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Hoyoung Joo

Effects of Mg^{2+} and light intensity on the growth and microcystin production of *Microcystis aeruginosa*

University of South-Eastern Norway
Faculty of Technology, Natural Sciences and Maritime Sciences
Department of Natural Sciences and Environmental Health
PO Box 235
NO-3603 Kongsberg, Norway

http://www.usn.no

This thesis is worth 60 study points

Abstract

Growth and toxin production of *Microcytis aeruginosa* PCC7806 (*M. aeruginosa*) grown in different Mg^{2+} concentrations and light intensities were investigated in this study. *M. aeruginosa* were cultured in four different Mg^{2+} concentrations ($MgSO_4$ content: 5, 12.5, 25 and 50 mg/L) in duplicate. Two experiments at low and high light intensities (8 $\mu Em^{-2}s^{-1}$ and 75 $\mu Em^{-2}s^{-1}$, respectively) were performed for 42 and 24 days, respectively. Mg^{2+} concentrations in media, pH, optical density, chlorophyll a concentrations, microcystin concentrations were analysed and morphology of *M. aeruginosa* cells observed during the experiments. Mg^{2+} concentrations in media were stable during the experiments at both light intensities. Optical density and chlorophyll a concentrations of each medium showed variation in onsets of the exponential growth phase at low light intensity. The biomass parameters had stronger positive correlation at low light intensity ($r > 0.974$) than at high light intensity ($r > 0.861$). Microcystin contents reached the highest value during the exponential growth phase and the stationary phase at low and high light intensity, respectively. It is suggested that Mg^{2+} concentrations may affect the cell division during the lag phase at low light intensity. This study reconfirms effects of light intensity on the growth and toxin production of *M. aeruginosa* like past studies.

Key words: *Microcystis aeruginosa*, Mg^{2+}, light intensity, growth, microcystin

Abbreviations

M. aeruginosa	*Microcystis aeruginosa*
MC	Microcystin
Adda	3-amino-9-methoxy-2, 6, 8-trimethyl-10phenyldeca-4, 6-dienoic acid
Chla	Chlorophyll a
Mg	Magnesium
ATP	Adenosine Triphosphate
N. muscorum	*Nostoc muscorum*
OD	Optical density
$MgSO_4$	Magnesium sulfate
Fe	Iron
NaHCO3	Sodium bicarbonate
Na_2SO_4	Sodium sulfate
SO_4^{2-}	Sulfate
$NaNO_3$	Sodium nitrate
K_2HPO_4	Dipotassium phosphate
$CaCl_2$	Calcium chloride
$FeCl_3$	Iron chloride
HCl	Hydrochloric acid
EDTA- Na_2	Disodium ethylenediaminetetraacetate dihydrate
NaOH	Sodium hydroxide
H_3BO_3	Boric acid
$MnCl_2 \cdot 4H_2O$	Manganese chloride
$(NH_4)_6 MoO_{24} \cdot 4H_2O$	Ammonium Molybdate Tetrahydrate
$ZnSO_4 \cdot 7H_2O$	Zinc sulfate
$CuSO_4 \cdot 5H_2O$	Copper sulfate
ELISA	Enzyme- linked immunosorbent assay
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
A. nidulans	*Anacystis nidulans*
CO_2	Carbon dioxide

Contents

List of Figures

List of Tables

Foreword

This work is a thesis of the master programme, Environmental Science at the University of South- Eastern Norway in 2019. This thesis assumes some knowledge of microbiology and chemistry. A number of experimental row data figures and tables are appended in Appendix 1 and 2.

I would like to thank my supervisor Synne Kleiven, who guided me patiently and stimulated my curiosity in this field. I am also grateful to Karin Brekke Li and Frode Bergan for their technical supports of the experiments. I appreciate the master fellow students for having discussion and fun together. I want to give special thanks to my family for their physical and mental supports. Without these people above, I could not challenge my journey in Norway.

Bø i Telemark/ 14th, May, 2019
Hoyoung Joo

1 Introduction

Cyanobacteria are gram- negative and photosynthetic prokaryotes that form blooms in brackish and fresh water environments all over the world (Potts and Whitton, 2000). Cyanobacterial blooms increasing likelihoods of adverse health risks on human and animals (Buratti et al., 2017). Bioaccumulations in crops and fruits are also detected after growing by irrigated water containing cyanotoxins (Romero-Oliva et al., 2014, Chen et al., 2012). *M. aeruginosa* produce hepatotoxic cyanotoxins called microcystins (MCs) (Sigee, 2005). The chemical structure of MCs is cyclic heptapeptides with cyclo (-D-Alanine-X-erythro-β-methyl-D-isoAspartic acid-Y-Adda-D-isoGlutamine-N-methyldehydro-Alanine) and X and Y are variable L- amino acids (De Figueiredo et al., 2004). More than 150 MCs that have different functional groups have been identified (Miles et al., 2014). One of the most frequent studied MCs is MC- LR (Puddick et al., 2014, De Figueiredo et al., 2004) (Figure 1 by Puddick et al. (2014)) with the variable amino acids leucine (L) and arginine (R). Effects of environmental conditions such as pH (Van Der Westhuizen and Eloff, 1983, McLachlan and Gorham, 1962), temperature (Paerl and Otten, 2013, Phelan and Downing, 2011, Coles and Jones, 2000), nutrients (Dai et al., 2016, Paerl and Otten, 2013, Yang et al., 2012), metals (Dai et al., 2016, Polyak et al., 2013) and light (Gonçalves et al., 2014, Yang et al., 2012, Wiedner et al., 2003, Kaebernick et al., 2000) have been studied and it has been observed that these parameters affect the growth and/or toxin production of cyanobacteria.

The functions of cyanotoxins have been discussed but many questions remain to validate the functions (Holland and Kinnear, 2013). MC production is significantly promoted or regulated by multiple environmental factors such as light intensity, sulfur and phosphorus (Jähnichen et al., 2011). *M. aeruginosa* may protect themselves by emitting information chemistry that leads to increase in MC production regardless of direct and indirect exposure of zooplankton (Jang et al., 2003). In terms of photosynthesis, *M. aeruginosa* may prevent photoinhibition of photosystem II by producing MC during low- light saturation periods (Phelan and Downing, 2011). Gan et al. (2012) showed significant effects of MC concentration on size of colonies of *M.*

aeruginosa spp. Allelopathic effects are reported when 50 µg MC/ mL produced by *M. aeruginosa* caused growth and nitrogenase inhibition of other cyanobacteria *Nostoc muscorum* (*N. muscorum*) and *Anabaena* (Singh et al., 2001). Li and Li (2012) reported that *M. aeruginosa* had more MC when they competed with other species.

Mg is the eighth richest element in the crust and the third most abundant elements in brine (Atkins et al., 2010) and Mg is used in various ways. Chla is a light- harvesting pigment that contains Mg^{2+} in the center of the cyclic structure of chla (Nelson, 2013). Also, Mg^{2+} plays important role in bacteriochlorophyll a,c and d, Calvin cycle (photosynthetic carbon reduction cycle) and ATP synthesis in cyanobacteria (Sigee, 2005). McSwain et al. (1976) showed that Mg^{2+} had an effect on protection of photosystem II through photooxidation of water and photoreduce $NADP^{+}$ which is an electron acceptor of photosynthetic electron transportation of *N. muscorum* (Strain 7119). Early study showed that Mg might be an important factor for cell division for gram- positive bacteria rather than gram- negative bacteria (Webb, 1949). A study in molecular biology supports the early studies on relationship between Mg and cell division because $MgATP^{2-}$ regulates cell growth through (Rubin, 2005).

The purpose of this study is to investigate the effects of Mg^{2+} concentrations and light intensities on the growth and MC production of *M. aeruginosa* grown in batch cultures. OD_{740nm}, chla concentrations, Mg^{2+} concentrations, MC concentrations of *M. aeruginosa*, pH in media were measured. Cellular morphology observations were also conducted.

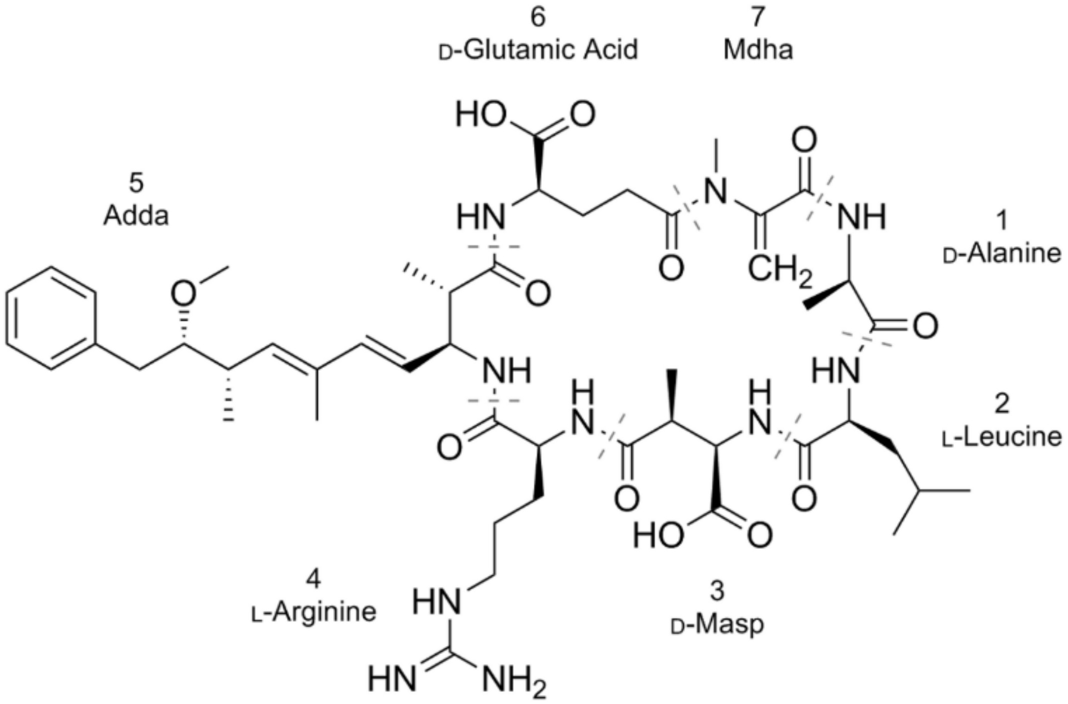


Fig 1. Chemical structure of MC- LR (Puddick et al., 2014). Masp is β-methyl-D-isoAspartic acid and Mdha is N-methyldehydro-Alanine.

2 Methods

2.1. Organism and growth conditions

Microcystis aeruginosa (PCC7806) was used in this study. *M. aeruginosa* was grown in batch cultures in 2 L glass flasks with 1.8 L of sterilized O2 media. (van Liere and Mur, 1978) There were 4 different modifications of the O2 media with different proportions of $MgSO_4$ (Table 2.1). The media were autoclaved at 120 °C for 20 minutes. The autoclaved medium was added 18mL of Fe solution and 9 mL sterile filtered (VWR Sterile Syringe Filter 0.2µm Cellulose Acetate) $NaHCO_3$ solution. 10 mL of *M. aeruginosa* strain was added to each glass bottle from a stationary pre culture. $MgSO_4$ was used as Mg source. The different Mg^{2+} treatments: 10, 25, 50 and 100% (100% of Mg^{2+} is the ordinary O2 medium working as control) were performed in duplicate. Na_2SO_4 was added in order not to change the amount of SO_4^{2-}. The work was done under controlled conditions in a cabinet.

Table 2. Composition of each media used in the growth experiments with Microcystis aeruginosa.

