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Growth and microcystin production by Microcystis aeruginosa in batch cultures at different iron concentrations

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1. Introduction

Shaping the earths biosphere, **cyanobacteria**, the oldest oxygenic photoautrophs (3, 5 billion years) have had an enormous impact. Because of their long evolutionary history and adaptions, they have developed many special functions (34). Before the evolution of oxygenic photosynthesis, iron (Fe) existed in its reduced lower vaulency form Fe (II), which is soluble at the circumnneutral pH range and therefor bioavailable. Between 2,3 and 2.2 10^9 years ago during the Proterozoic era when Fe (II) was in abundance, it is likely that the iron-rich photosynthetic electron transport chain evolved (14). It has been suggested that is was cyanobacteria who started the great oxidation event, supplying the earths atmosphere with oxygen (O_2) (24). Cyanobacteria are a group of photosynthetic bacteria that occurs naturally in watercourses. In some watercourses they occur in very large amounts, also called water-blooms. This can be a problem when the blooming appears in drinking water, or in water used for recreation, because of the ability cyanobacteria has to produce toxins (16). Two main types of toxins which are produced by cyanobacteria is potent inhibitors of nerve impulses (neurotoxins), and liver damaging toxins (hepatotoxins). These toxins have caused massive death among fish, birds, livestock and several human deaths (10). Humans are exposed through drinking water, dialysis and recreational activities (9). The toxin is also transferred via the food chain, i.e. through fish, mussels and larger crustaceans (20). Blooming of cyanobacteria is not caused by growth in the water surface, but by an accumulation of cells from the water volume below in the surface (40). With the global warming and increasing of water eutrophication, there is a growing concern about human exposure to microcystins (MCs), which are toxins produced by freshwater cyanobacteria species (51). Cyanobacteria, also known as blue-green algae, have a wide diversity, in soil, air and water. They will often bloom in eutrophic waters, but can also bloom in less nutritious watercourses (17). Cyanobacteria use CO_2 to make organic compounds such as sugars. They also produce O_2 during photosynthesis, and a variety of species can fix atmospheric nitrogen (N_2) into ammonium. An example is the cyanobacteria *Anabaena* who has genes that encodes for both proteins used for photosynthesis and nitrogen fixation. But a single cell cannot do both at the same time, because of the production of O_2 , which inactivate enzymes involved in nitrogen fixation. Instead of living as isolated cells, they form filamentous chains. In a filament most cells carry out only photosynthesis, while specialized cells called heterocyst`s carry out nitrogen fixation (21). The most important genera with toxin production is *Microcystis,*

Anabaena, *Aphanizomenon, Nodularia* and *Oscillatoria/Planktothrix* (40). It has been shown that cyanobacteria contains neurodegenerative non-protein amino acid, beta-N-methyl amino-L-alanine (BMAA) which might be the particular cause of neurodegenerative diseases like Parkinsonism dementia complex (PDC)/ Amyotrophic Lateral Sclerosis(ALS) (5, 43). In addition to these, BMAA has also been detected in the brains of Canadian patients with Alzheimer's disease (31). Cyanobacteria are directly consumed by people and samples of these cyanobacteria have been shown to contain BMAA in addition to MCs (22).

1.1 Microcystin

Microcystins (MCs) are a family of toxic cyclic heptapeptides that are produced by cyanobacteria (18, 34). These hepatotoxins are produced by the genera *Planktothrix*, *Microcystis*, *Aphanizomenon*, *Nostoc* and *Anabaena* (46). This group of toxins are named after the cyanobacteria *Microcystis* (18). They have the general formula (-D-Ala-$_L$-X-erythro-β-methyl-$_D$-isoAsp-$_L$-Y-Adda-$_D$-isoGlu-*N*-methyldehydro-Ala). The aminoacid who is considered to be responsible for the hepatotoxicity is Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) (figure 1.1) (12, 18). MC is a small cyclic peptide having a molecular weight of about 1000 Daltons (18). MCs are produced nonribosomally through a MC synthase complex (23). These toxins are assumed to be potential inhibitors of protein phosphatase type 1 and 2A (PP1 and PP2), by an initial non-covalent and reversible binding followed by a final covalent and irreversible linkage step (41). In addition the MCs could be skin and liver tumor promoters in animals (34). With this inhibition MCs causes oxidative stress, apoptosis and the disruption of many cellular functions (8). It has also been shown that MC results in neurodegeneration and Alzheimer disease (27).

A drinking water guide line of 1µg/L for MC-LR has been established by the World Health Organization (18). Earlier studies shows that there are some effects of various environmental conditions on the MCs synthesis, e.g. temperature, irradiance, macronutrients (nitrate, ammonium, phosphate), trace elements (iron and others), salinity, CO_2 and pH (41). There is a correlation between temperature and toxin production, with an optimal production at 25 °C and a decrease at lower or higher temperatures (49). When cells are added to new medium, they need some time before they reproduce, a period (lag phase) of intense metabolic activity and synthesis of enzymes and various biological molecules (44). It has been suggested that if the synthesis of microcystin requires energy (ATP), the variation of toxin production could be

explained by the energy state of the cyanobacterial cells (4). In addition to defense (39) and quorum sensing (13) there are a existing hypothesis, that the peptide toxin (MC) is an intracellular chelator (45).
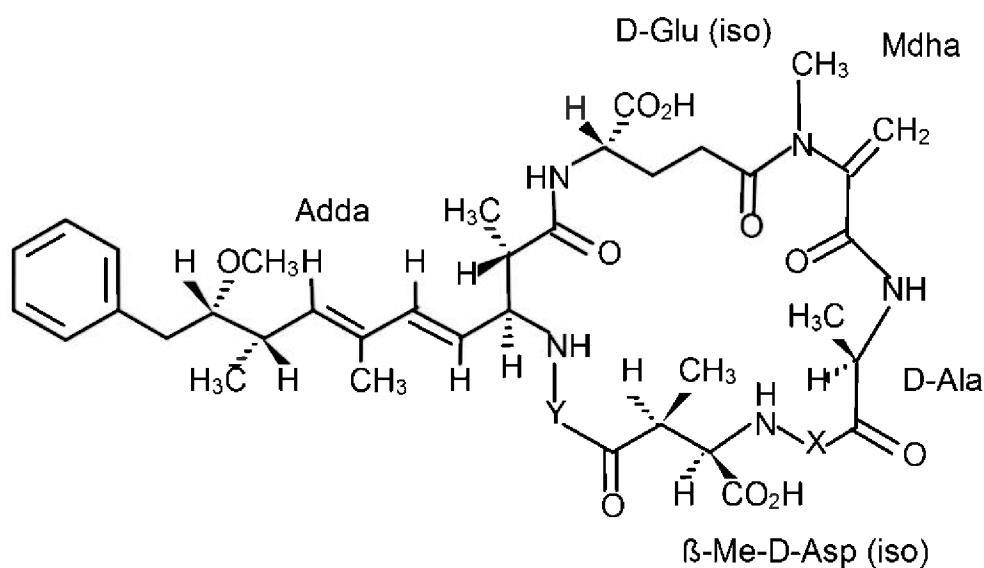
Figur 1.1: General structure of microcystins (MCYST), cyanobacterial heptapeptide hepatotoxins, showing the most frequently found variations. X and Z are variable L-amino acids (in MCYST-LR, X = L-Leusine (L) and Z = L-Arginine (R)). Adda is (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid and Mdha is N-methyldehydroalanine (Dha = dehydroalanine) (10).

1.2 Iron and microcystin

In addition to be involved in many biological functions, iron (Fe) is essential for the life of almost all living cells (25, 33). Bacteria needs a internal concentration of Fe in the micro molar range (28). Fe is a compound in many essential enzymes in living cells, therefore 10^5 ions are required per bacterial cell (37). Since Fe is involved in a numerous cellular processes, by being present in heme- and iron sulfur proteins. Fe availability is very important for growth, especially for photosynthetic organisms (15). Fe is required for major physiological functions such as photosynthesis, nitrogen assimilation, respiration and chlorophyll synthesis (19, 32). Iron-limitation can be a result of low total amounts of iron, or by low iron availability. The two main factors controlling bioavailability of iron are hydrolysis reactions of Fe $^{3+}$, and binding of Fe^{3+} to organic matter (50). In aqueous oxic environments Fe^{2+} is oxidized to Fe^{3+}, and under physiological pH`s, Fe $^{3+}$ will form highly insoluble hydroxides. The amount of free Fe^{3+} at pH 7 is not higher then about 10^3 ions per ml (6). Gram-negative

