321	9,9635782	3,85067E+11	2,92497E-11	25,9	15,8
			202	1,966568	9,96	7,43		1,08	1,966568338		3,85067E+11	5,10709E-12		0,0
			203	16,66109	9,96	7,43		0,90	16,66109296		3,85067E+11	4,32681E-11		0,0
1C	InFe	Sid	204	0	0,00				0	0	3,85067E+11	0	0,0	0,0
			205	0					0		3,85067E+11	0		0,0
			206	0					0		3,85067E+11	0		0,0
2A	PFe	WT	207	0	0,00				0	0	3,895E+11	0	0,0	0,0
			208	0					0		3,895E+11	0		0,0
			209	0					0		3,895E+11	0		0,0
2A	InFe	WT	210	0	0,00				0	0	3,895E+11	0	0,0	0,0
			211	0					0		3,895E+11	0		0,0
			212	0					0		3,895E+11	0		0,0
2B	PFe	PilA	213	0	0,00				0	0	3,95833E+11	0	0,0	0,0
			214	0					0		3,95833E+11	0		0,0
			215	0					0		3,95833E+11	0		0,0
2B	InFe	PilA	216	0	0,00				0	0	3,95833E+11	0	0,0	0,0
			217	0					0		3,95833E+11	0		0,0
			218	0					0		3,95833E+11	0		0,0
2C	PFe	Sid	219	0	0,00				0	0	3,88867E+11	0	0,0	0,0
			220	0					0		3,88867E+11	0		0,0
			221	0					0		3,88867E+11	0		0,0
2C	InFe	Sid	222	0	0,00				0	0	3,88867E+11	0	0,0	0,0
			223	0					0		3,88867E+11	0		0,0
			224	0					0		3,88867E+11	0		0,0
1A	PFe	WT	225	13,19672	8,81	3,80	43,198	1,15		6,6096366	4,782E+11		13,8	0,2

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
			226	6,683015	8,81	3,80		0,56	6,683014702		4,782E+11	1,39754E-11		0,0
			227	6,536258	8,81	3,80		0,60	6,536258476		4,782E+11	1,36685E-11		0,0
1A	InFe	WT	228	2,260584	8,73	12,04	137,93	0,54	2,260584289	1,7830708	4,782E+11	4,72728E-12	3,7	1,0
			229	22,61659	8,73	12,04		1,15			4,782E+11			0,0
			230	1,305557	8,73	12,04		0,62	1,305557329		4,782E+11	2,73015E-12		0,0
1B	PFe	PilA	231	6,094798	8,20	1,91	23,255	1,10	6,094797657	8,1987781	4,656E+11	1,30902E-11	17,6	3,3
			232	9,81224	8,20	1,91		0,85	9,812239596		4,656E+11	2,10744E-11		0,0
			233	8,689297	8,20	1,91		0,26	8,689296917		4,656E+11	1,86626E-11		0,0
1B	InFe	PilA	234	0	0,00				0	0	4,656E+11	0	0,0	0,0
			235	0					0		4,656E+11	0		0,0
			236	0					0		4,656E+11	0		0,0
1C	PFe	Sid	237	0	0,00				0	0	3,69E+11	0	0,0	0,0
			238	0					0		3,69E+11	0		0,0
			239	0					0		3,69E+11	0		0,0
1C	InFe	Sid	240	0	0,00				0	0	3,69E+11	0	0,0	0,0
			241	0					0		3,69E+11	0		0,0
			242	0					0		3,69E+11	0		0,0
2A	PFe	WT	243	0	0,00				0	0	3,702E+11	0	0,0	0,0
			244	0					0		3,702E+11	0		0,0
			245	0					0		3,702E+11	0		0,0
2A	InFe	WT	246	0	0,00				0	0	3,702E+11	0	0,0	0,0
			247	0					0		3,702E+11	0		0,0
			248	0					0		3,702E+11	0		0,0
2B	PFe	PilA	249	0					0		3,828E+11	0	0,0	0,0
			250	0					0		3,828E+11	0		0,0

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
			251	0					0		3,828E+11	0		0,0
2B	InFe	PilA	252	0					0		3,828E+11	0	0,0	0,0
			253	0					0		3,828E+11	0		0,0
			254	0					0		3,828E+11	0		0,0
2C	PFe	Sid	255	0					0		3,6E+11	0	0,0	0,0
			256	0					0		3,6E+11	0		0,0
			257	0					0		3,6E+11	0		0,0
2C	InFe	Sid	258	0					0		3,6E+11	0	0,0	0,0
			259	0					0		3,6E+11	0		0,0
			260	0					0		3,6E+11	0		0,0
1A	PFe	WT	261	14,30581	13,13	1,07	8,1443	1,10	14,30580869	13,13314	6,60167E+11	2,167E-11	19,9	1,3
			262	12,21112	13,13	1,07		0,86	12,21112391		6,60167E+11	1,8497E-11		0,0
			263	12,88249	13,13	1,07		0,23	12,88248798		6,60167E+11	1,9514E-11		0,0
1A	InFe	WT	264	6,951356	8,72	4,58	52,469	0,39	6,951356201	8,7222894	6,60167E+11	1,05297E-11	13,2	5,7
			265	5,295877	8,72	4,58		0,75	5,295877289		6,60167E+11	8,02203E-12		0,0
			266	13,91963	8,72	4,58		1,14	13,91963481		6,60167E+11	2,1085E-11		0,0
1B	PFe	PilA	267	25,87095	24,38	3,27	13,425	0,46	25,87095474	26,258051	6,8E+11	3,80455E-11	38,6	0,6
			268	26,64515	24,38	3,27		0,69	26,64514688		6,8E+11	3,9184E-11		0,0
			269	20,62835	24,38	3,27		1,15			6,8E+11			0,0
1B	InFe	PilA	270	6,487178	11,16	4,38	39,268	1,07	6,487178389	11,155505	6,8E+11	9,53997E-12	16,4	5,3
			271	11,80318	11,16	4,38		0,15	11,80318085		6,8E+11	1,73576E-11		0,0
			272	15,17616	11,16	4,38		0,92	15,17615508		6,8E+11	2,23179E-11		0,0

Culture	Fe type	Type	Filter #	56Fe, nM	Fe avg	Fe, StDev	Fe, RSD, %	Z (Grubb's test)	56Fe, nM (outliers omitted)	New Fe avg	Cells, avg per culture volume	Fe nM/cell	Fe avg E-12 nM/cell	Fe/cell E-12, StDev values
1C	PFe	Sid	273	16,99018	8,27	7,73	93,438	1,13	16,99017721	8,2724446	3,621E+11	4,69212E-11	22,8	17,4
			274	2,255944	8,27	8,27		0,73	2,255944274		3,621E+11	6,23017E-12		0,0
			275	5,571212	8,27	8,27		0,33	5,571212381		3,621E+11	1,53858E-11		0,0
1C	InFe	Sid	276	0					0		3,621E+11	0	0,0	0,0
			277	0					0		3,621E+11	0		0,0
			278	0					0		3,621E+11	0		0,0
2A	PFe	WT	279	0					0		3,66067E+11	0	0,0	0,0
			280	0					0		3,66067E+11	0		0,0
			281	0					0		3,66067E+11	0		0,0
2A	InFe	WT	282	0					0		3,66067E+11	0	0,0	0,0
			283	0					0		3,66067E+11	0		0,0
			284	0					0		3,66067E+11	0		0,0
2B	PFe	PilA	285	0					0		3,672E+11	0	0,0	0,0
			286	0					0		3,672E+11	0		0,0
			287	0					0		3,672E+11	0		0,0
2B	InFe	PilA	288	0					0		3,672E+11	0	0,0	0,0
			289	0					0		3,672E+11	0		0,0
			290	0					0		3,672E+11	0		0,0
2C	PFe	Sid	291	0					0		3,4E+11	0	0,0	0,0
			292	0					0		3,4E+11	0		0,0
			293	0					0		3,4E+11	0		0,0
2C	InFe	Sid	294	0					0		3,4E+11	0	0,0	0,0
			295	0					0		3,4E+11	0		0,0
			296	0					0		3,4E+11	0		0,0
END OF THE EXPERIMENT														

Table B-2 TFe ICP-MS data processing

Sample ID	TFe [uM]	Tfe average	StDev
Experiment 1			
Culture 1A 03.10.19 a	1,773637	1,722653763	0,036249
Culture 1A 03.10.19 b	1,7018		
Culture 1A 03.10.19 c	1,692524		
Culture 1B 03.10.19 a	5,039461	5,105513081	0,076095
Culture 1B 03.10.19 b	5,212115		
Culture 1B 03.10.19 c	5,064964		
Culture 1C 03.10.19 a	5,099338	5,04487703	0,061771
Culture 1C 03.10.19 b	4,958495		
Culture 1C 03.10.19 c	5,076799		
Culture 2A 03.10.19 a	4,903339	4,803137696	0,098554
Culture 2A 03.10.19 b	4,669138		
Culture 2A 03.10.19 c	4,836936		
Culture 2B 03.10.19 a	2,310866	2,350168013	0,03115
Culture 2B 03.10.19 b	2,352584		
Culture 2B 03.10.19 c	2,387054		
Culture 2C 03.10.19 a	0,365581	0,370565705	0,004772
Culture 2C 03.10.19 b	0,369118		
Culture 2C 03.10.19 c	0,376998		
Culture 1A 24.10.19 a	0,487473	0,475233014	0,008766
Culture 1A 24.10.19 b	0,470821		
Culture 1A 24.10.19 c	0,467406		
Culture 1B 24.10.19 a	1,847881	1,836762873	0,035081
Culture 1B 24.10.19 b	1,873077		
Culture 1B 24.10.19 c	1,789331		
Culture 1C 24.10.19 a	0,24882	0,244489642	0,00347
Culture 1C 24.10.19 b	0,244323		
Culture 1C 24.10.19 c	0,240325		
Culture 2A 24.10.19 a	1,041143	1,045190325	0,005801
Culture 2A 24.10.19 b	1,041034		
Culture 2A 24.10.19 c	1,053394		
Culture 2B 24.10.19 a	0,178752	0,181364826	0,002238
Culture 2B 24.10.19 b	0,184219		
Culture 2B 24.10.19 c	0,181123		
Culture 2C 24.10.19 a	0,163278	0,162559658	0,001961
Culture 2C 24.10.19 b	0,16452		
Culture 2C 24.10.19 c	0,159881		
Experiment 2			
Culture 1A 07.11.19 a	0,095292	0,094268677	0,000826
Culture 1A 07.11.19 b	0,094244		
Culture 1A 07.11.19 c	0,09327		
Culture 1B 07.11.19 a	0,102165	0,104062594	0,001447
Culture 1B 07.11.19 b	0,104346		