Composition	Mg^{2+} concentration in media			
	10%	25%	50%	100%
$NaNO_3$	500 mg/L			
K_2HPO_4	25 mg/L			
$MgSO_4$	5 mg/L	12.5 mg/L	25 mg/L	50 mg/l
$CaCl_2$	13 mg/L			
Na_2SO_4	25.9 mg/L	21.6 mg/L	14.4 mg/L	0 mg/L
Fe solution*	10 mL/L			
$NaHCO_3$	5 mL/L			
Microelement solution**	1 mL/L			

** Fe- solution composition*

10mL $FeCl_3$- solution (2.80g $FeCl_3$ in 100mL 0.1N HCl) and 9.5 mL EDTA solution (EDTA-Na_2 3.90g/L in 100mL NaOH) are mixed and filled up till 1000mL with distilled water.

***Microelement solution composition*

2.86g/L H_3BO_3, 1.81g/L $MnCl_2 \cdot 4H_2O$, 0.002g/L $(NH_4)_6 MoO_{24} \cdot 4H_2O$, 0.22g/L $ZnSO_4 \cdot 7H_2O$ and 0.08g/L $CuSO_4 \cdot 5H_2O$ are mixed and filled up to 1000mL.

Cultures were grown in an incubator (Termaks cabinet) at 25 °C with low light intensity (8 $\mu Em^{-2}s^{-1}$) during experiment 1 and higher light intensity (75 $\mu Em^{-2}s^{-1}$) during the second experiment (Fig.2). Light intensity was measured by using a photometer (LIGHT METER MODEL LI-250). The white light tubes (Nelson GARDEN® Art: 5590) above the bottles were covered by paper to regulate the light intensity in experiment 1. One light tube was used at each shelf in experiment 1 and two light tubes in experiment 2. The glass flasks were bubbled with air through 1 mL glass pipets and a VWR Sterile Syringe Cellulose Acetate Filter (0.2 µm). The glass flasks were moved systematically to different positions every second day. The experiment under low light intensity and higher light intensity were conducted for 42 days and 24 days, respectively.

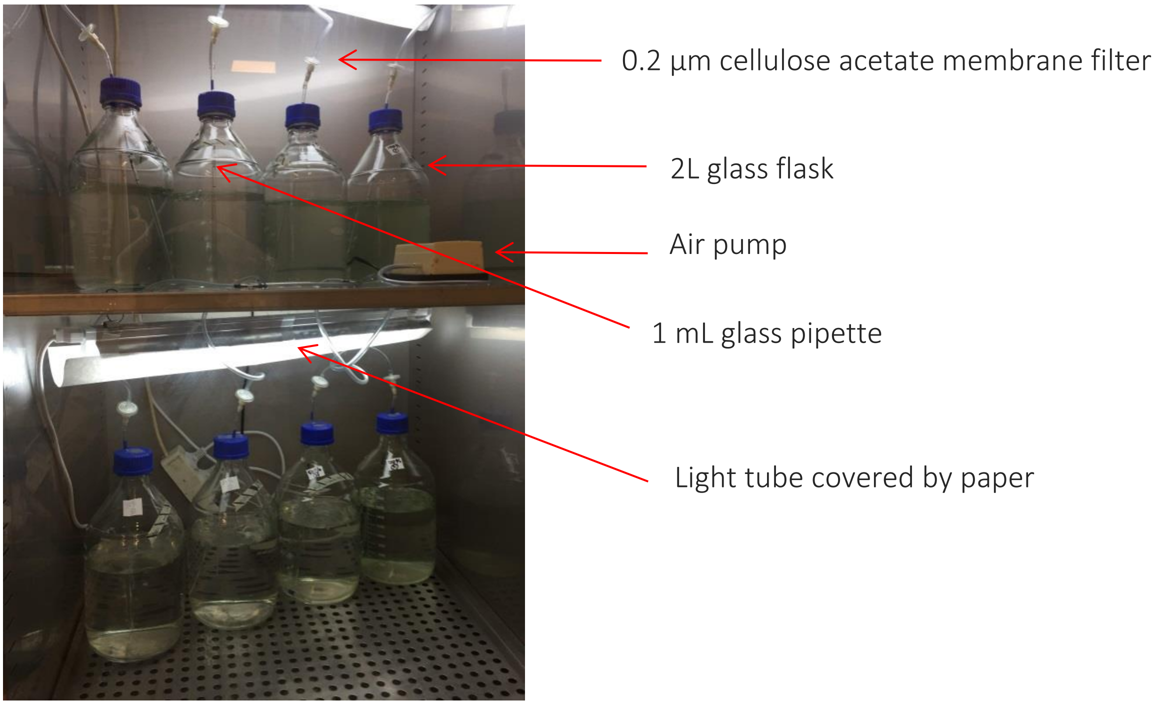


Fig.2. Experimental equipment for growing M. aeruginosa in the incubator (Photo by Hoyoung Joo).

2.2. Sampling

The sampling was performed every second day, taking 50 mL of each treatment by sterilized 50 mL pipets. 2 mL of each sample was frozen in glass tubes to measure MC concentrations and 5 mL was stored in plastic containers to measure Mg^{2+} concentrations. Samples for measuring chla concentrations were filtrated through Whatman GF/C glass microfibers filters. The filters were wrapped by aluminium foil to prevent samples from light and stored in a freezer (-18 °C).

2.3. Analysis

The morphology of the *Microcystis* cells was observed by using a hemocytometer (0.100 mm Tiefe Depth Profondeur) and a microscope (Olympus CX21) at 400 times magnifications.

OD was measured by a spectrometer (PerkinElmer UV/VIS Spectrometer Lambda 25) using 10 mm quartzite cuvette and 740 nm wavelength.

pH was measured by a pH meter (SevenCompact™S210).

Chlorophyll a was extracted by 90% acetone overnight. The chla concentrations were measured by the spectrometer after the samples were centrifuged at 4000 rpm for 10 minutes. The samples were added in 50 mm quartzite cuvette and measured by the spectrometer using 665 nm and 750 nm (Norges Standardiseringsforbund, 1983).

Before analyzing the Mg^{2+} concentrations, samples were filtrated by Pall Corporation Supor® 0.45 µm 25 mm PES filters to remove particles.Mg^{2+} concentrations were measured by atomic absorption spectrometer (PerkinElmer HGA900 Graphite Furnace). Standard curves were made by using standards of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 mg Mg^{2+}/L. The wavelength of the absorption was 285.2 nm (Standardiseringskommissionen I Sverige, 1993).

The concentration of MC in the samples was measured by using ELISA. Only selected samples were analyzed for MC content due to the expensive assay. MC ELISA kits

(Abraxis, Biosense laboratories 520011) were used for the measurement. MC concentrations were assessed by using ELISA Accu Reader at the wavelength 450 nm. In order to quantify the amount of MCs, the samples were diluted by 10, 20, 50, 100, 500 and 1000 times if needed to be inside the standard curve of the MC kit. Standard curves were made from 0, 0.15, 0.4, 1.0, 2.0 and 5.0 µg MC/L standards.

2.4. Calculation

Growth rates (k) and generation times (g) during the exponential growth phase were calculated by the following formulas (Stephenson, 2016). OD_{740nm} was used as the measure of biomass.

$\mathrm{k} = \frac{\log N - \log N_0}{t}$ (Changes in biomass per unit time)

$\mathrm{g} = \frac{\log 2}{k}$ (Time that takes to double the cell numbers)

t: duration of exponential growth phase (day)

N: Final biomass number of exponential growth phase

N_0: Initial biomass number of exponential growth phase

Chlorophyll a concentration (C) was calculated by the following (Norges Standardiseringsforbund, 1983).

$A_{665K} = A_{665} - A_{750}$

A_{665} and A_{750} are absorbencies at 665 nm and 750 nm wavelengths, respectively.

$$\mathrm{C} = \frac{10^4 \cdot \mathrm{e} \cdot \mathrm{A}_{665\mathrm{K}}}{89 \cdot \mathrm{V} \cdot \mathrm{l}}$$

e: volume of acetone extract (mL)

l: length of cuvette (mm)

V: filtrated volume (L)

89: coefficient of absorbency in 90 % acetone ($L\ g^{-1}\ cm^{-1}$)

2.5. Statistical analysis

Correlation between chla concentrations and OD_{740nm} was tested. Correlation coefficients (r) were calculated (Whitlock, 2015).

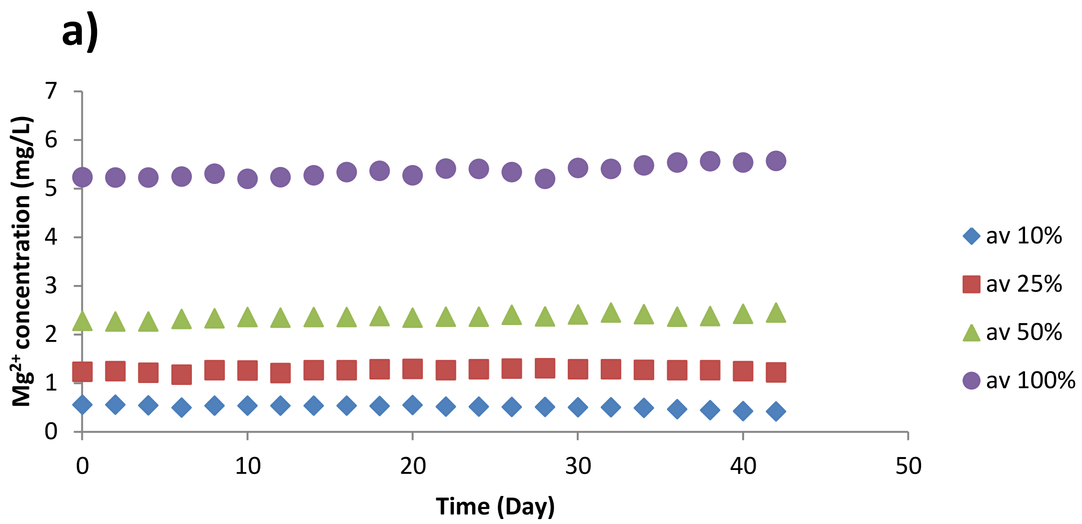
2.6. Source of possible errors

The morphology of the samples was observed except for Day 0 of the experiment 1. Plastic tubes were used to homogenize filters with chlorophyll instead of glass tubes. Chlorophyll was extracted by adding 10 mL 90 % acetone. Chlorophyll concentrations with more than 0.05 A_{750} were accepted in this study. Filtered volume might give influence on analyses (Appendix 1, 2).

3 Results

3.1. Mg^{2+} concentration in media

Mg^{2+} concentrations at low light intensity were stable and those at high light intensity showed decreasing trends during the experiments (Fig 3.1). Mg^{2+} concentrations in 10 % Mg^{2+} media at high light intensity dropped down from 0.5 to 0.1 mg/L in the end of the experiment.



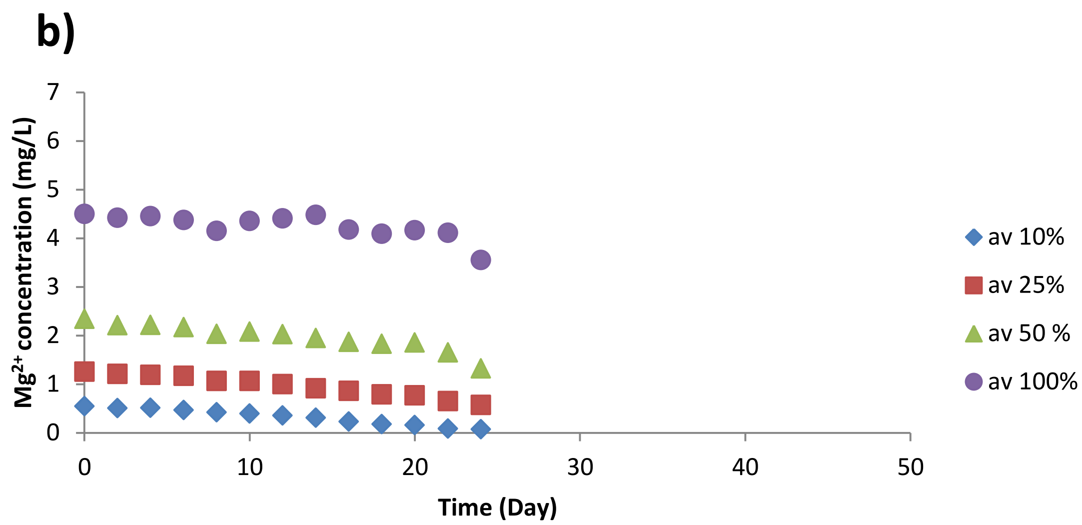


Fig 3.1. Mg^{2+} concentrations (mg/L) in media growing M. aeruginosa at a) low and b) high light intensity. Blue rhombus, red square, green triangle and purple circle show average values (av) of 10, 25, 50 and 100 % Mg^{2+}(mg/L) media, respectively (100 % equals normal O2 media).