bacteria, like Cyanobacteria are considered to have an iron-acquisition mechanism based on siderophores, which are specific Fe^{3+} chelating molecules that serve to capture Fe^{3+} from the environment and solubilize precipitated ferric hydroxides (25). By releasing chelators such as siderophores outside the cells, several algal species can accumulate iron (48). For most living organisms the oxidized form is insoluble and unavailable, and the reduced form highly toxic (47). With a severe iron-limitation in cyanobacteria, modifications of PSI and PSII influences the redox state of redox sensitive components of the electron transport chain, affecting the metabolic activity, which is regulated by the redox state of these components (30). Because of the insolubility of Fe^{3+}, it requires special mechanisms for iron acquisition. In addition to siderophores to chelate Fe^{3+} and transport iron in to the bacteria, they also use mechanism for receptor-dependent iron transport and iron- regulated gene expression (7). It has been shown that the Fe uptake by MC-producing strains is significantly higher than in non-MC-producing strains. Therefore it is assumed that the peptide toxin is an intracellular chelator which inactivate free cellular Fe^{2+}. Where MCs is produced by an enzyme (synthetase) whose activity is controlled by the amount of free Fe^{2+} available. Lacking this intracellular chelator (toxin), the non-toxin producing *Microcystis* strain must have a much lower cellular Fe^{2+} concentration, proved by an iron uptake system less efficient than in the toxin-producing *Microcystis* strain (45). Fur (ferric uptake regulator) is an iron-dependent transcriptional repressor, and is very important to many bacteria for maintaining iron homeostasis (3). Fur is a regulator that controls genes involved in siderophore-mediated iron uptake under iron-rich conditions. The regulator also controls genes involved in diverse cellular processes, metabolic pathways, acid tolerance, chemo taxis, the oxidative stress response, electron-transport systems, energy metabolism and virulence (36, 52). Fur binds ferrous ions when the intracellular Fe concentration is high. This binding induces a conformational change, which leads to activation (11). When Fur is activated, it can bind DNA, which is on the promoter region of all the genes involved in iron uptake. The binding will repress their expression and then limit the entry of iron into the cell (2).

There has been showed in earlier studies that the ability to produce toxin can change temporally and spatially at a specific site (26, 38). Previously, there has been proposed that the synthesis of the hepatotoxin MC is regulated by iron availability. The production of the toxin and factors regulation the production still remains unclear (1). A study has shown that iron has an essentially effect on growth and toxin production. With a lower concentration of

Fe, there was a much lower cell-growth, but the production of toxin was 20-40 % higher (29). There has been found a decrease in toxicity during iron-limited conditions (45). Use of batch culture versus continuous culture could be the reason for different results, because of possible influence on physiological behavior of *M. aeruginosa* (29). Under iron depletion it appears that the MCs producers remains viable for a longer period. A hypothesis about toxin production could be that the peptide really is a intracellular siderophore, produced to easier get their wanted and needed concentrations of Fe from the environment. Another hypothesis is that the toxin is produced to detoxify high Fe concentrations. There has also been shown a positive connection between ″energy state‵‵and MC content. The variation in MC production could therefore be explained by variations in the energy state of cells (4).

1.3 The aims of this study

The purpose of this study is to examine if and how Fe could influence growth and toxin-production of *Microcystis aeruginosa*. It is also interesting to investigate if growth of the toxin and non-toxic culture are affected differently. This can help us to explain the diversity and patches of non-toxic and toxic strains in cyanobacterial blooming. Because of MCs harmful effect on human health it is important to get a better understanding of the environmental and nutritional factors influence on this toxin production.

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2. Growth and microcystin production by *Microcystis aeruginosa* in batch cultures at different iron concentrations

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2.1 Abstract

In this study growth and toxin (microcystin) production by *Microcystis aeruginosa* at various iron (Fe) concentrations in the O2 medium, was examined. *Microcystis aeruginosa* PCC 7806 and MCYB⁻ non-toxic mutant were grown, in 2 l batch cultures. Low Fe concentrations (250 µg Fe/l) resulted in a decrease of growth for the PCC 7806 cultures, compared to growth in ordinary Fe concentrations (500 µg Fe/l). While adding 5000 µg Fe/l resulted in faster growth of both the MCYB⁻ and PCC 7806 cultures. This also resulted in a six-fold increase of microcystin content, compared to the medium containing 250 µg Fe/l. The MCYB⁻ cultures was almost not affected by the amount of Fe in the media. With only 50 µg Fe/l added to the medium, both the PCC 7806 and the MCYB⁻ cultures were unable to grow. Cell density and microcystin production increased as a result of increasing Fe concentration in the medium from the start of the experiment. Microcystin content increased as the Fe concentration decreased during the experiment under all treatments. This study found *M. aeruginosa* PCC 7806 to adapt slower under limiting conditions. While at higher Fe concentrations both cell density and microcystin content increased. Which shows that Fe concentration is an important factor for growth of *M. aeruginosa,* and that the trace metal has a high influence on microcystin production.

2.2 Introduction

Cyanobacteria are photoautotrophic microorganisms found in almost all types of illuminated environment, frequently occurring in eutrophic rivers, lakes and reservoirs (9, 25). They are known for their ability to produce a variety of toxins, where the hepatotoxin microcystin (MC) is the largest group (7). MCs are non-ribosomal cyclic heptapeptides, making a serious threat to human health (24). About 90 variants of MCs are identified. (41). Several environmental factors have been described to influence the biosynthesis of cyanotoxin. The effects of nutrients on MC production, such as nitrogen and phosphorus (39, 40), trace metals (Fe) (2, 16, 17, 34), temperature (2, 29, 37, 40), light (29, 35, 40) and pH (36) has been studied. Almost all studies suggest that the toxin production is highest under optimal growth conditions (30). There has been suggested that MC producing strains could grow faster in a nitrogen- and phosphorus rich freshwater, than the non-toxic strains (39). There is a correlation between temperature and toxin production, with an optimal production at 25 °C and a decrease at lower or higher temperatures (40). It has also been shown in continuous cultures that toxin production increases with light intensity up to 40 microeinsteins $m^{-2} s^{-1}$, while higher light intensities did not increase toxicity (35, 37, 40). Toxin production is correlated to light intensity below 40 microeinsteins $m^{-2}s^{-1}$ (42). This indicates that MC production is correlated to energy metabolism (22). Therefore all conditions affecting the energy level could have an effect on toxin production as well as growth (10). Iron (Fe) is essential for the survival and growth of almost all cells, because of its critical role in various metabolic processes, such as chlorophyll-*a* synthesis, respiration, photosynthesis, and nitrogen fixation (5, 8, 12, 21). With a severe iron-limitation in cyanobacteria, modifications of PSI and PSII occurs, which influences the redox state of redox sensitive components of the electron transport chain. This will have an effect on the metabolic activity (20). To maintain growth under Fe-limited conditions, bacteria have developed two basic responses. They scavenge Fe by synthesizing siderophores that solubilize Fe as siderophore-iron complexes (6). The second response is a reduced cellular demand for Fe, by altering the population of proteins, and reduce the number of proteins that contains Fe, or those where the synthesis require Fe. In cyanobacteria the mobile electron carrier flavodoxin replaces Fe-sulfur (S) - containing ferredoxin under Fe-limited conditions (13, 14, 31, 32). Although siderphores are synthesized in response to Fe-limitation, it has been shown that these siderophores are capable of binding other metal ions such as copper (Cu) (19). A transcriptional regulator of a number of genes involved in iron metabolism, called the ferric uptake regulator, may regulate the *mcy*

gene cluster, providing a relationship between toxin synthesis and Fe availability (26). The increased growth of cyanobacteria when supplemented with Fe, and the observerved changes in toxicity at different iron concentrations have led to several studies on the effect of iron on *M. aeruginosa* and the synthesis of MCs (1, 18, 26, 34). A suggested hypothesis is that the peptide toxin could be an intracellular chelator which inactivates free cellular Fe^{2+}. Where MC is produced by an enzyme (synthetase) whose activity is controlled by the amount of free Fe^{2+} present (34). Another finding showed MCs producing strains to remain viable for longer periods during iron depletion, than non-producing strains (17).

The purpose of this study is to examine if and how Fe could influence growth and toxin production of *Microcystis aeruginosa*. It is also interesting to investigate if growth of the toxic and non-toxic cultures is affected differently. This can help us to explain the diversity and patches of non-toxic and toxic strains in cyanobacterial blooming. Because of the harmful effects of MCs on human health it is important to get a better understanding of the environmental and nutritional factors influencing the toxin production.

Key words, *Microcystis,* microcystin, iron.

2.3 Material and methods

2.3.1 Growth conditions

M. aeruginosa PCC 7806, toxic strain and the non-toxic strain and *M. aeruginosa* (MCYB¯) were obtained from institute Pasteur and university of Berlin. Strains were grown in 2- L glass vessels as batch cultures. Strains from the same culture were used in all experiments. The incident light intensity was about 10 µmol photon $m^{-2} s^{-1}$, and the growth medium was O2 (38). The temperature was kept at 25 °C. The cultures were placed in a climate cabinet (Termaks), providing continuous illumination and light-intensity by Philips 36W/33 fluoroscent. The cultures where always handled with appropriate sterile techniques. 20 ml samples were collected from the 2-L vessels, every second day with sterile pipettes. The vessels were stirred by aeration. When *M. aeruginosa* was grown under Fe-limited conditions, the medium was supplied with 1/10 and ½ of the amount of Fe in standard O2 medium. Vessels were also made with medium supplied with two, five and ten times more Fe. The experimental vessels were always in pairs.