Sample ID	TFe [uM]	Tfe average	StDev
Culture 1B 07.11.19 c	0,105676		
Culture 1C 07.11.19 a	0,118899	0,118003116	0,002783
Culture 1C 07.11.19 b	0,114236		
Culture 1C 07.11.19 c	0,120874		
Culture 2A 07.11.19 a	0,033427	0,035282423	0,001342
Culture 2A 07.11.19 b	0,035866		
Culture 2A 07.11.19 c	0,036554		
Culture 2B 07.11.19 a	0,023359	0,023898744	0,000408
Culture 2B 07.11.19 b	0,02399		
Culture 2B 07.11.19 c	0,024347		
Culture 2C 07.11.19 a	0,038128	0,038264887	0,000864
Culture 2C 07.11.19 b	0,037281		
Culture 2C 07.11.19 c	0,039385		
Culture 1A 27.11.19 a	0,043502	0,043466657	0,000862
Culture 1A 27.11.19 b	0,042394		
Culture 1A 27.11.19 c	0,044504		
Culture 1B 27.11.19 a	0,043759	0,049532454	0,004085
Culture 1B 27.11.19 b	0,05259		
Culture 1B 27.11.19 c	0,052249		
Culture 1C 27.11.19 a	0,054907	0,056047224	0,000864
Culture 1C 27.11.19 b	0,056997		
Culture 1C 27.11.19 c	0,056238		
Culture 2A 27.11.19 a	0,006545	0,005926737	0,001055
Culture 2A 27.11.19 b	0,004442		
Culture 2A 27.11.19 c	0,006793		
Culture 2B 27.11.19 a	0,005166	0,006596228	0,001013
Culture 2B 27.11.19 b	0,007254		
Culture 2B 27.11.19 c	0,007369		
Culture 2C 27.11.19 a	0,005189	0,004655711	0,000731
Culture 2C 27.11.19 b	0,005156		
Culture 2C 27.11.19 c	0,003623		