3.2. pH in media

pH showed increasing trend after lag phase and reached highest values in stationary phase (Fig 3.2). pH at low light intensity started increasing at Day 10 which is the beginning of the exponential growth phase and flattened out from Day 28 at about pH 9. At high light intensity, pH started increasing at Day 4, which was earlier than at low light intensity. The highest pH was 9.3 at Day 32 and 10.7 at Day 8 at low and high light intensity, respectively. The largest difference in pH among treatments was 0.5 at Day 20 and 1.2 at Day 14 at low and high light intensity, respectively.

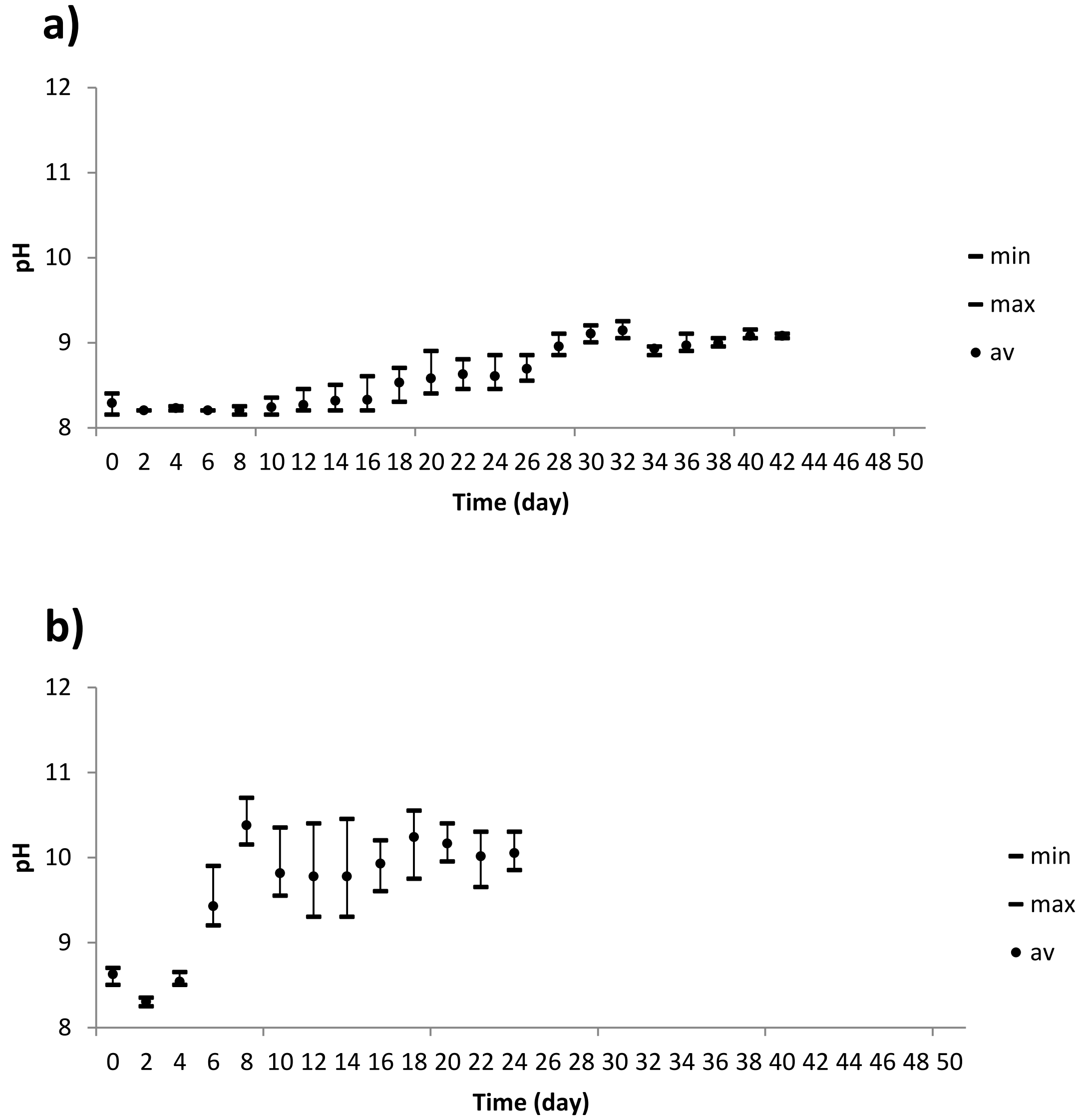


Fig 3.2. pH during the experiments with M. aeruginosa grew at a) low and b) high light intensity. Black horizontal line shows the maximum and minimum values. Black circles show the average pH values.

3.3. Biomass of *M. aeruginosa*

OD_{740nm} showed increasing trends in both the experiments but the periods of lag phase were different in length (Fig.3.3.1). At low light intensity the maximum duration of the lag phase was 12 days and OD_{740nm} increased exponentially. The periods of the exponential growth phase were during Day 8 to Day 22, Day 12 to Day 24, Day 8 to Day 20 and Day 6 to Day 16 in 10, 25, 50 and 100 % Mg^{2+} media, respectively. The exponential growth phase at high light intensity started at Day 2, which was earlier than

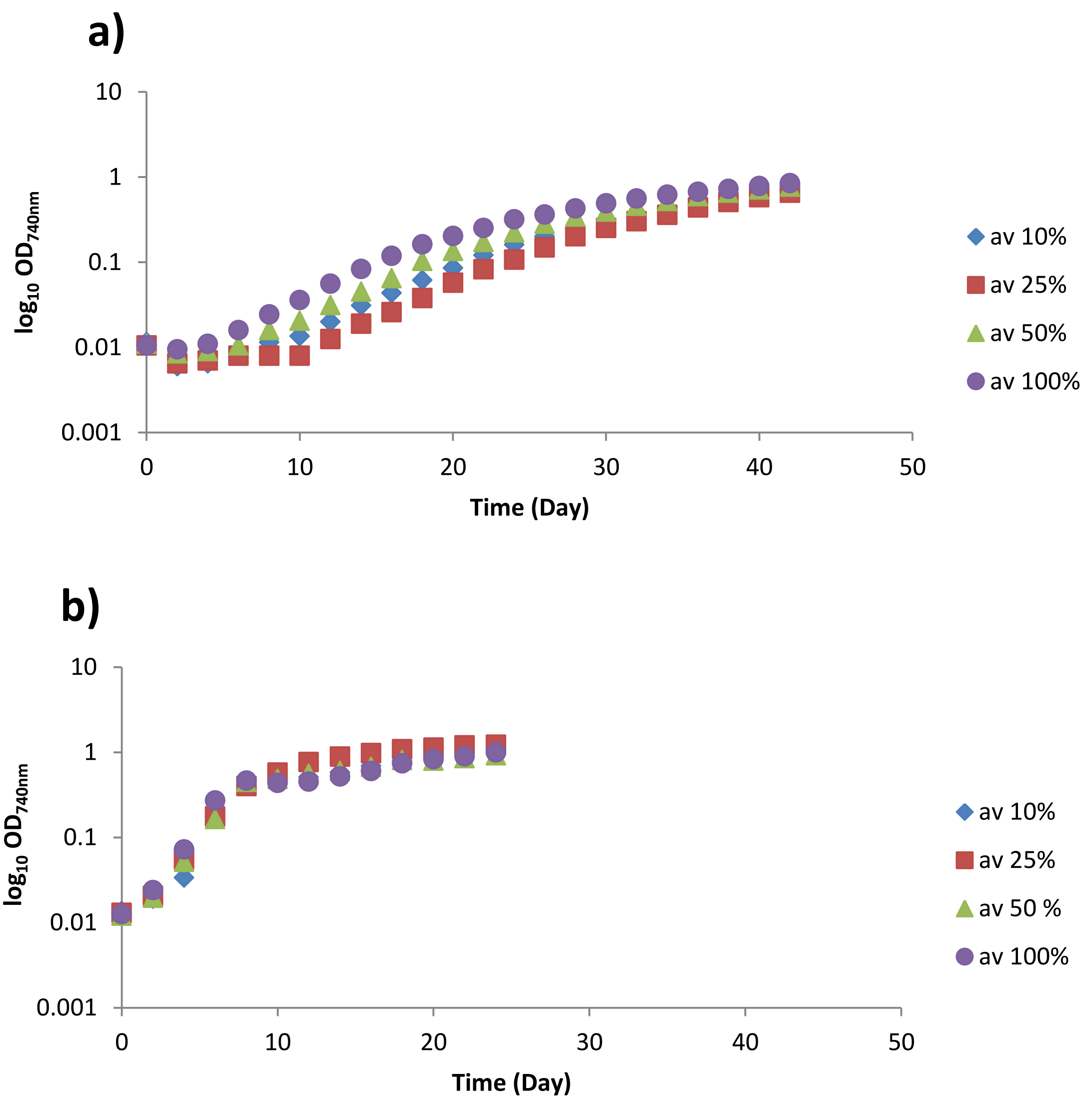


Fig 3.3.1. OD_{740nm} using logarithmic scale illustrating the growth of M. aeruginosa grown in different Mg^{2+} concentrations at a) low and b) high light intensity. Blue rhombus, red square, green triangle and purple circle show average values (av) of 10, 25, 50 and 100 % Mg^{2+}(mg/L) media, respectively (100 % equals normal O2 media).

at low light intensity. The growth of *M. aeruginosa* at high light intensity seemed to reach the stationary phase at Day 10. The maximum value of OD_{740nm} was 0.85 at Day 42 measured in 100 % Mg^{2+} media at low light intensity and 1.2 in 25 % Mg^{2+} media at Day 24 at high light intensity.

M. aeruginosa had higher growth rates and lower generation times during the exponential phases at high light intensity than those at low light intensity (Table. 3.3). The specific growth rate during the log phase was 0.1 in 25, 50, 100 % Mg^{2+} media at low light intensity and 0.3 in high light. The longest generation time was 4.1 days in 10 % Mg^{2+} media at low light intensity and the shortest one was 1.3 days at high light intensity and 50 % Mg^{2+} media.

Table3.1. Growth rates and generation times during the log phase accompanying the growth of M. aeruginosa in 10, 25, 50 and 100 % Mg^{2+} concentration media. Gray and white colors show low and high light intensity, respectively.

Mg^{2+} concentration	10%	25%	50%	100%
Growth rate (day^{-1})	0.1	0.1	0.1	0.1
	0.2	0.2	0.2	0.2
Generation time (day)	4.1	3.9	3.9	3.4
	1.4	1.4	1.3	1.4

Chla concentration is also regarded as one of the biomass parameters. Chla concentrations at both light intensities showed increasing trends and the treatment at higher intensity increased earlier (Fig 3.3.2). At low light intensity, chla concentration increased exponentially and flattened out at 1.2 mg/L from Day 28. On the other hand, chla concentrations at high light intensity started increasing at Day 8, which was earlier than at low light intensity. The maximum value of chla concentration was 1.3 mg /L in 100 % Mg^{2+} media at Day 40 and 3.6 mg/L in 25 % Mg^{2+} media at Day 22, at low and high light intensity, respectively. The Chla concentration in 25 % Mg^{2+} media increased from 1.1 mg/L to 3.6 mg /L during Day 18 to 20 at high light intensity.