2.3.2 Cyanobacteria biomass and chemical analysis

Samples for determination of cell density, protein, NO_3^-, Fe and PO_4^{3-} were taken every second day. Cell density was analyzed as optical density at 740 nm with a spectrophotometer (Perkin Elmer UV/Vis Spectrometer Lambda 20). Protein was analyzed by using the Lowry method (11), using triplicate samples (0,5 mL). The samples were collected in glass tubes, and stored frozen (-20°C). Before processing all samples were freeze-dried in acid-washed glass tubes. Each lyophilized sample was added distilled water (0,5 mL), and bovine serum albumin (BSA) used as a standard for calibration.

'Microcystin analyses

Microcystin concentration was measured by using ELISA- kit from, Abraxis, Biosense laboratories.

2.4 Results

Neither the MCYB⁻ nor the PCC 7806 cultures of *M. aeruginosa* grew under low Fe concentrations, with 50 µg Fe/l in the culture medium. Growth of the PCC 7806 cultures showed a different pattern than the MCYB⁻ cultures, with a greater difference in lag period between the treatments (fig. 1a and 1b). All the treatments started at the same optical density at day 0. The Fe concentration had a greater impact on growth of the PCC 7806 cultures, than on MCYB⁻ cultures. MCYB⁻ cultures at different Fe concentrations had equal long lag periods (fig. 1a). Differences occurred in the lag period between the PCC 7806 cultures. Lag period in treatment with 250 µg Fe/l lasted until day 17, and in the treatment with 500 µg Fe/l until day 7. The treatment with 5000 µg Fe/l showed a lag period of three days. The growth of MCYB⁻cultures was only slightly affected by differences in iron concentrations in the media (with growth rate (µ) in treatment with 250 µg Fe/l 0,033, 500 µg Fe/l 0,025 and 5000 µg Fe/l 0,028) (Fig 1a). The MC concentration increased with increasing Fe concentration to the medium from day 1 (fig. 2). In ordinary O2 medium (500 µg Fe/l) the cultures seem to have a similar Fe uptake, with equal changes in Fe content during the experiment (fig. 3a). In the medium with the PCC 7806 cultures and 10x Fe (5000 µg Fe/l) the Fe concentration was reduced to about zero at day 13. While the MCYB⁻ cultures appeared to be iron-depleted at day 19 (fig. 3b). In medium with higher Fe concentrations (5000 µg Fe/l) the MCYB⁻ cultures had a lower Fe uptake than the PCC 7806 culture. MC content increased in the cultures, as the iron concentration decreased (Fig. 4a and 4b).

2.5 Discussion

Our results showed 50 µg Fe/l to be too low for the bacteria to grow. It has been suggested that the highest toxin production is to be found under optimal conditions (30). In this study a increased Fe concentration in the medium, resulted in a higher cell density as well as a higher production of MCs. Results also showed MC content to increase, as the Fe concentration decreases during the experiments. Toxin production has been found to be affected by transcriptional regulation and the cellular availability of different substrates (33). Earlier studies have found growth of *M. aeruginosa* to be inhibited by limiting the trace element Fe (16), also shown in this study. Because of Fe being controlled by organic chelators, measurements of total Fe do not represent the Fe that is bio available (21). Previous experiments found that growth of the cyanobacteria and the content of MCs were maximal at the highest Fe concentration (15), similar to our findings. Both MCs and proteins per cell showed to be enhanced by an increase in iron concentration (2). There has been found a marked difference in the tolerance of iron-stress between the toxic and non-toxic strain of *M. aeruginosa*. The toxic strain kept its cells viability longer during growth than the non-toxic strain (17). A earlier study discovered a much lower cell-growth, but the production of toxin was 20-40 % higher under Fe-limited conditions (16). In our experiment MC content increased with increasing Fe concentration in the medium. We also found MC content to increase as the Fe concentration decreased during the experiments. The deviation from earlier studies under Fe-limitation could be attributed to different cyanobacteria strains, growth conditions or effects of undetected bacteria degrading MCs (15). There has been performed several studies on the transition from replete to limiting iron conditions (23, 27, 28). All studies have in common a decrease in photosynthesis and respiration genes. The toxic *M. aeruginosa* has been found to exhibited higher rates of Fe uptake than the non-toxic. Suggesting that the non-toxic strain is forced to maintain a lower cellular Fe content. There was similar findings in the media with maximal Fe concentration (5000 µg Fe/l) in this study concerning uptake, probably because of the absence of MCs. With less Fe it did not seem to be any differences between PCC 7806 and MCYB⁻ cultures in Fe uptake. It has been suggested that the toxin can give an advantage to the toxin producing *M. aeruginosa* under early stages of Fe stress, in addition to protect the cells against damages from oxidative stress (1). In cyanobacteria, which are obligate phototrophs, the photosynthetic electron transport pathway is the most important energy-transducing reaction in the cell. Fe plays an important

role in the synthesis of the phycobilin chromophore of the phycobiliproteins, and is an essential component of every membrane-bound protein complex of the light reaction (8). A positive connection between ˝energy state˝and MC content has been discovered (3). The variation in MC production could therefore be explained by variations in the energy state of cells. The uptake and conversion of compounds like nitrate (NO_3^-) could influence the energy state of the organism and thereby MC production. Since Fe being involved in essential metabolic processes, such as photosynthesis, it is not unlikely that both toxin production and protein synthesis increases with increased Fe concentration (3). In this study MC content increased as a response to an increased Fe concentration, indicating that toxin production could be regulated by the energetic state of cells. It has been suggested that MC production could be regulated by Fe concentration (34). Introducing MCs to be produced as an intracellular chelator which keeps the cellular level of free Fe^{2+} low, leading to a more efficient Fe uptake. In addition there was observed a decrease in MC quota per cell in the late exponential growth phase in an earlier study (44). This has been suggested to be attributed to an increase in MC binding to proteins in reactive oxygen species accumulating senescent cultures. MC has been suggested to be a protein-modulating metabolite and protectant against oxidative stress under high light and oxidative stress conditions. A binding is strengthened under high light and oxidative stress conditions, by a covalent interaction of cysteines and the N-methyldehydroalanine position of MC. (43). In co-culture experiments under favorable growth conditions, the non-toxin producing strain was found to dominate the toxin producing strain. There was also found a increase in the growth rate of the non-toxin producing strain and in the cellular MC content of the toxic producing strain. The differences between the two strain could be attributed the costs of producing MCs, and a possible cooperation between the strains (4). In this study both the PCC 7806 and MCYB⁻ cultures was affected by the amount of iron concentrations. An important finding was that the PCC 7806 culture lag phase increased with decreasing iron concentration in the medium. There was not observed any difference in the MCYB⁻ culture`s lag phase. The difference in lag periods for the toxin producing and non-toxin producing *M. aeruginosa* cultivated with different Fe concentrations has been observed earlier (17), and indicates that the toxin producing *M. aeruginosa* adapts slower to environmental changes. The non-toxin producer could therefore outgrow the toxic strain. This could be an additional explanation for a shift of the relation toxin/non-toxin-producing strains, and of patches with toxin production arias found in cyanobacterial blooms.

This study showed that growth of the PCC 7806 cultures was more affected than the MCYB⁻ cultures. This study found *M. aeruginosa* PCC 7806 to adapt slower under limiting conditions. While at higher Fe concentrations both cell density and MC content increased. In relation to human health, it is vital to focus on the cyanobacteria and the toxin production. Though many studies have been done, there is still need for more research. It is important that we get a better understanding of the function of the toxins, and how it's affected by environmental and nutritional factors, including the trace metal Fe.

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ATTACHMENTS

Attachment 1

Figure 1a: Optical density (OD 740 nm) of *M. aeruginosa* MCYB¯ grown in O2 medium at various iron concntrations (average values).

Figure 1b: Optical density (OD 740 nm) of *M. aeruginosa* PCC 7806 grown in O2 medium at various iron concntrations (average values).

Figure 2: µg microcystin/ mg protein in *M. aeruginosa* PCC 7806 grown in O2 medium at various iron concentrations (average values).

Figure 3a: Changes in iron concentrations (average values) during growth of *M. aeruginosa* PCC 7806 and MCYB¯ in O2 medium and 500 µg/l as the initial iron concentration.

Figure 3b: Changes in iron concentrations (average values) during growth of *M. aeruginosa* PCC 7806 and MCYB¯ in O2 medium and 5000 µg/l as the initial iron concentration.