Appendix D TFe graphs

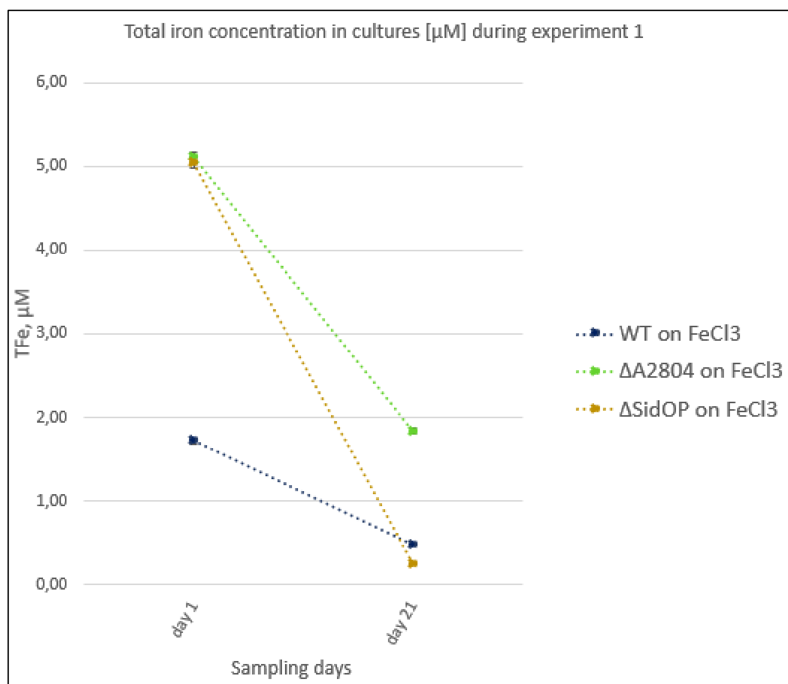

Figure D-1 Total iron concentrations in $FeCl_3$ cultures during experiment 1

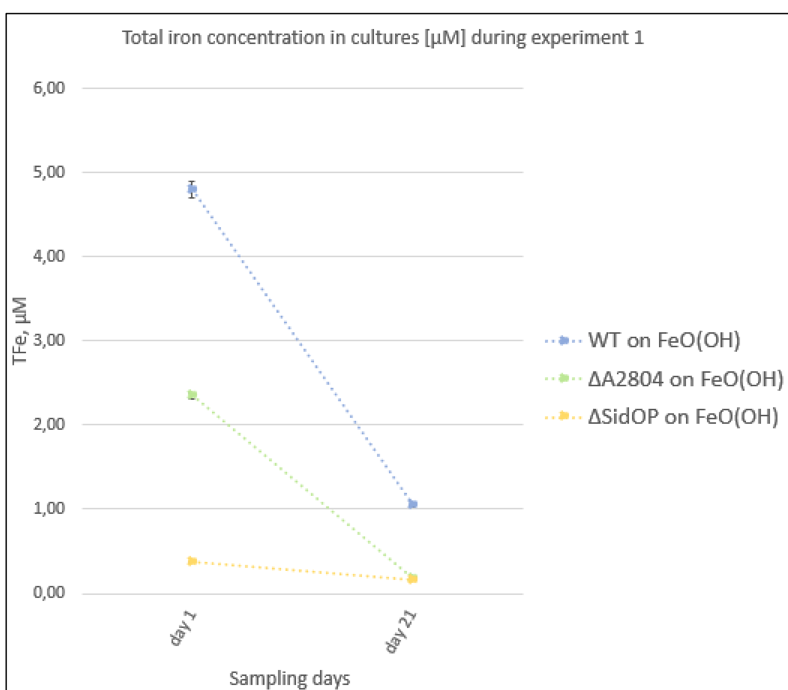

Figure D-2 Total iron concentrations in FeO(OH) cultures during experiment 1

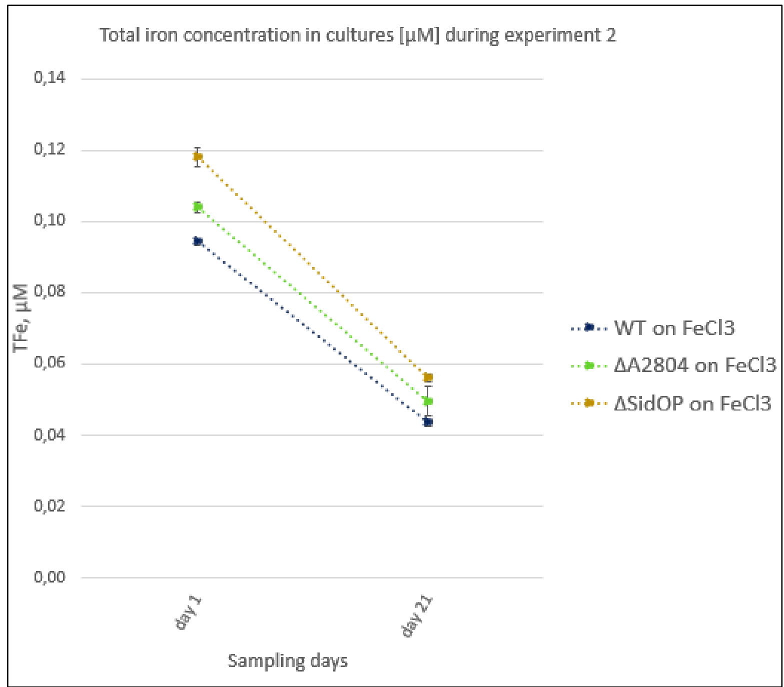

Figure D-3 Total iron concentrations in $FeCl_3$ cultures during experiment 2

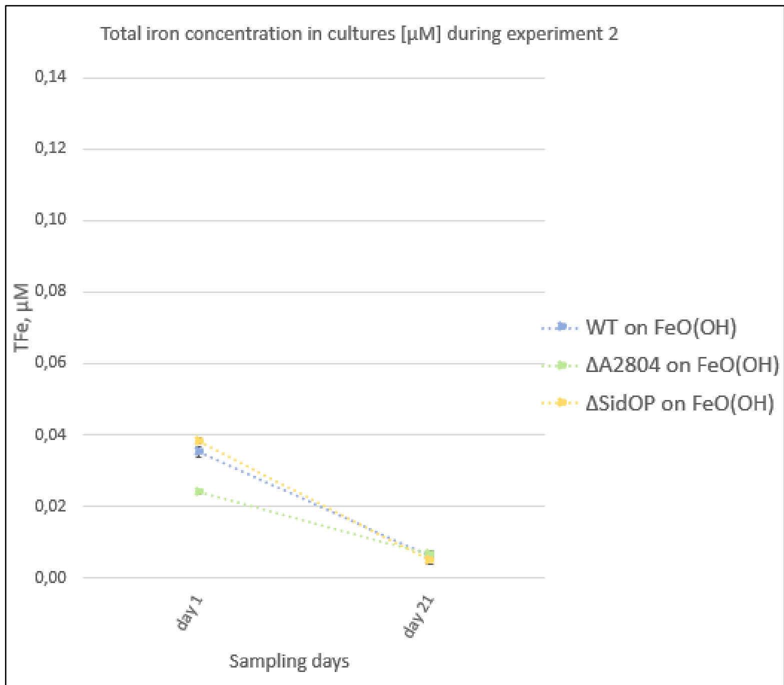

Figure D-4 Total iron concentrations in FeO(OH) cultures during experiment 2

Appendix E OD_{730} data processing

Date	Day	Culture	Parallell	OD	Average	StDev	No cells	No cells avg	StDev
03.10.2019	1	1A	a	0,007	0,006	0,002	103500000	102833333,3	623610
03.10.2019	1	1A	b	0,006			103000000		
03.10.2019	1	1A	c	0,004			102000000		
03.10.2019	1	1B	a	0,008	0,008	0,0006	104000000	104166666,7	235702
03.10.2019	1	1B	b	0,009			104500000		
03.10.2019	1	1B	c	0,008			104000000		
03.10.2019	1	1C	a	0,013	0,011	0,003	106500000	105333333,3	1027402
03.10.2019	1	1C	b	0,008			104000000		
03.10.2019	1	1C	c	0,011			105500000		
03.10.2019	1	2A	a	0,011	0,011	0,002	105500000	105500000	816497
03.10.2019	1	2A	b	0,009			104500000		
03.10.2019	1	2A	c	0,013			106500000		
03.10.2019	1	2B	a	0,004	0,004	0	102000000	102000000	0
03.10.2019	1	2B	b	0,004			102000000		
03.10.2019	1	2B	c	0,004			102000000		
03.10.2019	1	2C	a	0	0	0	100000000	100000000	0
03.10.2019	1	2C	b	0			100000000		
03.10.2019	1	2C	c	0			100000000		
09.10.2019	7	1A	a	0,104	0,102	0,002	152000000	151166666,7	623610
09.10.2019	7	1A	b	0,102			151000000		
09.10.2019	7	1A	c	0,101			150500000		
09.10.2019	7	1B	a	0,083	0,086	0,003	141500000	143000000	1224745
09.10.2019	7	1B	b	0,089			144500000		
09.10.2019	7	1B	c	0,086			143000000		
09.10.2019	7	1C	a	0,089	0,090	0,001	144500000	144833333,3	471405
09.10.2019	7	1C	b	0,091			145500000		
09.10.2019	7	1C	c	0,089			144500000		
09.10.2019	7	2A	a	0,061	0,061	0,001	130500000	130500000	408248
09.10.2019	7	2A	b	0,062			131000000		
09.10.2019	7	2A	c	0,060			130000000		
09.10.2019	7	2B	a	0,177	0,172	0,005	188500000	185833333,3	1929306
09.10.2019	7	2B	b	0,170			185000000		
09.10.2019	7	2B	c	0,168			184000000		
09.10.2019	7	2C	a	0,020	0,020	0,001	110000000	110166666,7	235702
09.10.2019	7	2C	b	0,021			110500000		
09.10.2019	7	2C	c	0,020			110000000		
16.10.2019	14	1A	a	0,404	0,405	0,001	302000000	302666666,7	471405
16.10.2019	14	1A	b	0,406			303000000		
16.10.2019	14	1A	c	0,406			303000000		

Date	Day	Culture	Parallell	OD	Average	StDev	No cells	No cells avg	StDev
16.10.2019	14	1B	a	0,244	0,242	0,0020	222000000	221000000	816497
16.10.2019	14	1B	b	0,242			221000000		
16.10.2019	14	1B	c	0,240			220000000		
16.10.2019	14	1C	a	0,169	0,169	0,002	184500000	184666666,7	623610
16.10.2019	14	1C	b	0,171			185500000		
16.10.2019	14	1C	c	0,168			184000000		
16.10.2019	14	2A	a	0,210	0,208	0,002	205000000	204000000	707107
16.10.2019	14	2A	b	0,207			203500000		
16.10.2019	14	2A	c	0,207			203500000		
16.10.2019	14	2B	a	0,236	0,237	0,003	218000000	218333333,3	1247219
16.10.2019	14	2B	b	0,240			220000000		
16.10.2019	14	2B	c	0,234			217000000		
16.10.2019	14	2C	a	0,235	0,231	0,004	217500000	215500000	1471960
16.10.2019	14	2C	b	0,230			215000000		
16.10.2019	14	2C	c	0,228			214000000		
23.10.2019	21	1A	a	0,599	0,596	0,003	399500000	398000000	1224745
23.10.2019	21	1A	b	0,596			398000000		
23.10.2019	21	1A	c	0,593			396500000		
23.10.2019	21	1B	a	0,420	0,420	0,004	310000000	310000000	1632993
23.10.2019	21	1B	b	0,424			312000000		
23.10.2019	21	1B	c	0,416			308000000		
23.10.2019	21	1C	a	0,318	0,316	0,002	259000000	258000000	816497
23.10.2019	21	1C	b	0,314			257000000		
23.10.2019	21	1C	c	0,316			258000000		
23.10.2019	21	2A	a	0,267	0,268	0,002	233500000	233833333,3	849837
23.10.2019	21	2A	b	0,266			233000000		
23.10.2019	21	2A	c	0,270			235000000		
23.10.2019	21	2B	a	0,088	0,0883	0,002	144000000	144166666,7	623610
23.10.2019	21	2B	b	0,090			145000000		
23.10.2019	21	2B	c	0,087			143500000		
23.10.2019	21	2C	a	0,380	0,381	0,001	290000000	290500000	408248
23.10.2019	21	2C	b	0,381			290500000		
23.10.2019	21	2C	c	0,382			291000000		
07.11.2019	1	1A	a	0,003	0,003	0,000	101500000	101500000	0
07.11.2019	1	1A	b	0,003			101500000		
07.11.2019	1	1A	c	0,003			101500000		
07.11.2019	1	1B	a	0,005	0,0057	0,001	102500000	102833333,3	235702

Date	Day	Culture	Parallell	OD	Average	StDev	No cells	No cells avg	StDev
07.11.2019	1	1B	b	0,006			103000000		
07.11.2019	1	1B	c	0,006			103000000		
07.11.2019	1	1C	a	0,005	0,005	0,000	102500000	102500000	0
07.11.2019	1	1C	b	0,005			102500000		
07.11.2019	1	1C	c	0,005			102500000		
07.11.2019	1	2A	a	0,013	0,0127	0,001	106500000	106333333,3	235702
07.11.2019	1	2A	b	0,012			106000000		
07.11.2019	1	2A	c	0,013			106500000		
07.11.2019	1	2B	a	0,01	0,009	0,001	105000000	104666666,7	235702
07.11.2019	1	2B	b	0,009			104500000		
07.11.2019	1	2B	c	0,009			104500000		
07.11.2019	1	2C	a	0,004	0,004	0,001	102000000	102166666,7	235702
07.11.2019	1	2C	b	0,004			102000000		
07.11.2019	1	2C	c	0,005			102500000		
13.11.2019	7	1A	a	0,03	0,032	0,002	115000000	115833333,3	849837
13.11.2019	7	1A	b	0,034			117000000		
13.11.2019	7	1A	c	0,031			115500000		
13.11.2019	7	1B	a	0,057	0,0573	0,001	128500000	128666666,7	235702
13.11.2019	7	1B	b	0,057			128500000		
13.11.2019	7	1B	c	0,058			129000000		
13.11.2019	7	1C	a	0,002	0,003	0,001	101000000	101333333,3	235702
13.11.2019	7	1C	b	0,003			101500000		
13.11.2019	7	1C	c	0,003			101500000		
13.11.2019	7	2A	a	0,005	0,0050	0,000	102500000	102500000	0
13.11.2019	7	2A	b	0,005			102500000		
13.11.2019	7	2A	c	0,005			102500000		
13.11.2019	7	2B	a	0,009	0,008	0,001	104500000	104166666,7	235702
13.11.2019	7	2B	b	0,008			104000000		
13.11.2019	7	2B	c	0,008			104000000		
13.11.2019	7	2C	a	0,005	0,005	0,001	102500000	102333333,3	235702
13.11.2019	7	2C	b	0,005			102500000		
13.11.2019	7	2C	c	0,004			102000000		
20.11.2019	14	1A	a	0,066	0,066	0,001	133000000	132833333,3	235702
20.11.2019	14	1A	b	0,066			133000000		
20.11.2019	14	1A	c	0,065			132500000		
20.11.2019	14	1B	a	0,059	0,0587	0,001	129500000	129333333,3	235702
20.11.2019	14	1B	b	0,059			129500000		
20.11.2019	14	1B	c	0,058			129000000		
20.11.2019	14	1C	a	0,005	0,005	0,000	102500000	102500000	0
20.11.2019	14	1C	b	0,005			102500000		
20.11.2019	14	1C	c	0,005			102500000		
20.11.2019	14	2A	a	0,007	0,0057	0,001	103500000	102833333,3	471405

Date	Day	Culture	Parallell	OD	Average	StDev	No cells	No cells avg	StDev
20.11.2019	14	2A	b	0,005			102500000		
20.11.2019	14	2A	c	0,005			102500000		
20.11.2019	14	2B	a	0,014	0,013	0,001	107000000	106333333,3	471405
20.11.2019	14	2B	b	0,012			106000000		
20.11.2019	14	2B	c	0,012			106000000		
20.11.2019	14	2C	a	0	0,000	0,000	100000000	100000000	0
20.11.2019	14	2C	b	0			100000000		
20.11.2019	14	2C	c	0			100000000		
27.11.2019	21	1A	a	0,189	0,188	0,001	194500000	194166666,7	235702
27.11.2019	21	1A	b	0,188			194000000		
27.11.2019	21	1A	c	0,188			194000000		
27.11.2019	21	1B	a	0,201	0,2000	0,001	200500000	200000000	408248
27.11.2019	21	1B	b	0,2			200000000		
27.11.2019	21	1B	c	0,199			199500000		
27.11.2019	21	1C	a	0,013	0,013	0,000	106500000	106500000	0
27.11.2019	21	1C	b	0,013			106500000		
27.11.2019	21	1C	c	0,013			106500000		
27.11.2019	21	2A	a	0,015	0,0153	0,001	107500000	107666666,7	235702
27.11.2019	21	2A	b	0,015			107500000		
27.11.2019	21	2A	c	0,016			108000000		
27.11.2019	21	2B	a	0,016	0,016	0,001	108000000	108000000	408248
27.11.2019	21	2B	b	0,015			107500000		
27.11.2019	21	2B	c	0,017			108500000		
27.11.2019	21	2C	a	0	0,000	0,000	100000000		0
27.11.2019	21	2C	b	0			100000000		
27.11.2019	21	2C	c	0			100000000		

Appendix F Statistical calculations

Table E-1 Results of two-way ANOVA for experiment 1 (cell concentrations)