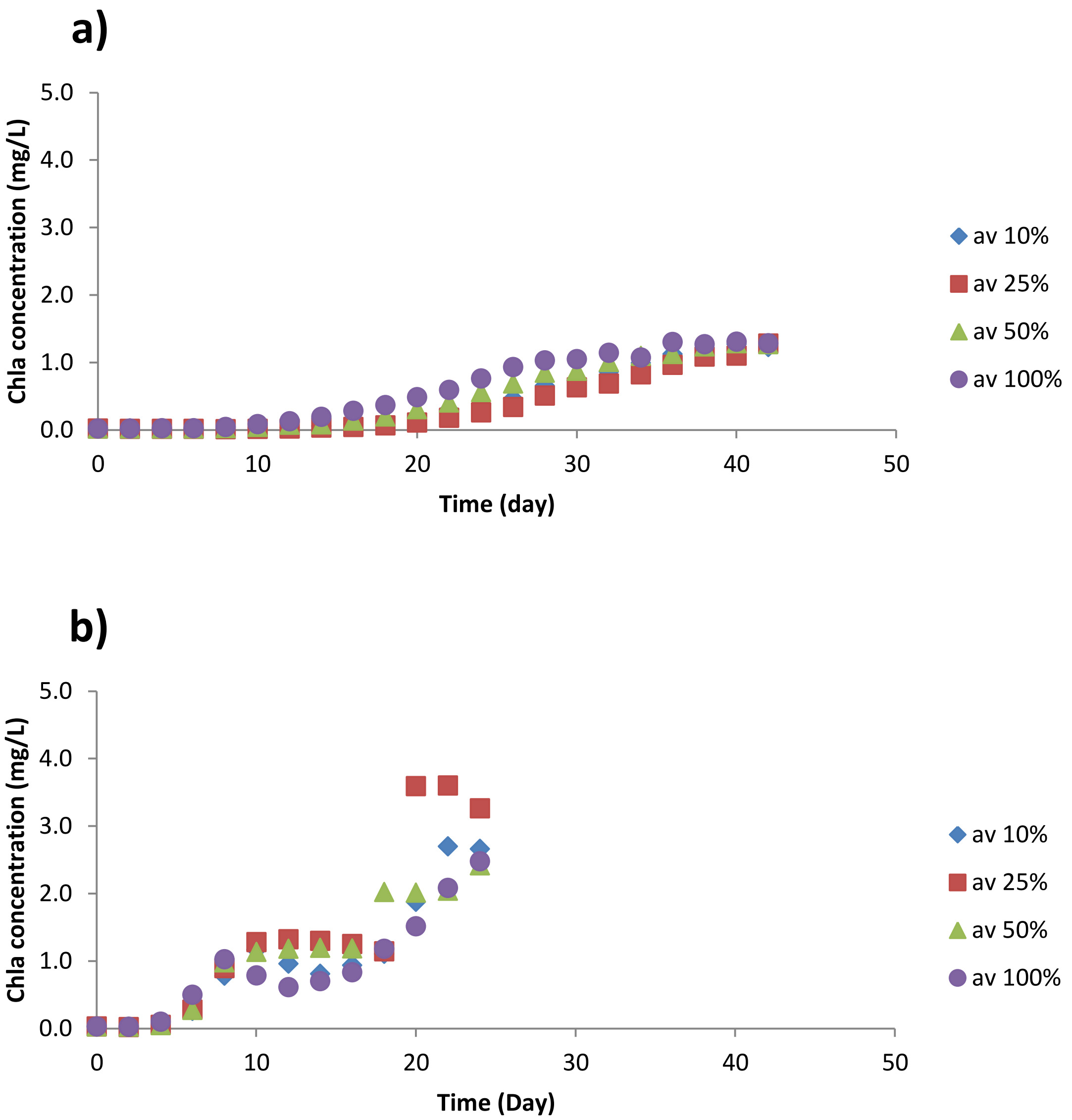


Fig 3.3.2. Changes in chla concentration (mg/L) as a measure of the biomass of M. aeruginosa grown at a) low and b) high light intensity. Blue rhombus, red square, green triangle and purple circle show average values (av) of 10, 25, 50 and 100 % Mg^{2+}(mg/L) media, respectively (100 % equals normal O2 media).

The biomass parameters had strong positive linear correlations at low light intensity (r< 0.974) and high light intensity (r< 0.861) (Fig 3.3.3 and Table 3.3).

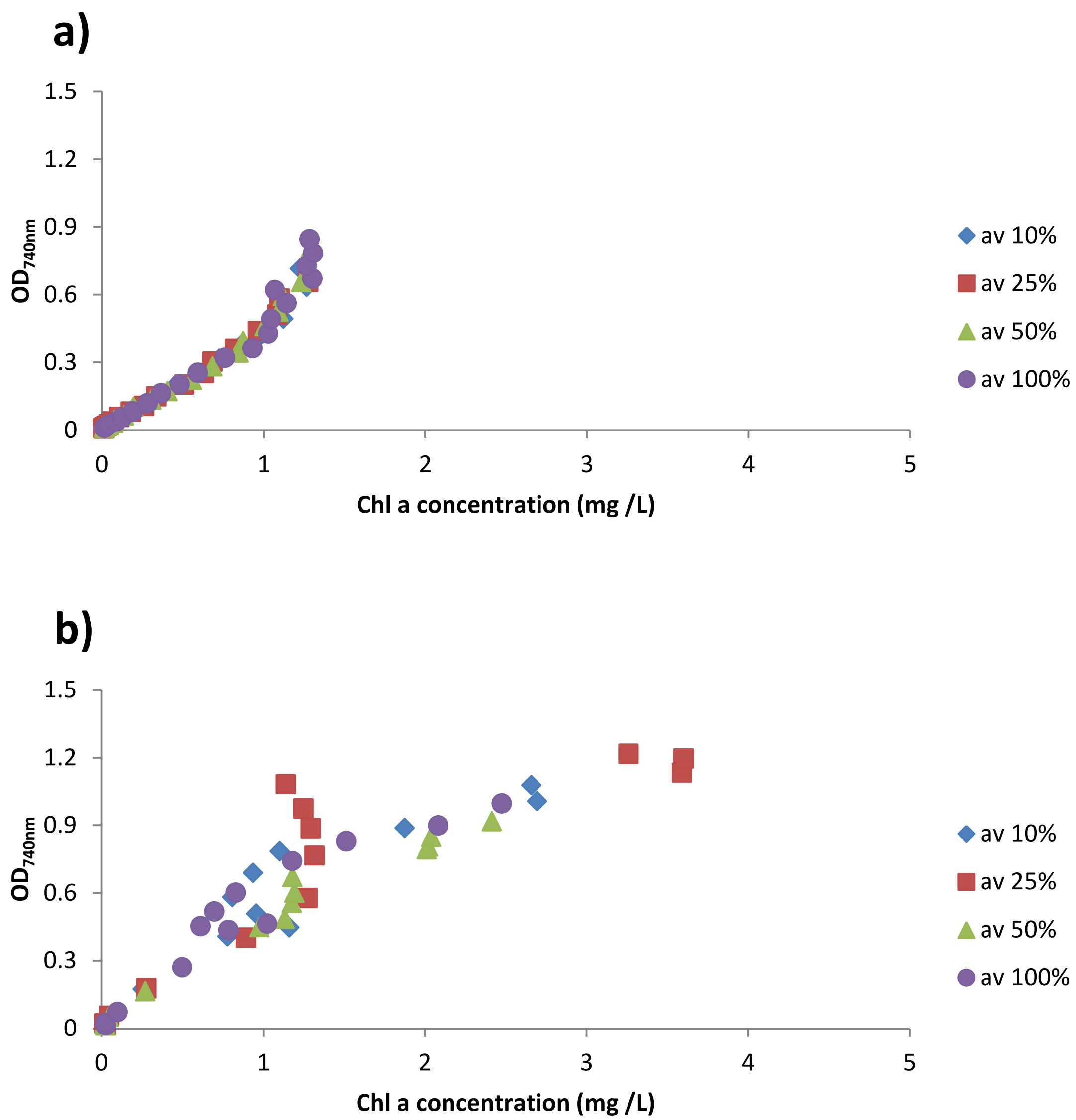


Fig 3.3.3. Correlation between chla concentration (mg/L) and OD_{740nm} of M. aeruginosa at a) low and b) high light intensity. Blue rhombus, red square, green triangle and purple circle show average values (av) of 10, 25, 50 and 100 % Mg^{2+}(mg/L) media, respectively (100 % equals normal O2 media).

Table 3.2. Correlation coefficients between chla concentration (mg/L) and OD_{740nm}. Grey zone shows the results at low light intensity and white shows at high light intensity. The sample numbers are 22 and 13 at low and high light intensity, respectively.

Parameters	Mg^{2+} concentration in media	r (correlation coefficient)	
(Chla, OD_{740nm})	10%	0.987	0.936
	25%	0.994	0.861
	50%	0.985	0.982
	100%	0.974	0.947

3.4. MC of *M. aeruginosa*

MC concentrations increased at both light intensities and MC concentration at low light intensity increased more slowly than at high light intensity (Fig. 3.4.1). While MC concentrations at low light intensity at Day 10 were less than 0.1 mg/L, those at high light intensity were more than 1.3 mg/L at Day 12. The maximum value of MC was 1.4 mg/L in 100 % Mg^{2+} media at Day 42 and 2.8 mg /L in 25 %Mg^{2+} at Day 24 at low and high light intensity, respectively.

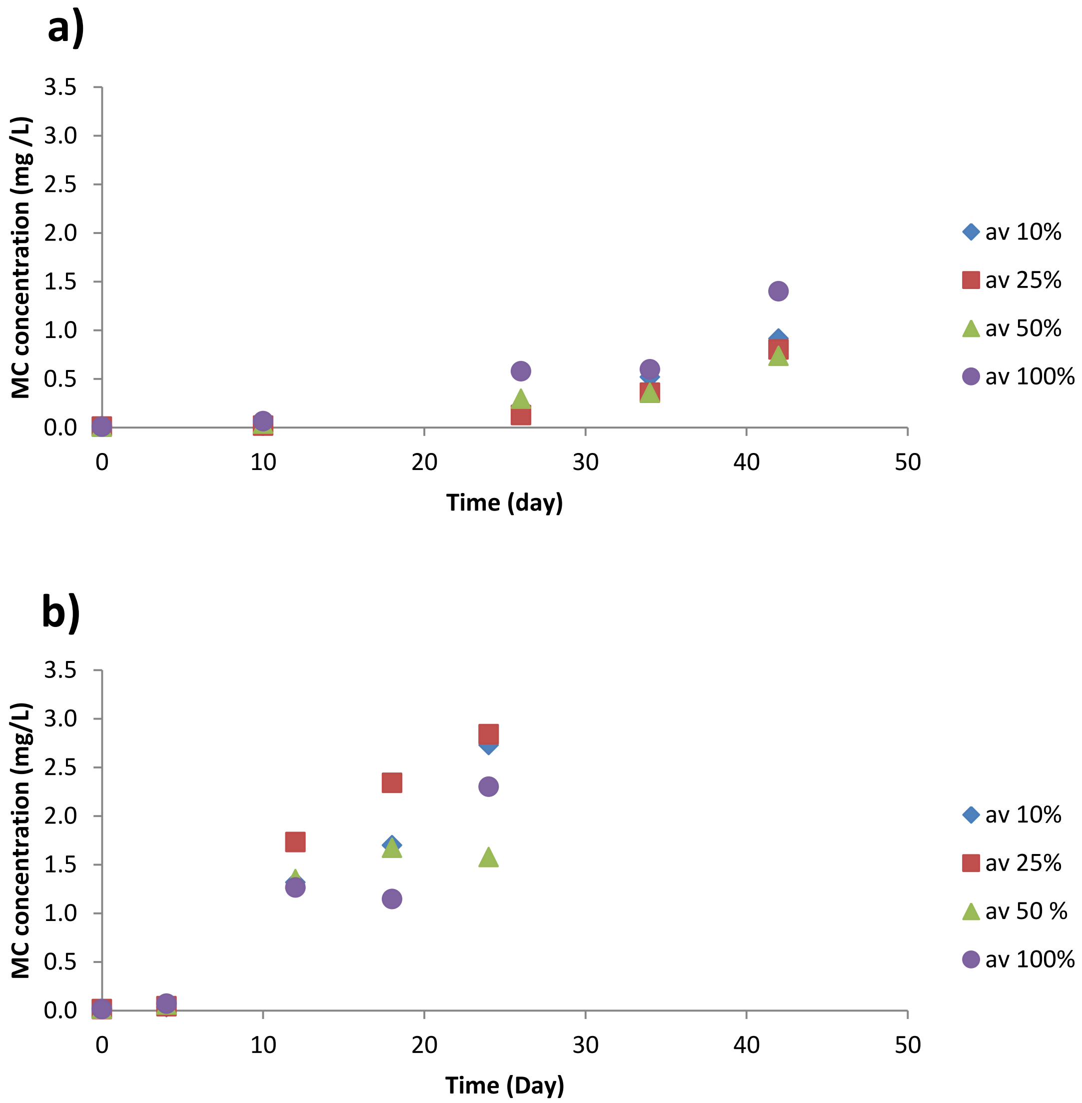


Fig 3.4.1. MC concentrations (mg/L) of M. aeruginosa at a) low and b) high light intensity. Blue rhombus, red square, green triangle and purple circle show average values (av) of 10, 25, 50 and 100 % Mg^{2+}(mg/L) media, respectively (100 % equals normal O2 media).