Figure 4a: Changes in iron concentrations (average values in µg/l) and microcystin content (µg microcystin/mg protein) of *M. aeruginosa* PCC 7806 grown in O2 medium and 5000 µg/l as the initial iron concentration.

Figure 4b: Changes in iron concentrations (average values in µg/l) and microcystin content (µg microcystin/mg protein) of *M. aeruginosa* PCC 7806 grown in O2 medium and 5000 µg/l as the initial iron concentration.

Attachment 2
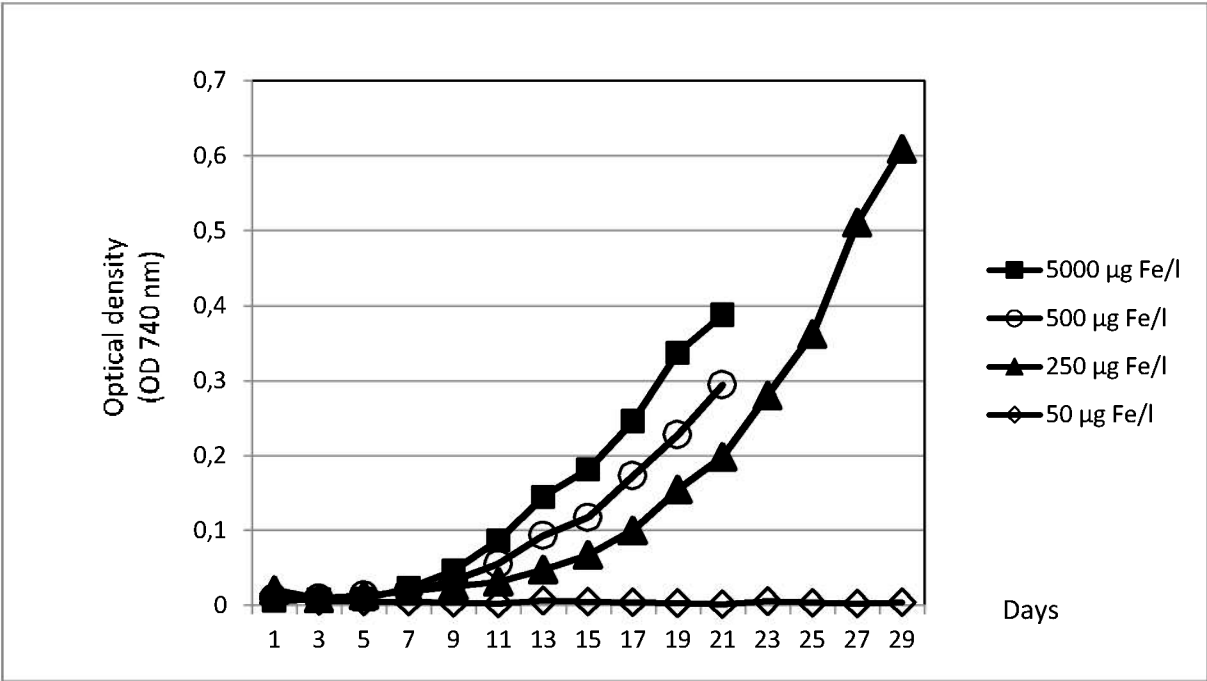