Two-way RM ANOVA	Matching: Stacked
Assume sphericity?	No
Alpha	0,05

Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	**Geisser-Greenhouse's epsilon**
Sampling day x Organism and iron source	20,63	<0,0001	****	Yes	
Sampling day	69,07	<0,0001	****	Yes	0,8016
Organism and iron source	10,29	<0,0001	****	Yes	
Subject	0,003970	0,2532	ns	No	
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Sampling day x Organism and iron source	0,3768	15	0,02512	F (15, 36) = 5464	P<0,0001
Sampling day	1,261	3	0,4205	F (2,405, 28,86) = 91468	P<0,0001
Organism and iron source	0,1879	5	0,03758	F (5, 12) = 6220	P<0,0001
Subject	7,250e-005	12	6,042e-006	F (12, 36) = 1,314	P=0,2532
Residual	0,0001655	36	4,597e-006		

Data summary	
Number of columns (Organism and iron source)	6
Number of rows (Sampling day)	4
Number of subjects (Subject)	18
Number of missing values	0

Table E-2 Results of analysis of variance between cell concentrations in cultures on different sampling days during experiment 1 (based on two-way ANOVA)

Number of families	*6*
Number of comparisons per family	*6*
Alpha	*0,05*

Tukey's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Significant?**	**Summary**	**Adjusted P Value**
WT FeCl3					
Day 1 vs. Day 7	-0,09667	-0,09898 to -0,09436	Yes	****	<0,0001
Day 1 vs. Day 14	-0,3997	-0,4097 to -0,3896	Yes	****	<0,0001
Day 1 vs. Day 21	-0,5903	-0,5964 to -0,5842	Yes	****	<0,0001
Day 7 vs. Day 14	-0,303	-0,3136 to -0,2924	Yes	****	<0,0001
Day 7 vs. Day 21	-0,4937	-0,4998 to -0,4876	Yes	****	<0,0001
Day 14 vs. Day 21	-0,1907	-0,2068 to -0,1745	Yes	****	<0,0001
ΔA2804 FeCl3					
Day 1 vs. Day 7	-0,07767	-0,08773 to -0,06760	Yes	****	<0,0001
Day 1 vs. Day 14	-0,2337	-0,2420 to -0,2253	Yes	****	<0,0001
Day 1 vs. Day 21	-0,4117	-0,4257 to -0,3976	Yes	****	<0,0001
Day 7 vs. Day 14	-0,156	-0,1734 to -0,1386	Yes	****	<0,0001
Day 7 vs. Day 21	-0,334	-0,3484 to -0,3196	Yes	****	<0,0001
Day 14 vs. Day 21	-0,178	-0,1919 to -0,1641	Yes	****	<0,0001
ΔSidOP FeCl3					
Day 1 vs. Day 7	-0,079	-0,09342 to -0,06458	Yes	***	0,0004
Day 1 vs. Day 14	-0,1587	-0,1738 to -0,1435	Yes	****	<0,0001
Day 1 vs. Day 21	-0,3053	-0,3076 to -0,3030	Yes	****	<0,0001
Day 7 vs. Day 14	-0,07967	-0,08198 to -0,07736	Yes	****	<0,0001
Day 7 vs. Day 21	-0,2263	-0,2386 to -0,2141	Yes	****	<0,0001
Day 14 vs. Day 21	-0,1467	-0,1595 to -0,1338	Yes	****	<0,0001
WT FeO(OH)					

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Day 1 vs. Day 7	-0,05	-0,06200 to -0,03800	Yes	**	0,0021
Day 1 vs. Day 14	-0,197	-0,2076 to -0,1864	Yes	****	<0,0001
Day 1 vs. Day 21	-0,2567	-0,2590 to -0,2544	Yes	****	<0,0001
Day 7 vs. Day 14	-0,147	-0,1550 to -0,1390	Yes	****	<0,0001
Day 7 vs. Day 21	-0,2067	-0,2189 to -0,1944	Yes	****	<0,0001
Day 14 vs. Day 21	-0,05967	-0,07189 to -0,04745	Yes	***	0,0009
ΔA2804 FeO(OH)					
Day 1 vs. Day 7	-0,1677	-0,1866 to -0,1488	Yes	****	<0,0001
Day 1 vs. Day 14	-0,2327	-0,2449 to -0,2204	Yes	****	<0,0001
Day 1 vs. Day 21	-0,08433	-0,09044 to -0,07822	Yes	****	<0,0001
Day 7 vs. Day 14	-0,065	-0,08727 to -0,04273	Yes	**	0,0063
Day 7 vs. Day 21	0,08333	0,06360 to 0,1031	Yes	**	0,0019
Day 14 vs. Day 21	0,1483	0,1422 to 0,1544	Yes	****	<0,0001
ΔSidOP FeO(OH)					
Day 1 vs. Day 7	-0,02033	-0,02264 to -0,01802	Yes	****	<0,0001
Day 1 vs. Day 14	-0,231	-0,2454 to -0,2166	Yes	****	<0,0001
Day 1 vs. Day 21	-0,381	-0,3850 to -0,3770	Yes	****	<0,0001
Day 7 vs. Day 14	-0,2107	-0,2258 to -0,1955	Yes	****	<0,0001
Day 7 vs. Day 21	-0,3607	-0,3653 to -0,3560	Yes	****	<0,0001
Day 14 vs. Day 21	-0,15	-0,1683 to -0,1317	Yes	****	<0,0001

Table E-3 Results of analysis of variance between cell concentrations in different cultures during experiment 1 (based on two-way ANOVA)

Number of families	*4*
Number of comparisons per family	*15*
Alpha	*0,05*

Tukey's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Significant?**	**Summary**	**Adjusted P Value**
Day 1					
WT FeCl3 vs. ΔA2804 FeCl3	-0,002667	-0,008755 to 0,003421	No	ns	0,2933
WT FeCl3 vs. ΔSidOP FeCl3	-0,005	-0,01404 to 0,004043	No	ns	0,2331
WT FeCl3 vs. WT FeO(OH)	-0,005333	-0,01248 to 0,001818	No	ns	0,1203
WT FeCl3 vs. ΔA2804 FeO(OH)	0,001667	-0,005651 to 0,008984	No	ns	0,5647
WT FeCl3 vs. ΔSidOP FeO(OH)	0,005667	-0,001651 to 0,01298	No	ns	0,0814
ΔA2804 FeCl3 vs. ΔSidOP FeCl3	-0,002333	-0,01341 to 0,008747	No	ns	0,6735
ΔA2804 FeCl3 vs. WT FeO(OH)	-0,002667	-0,01113 to 0,005799	No	ns	0,4483
ΔA2804 FeCl3 vs. ΔA2804 FeO(OH)	0,004333	0,001568 to 0,007099	Yes	*	0,0208
ΔA2804 FeCl3 vs. ΔSidOP FeO(OH)	0,008333	0,005568 to 0,01110	Yes	**	0,0061
ΔSidOP FeCl3 vs. WT FeO(OH)	-0,000333	-0,009378 to 0,008711	No	ns	>0,9999
ΔSidOP FeCl3 vs. ΔA2804 FeO(OH)	0,006667	-0,005388 to 0,01872	No	ns	0,151
ΔSidOP FeCl3 vs. ΔSidOP FeO(OH)	0,01067	-0,001388 to 0,02272	No	ns	0,0632
WT FeO(OH) vs. ΔA2804 FeO(OH)	0,007	-0,002580 to 0,01658	No	ns	0,0908
WT FeO(OH) vs. ΔSidOP FeO(OH)	0,011	0,001420 to 0,02058	Yes	*	0,0383
ΔA2804 FeO(OH) vs. ΔSidOP FeO(OH)	0,004		Yes	****	<0,0001
Day 7					
WT FeCl3 vs. ΔA2804 FeCl3	0,01633	0,005210 to 0,02746	Yes	*	0,0173
WT FeCl3 vs. ΔSidOP FeCl3	0,01267	0,007211 to 0,01812	Yes	**	0,0028

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
WT FeCl3 vs. WT FeO(OH)	0,04133	0,03588 to 0,04678	Yes	****	<0,0001
WT FeCl3 vs. ΔA2804 FeO(OH)	-0,06933	-0,08887 to -0,04980	Yes	**	0,003
WT FeCl3 vs. ΔSidOP FeO(OH)	0,082	0,07591 to 0,08809	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔSidOP FeCl3	-0,003667	-0,01557 to 0,008236	No	ns	0,5126
ΔA2804 FeCl3 vs. WT FeO(OH)	0,025	0,01268 to 0,03732	Yes	**	0,0094
ΔA2804 FeCl3 vs. ΔA2804 FeO(OH)	-0,08567	-0,1026 to -0,06877	Yes	***	0,0004
ΔA2804 FeCl3 vs. ΔSidOP FeO(OH)	0,06567	0,05216 to 0,07918	Yes	***	0,0006
ΔSidOP FeCl3 vs. WT FeO(OH)	0,02867	0,02444 to 0,03289	Yes	****	<0,0001
ΔSidOP FeCl3 vs. ΔA2804 FeO(OH)	-0,082	-0,1026 to -0,06139	Yes	**	0,0022
ΔSidOP FeCl3 vs. ΔSidOP FeO(OH)	0,06933	0,06503 to 0,07363	Yes	****	<0,0001
WT FeO(OH) vs. ΔA2804 FeO(OH)	-0,1107	-0,1317 to -0,08963	Yes	***	0,0003
WT FeO(OH) vs. ΔSidOP FeO(OH)	0,04067	0,03705 to 0,04429	Yes	****	<0,0001
ΔA2804 FeO(OH) vs. ΔSidOP FeO(OH)	0,1513	0,1293 to 0,1734	Yes	****	<0,0001
Day 14					
WT FeCl3 vs. ΔA2804 FeCl3	0,1633	0,1561 to 0,1706	Yes	****	<0,0001
WT FeCl3 vs. ΔSidOP FeCl3	0,236	0,2305 to 0,2415	Yes	****	<0,0001
WT FeCl3 vs. WT FeO(OH)	0,1973	0,1912 to 0,2035	Yes	****	<0,0001
WT FeCl3 vs. ΔA2804 FeO(OH)	0,1687	0,1565 to 0,1808	Yes	****	<0,0001
WT FeCl3 vs. ΔSidOP FeO(OH)	0,1743	0,1594 to 0,1893	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔSidOP FeCl3	0,07267	0,06552 to 0,07982	Yes	****	<0,0001
ΔA2804 FeCl3 vs. WT FeO(OH)	0,034	0,02668 to 0,04132	Yes	***	0,0002
ΔA2804 FeCl3 vs. ΔA2804 FeO(OH)	0,005333	-0,005564 to 0,01623	No	ns	0,3135
ΔA2804 FeCl3 vs. ΔSidOP FeO(OH)	0,011	-0,002139 to 0,02414	No	ns	0,081
ΔSidOP FeCl3 vs. WT FeO(OH)	-0,03867	-0,04504 to -0,03229	Yes	****	<0,0001