The ratio between MC and *M. aeruginosa* biomass measured as OD_{740nm} increased at around the beginning of the exponential growth phase at low light intensity and at stationary phase at high light intensity (Fig 3.4.2). The highest value was 2.5mg/L in 25 % Mg^{2+} media at Day 10 and 2.8 mg/L in 100 % Mg^{2+} media at Day 12 at low and high light intensity, respectively.

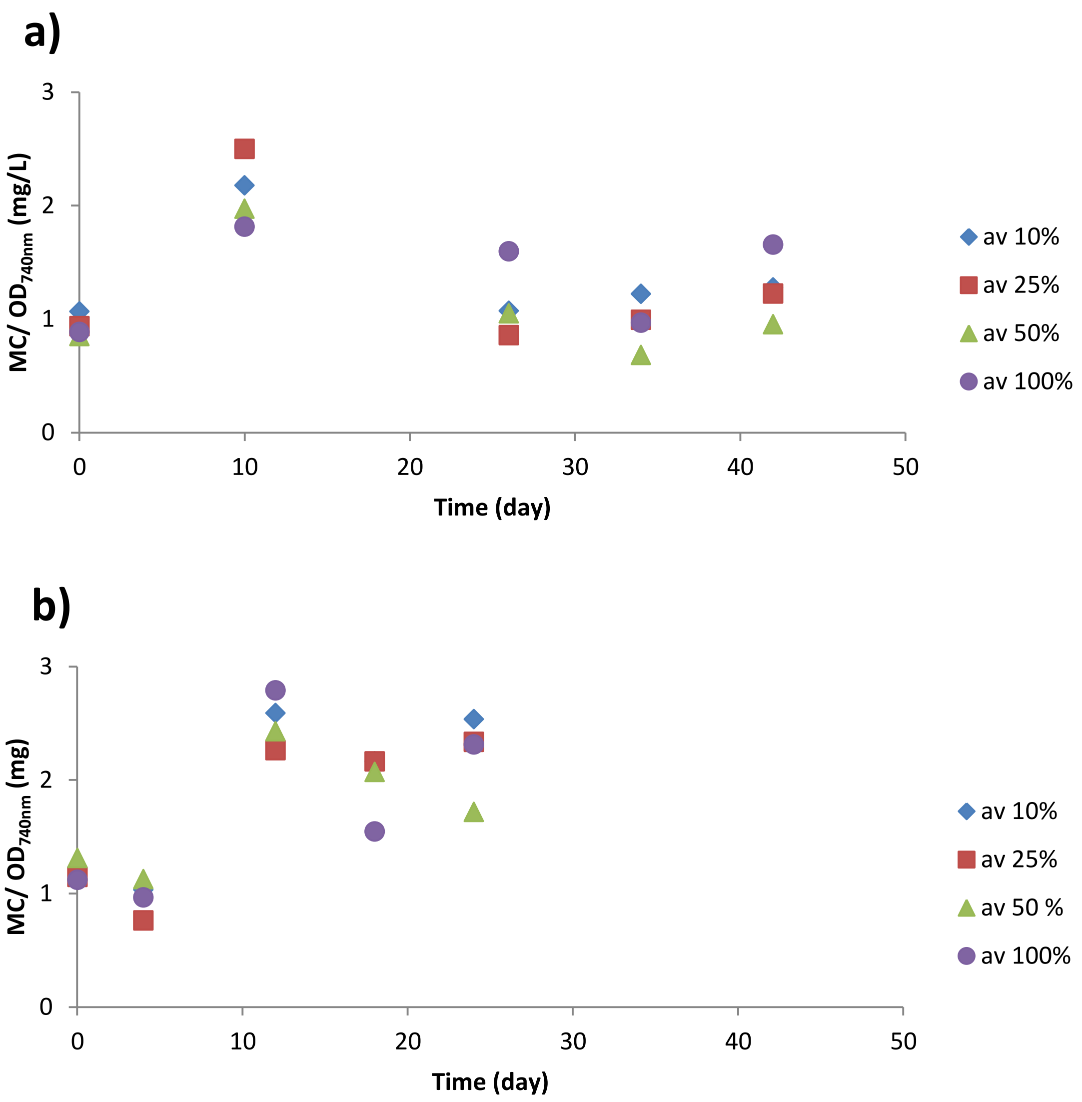
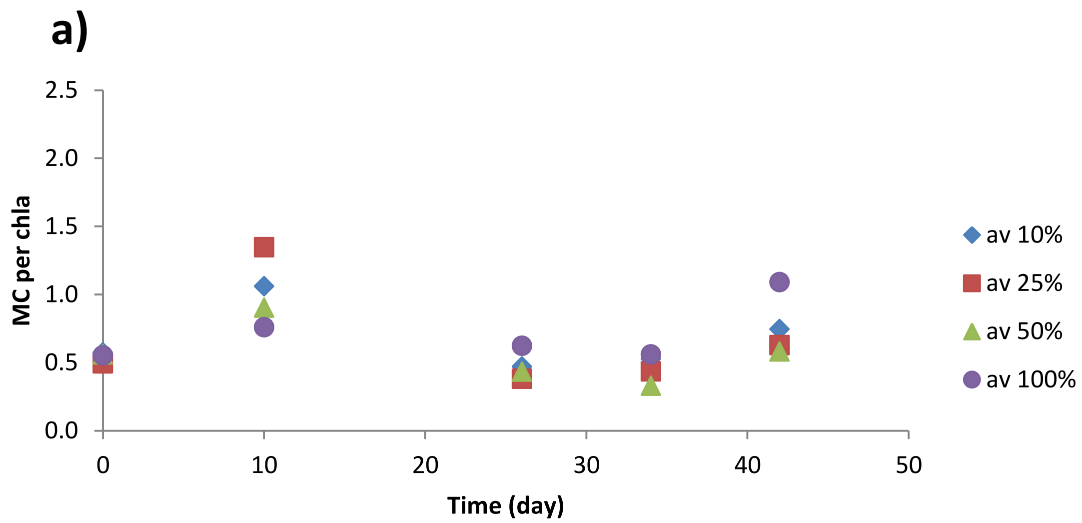


Fig 3.4.2. MC concentration (mg/L) in relation to OD_{740nm} showing the toxin content of M. aeruginosa at low and high light intensity. Blue rhombus, red square, green triangle and purple circle show average values (av) of 10, 25, 50 and 100 % Mg^{2+}(mg/L) media, respectively (100 % equals normal O2 media).

The ratio between MC concentration and *M. aeruginosa* biomass measured as chla concentration showed peaks during the exponential growth phase except for 100 % Mg^{2+} media at low light intensity and the stationary growth phase at high light intensity (Fig. 3.4.3). The maximum values were 1.3 at Day 12 and 2.1 at Day 12 and Day 18 at low and high light intensity, respectively.



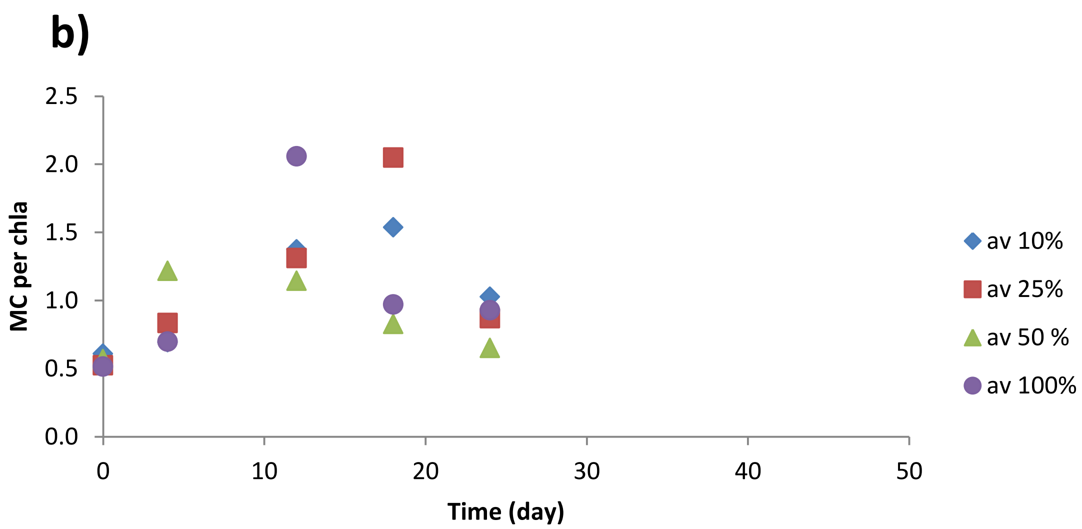


Fig. 3.4.3. MC concentration (mg/L) in relation to chla concentration (mg/L) accompanying the growth of M. aeruginosa at a) low and b) high light intensity. Blue rhombus, red square, green triangle and purple circle show average values (av) of 10, 25, 50 and 100 % Mg^{2+}(mg/L) media, respectively (100 % equals normal O2 media).

3.5. Morphology of *M. aeruginosa*

Most *M. aeruginosa* had spherical cells, existed alone as separate cells and the color was green regardless of light intensity. Some existed as colonies and the sizes of the colonies varied. When *M. aeruginosa* was grown at high light intensity, some of them had yellowish color during the experiment (Appendix 3).

4 Discussion

4.1. Effects of light intensity for Mg^{2+} uptake of *M. aeruginosa*

The result of Mg^{2+} concentrations indicates that higher light intensity may affect the Mg^{2+} uptake by *M. aeruginosa* (Fig 3.1. b) and low amounts of Mg^{2+} might be taken up by *M. aeruginosa* at low light intensity (Fig 3. 2. a) Mg^{2+} contents of *M. aeruginosa* should be measured to clarify the Mg^{2+} uptake by *M. aeruginosa*. More Mg^{2+} may be taken up to maintain photosynthetic pigments during the stationary phase and death phase of *M. aeruginosa* at high light intensity. Mg^{2+} was reported as a protector of photosystem II from water- photooxidation and a mediator to convey electrons from water to $NADP^+$ (McSwain et al., 1976). Higher light intensities attributes the damage of photosynthetic electron transport (Whitelam and Cold, 1983). Through these previous studies, it may be considered that Mg^{2+} is used for keeping light- inducing electron transport.