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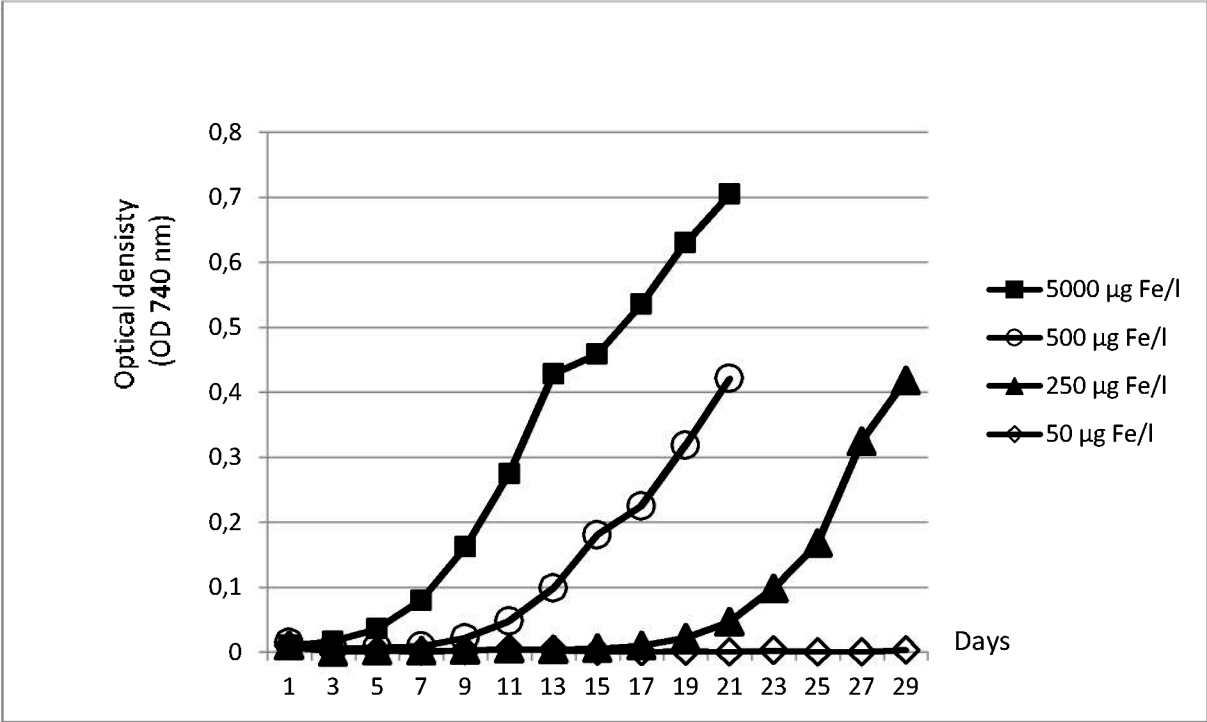
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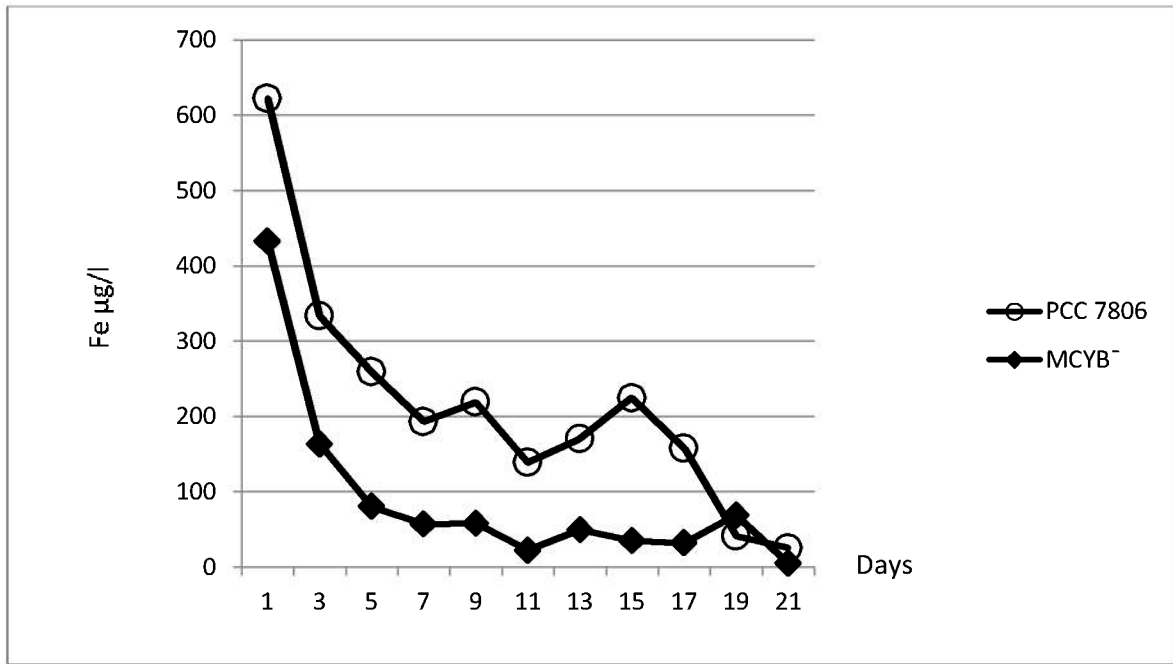
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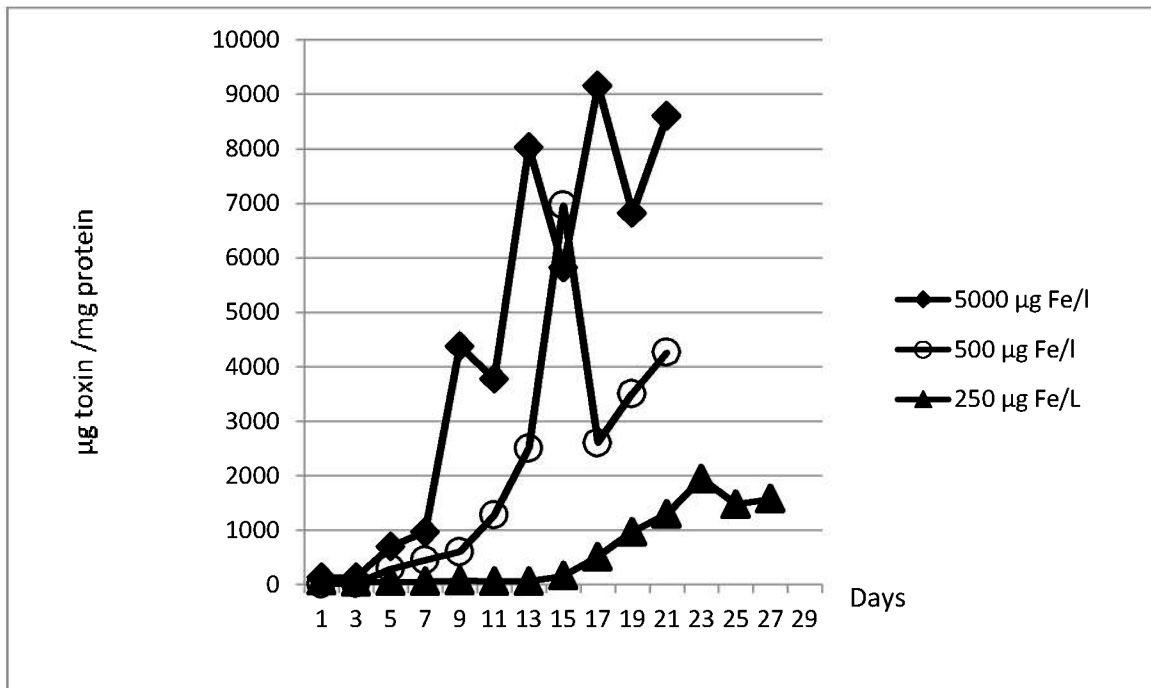
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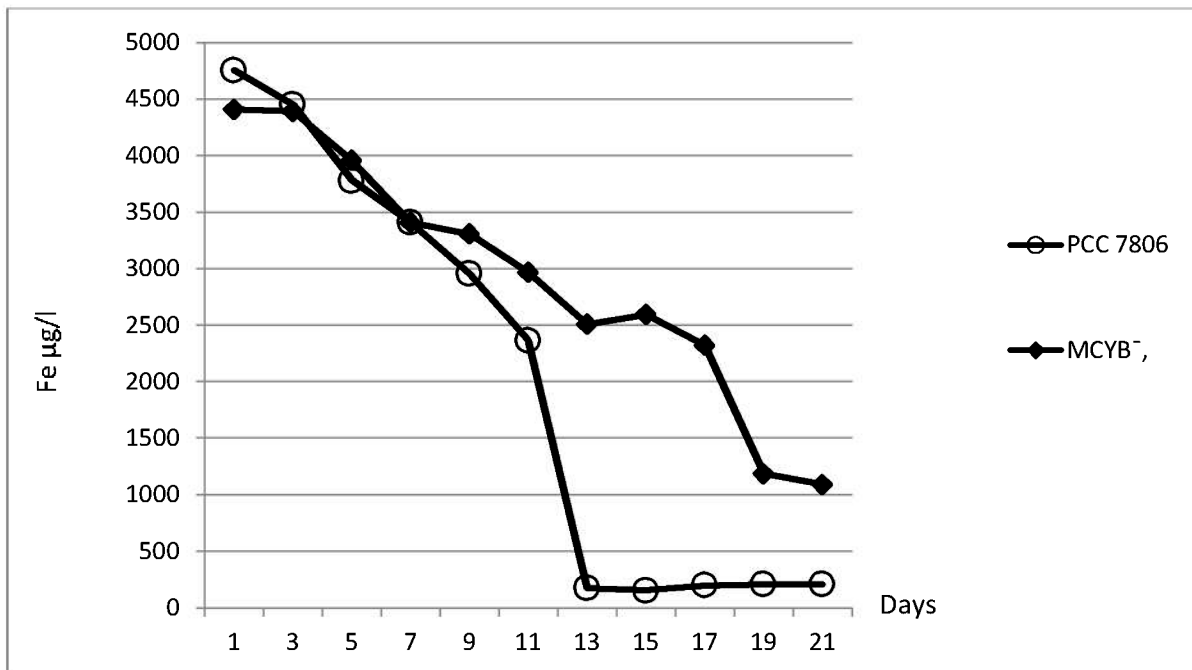
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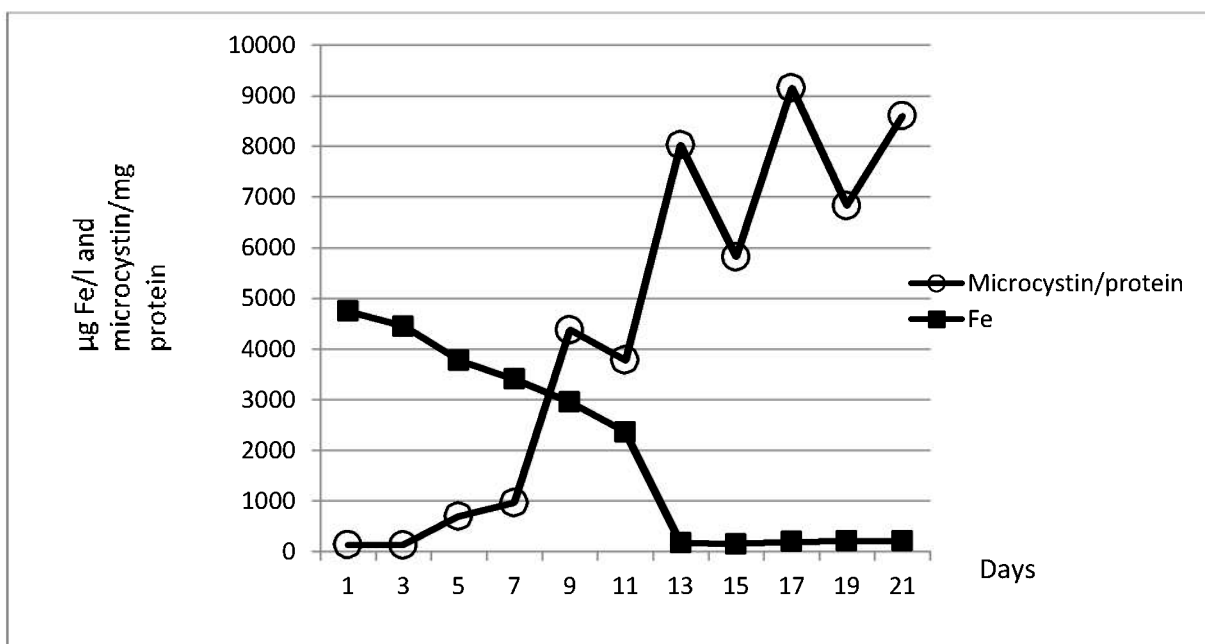
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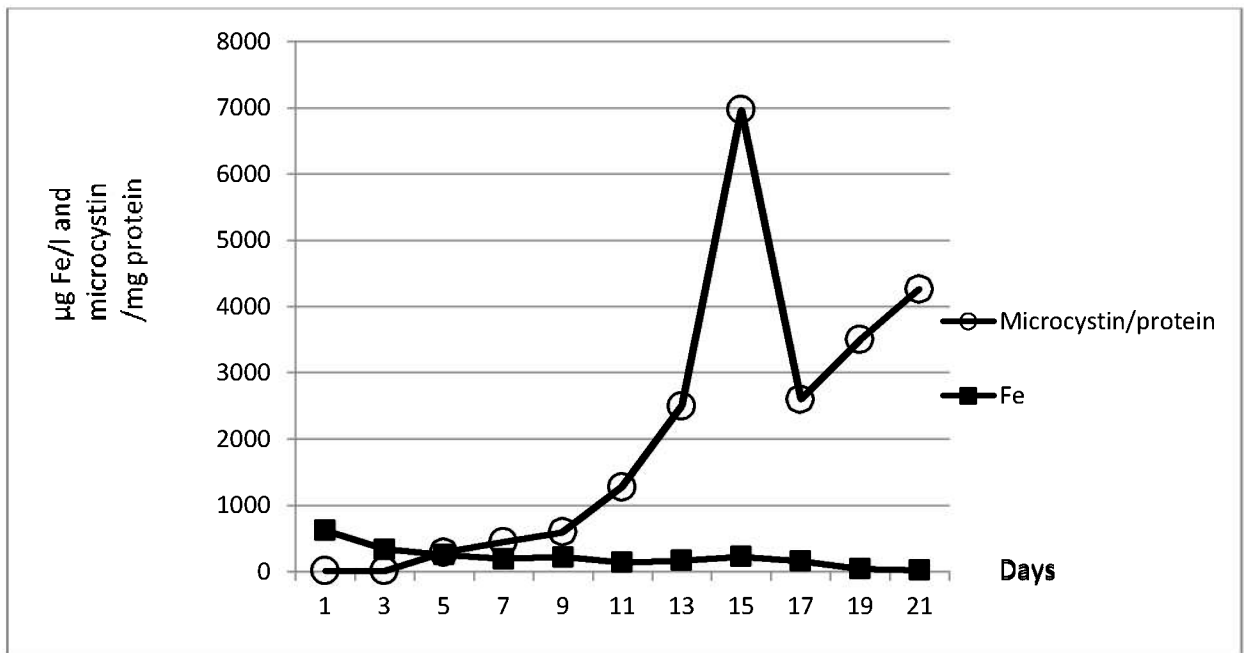
Figure 4b