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
ΔSidOP FeCl3 vs. ΔA2804 FeO(OH)	-0,06733	-0,07871 to -0,05596	Yes	***	0,0002
ΔSidOP FeCl3 vs. ΔSidOP FeO(OH)	-0,06167	-0,07564 to -0,04770	Yes	**	0,0012
WT FeO(OH) vs. ΔA2804 FeO(OH)	-0,02867	-0,03976 to -0,01758	Yes	**	0,0029
WT FeO(OH) vs. ΔSidOP FeO(OH)	-0,023	-0,03655 to -0,009453	Yes	*	0,0119
ΔA2804 FeO(OH) vs. ΔSidOP FeO(OH)	0,005667	-0,007460 to 0,01879	No	ns	0,4382
Day 21					
WT FeCl3 vs. ΔA2804 FeCl3	0,176	0,1617 to 0,1903	Yes	****	<0,0001
WT FeCl3 vs. ΔSidOP FeCl3	0,28	0,2693 to 0,2907	Yes	****	<0,0001
WT FeCl3 vs. WT FeO(OH)	0,3283	0,3177 to 0,3390	Yes	****	<0,0001
WT FeCl3 vs. ΔA2804 FeO(OH)	0,5077	0,4965 to 0,5188	Yes	****	<0,0001
WT FeCl3 vs. ΔSidOP FeO(OH)	0,215	0,2027 to 0,2273	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔSidOP FeCl3	0,104	0,08911 to 0,1189	Yes	****	<0,0001
ΔA2804 FeCl3 vs. WT FeO(OH)	0,1523	0,1376 to 0,1671	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔA2804 FeO(OH)	0,3317	0,3158 to 0,3476	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔSidOP FeO(OH)	0,039	0,02162 to 0,05638	Yes	**	0,0087
ΔSidOP FeCl3 vs. WT FeO(OH)	0,04833	0,04042 to 0,05624	Yes	****	<0,0001
ΔSidOP FeCl3 vs. ΔA2804 FeO(OH)	0,2277	0,2205 to 0,2348	Yes	****	<0,0001
ΔSidOP FeCl3 vs. ΔSidOP FeO(OH)	-0,065	-0,07245 to -0,05755	Yes	****	<0,0001
WT FeO(OH) vs. ΔA2804 FeO(OH)	0,1793	0,1719 to 0,1868	Yes	****	<0,0001
WT FeO(OH) vs. ΔSidOP FeO(OH)	-0,1133	-0,1212 to -0,1055	Yes	****	<0,0001
ΔA2804 FeO(OH) vs. ΔSidOP FeO(OH)	-0,2927	-0,2981 to -0,2872	Yes	****	<0,0001

Table E-4 Results of two-way ANOVA for experiment 2 (cell concentrations)

Two-way RM ANOVA	Matching: Stacked
Assume sphericity?	No
Alpha	0,05

Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	**Geisser-Greenhouse's epsilon**
Sampling day x Organism and iron source	39,23	<0,0001	****	Yes	
Sampling day	22,36	<0,0001	****	Yes	0,7552
Organism and iron source	38,40	<0,0001	****	Yes	
Subject	0,003417	0,4126	ns	No	

ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Sampling day x Organism and iron source	0,07845	15	0,005230	F (15, 36) = 9823	P<0,0001
Sampling day	0,04470	3	0,01490	F (2,266, 27,19) = 27988	P<0,0001
Organism and iron source	0,07677	5	0,01535	F (5, 12) = 26964	P<0,0001
Subject	6,833e-006	12	5,694e-007	F (12, 36) = 1,070	P=0,4126
Residual	1,917e-005	36	5,324e-007		

Data summary	
Number of columns (Organism and iron source)	6
Number of rows (Sampling day)	4
Number of subjects (Subject)	18
Number of missing values	0

Table E-5 Results of analysis of variance between cell concentrations in cultures on different sampling days during experiment 2 (based on two-way ANOVA)

Number of families	*6*
Number of comparisons per family	*6*
Alpha	*0,05*

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
WT FeCl3					
Day 1 vs. Day 7	-0,02867	-0,03699 to -0,02034	Yes	**	0,0041
Day 1 vs. Day 14	-0,06267	-0,06498 to -0,06036	Yes	****	<0,0001
Day 1 vs. Day 21	-0,1853	-0,1876 to -0,1830	Yes	****	<0,0001
Day 7 vs. Day 14	-0,034	-0,04200 to -0,02600	Yes	**	0,0019
Day 7 vs. Day 21	-0,1567	-0,1667 to -0,1466	Yes	****	<0,0001
Day 14 vs. Day 21	-0,1227	-0,1250 to -0,1204	Yes	****	<0,0001
ΔA2804 FeCl3					
Day 1 vs. Day 7	-0,05167	-0,05398 to -0,04936	Yes	****	<0,0001
Day 1 vs. Day 14	-0,053	-0,05700 to -0,04900	Yes	****	<0,0001
Day 1 vs. Day 21	-0,1943	-0,2004 to -0,1882	Yes	****	<0,0001
Day 7 vs. Day 14	-0,001333	-0,005953 to 0,003286	No	ns	0,4061
Day 7 vs. Day 21	-0,1427	-0,1488 to -0,1366	Yes	****	<0,0001
Day 14 vs. Day 21	-0,1413	-0,1436 to -0,1390	Yes	****	<0,0001
ΔSidOP FeCl3					
Day 1 vs. Day 7	0,002333	2,368e-005 to 0,004643	Yes	*	0,049
Day 1 vs. Day 14	0				
Day 1 vs. Day 21	-0,008		Yes	****	<0,0001
Day 7 vs. Day 14	-0,002333	-0,004643 to -2,368e-005	Yes	*	0,049
Day 7 vs. Day 21	-0,01033	-0,01264 to -0,008024	Yes	**	0,0015
Day 14 vs. Day 21	-0,008		Yes	****	<0,0001
WT FeO(OH)					
Day 1 vs. Day 7	0,007667	0,005357 to 0,009976	Yes	**	0,0046

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Day 1 vs. Day 14	0,007	0,003000 to 0,01100	Yes	*	0,0169
Day 1 vs. Day 21	-0,002667	-0,004976 to -0,0003570	Yes	*	0,0379
Day 7 vs. Day 14	-0,000667	-0,005286 to 0,003953	No	ns	0,7683
Day 7 vs. Day 21	-0,01033	-0,01264 to -0,008024	Yes	**	0,0015
Day 14 vs. Day 21	-0,009667	-0,01578 to -0,003556	Yes	*	0,0205
ΔA2804 FeO(OH)					
Day 1 vs. Day 7	0,001	0,001000 to 0,001000	Yes	****	<0,0001
Day 1 vs. Day 14	-0,003333	-0,005643 to -0,001024	Yes	*	0,0246
Day 1 vs. Day 21	-0,006667	-0,01129 to -0,002047	Yes	*	0,0246
Day 7 vs. Day 14	-0,004333	-0,006643 to -0,002024	Yes	*	0,0148
Day 7 vs. Day 21	-0,007667	-0,01229 to -0,003047	Yes	*	0,0187
Day 14 vs. Day 21	-0,003333	-0,009444 to 0,002777	No	ns	0,1526
ΔSidOP FeO(OH)					
Day 1 vs. Day 7	-0,000333	-0,004953 to 0,004286	No	ns	0,952
Day 1 vs. Day 14	0,004333	0,002024 to 0,006643	Yes	*	0,0148
Day 1 vs. Day 21	0,004333	0,002024 to 0,006643	Yes	*	0,0148
Day 7 vs. Day 14	0,004667	0,002357 to 0,006976	Yes	*	0,0128
Day 7 vs. Day 21	0,004667	0,002357 to 0,006976	Yes	*	0,0128
Day 14 vs. Day 21	0				

Table E-6 Results of analysis of variance between cell concentrations in different cultures during experiment 2 (based on two-way ANOVA)

Number of families	*4*
Number of comparisons per family	*15*
Alpha	*0,05*

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Day 1					
WT FeCl3 vs. ΔA2804 FeCl3	-0,002667	-0,005432 to 9,897e-005	No	ns	0,0536
WT FeCl3 vs. ΔSidOP FeCl3	-0,002		Yes	****	<0,0001
WT FeCl3 vs. WT FeO(OH)	-0,009667	-0,01243 to -0,006901	Yes	**	0,0038
WT FeCl3 vs. ΔA2804 FeO(OH)	-0,006333	-0,009099 to -0,003568	Yes	*	0,0105
WT FeCl3 vs. ΔSidOP FeO(OH)	-0,001333	-0,004099 to 0,001432	No	ns	0,1919
ΔA2804 FeCl3 vs. ΔSidOP FeCl3	0,0006667	-0,002099 to 0,003432	No	ns	0,5302
ΔA2804 FeCl3 vs. WT FeO(OH)	-0,007	-0,009235 to -0,004765	Yes	***	0,0007
ΔA2804 FeCl3 vs. ΔA2804 FeO(OH)	-0,003667	-0,005902 to -0,001431	Yes	**	0,0087
ΔA2804 FeCl3 vs. ΔSidOP FeO(OH)	0,001333	-0,0009021 to 0,003569	No	ns	0,2267
ΔSidOP FeCl3 vs. WT FeO(OH)	-0,007667	-0,01043 to -0,004901	Yes	**	0,0075
ΔSidOP FeCl3 vs. ΔA2804 FeO(OH)	-0,004333	-0,007099 to -0,001568	Yes	*	0,0208
ΔSidOP FeCl3 vs. ΔSidOP FeO(OH)	0,0006667	-0,002099 to 0,003432	No	ns	0,5302
WT FeO(OH) vs. ΔA2804 FeO(OH)	0,003333	0,001098 to 0,005569	Yes	*	0,0123
WT FeO(OH) vs. ΔSidOP FeO(OH)	0,008333	0,006098 to 0,01057	Yes	***	0,0004
ΔA2804 FeO(OH) vs. ΔSidOP FeO(OH)	0,005	0,002765 to 0,007235	Yes	**	0,0027
Day 7					
WT FeCl3 vs. ΔA2804 FeCl3	-0,02567	-0,03455 to -0,01679	Yes	**	0,0051
WT FeCl3 vs. ΔSidOP FeCl3	0,029	0,02012 to 0,03788	Yes	**	0,004
WT FeCl3 vs. WT FeO(OH)	0,02667	0,01670 to 0,03664	Yes	**	0,008
WT FeCl3 vs. ΔA2804 FeO(OH)	0,02333	0,01445 to 0,03221	Yes	**	0,0061
WT FeCl3 vs. ΔSidOP FeO(OH)	0,027	0,01812 to 0,03588	Yes	**	0,0047