Another suggestion for the future experiments to investigate effects of Mg^{2+} concentrations is to use less Mg^{2+} concentrations for growth of *M. aeruginosa*. Utkilen (1982) conducted his experiments by increasing Mg^{2+} concetrations from 2µM to 1mM Mg^{2+}. He showed that growth rates of *Anacystis nidulans* (*A. nidulans*) reached the stationary value 0.2 h^{-1} at higher Mg^{2+} concentrations than 5 µM. This study used 20.3, 50.7, 101, 202 µM Mg^{2+} concentrations in the media (0.5, 1.2, 2.5 and 4.9 mg Mg^{2+}/L) and the concentrations might be too high to affect growth of *M. aeruginosa*. Zhao et al. (2011) investigated relationship between growth of *M. aeruginosa* and Mg^{2+} concentrations (0, 2, 7, 10 and 20 mg Mg^{2+}/L) in media. They observed less biomass in 0 mg Mg^{2+} /L media. It could be suggested from the opinions above that Mg^{2+}concentrations less than 5 µM should be used to analyze the effect of Mg^{2+} concentrations on growth of *M. aeruginosa*.

4.2. Effects of light and Mg^{2+} on growth of *M. aeruginosa*

The results of OD_{740nm} suggest that Mg^{2+} concentrations affects the onset of exponential growth phase at low light intensity while the growth curves at high light intensity show no differences in onsets of exponential growth phase (Fig 3.3.1). A possible explanation of different onsets of exponential growth phase is that Mg^{2+} can be a limiting factor of dividing cells during lag phase at low light intensity. It is indicated that Mg^{2+} influences the cell size and cell division as a common mechanism (Utkilen, 1982). The more light intensity *M. aeruginosa* is exposed to, the less Mg^{2+} concentrations seem to influence the cell divisions. The exponential growth phase at high light intensity started at the same day regardless of Mg^{2+} concentrations in media. Higher light intensities between 21 to 145 $\mu Em^{-2}s^{-1}$ facilitate the growth of *M. aeruginosa* UV-006 (Van Der Westhuizen and Eloff, 1985). The growth rate during the exponential phase indicate that at least light intensity affects the growth rate of *M. aeruginosa* but the effects of Mg^{2+} concentrations could be discussed. Light intensity and Mg^{2+} concentrations can be important factors for cell division during the growth of *M. aeruginosa*. It is reported that there is no significant differences between the growth curves of MC producing PCC7806 and MC- deficient mutants (Hesse et al., 2001). Effects of MC on the growth of *M. aeruginosa* can therefore be ignored.

The chla concentrations indicate that chla concentrations increase at higher light intensity in this study (Fig 3.3.2). Kaebernick et al (2000) observed decreasing trend of chla concentrations at light intensities from 16 to 68 $\mu Em^{-2}s^{-1}$. In addition, decrease in chla contents of *M. aeruginosa* between 4 and 110 $\mu Em^{-2}s^{-1}$ (Hesse et al., 2001) and between 20 and 565 $\mu Em^{-2}s^{-1}$ (Raps et al., 1983) have been reported. A possible reason could be the extraction limit of chla in 90 % acetone. At low light intensity, chla concentrations reached a threshold at around 1.2 mg/L. Also, chla concentrations in 50 and 100 % Mg^{2+} media at high light intensity reached stable value at around 1.2mg/L. It is speculated that chla of these media might have been extracted less effectively. It is suggested that the volume of acetone should be changed, depending on the amount of chla. Another suggestion is to filter sample volumes that do not clog the filter. Myers et al. (2013) suggested that OD can be better biomass parameter compared to pigment concentration.

The results of linear correlation between OD_{740nm} and chla concentrations indicate strong correlations (Fig 3.3.3, Table 3.2). Only the chla concentration in 25 % Mg^{2+}

concentration showed a correlation factor below 0.9 (Table 3.2). A possible reason is that the chla concentrations had stable values during Day 10 to Day 18 (Fig 3.3.2 b). Sun et al. (2012) reported strong correlations between chla concentrations and biomass of *M. aeruginosa* FACHB- 905 grown under laboratory conditions. Phytoplankton biomass and chla concentrations showed significantly positive correlations in a field study by Vörös and Padisák (1991).

Previous studies indicated that high pH may decrease the growth rate of *M. aeruginosa*. *M. aeruginosa* had the lowest growth rate at pH 10.5 compared to the growth rates at pH 9.0, 9.5 and 10.0. They had the highest growth rate at pH 9.0 (Van Der Westhuizen and Eloff, 1983). pH increased up to pH 10.7 at high light intensity because of the continuous CO_2 addition by the air pump (Fig 3.2). Sigee (2005) mentioned that *M. aeruginosa* can form blooms at pH 11 in fresh water. Mg^{2+} concentrations in media may affect pH because Mg^{2+} facilitates kinetics of RuBisCO which is an enzyme of Calvin cycle that associates with carbon fixation (Lodish H et al., 2000). CO_2 is converted to alkaline bicarbonate, during the carbon metabolism of Calvin cycle (Madigan et al., 2012).

4.4. Effects of light and Mg^{2+} on MC contents

The results of this study for MC indicate that MC concentrations increased concomitant with increase of light intensities (Fig 3.4.1). The higher light intensity *M. aeruginosa* is exposed to, the more MCs are produced (Wiedner et al., 2003). Results of OD and chla in this study may be a support the findings by Oh et al. (2001) that MC concentrations significantly correlate to chla concentrations and phytoplankton number.

MC contents of *M. aeruginosa* (MC concentrations to OD and chla) in this study increased during the exponential growth phase or stationary phase (Fig 3.4.2 and 3.4.3). Although this study reports only 5 days for each experiment, it supports the findings by Lee et al. (2000) that maximum MC contents of *M.aeruginosa* occurs during the exponential growth phase and decrease during the stationary phase. El Semary (2010) also showed the increase in MC during the stationary phase of *M. aeruginosa*.

The observation of this study indicates that maximum MC contents are found at high light intensity (Fig 3.4.2 and 3.4.3). Wiedner et al (2003) observed that maximum MC contents was measured at a light intenisty of 126 $\mu Em^{-2} s^{-1}$ in continuous culture systems compared to MC contents at light intensity of 29 $\mu Em^{-2} s^{-1}$. Kaebernick et al (2000) also showed maximum MC content (3.43 ± 1.04 10^{-5} pmol/cell) at 68 $\mu E\ m^{-2}s^{-1}$ in batch culture of *M. aeruginosa*.

4.5. Effects of light and Mg^{2+} for morphology of *M. aeruginosa* cells

The results of morphology indicated that light intensity might cause chlorosis at high light intensity (Appendix 4). Chlorosis may have happened at a light intensity higher than 37 $\mu Em^{-2}s^{-1}$ that was found to damage phycobilisomes and photosystems by Phelan and Downing (2011). Also proportion of photosynthetic pigments caused the color variations of each cell because *M. aeruginosa* have chlorophyll a, bacteriochlorophylls and carotenoids (Sigee, 2005). It is necessary to measure other photosynthetic pigments to check the proportion. Effects of Mg^{2+} on morphology of *M. aeruginosa* cells appear to be unclear. Some studies, including this study, implied Mg^{2+} influence on cell division and growth of various organisms such as bacilli and cyanobacterium *A. nidulans* (Rubin, 2005, Utkilen, 1982, Webb, 1949). It is necessary to observe cellular tissues by more precise microscope to check morphological changes.

5 Conclusion

The results showed some effects of Mg^{2+} and light intensity on the growth and MC production of *M. aeruginosa*. Mg^{2+} affected the cell division in the lag phase of *M. aeruginosa* and the time of lag phase. Higher light intensity facilitates the growth of *M. aeruginosa*. Chla concentrations and OD_{740nm} showed strong correlations at both high and low light intensities. Higher light intensity also increases MC concentrations. However, effects of Mg^{2+} concentrations on MC concentrations were not figured out. Cells of *M. aeruginosa* can have risks of chlorosis at high light intensity.

Further studies are necessities to figure out the Mg^{2+} cellular contents of *M. aeruginosa* to clarify the cellular usage of Mg^{2+}. It may be interesting to observe cellular changes by using microscope with larger magnification. Using media less Mg^{2+} concentration than 5 µM in media may provide more insight in the growth and MC production of *M. aeruginosa*.

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Appendix

Contents

Appendix 1

Appendix 1: Experimental data at low light intensity

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
05.09.18	0	①10%	8.3	0.012	0.5	42.1	0.052	0.011	0.041	0.022	0.013
		②10%	8.3	0.011	0.6	45	0.048	0.007	0.041	0.021	0.011
		①25%	8.3	0.011	1.2	42.6	0.053	0.013	0.039	0.020	0.011
		②25%	8.3	0.01	1.2	44.8	0.05	0.011	0.039	0.019	0.008
		①50%	8.2	0.011	2.3	42.8	0.045	0.014	0.031	0.016	0.009
		②50%	8.4	0.011	2.2	42.9	0.043	0.011	0.032	0.017	0.009
		①100%	8.1	0.011	5.2	42.6	0.047	0.007	0.04	0.021	0.010
		②100%	8.2	0.01	5.2	41.8	0.028	0.004	0.024	0.013	0.009
07.09.18	2	①10%	8.2	0.006	0.6	40.7	0.041	0.014	0.027	0.015	
		②10%	8.2	0.006	0.5	41.3	0.051	0.013	0.038	0.021	
		①25%	8.2	0.006	1.3	40.9	0.039	0.01	0.03	0.016	
		②25%	8.2	0.007	1.2	43.9	0.041	0.009	0.032	0.016	
		①50%	8.2	0.009	2.3	40	0.039	0.007	0.033	0.018	
		②50%	8.2	0.008	2.2	41.2	0.036	0.007	0.028	0.016	
		①100%	8.2	0.01	5.2	43.5	0.035	0.007	0.028	0.014	
		②100%	8.2	0.009	5.2	41.5	0.058	0.016	0.042	0.022	

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
09.09.18	4	①10%	8.2	0.006	0.5	43.1	0.034	0.006	0.028	0.015	
		②10%	8.2	0.007	0.5	41	0.043	0.016	0.027	0.015	
		①25%	8.3	0.007	1.2	41.3	0.041	0.009	0.027	0.017	
		②25%	8.2	0.007	1.2	41.2	0.036	0.011	0.026	0.014	
		①50%	8.2	0.01	2.3	41	0.042	0.009	0.032	0.018	
		②50%	8.2	0.008	2.2	41	0.049	0.013	0.036	0.020	
		①100%	8.3	0.012	5.2	41.2	0.062	0.011	0.051	0.028	
		②100%	8.2	0.01	5.3	41.2	0.035	0.006	0.029	0.016	
11.09.18	6	①10%	8.2	0.009	0.5	41	0.032	0.007	0.025	0.014	
		②10%	8.2	0.008	0.5	43.7	0.037	0.008	0.029	0.015	
		①25%	8.2	0.009	1.1	41.1	0.03	0.007	0.023	0.013	
		②25%	8.2	0.007	1.2	43.8	0.03	0.007	0.022	0.011	
		①50%	8.2	0.012	2.3	43.5	0.05	0.006	0.044	0.022	
		②50%	8.2	0.009	2.3	41.2	0.037	0.008	0.028	0.016	
		①100%	8.2	0.018	5.2	41.1	0.064	0.012	0.053	0.029	
		②100%	8.2	0.014	5.2	41.2	0.055	0.014	0.041	0.022	
13.09.18	8	①10%	8.2	0.013	0.5	43.1	0.044	0.008	0.036	0.019	
		②10%	8.2	0.01	0.5	41.3	0.044	0.016	0.029	0.016	
		①25%	8.2	0.009	1.3	41.3	0.035	0.012	0.023	0.013	
		②25%	8.1	0.007	1.3	41	0.026	0.006	0.023	0.011	
		①50%	8.2	0.02	2.3	41.2	0.063	0.008	0.055	0.030	
		②50%	8.2	0.012	2.3	41.2	0.038	0.004	0.034	0.019	
		①100%	8.3	0.03	5.3	41.2	0.089	0.004	0.085	0.047	
		②100%	8.2	0.019	5.3	41.2	0.079	0.005	0.074	0.040	