Attachment 3

Date:	Species:	OD (740 nm)	Protein (µg/ml)	Microcystin (µg/l)	Po4 (µg/l)	Fe (µg/l)
07.jun	MCYB⁻	0.006	0,118		3610	485
	PCC 7806	0,002			3705	704
	PCC 7806	0,003	0,119	1,3	3545	478
	PCC 7806	0,003	0,115	1,3	3605	437
09.jun	MCYB⁻	0.008	0,127		3700	309
	PCC 7806	0,003			3615	484
	PCC 7806	0,0045	0,137	1,9	3510	467
	PCC 7806	0,005	0,122	1,5	3455	283
11.jun	MCYB⁻	0,013	0,146		3505	432
	PCC 7806	0,006			2830	242
	PCC 7806	0,01	0,123	5	3550	207
	PCC 7806	0,014	0,133	4,2	3375	295
13.jun	MCYB⁻	0,013	0,141		3725	218
	PCC 7806	0,006			3665	226
	PCC 7806	0,011	0,151	18,5	3605	188
	PCC 7806	0,011	0,126	20	3635	188
15.jun	MCYB⁻	0,022	0,125		3760	425
	PCC 7806	0,004			3840	684
	PCC 7806	0,022	0,1	16	3415	277
	PCC 7806	0,02	0,208	22,8	3780	218
17.jun	MCYB⁻	0,034	0,107		3965	168
	PCC 7806	0,004			3765	219
	PCC 7806	0,044	0,096	48,3	3630	160
	PCC 7806	0,038	0,142	42,7	3570	130
19.jun	MCYB⁻	0,054	0,118		3695	163
	PCC 7806	0,004			3950	226
	PCC 7806	0,075	0,107	48,6	3540	147
	PCC 7806	0,065	0,125	115,8	3600	119
21.jun	MCYB⁻	0,085	0,142		3280	136
	PCC 7806	0,004			3975	252
	PCC 7806	0,139	0,186	152,6	3425	136
	PCC 7806	0,11	0,186	179,9	3315	112
23.jun	MCYB⁻	0,121	0,155		3485	256
	PCC 7806	0,005			4175	262
	PCC 7806	0,377	0,191	320,3	3275	110
	PCC 7806	0,224	0,144	246,1	3335	93
25.jun	MCYB⁻	0,166	0,201		3415	92
	PCC 7806	0,006			3970	248
	PCC 7806	0,295	0,21	344,3	2780	91
	PCC 7806	0,203	0,127	360,9	3340	116
27.jun	MCYB⁻	0,211	0,205		3850	111
	PCC 7806	0,005			4125	235
	PCC 7806	0,377	0,243	413,3	3125	55
	PCC 7806	0,244	0,174	344,8	3205	86
29.jun	MCYB⁻	0,257	0,214		3270	100
	PCC 7806	0,006			4165	

	PCC 7806	0,458	0,235	396,8	3455	
	PCC 7806	0,217	0,202	266,3	3175	
02.jul	MCYB⁻	0,346	0,222		3665	
	PCC 7806	0,007			3805	
	PCC 7806	0,603	0,228	771,6	2950	
	PCC 7806	0,194	0,16	242	3070	
04.jul	MCYB⁻	0,413	0,228		3145	
	PCC 7806	0,005			4295	
	PCC 7806	0,689	0,268	520	2865	
	PCC 7806	0,161	0,13	314,4	3205	
06.jul	MCYB⁻	0,514	0,264		3110	85
	PCC 7806	0,01			2285	249
	PCC 7806	0,803		621,4	2900	72
	PCC 7806	0,179	0,155	250,5	3450	74
11.jul	MCYB⁻	0,711				
	PCC 7806					
	PCC 7806	0,988				
	PCC 7806					

Attachment 4

Date:	Species	Fe (day 1)	OD (740 nm)	Protein (µg/l)	Microcystin (µg/l)	PO₄⁻ (µg/l)	Fe (µg/l)
17.sep	MCYB⁻	50 µg Fe/l	0,01	0,01		3860	78
		50 µg Fe/l	0,002	0,002		3565	90
		250 µg Fe/l	0,021	0,021		3675	225
		250 µg Fe/l	0,02	0,02		3620	219
	PCC 7806	50 µg Fe/l	0,016	0,016		3930	9
		50 µg Fe/l	0,003	0,003		3735	7
		250 µg Fe/l	0,009	0,009	5	3615	172
		250 µg Fe/l	0,01	0,01	5,5	3570	181
19.sep	MCYB⁻	50 µg Fe/l	0,006	0,006		4190	0
		50 µg Fe/l	0,006	0,006		3705	82
		250 µg Fe/l	0,01	0,01		3745	78
		250 µg Fe/l	0,008	0,008		3690	0
	PCC 7806	50 µg Fe/l	0,004	0,004		3865	0
		50 µg Fe/l	0	0		379	25
		250 µg Fe/l	0	0	4,5	3515	49
		250 µg Fe/l	0	0	3,3	3605	49
21.sep	MCYB⁻	50 µg Fe/l	0,005	0,005		4005	46
		50 µg Fe/l	0,005	0,005		3580	35
		250 µg Fe/l	0,012	0,012		5545	45
		250 µg Fe/l	0,015	0,015		4070	44
	PCC 7806	50 µg Fe/l	0,002	0,002		3625	20
		50 µg Fe/l	0,002	0,002		3980	21
		250 µg Fe/l	0,002	0,002		3170	70
		250 µg Fe/l	0,002	0,002		3520	59
23.sep	MCYB⁻	50 µg Fe/l	0,004	0,004		398	0

Date	Strain						
		50 µg Fe/l	0,006	0,006		3805	0
		250 µg Fe/l	0,015	0,015		3710	76
		250 µg Fe/l	0,023	0,023		4130	67
	PCC 7806	50 µg Fe/l	0,001	0,001		3625	0
		50 µg Fe/l	0,001	0,001		4050	0
		250 µg Fe/l	0,001	0,001		3790	60
		250 µg Fe/l	0,002	0,002		3820	39
25.sep	MCYB⁻	500 µg Fe/l	0,003	0,003		4010	0
		500 µg Fe/l	0,004	0,004		3690	10
		250 µg Fe/l	0,018	0,018		3740	36
		250 µg Fe/l	0,033	0,033		3830	31
	PCC 7806	50 µg Fe/l	0,001	0,001		3825	28
		50 µg Fe/l	0,003	0,003		4065	67
		250 µg Fe/l	0,003	0,003	4,9	3620	65
		250 µg Fe/l	0,003	0,003	4,7	3955	87
27.sep	MCYB⁻	50 µg Fe/l	0,001	0,001		4210	39
		50 µg Fe/l	0,004	0,004		3540	40
		250 µg Fe/l	0,016	0,016		4220	52
		250 µg Fe/l	0,047	0,047		3880	84
	PCC 7806	50 µg Fe/l	0,005	0,005		3725	26
		50 µg Fe/l	0,005	0,005		3805	39
		250 µg Fe/l	0,004	0,004		3690	54
		250 µg Fe/l	0,005	0,005		3930	39
29.sep	MCYB⁻	50 µg Fe/l	0,005	0,005		4275	16
		50 µg Fe/l	0,008	0,008		3790	0
		250 µg Fe/l	0,025	0,025		3945	43
		250 µg Fe/l	0,07	0,07		3910	27
	PCC 7806	50 µg Fe/l	0,005	0,005		3720	39
		50 µg Fe/l	0,002	0,002		4040	4
		250 µg Fe/l	0,004	0,004	5,5	3900	25
		250 µg Fe/l	0,004	0,004	4,5	3990	44
01.okt	MCYB⁻	50 µg Fe/l	0,005	0,005		4255	88
		50 µg Fe/l	0,005	0,005		3755	55
		250 µg Fe/l	0,03	0,03		3630	46
		250 µg Fe/l	0,104	0,104		3545	29
	PCC 7806	50 µg Fe/l	0,001	0,001		3600	52
		50 µg Fe/l	0,001	0,001		3925	30
		250 µg Fe/l	0,006	0,006	20,9	3760	31
		250 µg Fe/l	0,005	0,005	8,5	3780	34
03.okt	MCYB⁻	50 µg Fe/l	0,004	0,004		4000	20
		50 µg Fe/l	0,004	0,004		3635	43
		250 µg Fe/l	0,04	0,04		3725	75
		250 µg Fe/l	0,16	0,16		3760	28
	PCC 7806	50 µg Fe/l	0	0		3880	26
		50 µg Fe/l	0,001	0,001		3955	21
		250 µg Fe/l	0,015	0,015	20,2	3695	36
		250 µg Fe/l	0,005	0,005	54,5	3950	0
05.okt	MCYB⁻	50 µg Fe/l	0,003	0,003		4120	84
		50 µg Fe/l	0,003	0,003		3920	6
		250 µg Fe/l	0,065	0,065		3685	43