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
ΔA2804 FeCl3 vs. ΔSidOP FeCl3	0,05467	0,05243 to 0,05690	Yes	****	<0,0001
ΔA2804 FeCl3 vs. WT FeO(OH)	0,05233	0,04957 to 0,05510	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔA2804 FeO(OH)	0,049	0,04676 to 0,05124	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔSidOP FeO(OH)	0,05267	0,05043 to 0,05490	Yes	****	<0,0001
ΔSidOP FeCl3 vs. WT FeO(OH)	-0,002333	-0,005099 to 0,0004323	No	ns	0,0692
ΔSidOP FeCl3 vs. ΔA2804 FeO(OH)	-0,005667	-0,007902 to -0,003431	Yes	**	0,0016
ΔSidOP FeCl3 vs. ΔSidOP FeO(OH)	-0,002	-0,004235 to 0,0002355	No	ns	0,0717
WT FeO(OH) vs. ΔA2804 FeO(OH)	-0,003333	-0,006099 to -0,0005677	Yes	*	0,0348
WT FeO(OH) vs. ΔSidOP FeO(OH)	0,0003333	-0,002432 to 0,003099	No	ns	0,8866
ΔA2804 FeO(OH) vs. ΔSidOP FeO(OH)	0,003667	0,001431 to 0,005902	Yes	**	0,0087
Day 14					
WT FeCl3 vs. ΔA2804 FeCl3	0,007	0,004765 to 0,009235	Yes	***	0,0007
WT FeCl3 vs. ΔSidOP FeCl3	0,06067	0,05790 to 0,06343	Yes	****	<0,0001
WT FeCl3 vs. WT FeO(OH)	0,06	0,05570 to 0,06430	Yes	****	<0,0001
WT FeCl3 vs. ΔA2804 FeO(OH)	0,053	0,04870 to 0,05730	Yes	****	<0,0001
WT FeCl3 vs. ΔSidOP FeO(OH)	0,06567	0,06290 to 0,06843	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔSidOP FeCl3	0,05367	0,05090 to 0,05643	Yes	****	<0,0001
ΔA2804 FeCl3 vs. WT FeO(OH)	0,053	0,04870 to 0,05730	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔA2804 FeO(OH)	0,046	0,04170 to 0,05030	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔSidOP FeO(OH)	0,05867	0,05590 to 0,06143	Yes	****	<0,0001
ΔSidOP FeCl3 vs. WT FeO(OH)	-0,0006667	-0,006198 to 0,004865	No	ns	0,8866
ΔSidOP FeCl3 vs. ΔA2804 FeO(OH)	-0,007667	-0,01320 to -0,002135	Yes	*	0,0264
ΔSidOP FeCl3 vs. ΔSidOP FeO(OH)	0,005		Yes	****	<0,0001
WT FeO(OH) vs. ΔA2804 FeO(OH)	-0,007	-0,01147 to -0,002529	Yes	*	0,0103
WT FeO(OH) vs. ΔSidOP FeO(OH)	0,005667	0,0001354 to 0,01120	Yes	*	0,0477

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
ΔA2804 FeO(OH) vs. ΔSidOP FeO(OH)	0,01267	0,007135 to 0,01820	Yes	*	0,0105
Day 21					
WT FeCl3 vs. ΔA2804 FeCl3	-0,01167	-0,01529 to -0,008047	Yes	**	0,0014
WT FeCl3 vs. ΔSidOP FeCl3	0,1753	0,1726 to 0,1781	Yes	****	<0,0001
WT FeCl3 vs. WT FeO(OH)	0,173	0,1708 to 0,1752	Yes	****	<0,0001
WT FeCl3 vs. ΔA2804 FeO(OH)	0,1723	0,1687 to 0,1760	Yes	****	<0,0001
WT FeCl3 vs. ΔSidOP FeO(OH)	0,1883	0,1856 to 0,1911	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔSidOP FeCl3	0,187	0,1822 to 0,1918	Yes	****	<0,0001
ΔA2804 FeCl3 vs. WT FeO(OH)	0,1847	0,1810 to 0,1883	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔA2804 FeO(OH)	0,184	0,1801 to 0,1879	Yes	****	<0,0001
ΔA2804 FeCl3 vs. ΔSidOP FeO(OH)	0,2	0,1952 to 0,2048	Yes	****	<0,0001
ΔSidOP FeCl3 vs. WT FeO(OH)	-0,002333	-0,005099 to 0,0004323	No	ns	0,0692
ΔSidOP FeCl3 vs. ΔA2804 FeO(OH)	-0,003	-0,007790 to 0,001790	No	ns	0,1207
ΔSidOP FeCl3 vs. ΔSidOP FeO(OH)	0,013		Yes	****	<0,0001
WT FeO(OH) vs. ΔA2804 FeO(OH)	-0,0006667	-0,004286 to 0,002953	No	ns	0,8937
WT FeO(OH) vs. ΔSidOP FeO(OH)	0,01533	0,01257 to 0,01810	Yes	***	0,0001
ΔA2804 FeO(OH) vs. ΔSidOP FeO(OH)	0,016	0,01121 to 0,02079	Yes	**	0,0045

Table E-7 Results of two-way ANOVA for experiment 1 (iron concentrations)

Mixed-effects model (REML)	Matching: Stacked
Assume sphericity?	No
Alpha	0,05

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0,05)?**	**F (DFn, DFd)**	**Geisser-Greenhouse's epsilon**
Sampling day	0,0010	**	Yes	F (1,421, 23,22) = 11,35	0,4738
Organism and iron source	<0,0001	****	Yes	F (11, 24) = 30,21	
Sampling day x Organism and iron source	<0,0001	****	Yes	F (33, 49) = 6,749	

Random effects	SD	Variance
20 uM iron	1,142e-010	1,304e-020
Residual	3,577e-010	1,280e-019

Was the matching effective?	
Chi-square, df	0,5875, 1
P value	0,4434
P value summary	ns
Is there significant matching (P < 0.05)?	No

Data summary	
Number of columns (Organism and iron source)	12
Number of rows (Sampling day)	4
Number of subjects (20 uM iron)	36
Number of missing values	23

Table E-8 Results of anaylsis of variance between iron concentrations in cultures on different sampling days during experiment 1 (based on two-way ANOVA)

Number of families	*12*
Number of comparisons per family	*6*
Alpha	*0,05*

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Particulate WT FeCl3					
Day 1 vs. Day 7	-3,03E-10	-2,146e-009 to 1,539e-009	No	ns	0,311
Day 1 vs. Day 14	-8,30E-11	-5,452e-010 to 3,793e-010	No	ns	0,2872
Day 1 vs. Day 21	-4,53E-11	-9,132e-010 to 8,226e-010	No	ns	0,7406
Day 7 vs. Day 14	2,20E-10	-5,080e-010 to 9,489e-010	No	ns	0,3818
Day 7 vs. Day 21	2,58E-10	-9,662e-011 to 6,128e-010	No	ns	0,091
Day 14 vs. Day 21	3,76E-11	-3,504e-010 to 4,256e-010	No	ns	0,9
Intracellular WT FeCl3					
Day 1 vs. Day 7	1,45E-10	-1,867e-009 to 2,157e-009	No	ns	0,6112
Day 1 vs. Day 14	3,27E-11	-4,110e-010 to 4,764e-010	No	ns	0,9492
Day 1 vs. Day 21	9,39E-11	-2,818e-009 to 3,006e-009	No	ns	0,8892
Day 7 vs. Day 14	-1,13E-10	-8,434e-010 to 6,185e-010	No	ns	0,3309
Day 7 vs. Day 21	-5,12E-11				
Day 14 vs. Day 21	6,12E-11	-9,326e-010 to 1,055e-009	No	ns	0,6759
Particulate ΔA2804 FeCl3					
Day 1 vs. Day 7	-8,86E-10	-1,200e-008 to 1,022e-008	No	ns	0,5698
Day 1 vs. Day 14	-3,87E-10				
Day 1 vs. Day 21	-1,95E-09				
Day 7 vs. Day 14	5,00E-10	-1,754e-008 to 1,854e-008	No	ns	0,9203
Day 7 vs. Day 21	-1,07E-09	-1,888e-008 to 1,675e-008	No	ns	0,6871
Day 14 vs. Day 21	-1,57E-09	-1,818e-009 to -1,316e-009	Yes	*	0,0211
Intracellular ΔA2804 FeCl3					