Appendix 1

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
15.09.18	10	①10%	8.2	0.016	0.5	43.4	0.09	0.019	0.071	0.037	0.031
		②10%	8.2	0.011	0.5	43.4	0.044	0.009	0.035	0.018	0.028
		①25%	8.2	0.011	1.2	43.2	0.041	0.005	0.036	0.019	0.023
		②25%	8.1	0.005	1.3	41.2	0.024	0.004	0.02	0.011	0.017
		①50%	8.3	0.027	2.4	41.5	0.118	0.008	0.11	0.059	0.051
		②50%	8.2	0.014	2.3	41.3	0.064	0.007	0.056	0.031	0.030
		①100%	8.4	0.046	5.1	41.2	0.208	0.007	0.201	0.110	0.080
		②100%	8.3	0.026	5.3	41.1	0.12	0.006	0.113	0.062	0.050
17.09.18	12	①10%	8.2	0.024	0.5	41.1	0.081	0.007	0.074	0.040	
		②10%	8.2	0.016	0.5	41	0.06	0.007	0.053	0.029	
		①25%	8.2	0.017	1.2	43	0.054	0.003	0.051	0.026	
		②25%	8.2	0.008	1.2	41.3	0.031	0.005	0.025	0.014	
		①50%	8.4	0.044	2.4	41.3	0.196	0.007	0.189	0.104	
		②50%	8.2	0.019	2.3	43.4	0.089	0.007	0.081	0.042	
		①100%	8.5	0.072	5.2	41.1	0.291	0.006	0.286	0.157	
		②100%	8.4	0.04	5.3	43.5	0.202	0.007	0.194	0.099	
19.09.18	14	①10%	8.3	0.038	0.5	40.9	0.143	0.011	0.132	0.072	
		②10%	8.2	0.024	0.5	41.5	0.077	0.008	0.069	0.037	
		①25%	8.2	0.024	1.3	41	0.083	0.006	0.077	0.042	
		②25%	8.2	0.014	1.2	43.5	0.055	0.016	0.039	0.020	
		①50%	8.4	0.064	2.4	41	0.215	0.009	0.206	0.113	
		②50%	8.2	0.026	2.3	43.5	0.095	0.009	0.086	0.044	
		①100%	8.6	0.109	5.2	43	0.492	0.009	0.483	0.252	
		②100%	8.4	0.058	5.4	41	0.258	0.013	0.244	0.134	

Appendix 1

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
21.09.18	16	①10%	8.3	0.056	0.5	43	0.209	0.01	0.199	0.104	
		②10%	8.2	0.031	0.5	41.1	0.129	0.011	0.118	0.065	
		①25%	8.3	0.035	1.3	41.3	0.118	0.01	0.108	0.059	
		②25%	8.2	0.017	1.3	43.6	0.071	0.013	0.058	0.029	
		①50%	8.5	0.094	2.4	43.8	0.385	0.008	0.377	0.193	
		②50%	8.2	0.036	2.3	41.5	0.167	0.012	0.156	0.083	
		①100%	8.7	0.154	5.3	41.1	0.681	0.009	0.672	0.368	
		②100%	8.5	0.085	5.4	41.1	0.378	0.016	0.362	0.198	
23.09.18	18	①10%	8.8	0.078	0.5	40.8	0.304	0.01	0.294	0.161	
		②10%	8.3	0.045	0.5	43.4	0.181	0.012	0.169	0.089	
		①25%	8.4	0.052	1.3	41	0.179	0.009	0.171	0.093	
		②25%	8.2	0.024	1.3	41	0.091	0.015	0.076	0.042	
		①50%	8.7	0.148	2.4	41	0.491	0.008	0.483	0.265	
		②50%	8.4	0.059	2.4	41	0.242	0.009	0.223	0.128	
		①100%	8.8	0.211	5.2	41.8	0.893	0.015	0.878	0.470	
		②100%	8.6	0.115	5.5	41	0.485	0.009	0.476	0.261	
25.09.18	20	①10%	8.6	0.113	0.5	41	0.432	0.01	0.422	0.232	
		②10%	8.4	0.059	0.5	41	0.262	0.018	0.244	0.134	
		①25%	8.5	0.082	1.3	43.5	0.323	0.018	0.305	0.156	
		②25%	8.3	0.033	1.3	41	0.118	0.006	0.112	0.061	
		①50%	8.8	0.193	2.4	41	0.848	0.012	0.835	0.458	
		②50%	8.5	0.078	2.3	43.4	0.308	0.007	0.3	0.157	
		①100%	9.1	0.257	5.2	41	1.166	0.014	1.152	0.631	
		②100%	8.7	0.15	5.4	41	0.619	0.012	0.607	0.333	

Appendix 1

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
27.09.18	22	①10%	8.7	0.159	0.5	42.8	0.75	0.011	0.738	0.386	
		②10%	8.5	0.083		41.2	0.417	0.019	0.398	0.218	
		①25%	8.6	0.119	1.3	43.6	0.52	0.01	0.511	0.261	
		②25%	8.3	0.046	1.2	41.2	0.181	0.008	0.173	0.095	
		①50%	8.8	0.242	2.4	41	1.03	0.012	1.018	0.558	
		②50%	8.5	0.102	2.4	43.5	0.507	0.012	0.495	0.253	
		①100%	8.9	0.316	5.3	41.5	1.331	0.009	1.323	0.708	
		②100%	8.7	0.191	5.5	41	0.89	0.014	0.876	0.480	
27.09.18	24	①10%	8.7	0.209	0.5	43.1	1.02	0.02	1.001	0.523	
		②10%	8.5	0.113	0.5	41	0.468	0.007	0.461	0.253	
		①25%	8.6	0.154	1.3	41	0.693	0.02	0.673	0.369	
		②25%	8.3	0.061	1.3	41	0.29	0.011	0.279	0.153	
		①50%	8.8	0.308	2.4	41	1.481	0.018	1.464	0.802	
		②50%	8.5	0.138	2.4	41	0.59	0.018	0.572	0.314	
		①100%	8.9	0.394	5.4	43.5	1.661	0.016	1.645	0.840	
		②100%	8.8	0.245	5.4	41	1.265	0.021	1.244	0.682	
29.09.18	26	①10%	8.7	0.259	0.5	41	1.161	0.015	1.146	0.628	0.312
		②10%	8.4	0.145	0.5	41	0.549	0.013	0.536	0.294	0.121
		①25%	8.8	0.21	1.3	41.5	0.927	0.012	0.915	0.489	0.166
		②25%	8.4	0.089	1.3	41.2	0.342	0.008	0.335	0.183	0.090
		①50%	8.9	0.38	2.4	43.3	1.79	0.024	1.766	0.923	0.521
		②50%	8.6	0.184	2.4	41	0.827	0.012	0.814	0.446	0.070
		①100%	8.9	0.437	5.3	41	2.049	0.028	2.02	1.107	0.786
		②100%	8.8	0.289	5.4	41	1.395	0.019	1.375	0.754	0.372

Appendix 1

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
01.10.18	28	①10%	9	0.32	0.5	43.1	1.668	0.028	1.641	0.857	
		②10%	8.9	0.187	0.5	41	0.833	0.012	0.822	0.450	
		①25%	9	0.281	1.3	41.3	1.385	0.022	1.363	0.747	
		②25%	8.7	0.123	1.3	43.6	0.542	0.011	0.531	0.271	
		①50%	9.1	0.455	2.3	41	2.075	0.04	2.035	1.115	
		②50%	8.9	0.231	2.4	41	1.073	0.019	1.054	0.578	
		①100%	9.2	0.516	5.1	41	2.278	0.039	2.239	1.227	
		②100%	9	0.341	5.3	43	1.624	0.03	1.594	0.833	
03.10.18	30	①10%	9.2	0.396	0.5	41	1.634	0.018	1.616	0.885	
		②10%	9	0.236	0.5	41	1.049	0.011	1.038	0.569	
		①25%	9.1	0.347	1.2	43.5	1.747	0.018	1.729	0.883	
		②25%	8.9	0.159	1.3	41	0.762	0.015	0.746	0.381	
		①50%	9.2	0.511	2.4	41	2.063	0.029	2.033	1.114	
		②50%	9	0.278	2.4	41.1	1.168	0.011	1.157	0.634	
		①100%	9.3	0.582	5.2	41.1	2.329	0.026	2.302	1.262	
		②100%	9.1	0.401	5.6	41	1.535	0.02	1.516	0.831	
05.10.18	32	①10%	9.2	0.464	0.5	42.5	1.992	0.024	1.967	1.028	
		②10%	9.1	0.291	0.5	42.1	1.313	0.013	1.3	0.696	
		①25%	9.2	0.408	1.2	41.5	1.748	0.02	1.729	0.925	
		②25%	8.9	0.199	1.3	41	0.823	0.013	0.81	0.444	
		①50%	9.3	0.585	2.4	41	2.384	0.025	2.359	1.293	
		②50%	9.1	0.336	2.5	43.6	1.424	0.016	1.408	0.719	
		①100%	9.3	0.661	5.3	43.5	2.433	0.03	2.403	1.227	
		②100%	9.2	0.463	5.5	41.1	1.96	0.024	1.936	1.061	

Appendix 1

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
07.10.18	34	①10%	9	0.519	0.4	40.8	2.171	0.03	2.141	1.174	0.760
		②10%	8.9	0.333	0.5	41	1.492	0.022	1.471	0.806	0.281
		①25%	9	0.475	1.2	41	2.12	0.023	2.097	1.149	0.507
		②25%	8.7	0.248	1.3	43.1	0.968	0.013	0.955	0.499	0.211
		①50%	9	0.657	2.4	43.1	2.454	0.039	2.415	1.262	0.484
		②50%	8.9	0.393	2.5	41	1.712	0.021	1.69	0.926	0.234
		①100%	9	0.724	5.3	41	2.437	0.03	2.407	1.319	0.724
		②100%	8.9	0.517	5.6	40.8	1.518	0.017	1.501	0.823	0.475
09.10.18	36	①10%	9	0.596	0.4	41	2.3	0.019	2.281	1.250	
		②10%	8.9	0.391	0.5	41.1	1.842	0.021	1.821	0.998	
		①25%	9	0.564	1.2	43.6	2.368	0.021	2.347	1.199	
		②25%	8.8	0.312	1.3	41.1	1.348	0.016	1.332	0.730	
		①50%	9	0.732	2.2	41.5	2.43	0.022	2.408	1.289	
		②50%	8.9	0.456	2.5	41.4	1.758	0.017	1.741	0.954	
		①100%	9.2	0.77	5.3	41.2	2.448	0.024	2.424	1.328	
		②100%	9	0.572	5.7	41.2	2.368	0.034	2.334	1.279	
11.10.18	38	①10%	9	0.664	0.4	43.1	2.431	0.038	2.393	1.250	
		②10%	9	0.449	0.5	43	1.87	0.018	1.852	0.968	
		①25%	9	0.647	1.2	43.6	2.43	0.034	2.396	1.224	
		②25%	8.9	0.376	1.3	40.8	1.755	0.028	1.727	0.947	
		①50%	9	0.795	2.3	41	2.451	0.031	2.421	1.327	
		②50%	8.9	0.517	2.5	41	2.098	0.017	2.082	1.141	
		①100%	9.1	0.825	5.3	43	2.461	0.027	2.435	1.272	
		②100%	9	0.629	5.8	41	2.327	0.02	2.307	1.264	

Appendix 1

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
13.10.18	40	①10%	9.2	0.744	0.4	41	2.467	0.025	2.443	1.339	
		②10%	9.1	0.526	0.5	41.1	2.213	0.033	2.181	1.195	
		①25%	9.1	0.721	1.2	43	2.427	0.032	2.395	1.252	
		②25%	9	0.444	1.3	43.5	1.871	0.016	1.855	0.948	
		①50%	9.1	0.849	2.3	41.1	2.436	0.028	2.409	1.320	
		②50%	9	0.578	2.5	41	2.298	0.026	2.272	1.245	
		①100%	9.1	0.874	5.3	41.1	2.456	0.03	2.426	1.330	
		②100%	9	0.695	5.7	41	2.366	0.022	2.344	1.285	
15.10.18	42	①10%	9.1	0.832	0.4	41.5	2.43	0.032	2.398	1.283	1.148
		②10%	9.1	0.599	0.5	44	2.316	0.028	2.288	1.169	0.681
		①25%	9.1	0.798	1.1	41.8	2.451	0.03	2.42	1.295	1.161
		②25%	9	0.513	1.3	41.8	2.049	0.025	2.356	1.261	0.442
		①50%	9.1	0.905	2.3	41.5	2.43	0.038	2.392	1.280	0.824
		②50%	9	0.639	2.6	42	2.382	0.025	2.356	1.261	0.648
		①100%	9.1	0.934	5.4	42.2	2.457	0.032	2.425	1.297	1.732
		②100%	9.1	0.758	5.8	42	2.411	0.033	2.378	1.272	1.069