		250 µg Fe/l	0,243	0,243		3415	23
	PCC 7806	50 µg Fe/l	0,001	0,001		3535	21
		50 µg Fe/l	0,001	0,001		3985	18
		250 µg Fe/l	0,033	0,033	93,1	3750	40
		250 µg Fe/l	0,011	0,011	46,4	4005	34
07.okt	MCYB⁻	50 µg Fe/l	0,002	0,002		4180	0
		50 µg Fe/l	0,001	0,001		4255	27
		250 µg Fe/l	0,092	0,092		4040	0
		250 µg Fe/l	0,304	0,304		3880	23
	PCC 7806	50 µg Fe/l	0	0		4140	0
		50 µg Fe/l	0	0		4430	70
		250 µg Fe/l	0,065	0,065	202,4	4160	0
		250 µg Fe/l	0,027	0,027	75,9	4265	29
09.okt	MCYB⁻	50 µg Fe/l	0,005	0,005		4745	0
		50 µg Fe/l	0,006	0,006		4235	0
		250 µg Fe/l	0,153	0,153		3840	11
		250 µg Fe/l	0,407	0,407		3685	13
	PCC 7806	50 µg Fe/l	0,002	0,002		4200	0
		50 µg Fe/l	0,003	0,003		4300	0
		250 µg Fe/l	0,135	0,135	236,5	3970	48
		250 µg Fe/l	0,06	0,06	164,1	3995	45
11.okt	MCYB⁻	50 µg Fe/l	0,003	0,003		4700	25
		50 µg Fe/l	0,004	0,004		4345	0
		250 µg Fe/l	0,223	0,223		3515	31
		250 µg Fe/l	0,5	0,5		3175	24
	PCC 7806	50 µg Fe/l	0	0		3970	0
		50 µg Fe/l	0	0		4440	14
		250 µg Fe/l	0,213	0,213	646,5	3880	24
		250 µg Fe/l	0,122	0,122	604,7	3770	0
14.okt	MCYB⁻	50 µg Fe/l	0,002	0,002		4450	85
		50 µg Fe/l	0,003	0,003		4310	56
		250 µg Fe/l	0,35	0,35		3995	0
		250 µg Fe/l	0,67	0,67		3260	37
	PCC 7806	50 µg Fe/l	0	0		3515	0
		50 µg Fe/l	0	0		4715	16
		250 µg Fe/l	0,374	0,374	433,2	4045	0
		250 µg Fe/l	0,274	0,274	284,7	3910	38
16.okt	MCYB⁻	50 µg Fe/l	0,002	0,002		4520	0
		50 µg Fe/l	0,006	0,006		4220	0
		250 µg Fe/l	0,429	0,429		3570	0
		250 µg Fe/l	0,78	0,78		2570	49
	PCC 7806	50 µg Fe/l	0,002	0,002		3915	13
		50 µg Fe/l	0,004	0,004		4365	0
		250 µg Fe/l	0,465	0,465	133,8	3545	0
		250 µg Fe/l	0,37	0,37	128,2	3545	30

Attachment 5

Date:	Species	Fe (Day 1)	OD (740 nm)	Protein	Microcystin (µg/l)	PO$_4^-$ (µg/l)	Fe (µg/l)
02.nov	MCYB$^-$	1000 µg Fe/l	0	0,113		3657,5	1067
		1000 µg Fe/l	0	0,073		3773,5	1082
		2500 µg Fe/l	0	0,066		3620,5	1975
		2500 µg Fe/l	0	0,069		3695,5	2209
	PCC 7806	1000 µg Fe/l	0	0,084	7,8	4448	1205
		1000 µg Fe/l	0	0,081	30,2	4522	858
		2500 µg Fe/l	0	0,119	11,2	4114	2210
		2500 µg Fe/l	0	0,094	27,2	4579,5	2371
04.nov	MCYB$^-$	1000 µg Fe/l	0,025	0,088		3824	633
		1000 µg Fe/l	0,012	0,066		4037,25	583
		2500 µg Fe/l	0,022	0,066		3685	1300
		2500 µg Fe/l	0,012	0,104		4132,5	1361
	PCC 7806	1000 µg Fe/l	0,004	0,084	30,8	4226,85	934
		1000 µg Fe/l	0,001	0,061	24,7	4321	541
		2500 µg Fe/l	0,024	0,069	24,8	3974	1593
		2500 µg Fe/l	0,023	0,082	27,7	3835,5	1709
06.nov	MCYB$^-$	1000 µg Fe/l	0,034	0,071		3361,6	720
		1000 µg Fe/l	0,028	0,066		3789,7	501
		2500 µg Fe/l	0,036	0,07		3406	920
		2500 µg Fe/l	0,027	0,071		3931	1225
	PCC 7806	1000 µg Fe/l	0,035	0,085	83	4888,5	780
		1000 µg Fe/l	0,03	0,073	40,4	4850	762
		2500 µg Fe/l	0,043	0,072	76,5	4211,8	1184
		2500 µg Fe/l	0,026	0,07	100,2	4213,5	1231
08.nov	MCYB$^-$	1000 µg Fe/l	0,056	0,071		3857,5	692
		1000 µg Fe/l	0,042	0,087		4028,5	279
		2500 µg Fe/l	0,053	0,097		3484,25	1023
		2500 µg Fe/l	0,041	0,069		4235	1216
	PCC 7806	1000 µg Fe/l	0,066	0,076	192,4	4524,5	752
		1000 µg Fe/l	0,06	0,069	158	4699	423
		2500 µg Fe/l	0,052	0,082	72,2	3410	1026
		2500 µg Fe/l	0,041	0,089	94,4	4168,5	1382
10.nov	MCYB$^-$	1000 µg Fe/l	0,092	0,106		3809,5	211
		1000 µg Fe/l	0,065	0,109		3810	121
		2500 µg Fe/l	0,082	0,095		3360	502
		2500 µg Fe/l	0,07	0,092		4006,5	897
	PCC 7806	1000 µg Fe/l	0,12	0,076	325,6	4455,5	378
		1000 µg Fe/l	0,114	0,09	282,3	4646,5	340
		2500 µg Fe/l	0,113	0,103	293,3	3979,5	948
		2500 µg Fe/l	0,104	0,101	247,9	4239	1215
12.nov	MCYB$^-$	1000 µg Fe/l	0,148	0,089		3254	178
		1000 µg Fe/l	0,103	0,101		3110	148
		2500 µg Fe/l	0,137	0,113		2434	404
		2500 µg Fe/l	0,122	0,123		2700,5	502
	PCC 7806	1000 µg Fe/l	0,208	0,089	589,9	3947	267
		1000 µg Fe/l	0,197	0,087	550,6	3817,5	295