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Day 1 vs. Day 7	-5,34E-11	-2,220e-010 to 1,152e-010	No	ns	0,1667
Day 1 vs. Day 14	-9,46E-11	-5,101e-010 to 3,209e-010	No	ns	0,2294
Day 1 vs. Day 21	-1,36E-10	-3,433e-010 to 7,236e-011	No	ns	0,0816
Day 7 vs. Day 14	-4,12E-11	-1,634e-010 to 8,092e-011	No	ns	0,3286
Day 7 vs. Day 21	-8,21E-11	-1,678e-010 to 3,646e-012	No	ns	0,0543
Day 14 vs. Day 21	-4,09E-11	-9,015e-011 to 8,447e-012	No	ns	0,0714
Particulate ΔSidOP FeCl3					
Day 1 vs. Day 7	-2,90E-09	-6,302e-009 to 4,972e-010	No	ns	0,0612
Day 1 vs. Day 14	-3,92E-09	-1,106e-008 to 3,226e-009	No	ns	0,1513
Day 1 vs. Day 21	-2,02E-09	-6,111e-009 to 2,081e-009	No	ns	0,1081
Day 7 vs. Day 14	-1,01E-09	-2,807e-008 to 2,605e-008	No	ns	0,8513
Day 7 vs. Day 21	8,88E-10				
Day 14 vs. Day 21	1,90E-09	-3,751e-008 to 4,131e-008	No	ns	0,7699
Intracellular ΔSidOP FeCl3					
Day 1 vs. Day 7	-1,70E-10	-7,240e-010 to 3,838e-010	No	ns	0,1718
Day 1 vs. Day 14	-5,24E-11	-6,065e-010 to 5,017e-010	No	ns	0,5012
Day 1 vs. Day 21	-6,73E-11	-2,453e-010 to 1,108e-010	No	ns	0,1403
Day 7 vs. Day 14	1,18E-10	-5,468e-011 to 2,900e-010	No	ns	0,1023
Day 7 vs. Day 21	1,03E-10	1,558e-012 to 2,041e-010	Yes	*	0,0486
Day 14 vs. Day 21	-1,49E-11	-1,310e-010 to 1,013e-010	No	ns	0,8169
Particulate WT FeO(OH)					
Day 1 vs. Day 7	-1,36E-09	-3,914e-009 to 1,200e-009	No	ns	0,1003
Day 1 vs. Day 14	2,43E-10	-2,180e-009 to 2,666e-009	No	ns	0,4781
Day 1 vs. Day 21	3,89E-10	9,553e-011 to 6,815e-010	Yes	*	0,029
Day 7 vs. Day 14	1,60E-09				
Day 7 vs. Day 21	1,75E-09	-1,190e-009 to 4,680e-009	No	ns	0,0895
Day 14 vs. Day 21	1,45E-10	-1,615e-009 to 1,905e-009	No	ns	0,5558
Intracellular WT FeO(OH)					

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Day 1 vs. Day 7	-4,55E-11	-6,793e-010 to 5,883e-010	No	ns	0,9526
Day 1 vs. Day 14	-9,36E-12	-7,341e-010 to 7,154e-010	No	ns	0,9997
Day 1 vs. Day 21	2,90E-11	-2,369e-009 to 2,427e-009	No	ns	0,9905
Day 7 vs. Day 14	3,61E-11	-2,072e-010 to 2,795e-010	No	ns	0,7557
Day 7 vs. Day 21	7,45E-11	-8,243e-010 to 9,733e-010	No	ns	0,5541
Day 14 vs. Day 21	3,84E-11	-5,202e-010 to 5,970e-010	No	ns	0,6313
Particulate ΔA2804 FeO(OH)					
Day 1 vs. Day 7	-4,66E-10	-2,347e-009 to 1,416e-009	No	ns	0,2118
Day 1 vs. Day 14	-8,14E-10	-1,416e-009 to -2,114e-010	Yes	*	0,0315
Day 1 vs. Day 21	-2,75E-10	-2,235e-009 to 1,685e-009	No	ns	0,3598
Day 7 vs. Day 14	-3,48E-10	-2,161e-009 to 1,465e-009	No	ns	0,2697
Day 7 vs. Day 21	1,91E-10	-8,937e-010 to 1,275e-009	No	ns	0,6743
Day 14 vs. Day 21	5,39E-10	-1,034e-009 to 2,112e-009	No	ns	0,1544
Intracellular ΔA2804 FeO(OH)					
Day 1 vs. Day 7	-1,79E-10	-9,274e-010 to 5,701e-010	No	ns	0,5092
Day 1 vs. Day 14	-1,64E-10	-8,818e-010 to 5,529e-010	No	ns	0,5313
Day 1 vs. Day 21	1,20E-10	-2,761e-009 to 3,000e-009	No	ns	0,8206
Day 7 vs. Day 14	1,42E-11	-1,945e-010 to 2,228e-010	No	ns	0,959
Day 7 vs. Day 21	2,98E-10	-2,656e-010 to 8,618e-010	No	ns	0,1006
Day 14 vs. Day 21	2,84E-10	-5,975e-010 to 1,165e-009	No	ns	0,164
Particulate ΔSidOP FeO(OH)					
Day 1 vs. Day 7	-7,03E-11				
Day 1 vs. Day 14	-1,14E-10				
Day 1 vs. Day 21	-4,03E-11	-5,334e-010 to 4,527e-010	No	ns	0,5596
Day 7 vs. Day 14	-4,40E-11	-1,153e-010 to 2,736e-011	No	ns	0,0863
Day 7 vs. Day 21	3,00E-11	-7,329e-010 to 7,928e-010	No	ns	0,837

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Day 14 vs. Day 21	7,40E-11	-6,241e-010 to 7,721e-010	No	ns	0,4576
Intracellular ΔSidOP FeO(OH)					
Day 1 vs. Day 7	-7,24E-11				
Day 1 vs. Day 14	-7,70E-11				
Day 1 vs. Day 21	-1,29E-11				
Day 7 vs. Day 14	-4,58E-12	-1,156e-009 to 1,147e-009	No	ns	0,9996
Day 7 vs. Day 21	5,95E-11	-4,029e-011 to 1,593e-010	No	ns	0,0893
Day 14 vs. Day 21	6,41E-11	-1,124e-009 to 1,252e-009	No	ns	0,728

Table E-9 Results of analysis of variance between iron concentrations in different cultures during experiment 1 (based on two-way ANOVA)

Number of families	*4*
Number of comparisons per family	*66*
Alpha	*0,05*

Tukey's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Significant?**	**Summary**	**Adjusted P Value**
Day 1					
Particulate WT FeCl3 vs. Intracellular WT FeCl3	-5,04E-11	-1,076e-009 to 9,758e-010	No	ns	0,9998
Particulate ΔA2804 FeCl3 vs. Intracellular ΔA2804 FeCl3	1,81E-10	1,389e-010 to 2,226e-010	Yes	***	0,0006
Particulate ΔSidOP FeCl3 vs. Intracellular ΔSidOP FeCl3	3,34E-10	-1,034e-009 to 1,701e-009	No	ns	0,5499
Particulate WT FeO(OH) vs. Intracellular WT FeO(OH)	6,21E-10	-3,157e-010 to 1,557e-009	No	ns	0,1485
Particulate ΔA2804 FeO(OH) vs. Intracellular ΔA2804 FeO(OH)	2,08E-10	-7,461e-010 to 1,163e-009	No	ns	0,6215
Particulate ΔSidOP FeO(OH) vs. Intracellular ΔSidOP FeO(OH)	2,46E-12				
Day 7					
Particulate WT FeCl3 vs. Intracellular WT FeCl3	3,98E-10	-5,431e-010 to 1,340e-009	No	ns	0,2438
Particulate ΔA2804 FeCl3 vs. Intracellular ΔA2804 FeCl3	1,01E-09	-4,431e-009 to 6,459e-009	No	ns	0,7234
Particulate ΔSidOP FeCl3 vs. Intracellular ΔSidOP FeCl3	3,07E-09	2,902e-009 to 3,230e-009	Yes	****	<0,0001
Particulate WT FeO(OH) vs. Intracellular WT FeO(OH)	1,93E-09	1,238e-009 to 2,626e-009	Yes	**	0,0084
Particulate ΔA2804 FeO(OH) vs. Intracellular ΔA2804 FeO(OH)	4,95E-10	-4,188e-010 to 1,409e-009	No	ns	0,1678
Particulate ΔA2804 FeO(OH) vs. Particulate ΔSidOP FeO(OH)	6,96E-10	-2,692e-010 to 1,660e-009	No	ns	0,0935
Particulate ΔSidOP FeO(OH) vs. Intracellular ΔSidOP FeO(OH)	3,99E-13	-5,311e-011 to 5,391e-011	No	ns	>0,9999
Day 14					
Particulate WT FeCl3 vs. Intracellular WT FeCl3	6,53E-11	-2,073e-010 to 3,379e-010	No	ns	0,8215
Particulate ΔA2804 FeCl3 vs. Intracellular ΔA2804 FeCl3	4,73E-10	8,487e-011 to 8,610e-010	Yes	*	0,0381
Particulate ΔSidOP FeCl3 vs. Intracellular ΔSidOP FeCl3	4,20E-09	-7,920e-009 to 1,631e-008	No	ns	0,3366
Particulate WT FeO(OH) vs. Intracellular WT FeO(OH)	3,68E-10	1,840e-010 to 5,521e-010	Yes	**	0,0075
Particulate ΔA2804 FeO(OH) vs. Intracellular ΔA2804 FeO(OH)	8,57E-10	5,273e-010 to 1,188e-009	Yes	**	0,0037
Particulate ΔSidOP FeO(OH) vs. Intracellular ΔSidOP FeO(OH)	3,98E-11	-5,433e-010 to 6,229e-010	No	ns	0,9968

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Day 21					
Particulate WT FeCl3 vs. Intracellular WT FeCl3	8,89E-11	-3,244e-010 to 5,021e-010	No	ns	0,6305
Particulate ΔA2804 FeCl3 vs. Intracellular ΔA2804 FeCl3	2,00E-09	1,843e-009 to 2,154e-009	Yes	***	0,0004
Particulate ΔSidOP FeCl3 vs. Intracellular ΔSidOP FeCl3	2,28E-09	1,610e-009 to 2,952e-009	Yes	*	0,015
Particulate WT FeO(OH) vs. Intracellular WT FeO(OH)	2,61E-10	-3,303e-010 to 8,525e-010	No	ns	0,2268
Particulate ΔA2804 FeO(OH) vs. Intracellular ΔA2804 FeO(OH)	6,03E-10	-2,040e-010 to 1,409e-009	No	ns	0,0873
Particulate ΔSidOP FeO(OH) vs. Intracellular ΔSidOP FeO(OH)	2,99E-11	-1,808e-010 to 2,407e-010	No	ns	0,8717

Table E-10 Results of two-way ANOVA for experiment 2 (iron concentrations)

Mixed-effects model (REML)	Matching: Stacked
Assume sphericity?	No
Alpha	0,05

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0,05)?**	**F (DFn, DFd)**	**Geisser-Greenhouse's epsilon**
Sampling day	<0,0001	****	Yes	F (1,618, 35,61) = 19,74	0,5395
Organism and iron source	<0,0001	****	Yes	F (11, 24) = 12,96	
Sampling day x Organism and iron source	<0,0001	****	Yes	F (33, 66) = 9,737	

Random effects	SD	Variance
100 nM iron	5,612e-012	3,150e-023
Residual	1,097e-011	1,203e-022