Appendix 2

Appendix 2: Experimental data at high light intensity

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
26.10.18	0	①10%	8.6	0.014	0.6	41.6	0.075	0.02	0.055	0.029	0.017
		②10%	8.7	0.013	0.5	41.9	0.071	0.024	0.047	0.025	0.017
		①25%	8.7	0.013	1.3	44	0.073	0.012	0.061	0.031	0.015
		②25%	8.6	0.013	1.2	41.8	0.071	0.023	0.048	0.026	0.015
		①50%	8.5	0.012	2.4	41.8	0.063	0.014	0.05	0.027	0.015
		②50%	8.5	0.012	2.3	41.9	0.077	0.023	0.054	0.029	0.017
		①100%	8.7	0.012	4.5	44	0.06	0.012	0.048	0.024	0.017
		②100%	8.7	0.013	4.5	41.8	0.073	0.017	0.056	0.030	0.011
28.10.18	2	①10%	8.3	0.021	0.5	40.9	0.056	0.012	0.045	0.024	
		②10%	8.3	0.017	0.5	41	0.047	0.009	0.039	0.021	
		①25%	8.3	0.022	1.2	41	0.042	0.008	0.034	0.019	
		②25%	8.4	0.019	1.2	43.2	0.048	0.009	0.04	0.021	
		①50%	8.3	0.022	2.2	41	0.045	0.007	0.038	0.021	
		②50%	8.2	0.017	2.2	41	0.055	0.012	0.043	0.023	
		①100%	8.3	0.025	4.4	43.5	0.059	0.009	0.05	0.026	
		②100%	8.3	0.023	4.4	41	0.05	0.005	0.044	0.024	

Appendix 2

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
30.10.18	4	①10%	8.6	0.034	0.5	43.6	0.157	0.018	0.139	0.071	0.049
		②10%	8.4	0.033	0.5	40.4	0.069	0.017	0.052	0.029	0.020
		①25%	8.6	0.068	1.2	41.4	0.127	0.01	0.117	0.064	0.054
		②25%	8.4	0.041	1.2	41.5	0.073	0.007	0.066	0.035	0.029
		①50%	8.6	0.065	2.2	43.9	0.129	0.009	0.12	0.061	0.070
		②50%	8.4	0.038	2.2	41.5	0.073	0.009	0.063	0.034	0.046
		①100%	8.8	0.081	4.4	43.3	0.238	0.013	0.225	0.117	0.084
		②100%	8.5	0.063	4.5	41.7	0.164	0.012	0.152	0.081	0.055
01.11.18	6	①10%	9.7	0.243	0.5	41.7	0.75	0.014	0.736	0.394	
		②10%	8.8	0.105	0.5	44.2	0.242	0.009	0.233	0.119	
		①25%	9.6	0.227	1.1	44	0.819	0.01	0.81	0.413	
		②25%	8.8	0.125	1.2	41.8	0.275	0.014	0.261	0.140	
		①50%	9.8	0.204	2.2	41.9	0.706	0.011	0.696	0.372	
		②50%	8.9	0.123	2.2	41.9	0.33	0.013	0.317	0.170	
		①100%	10.3	0.316	4.3	44.2	1.237	0.017	1.22	0.623	
		②100%	9.5	0.224	4.4	42	0.72	0.016	0.704	0.377	
03.11.18	8	①10%	10.8	0.527	0.4	43.5	2.153	0.021	2.132	1.089	
		②10%	9.5	0.288	0.4	41.5	0.892	0.011	0.881	0.471	
		①25%	10.6	0.475	1.1	41.5	2.072	0.017	2.055	1.100	
		②25%	9.8	0.328	1.0	41.3	1.269	0.015	1.254	0.687	
		①50%	10.8	0.535	2.0	41.5	2.201	0.019	2.182	1.167	
		②50%	10.1	0.361	2.1	41.5	1.49	0.022	1.468	0.785	
		①100%	10.5	0.536	4.3	41.2	2.283	0.017	2.266	1.242	
		②100%	10.9	0.391	4.0	41.2	1.493	0.018	1.475	0.809	

Appendix 2

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
05.11.18	10	①10%	9	0.375	0.4	41	1.489	0.042	1.447	0.793	
		②10%	10.6	0.519	0.4	43.2	2.963	0.027	2.936	1.534	
		①25%	10.3	0.561	1.1	44	2.5	0.039	2.46	1.256	
		②25%	10.4	0.591	1.1	41.3	2.383	0.018	2.365	1.296	
		①50%	8.9	0.405	2.1	43.7	2.049	0.052	1.996	1.020	
		②50%	10.2	0.564	2.1	41.7	2.383	0.026	2.336	1.250	
		①100%	10.4	0.555	4.3	41.5	2.264	0.051	2.213	1.184	
		②100%	8.7	0.317	4.4	41.5	0.738	0.019	0.719	0.385	
07.11.18	12	①10%	8.9	0.344	0.4	43.2	1.231	0.109	1.122	0.586	1.322
		②10%	10.8	0.672	0.3	41.1	2.474	0.051	2.423	1.328	1.309
		①25%	10.2	0.754	1.0	41.1	2,446	0.053	2.393	1.312	1.777
		②25%	10.6	0.777	1.0	41.1	2.465	0.044	2.421	1.327	1.686
		①50%	9.4	0.445	2.0	41.1	2.005	0.084	1.921	1.053	1.378
		②50%	9.7	0.667	2.0	41.2	2.428	0.048	2.38	1.305	1.321
		①100%	9.8	0.586	4.3	44	2.163	0.071	2.092	1.068	1.640
		②100%	8.8	0.319	4.5	43.9	0.331	0.022	0.308	0.157	0.883
09.11.18	14	①10%	9.2	0.358	0.3	41	0.867	0.111	0.756	0.414	
		②10%	10.5	0.804	0.3	43.6	2.433	0.079	2.354	1.202	
		①25%	10.7	0.889	1.0	41.1	2.424	0.068	2.357	1.292	
		②25%	10.2	0.881	0.8	41.1	2.438	0.067	2.371	1.299	
		①50%	9.5	0.49	1.9	41	2.157	0.096	2.061	1.130	
		②50%	9.5	0.71	2.0	41.5	2.435	0.079	2.356	1.260	
		①100%	9.7	0.666	4.4	41.2	2.266	0.103	2.163	1.186	
		②100%	8.9	0.368	4.5	43.2	0.442	0.036	0.406	0.212	

Appendix 2

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
11.11.18	16	①10%	9.4	0.458	0.3	40.9	1.324	0.119	1.206	0.661	
		②10%	10.2	0.917	0.2	43.5	2.483	0.109	2.374	1.212	
		①25%	10.2	1.019	0.9	43.8	2.499	0.102	2.396	1.224	
		②25%	10	0.927	0.8	41.7	2.48	0.092	2.388	1.277	
		①50%	10	0.667	1.8	41.2	2.337	0.125	2.212	1.213	
		②50%	9.2	0.673	2.0	41.5	2.275	0.117	2.158	1.155	
		①100%	10.7	0.741	3.9	41.2	2.244	0.109	2.134	1.170	
		②100%	9.7	0.459	4.4	41.3	0.952	0.059	0.894	0.490	
13.11.18	18	①10%	10.5	0.574	0.2	41	2.023	0.159	1.864	1.022	1.310
		②10%	10.1	0.996	0.1	43.9	2.487	0.163	2.323	1.186	2.084
		①25%	11	1.153	0.8	43.8	2.496	0.115	2.381	1.216	2.560
		②25%	10.1	1.01	0.7	41.5	2.058	0.067	1.922	1.066	2.118
		①50%	10.4	0.857	1.6	20.6	2.467	0.144	2.323	2.486	2.034
		②50%	9.1	0.757	2.0	21.2	1.543	0.09	1.453	1.554	1.309
		①100%	10	0.804	4.0	21.1	1.266	0.058	1.207	1.292	1.367
		②100%	10.7	0.678	4.2	21	1.032	0.032	1	1.070	0.922
15.11.18	20	①10%	10.5	0.703	0.2	20.5	1.302	0.066	1.236	1.323	
		②10%	10.3	1.07	0.1	21.5	2.467	0.087	2.38	2.431	
		①25%	10.8	1.236	0.9	12.1	2.259	0.035	2.224	4.164	
		②25%	9.8	1.029	0.7	12.1	1.647	0.037	1.611	3.016	
		①50%	10.7	1.008	1.7	10	1.439	0.025	1.414	3.178	
		②50%	9.2	0.581	2.0	20.5	0.858	0.069	0.789	0.844	
		①100%	9.6	0.854	4.0	21	1.37	0.065	1.305	1.396	
		②100%	10.4	0.805	4.3	19.5	1.484	0.031	1.453	1.633	

Appendix 2

Date (day. month .year)	Day	Sample	pH	OD_{740nm}	Mg^{2+} concentration (mg/L)	Filtrated volume (mL)	A665	A750	A655K	Chl a concentration (mg/L)	MC concentration (mg/L)
17.11.18	22	①10%	10.1	0.846	0.1	10.2	0.895	0.027	0.869	1.952	
		②10%	10.5	1.165	0.0	11.5	1.882	0.045	1.837	3.439	
		①25%	10.9	1.324	0.6	11.8	2.369	0.048	2.321	4.347	
		②25%	9.4	1.067	0.7	11	1.442	0.044	1.398	2.856	
		①50%	10.4	1.138	1.3	11.5	1.816	0.029	1.787	3.346	
		②50%	8.9	0.561	2.0	14	0.502	0.046	0.456	0.733	
		①100%	9.7	0.816	4.1	14.2	0.983	0.039	0.945	1.516	
		②100%	10.2	0.979	4.1	12.5	1.558	0.025	1.533	2.649	
19.11.20	24	①10%	10	0.924	0.1	12	1.162	0.04	1.122	2.101	2.154
		②10%	10.6	1.228	0.0	11	1.608	0.033	1.575	3.218	3.303
		①25%	10.6	1.38	0.5	13	2.447	0.058	2.388	4.128	3.253
		②25%	9.1	1.053	0.6	12.5	1.436	0.052	1.385	2.394	2.426
		①50%	11	1.219	0.7	10.5	2.053	0.042	2.011	4.109	1.387
		②50%	8.9	0.615	2.0	10.8	0.388	0.033	0.354	0.724	1.762
		①100%	9.5	0.886	4.0	11	0.917	0.035	0.882	1.802	2.280
		②100%	10.7	1.104	3.1	10.5	1.566	0.024	1.542	3.150	2.322

Appendix 3: Color status of cells of *M. aeruginosa* at high light intensity in duplicates of 10, 25, 50 and 100 % Mg^{2+} media in duplicate. G: green, Y: yellow, YG: yellow green.

Day	①10%	②10%	①25%	②25%	①50%	②50%	①100%	②100%
0	G	G	G	G	G	G	G	G
2	G	G	G	G	G	G	G	G
4	G	G	G	G	G	G	G	G
6	G	G	G	G	G	G	G	G
8	G	G	G	G	G	G	YG	YG
10	G	G	G	G	G	G	G	G
12	YG	G	G	G	YG	G	G	YG
14	Y	G	G	G	YG	G	YG	Y
16	YG	G	G	G	G	G	G	YG
18	YG	G	G	G	G	G	G	G
20	G	G	G	G	G	YG	YG	G
22	G	G	G	G	G	YG	YG	G
24	G	G	G	G	G	YG	YG	G