		2500 µg Fe/l	0,208	0,088	545,6	2887,5	98
		2500 µg Fe/l	0,184	0,137	448,6	3264	1105
14.nov	MCYB⁻	1000 µg Fe/l	0,217	0,111		2863,5	137
		1000 µg Fe/l	0,155	0,148		2875,5	98
		2500 µg Fe/l	0,199	0,131		2146	577
		2500 µg Fe/l	0,184	0,123		2880	577
	PCC 7806	1000 µg Fe/l	0,315	0,085	630,2	3502	220
		1000 µg Fe/l	0,3	0,129	662,8	3073,5	200
		2500 µg Fe/l	0,304	0,092	708,3	3531	267
		2500 µg Fe/l	0,281	0,156	819,3	3288,5	1026
16.nov	MCYB⁻	1000 µg Fe/l	0,279	0,138		2832	118
		1000 µg Fe/l	0,207	0,17		2880,85	109
		2500 µg Fe/l	0,262	0,181		2350,5	280
		2500 µg Fe/l	0,248	0,136		2729,5	563
	PCC 7806	1000 µg Fe/l	0,392	0,109	597,9	3239	151
		1000 µg Fe/l	0,399	0,136	1172,2	3493,5	228
		2500 µg Fe/l	0,4	0,134	955,2	2956,5	270
		2500 µg Fe/l	0,372	0,201	1115,9	3022	943
18.nov	MCYB⁻	1000µg Fe/l	0,374	0,191		2200	62
		1000 µg Fe/l	0,286	0,196		2272,5	67
		2500 µg Fe/l	0,34	0,212		2203,5	247
		2500 µg Fe/l	0,335	0,169		2416	532
	PCC 7806	1000 µg Fe/l	0,534	0,162	2080,7	3351	74
		1000 µg Fe/l	0,517	0,171	1847,1	3114	225
		2500 µg Fe/l	0,525	0,192	1595,9	2622	275
		2500 µg Fe/l	0,484	0,23	1736,4	3091,5	887
20.nov	MCYB⁻	1000 µg Fe/l	0,437	0,25		2251	263
		1000 µg Fe/l	0,299	0,239		837	11
		2500 µg Fe/l	0,427	0,223		1591,95	254
		2500 µg Fe/l	0,418	0,227		2514	529
	PCC 7806	1000 µg Fe/l	0,625	0,172	1790,7	2756,5	106
		1000 µg Fe/l	0,607	0,225	1894,2	3287	290
		2500 µg Fe/l	0,616	0,223	1772,3	2499	194
		2500 µg Fe/l	0,584	0,278	1650,3	2700,5	790
22.nov	MCYB⁻	1000 µg Fe/l	0,483	0,251		2508,5	150
		1000 µg Fe/l	0,266	0,25		1301,5	89
		2500 µg Fe/l	0,479	0,256		1779,75	330
		2500 µg Fe/l	0,473	0,241		2653,5	551
	PCC 7806	1000 µg Fe/l	0,696	0,186	2576,4	2846,5	93
		1000 µg Fe/l	0,682	0,214	1894,2	2940	178
		2500 µg Fe/l	0,692	0,255	1719,5	2530,35	394
		2500 µg Fe/l	0,663	0,256	1914,5	2913	743

Attachment 6

Date:	Species	Fe (day 1):	OD (740 nm)	Protein (µg/l)	Microcystin (µg/l)	Po4 (µg/l)	Fe (µg/l)
28.nov	MCYB⁻	500 µg Fe/l	0,011	0,074		3866	411
		500 µg Fe/l	0,008	0,07		3385,5	455
		5000 µg Fe/l	0,006	0,063		3940,5	4247
		5000 µg Fe/l	0,007	0,082		3314,5	4569
	PCC 7806	500 µg Fe/l	0,014	0,074	0,9	4093	747
		500 µg Fe/l	0,015	0,09	0,9	3585	498
		5000 µg Fe/l	0,01	0,076	9,8	3916	4566
		5000 µg Fe/l	0,011	0,066	9,2	3487,5	4939
30.nov	MCYB⁻	500 µg Fe/l	0,01	0,094		3850,5	184
		500 µg Fe/l	0,009	0,074		3073,35	142
		5000 µg Fe/l	0,008	0,072		3469	4164
		5000 µg Fe/l	0,007	0,084		3012	4619
	PCC 7806	500 µg Fe/l	0,005	0,083	0,9	3707	425
		500 µg Fe/l	0,006	0,072	0,9	3702	242
		5000 µg Fe/l	0,017	0,073	8,7	3735,5	4223
		5000 µg Fe/l	0,016	0,064	8,7	3441,5	4674
02.des	MCYB⁻	500 µg Fe/l	0,013	0,077		3937	107
		500 µg Fe/l	0,014	0,068		3008,5	54
		5000 µg Fe/l	0,007	0,082		3583,5	4128
		5000 µg Fe/l	0,01	0,068		2742	3785
	PCC 7806	500 µg Fe/l	0,013	0,067	20,6	3775	353
		500 µg Fe/l	0,001	0,078	21	3518	165
		5000 µg Fe/l	0,038	0,078	59,5	3213,75	3554
		5000 µg Fe/l	0,034	0,079	49,9	2915,15	3999
04.des	MCYB⁻	500 µg Fe/l	0,02	0,104		3682	68
		500 µg Fe/l	0,017	0,09		2800,2	46
		5000 µg Fe/l	0,022	0,089		3414	3243
		5000 µg Fe/l	0,024	0,07		2740	3572
	PCC 7806	500 µg Fe/l	0,011	0,077	40,9	3937,5	300
		500 µg Fe/l	0,007	0,073	26,3	3606,5	86
		5000 µg Fe/l	0,087	0,088	71,6	3178,5	3313
		5000 µg Fe/l	0,072	0,063	69,9	2751,85	3500
06.des	MCYB⁻	500 µg Fe/l	0,037	0,083		3773	76
		500 µg Fe/l	0,028	0,099		3218	40
		5000 µg Fe/l	0,045	0,134		3530,5	3289
		5000 µg Fe/l	0,047	0,096		2761,5	3320
	PCC 7806	500 µg Fe/l	0,026	0,083	34	3893,5	346
		500 µg Fe/l	0,017	0,084	65,8	3572,5	92
		5000 µg Fe/l	0,174	0,12	650,7	3145,5	2905
		5000 µg Fe/l	0,149	0,138	459,6	2954	3004
08.des	MCYB⁻	500 µg Fe/l	0,066	0,103		3807,5	29
		500 µg Fe/l	0,044	0,097		3165,5	15
		5000 µg Fe/l	0,085	0,136		3171	2937
		5000 µg Fe/l	0,088	0,093		2696	2993
	PCC 7806	500 µg Fe/l	0,062	0,096	139,3	3720	243
		500 µg Fe/l	0,033	0,092	100,5	3951,5	35

		5000 µg Fe/l	0,295	0,233	611	3037	2133
		5000 µg Fe/l	0,254	0,121	597,5	2729,5	2590
10.des	MCYB⁻	500 µg Fe/l	0,112	0,057		3852,5	29
		500 µg Fe/l	0,074	0,03		3037	70
		5000 µg Fe/l	0,14	0,059		2969,5	2519
		5000 µg Fe/l	0,149	0,074		2152,5	2494
	PCC 7806	500 µg Fe/l	0,126	0,061	192,2	3560	189
		500 µg Fe/l	0,07	0,047	86,5	3829,5	152
		5000 µg Fe/l	0,449	0,097	975,7	2480	90
		5000 µg Fe/l	0,407	0,082	491,6	2047,5	256
12.des	MCYB⁻	500 µg Fe/l	0,142	0,085		3704,5	39
		500 µg Fe/l	0,092	0,085		2987,5	31
		5000 µg Fe/l	0,181	0,086		2887,5	2377
		5000µg Fe/l	0,182	0,061		2286,5	2812
	PCC 7806	500µg Fe/l	0,181	0,104	653,8	3426,5	247
		500 µg Fe/l	0,179	0,05	382,4	3570,5	202
		5000µg Fe/l	0,495	0,099	473,6	2336,5	189
		5000 µg Fe/l	0,422	0,106	726,4	3557,5	115
14.des	MCYB⁻	500 µg Fe/l	0,21	0,075		2284,5	32
		500 µg Fe/l	0,136	0,079		1988	32
		5000 µg Fe/l	0,248	0,105		3197,5	2225
		5000 µg Fe/l	0,244	0,075		2816	2418
	PCC 7806	500 µg Fe/l	0,283	0,078	286,7	2593	248
		500 µg Fe/l	0,166	0,07	106,4	1519,5	67
		5000 µg Fe/l	0,573	0,133	1212,8	3301,5	245
		5000µg Fe/l	0,498	0,099	910,4	2044	152
16.des	MCYB⁻	500 µg Fe/l	0,274	0,096		1802,5	40
		500 µg Fe/l	0,181	0,075		3161,5	97
		5000 µg Fe/l	0,33	0,133		2727,5	2057
		5000 µg Fe/l	0,344	0,12		1797,5	315
	PCC 7806	500 µg Fe/l	0,389	0,078	394,7	3656,5	54
		500 µg Fe/l	0,247	0,137	266,4	3557,5	28
		5000 µg Fe/l	0,668	0,267	2093,4	2284,5	241
		5000 µg Fe/l	0,592	0,26	1510,5	1988	174
18.des	MCYB⁻	500 µg Fe/l	0,349	0,09		3197,5	8
		500 µg Fe/l	0,239	0,068		2816	2
		5000 µg Fe/l	0,399	0,204		2593	1983
		5000 µg Fe/l	0,376	0,195		1519,5	194
	PCC 7806	500 µg Fe/l	0,499	0,214	987,5	3301,5	32
		500 µg Fe/l	0,343	0,247	965	3518	18
		5000 µg Fe/l	0,743	0,239	2665,5	2044	201
		5000 µg Fe/l	0,667	0,197	1193,5	1802,5	217