Was the matching effective?	
Chi-square, df	4,438, 1
P value	0,0351
P value summary	*
Is there significant matching (P < 0.05)?	Yes

Data summary	
Number of columns (Organism and iron source)	12
Number of rows (Sampling day)	4
Number of subjects (100 nM iron)	36
Number of missing values	6

Table E-11 Results of analysis of variance between iron concentrations in cultures on different sampling days during experiment 2 (based on two-way ANOVA)

Number of families	*12*
Number of comparisons per family	*6*
Alpha	*0,05*

Tukey's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Significant?**	**Summary**	**Adjusted P Value**
Particulate WT FeCl3					
Day 1 vs. Day 7	1,03E-10	-5,412e-011 to 2,597e-010	No	ns	0,1102
Day 1 vs. Day 14	1,35E-10	-9,536e-010 to 1,224e-009	No	ns	0,3998
Day 1 vs. Day 21	1,29E-10	-9,879e-011 to 3,574e-010	No	ns	0,1427
Day 7 vs. Day 14	3,26E-11	-3,623e-010 to 4,275e-010	No	ns	0,556
Day 7 vs. Day 21	2,65E-11	-8,515e-011 to 1,382e-010	No	ns	0,5117
Day 14 vs. Day 21	-6,07E-12	-2,806e-011 to 1,592e-011	No	ns	0,1905
Intracellular WT FeCl3					
Day 1 vs. Day 7	1,10E-11	-8,747e-011 to 1,094e-010	No	ns	0,8627
Day 1 vs. Day 14	2,67E-11	-7,106e-010 to 7,640e-010	No	ns	0,86
Day 1 vs. Day 21	1,73E-11	-1,061e-010 to 1,406e-010	No	ns	0,7818
Day 7 vs. Day 14	1,58E-11	-2,629e-010 to 2,944e-010	No	ns	0,71
Day 7 vs. Day 21	6,27E-12	-3,142e-011 to 4,396e-011	No	ns	0,7026
Day 14 vs. Day 21	-9,48E-12	-1,443e-010 to 1,253e-010	No	ns	0,6216
Particulate ΔA2804 FeCl3					
Day 1 vs. Day 7	-2,18E-11	-1,127e-010 to 6,920e-011	No	ns	0,5078
Day 1 vs. Day 14	-6,07E-12	-5,807e-011 to 4,593e-011	No	ns	0,8485
Day 1 vs. Day 21	-2,71E-11	-2,029e-010 to 1,488e-010	No	ns	0,3307
Day 7 vs. Day 14	1,57E-11	-5,515e-011 to 8,652e-011	No	ns	0,5506
Day 7 vs. Day 21	-5,32E-12	-1,990e-010 to 1,883e-010	No	ns	0,9216
Day 14 vs. Day 21	-2,10E-11	-8,975e-011 to 4,774e-011	No	ns	0,1726

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Intracellular ΔA2804 FeCl3					
Day 1 vs. Day 7	-4,71E-11	-7,684e-011 to -1,744e-011	Yes	*	0,0204
Day 1 vs. Day 14	0				
Day 1 vs. Day 21	-1,64E-11	-4,218e-011 to 9,366e-012	No	ns	0,1161
Day 7 vs. Day 14	4,71E-11	1,744e-011 to 7,684e-011	Yes	*	0,0204
Day 7 vs. Day 21	3,07E-11	5,842e-012 to 5,563e-011	Yes	*	0,0333
Day 14 vs. Day 21	-1,64E-11	-4,218e-011 to 9,366e-012	No	ns	0,1161
Particulate ΔSidOP FeCl3					
Day 1 vs. Day 7	5,21E-12	-3,677e-010 to 3,782e-010	No	ns	0,9859
Day 1 vs. Day 14	3,11E-11	2,380e-011 to 3,837e-011	Yes	*	0,0211
Day 1 vs. Day 21	8,24E-12	-2,449e-010 to 2,614e-010	No	ns	0,8871
Day 7 vs. Day 14	2,59E-11	-5,135e-011 to 1,031e-010	No	ns	0,332
Day 7 vs. Day 21	3,03E-12	-8,922e-011 to 9,528e-011	No	ns	0,9946
Day 14 vs. Day 21	-2,29E-11	-1,082e-010 to 6,255e-011	No	ns	0,4464
Intracellular ΔSidOP FeCl3					
Day 1 vs. Day 7	0				
Day 1 vs. Day 14	0				
Day 1 vs. Day 21	0				
Day 7 vs. Day 14	0				
Day 7 vs. Day 21	0				
Day 14 vs. Day 21	0				
Particulate WT FeO(OH)					
Day 1 vs. Day 7	3,87E-11	2,783e-012 to 7,455e-011	Yes	*	0,0449
Day 1 vs. Day 14	3,87E-11	2,783e-012 to 7,455e-011	Yes	*	0,0449
Day 1 vs. Day 21	3,87E-11	2,783e-012 to 7,455e-011	Yes	*	0,0449
Day 7 vs. Day 14	0				
Day 7 vs. Day 21	0				
Day 14 vs. Day 21	0				
Intracellular WT FeO(OH)					
Day 1 vs. Day 7	0				
Day 1 vs. Day 14	0				
Day 1 vs. Day 21	0				
Day 7 vs. Day 14	0				
Day 7 vs. Day 21	0				
Day 14 vs. Day 21	0				
Particulate ΔA2804 FeO(OH)					

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Day 1 vs. Day 7	0				
Day 1 vs. Day 14	0				
Day 1 vs. Day 21	0				
Day 7 vs. Day 14	0				
Day 7 vs. Day 21	0				
Day 14 vs. Day 21	0				
Intracellular ΔA2804 FeO(OH)					
Day 1 vs. Day 7	0				
Day 1 vs. Day 14	0				
Day 1 vs. Day 21	0				
Day 7 vs. Day 14	0				
Day 7 vs. Day 21	0				
Day 14 vs. Day 21	0				
Particulate ΔSidOP FeO(OH)					
Day 1 vs. Day 7	1,11E-11	-5,810e-012 to 2,804e-011	No	ns	0,081
Day 1 vs. Day 14	1,11E-11	-5,810e-012 to 2,804e-011	No	ns	0,081
Day 1 vs. Day 21	1,11E-11	-5,810e-012 to 2,804e-011	No	ns	0,081
Day 7 vs. Day 14	0				
Day 7 vs. Day 21	0				
Day 14 vs. Day 21	0				
Intracellular ΔSidOP FeO(OH)					
Day 1 vs. Day 7	0				
Day 1 vs. Day 14	0				
Day 1 vs. Day 21	0				
Day 7 vs. Day 14	0				
Day 7 vs. Day 21	0				
Day 14 vs. Day 21	0				

Table E-12 Results of analysis of variance between iron concentrations in different cultures during experiment 2 (based on two-way ANOVA)

Number of families	*4*
Number of comparisons per family	*66*
Alpha	*0,05*

Tukey's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Significant?**	**Summary**	**Adjusted P Value**
Day 1					
Particulate WT FeCl3 vs. Intracellular WT FeCl3	1,19E-10	-1,276e-010 to 3,651e-010	No	ns	0,3468
Particulate ΔA2804 FeCl3 vs. Intracellular ΔA2804 FeCl3	1,15E-11	-5,154e-011 to 7,462e-011	No	ns	0,7339
Particulate ΔSidOP FeCl3 vs. Intracellular ΔSidOP FeCl3	3,11E-11	1,887e-011 to 4,331e-011	Yes	*	0,0211
Particulate WT FeO(OH) vs. Intracellular WT FeO(OH)	3,87E-11	-2,151e-011 to 9,885e-011	No	ns	0,091
Particulate ΔA2804 FeO(OH) vs. Intracellular ΔA2804 FeO(OH)	0				
Particulate ΔSidOP FeO(OH) vs. Intracellular ΔSidOP FeO(OH)	1,11E-11	-1,727e-011 to 3,950e-011	No	ns	0,1397
Day 7					
Particulate WT FeCl3 vs. Intracellular WT FeCl3	2,69E-11	-8,963e-011 to 1,435e-010	No	ns	0,8769
Particulate ΔA2804 FeCl3 vs. Intracellular ΔA2804 FeCl3	-1,39E-11	-8,547e-011 to 5,777e-011	No	ns	0,9025
Particulate ΔSidOP FeCl3 vs. Intracellular ΔSidOP FeCl3	2,59E-11	-9,043e-011 to 1,422e-010	No	ns	0,6076
Particulate WT FeO(OH) vs. Intracellular WT FeO(OH)	0				
Particulate ΔA2804 FeO(OH) vs. Intracellular ΔA2804 FeO(OH)	0				
Particulate ΔSidOP FeO(OH) vs. Intracellular ΔSidOP FeO(OH)	0				
Day 14					
Particulate WT FeCl3 vs. Intracellular WT FeCl3	1,01E-11	-2,195e-011 to 4,214e-011	No	ns	0,1713
Particulate ΔA2804 FeCl3 vs. Intracellular ΔA2804 FeCl3	1,76E-11	-7,065e-012 to 4,228e-011	No	ns	0,0952
Particulate ΔSidOP FeCl3 vs. Intracellular ΔSidOP FeCl3	0				
Particulate WT FeO(OH) vs. Intracellular WT FeO(OH)	0				
Particulate ΔA2804 FeO(OH) vs. Intracellular ΔA2804 FeO(OH)	0				
Particulate ΔSidOP FeO(OH) vs. Intracellular ΔSidOP FeO(OH)	0				
Day 21					

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Particulate WT FeCl3 vs. Intracellular WT FeCl3	6,68E-12	-3,143e-011 to 4,479e-011	No	ns	0,8266
Particulate ΔA2804 FeCl3 vs. Intracellular ΔA2804 FeCl3	2,22E-11	-1,501e-011 to 5,943e-011	No	ns	0,1378
Particulate ΔSidOP FeCl3 vs. Intracellular ΔSidOP FeCl3	2,29E-11	-1,058e-010 to 1,515e-010	No	ns	0,7517
Particulate WT FeO(OH) vs. Intracellular WT FeO(OH)	0				
Particulate ΔA2804 FeO(OH) vs. Intracellular ΔA2804 FeO(OH)	0				
Particulate ΔSidOP FeO(OH) vs. Intracellular ΔSidOP FeO(OH)	0				

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