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Research Report Robust algae for aquaculture

*Effect of N and P concentrations on biomass productivity and cell composition of Rhodomonas sp.*

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Robust algae for aquaculture

Effect of N and P concentrations on biomass productivity and cell composition of *Rhodomonas sp.*

HZ University of Applied Sciences

Delta Academy

Water Management

Aquaculture in Delta Areas Research Group

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# Preface

This report is the result as well as a summary of my final thesis internship, which is the last part of my bachelor program Aquatic Ecotechnology at HZ University of Applied Sciences. I have been working on the thesis internship in the Aquaculture in Delta Areas research group of HZ from the end of January and it’s finished at the end of June according to the schedule.

The main goal of this thesis is to find the answer to the research question: “what is the effect of N and P concentrations on biomass productivity and cell composition of *Rhodomonas sp.*?” and finally give suggestions to Fry Marine on how much nutrients they should apply in their cultivation medium for *Rhodomonas sp.*. To achieve this, a great quantity of time has been spent on doing experiments, collecting data and making analyses. Many problems occurred during these processes and some really made me desperate especially those happened in the last stage of the experiment when time is pressing. Fortunately, I got help from many people during the very hard times for me, and my internship would not have been accomplished smoothly without their help.

Therefore, I would like to express my gratitude to some certain people here. Firstly, I want to give my special thanks to my supervisor Christos Latsos. I’m a slow leaner and it's always a challenge for me to operate a machine, so I’m really grateful for Chris’s patience to tell me something again and again, and his kindness to be always willing to help with the problems happened in the experiment no matter how small things they are. I also want to express my respect for him because he can always give me suggestions and solve problems in a very professional way that I can learn a lot from. I also want to thank my mentor and examiner Alco Nijssen for his advice and help, especially the encouragement when I was downhearted. Besides, I’m always so grateful for my two tutors Jouke Heringa and Mitra Vaskoska for their cares from the beginning of my journey in the Netherlands.

In addition, I’m also grateful for other teachers and fellow students in our research group such as Gabrielle Verbeeke and David Radzikovsky, who also have helped me a lot.

Last but not least, I want to thank my family for always trying their best to support me although we have the time difference of 6 hours, every time talking with them on phone is the warmest thing for me.

# 

# Abstract

Microalgae can produce a wide range of valuable metabolites such as proteins, lipids and pigments, so they can be used in many ways. For aquaculture industry, microalgae are usually used as live feed because of their high nutritional level. Fry Marine(Vlissingen, NL) is a hatchery for marine feed and flatfish, they create a 'food-chain' for their flatfish which starts from cultivating *Rhodomonas* in photobioreactors. The nutrients (mainly Nitrogen and Phosphorus) that are mainly used for algae cultivation are expensive chemicals, and extra nutrients in the harvest/waste of algae cultivation can be harmful for fish/environment. Since they are artificially added into the medium in continuous systems, it is possible to find a more cost-effective as well as environmentally friendly way to cultivate the algae by optimizing the nutrient application. Previous research on *Rhodomonas Baltica*  has shown that a medium with 0.54 g/L Nitrate and 0.034 g/L Phosphate is for growing the algae with the maximum productivity (0.486 g/L/day) and the minimum waste of nutrients in a continuous system. Based on the results of previous research, this study has demonstrated that cultivating *Rhodomonas sp.* with a 10x medium (0.54 g/L Nitrate and 0.034 g/L Phosphate) is optimal to achieve the highest productivity of 0.837 g/L/d and leave the least amount of unused nutrients in the harvest. Besides, since it’s known that nitrogen starvation often stimulates the accumulation of carbohydrates and lipids, a medium without nitrogen (N starvation) and another one without phosphorus (P starvation) were applied after the biomass production phase to text their effects on the biochemical composition of *Rhodomonas sp.* cells. Protein, phycobiliprotein and pigments content in the algae cell were measured every few hours for the phase of N and P starvations, and it was suggested not to apply a period of N starvation after the harvest of *Rhodomonas sp.* because it would cause decreases on the contents of protein, phycobiliprotein and chlorophyll, P starvation medium can be used to store the algae culture after the harvest because cells could survive in this case without changing their composition.

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# List of abbreviations

|  |  |
| --- | --- |
| Chl a | Chlorophyll a |
| Chl b | Chlorophyll b |
| Chltot | Total chlorophyll |
| Carottot | Total carotenoids |
| DW | Dry weight |
| dH2O | Demineralized water |
| N- | Nitrogen starvation |
| P- | Phosphorus starvation |

# 1. Introduction

Microalgae are microscopic algae, representing one of the most diverse groups of microorganisms in freshwater and marine systems (Fu, et al., 2017). They are unicellular organisms capable of photosynthesis which convert solar energy to chemical energy. Microalgae are important for the life on earth since they produce about half of the atmospheric oxygen (Cardozo, et al., 2007). A wide range of valuable metabolites such as proteins, lipids and pigments can be produced from microalgae (Department of Biotechnology, 2012), and more than 15,000 novel compounds from microalgae have been chemically determined as a whole (Cardozo, et al., 2007), so they can be applied in many ways. Microalgae were firstly used 2000 years ago by Chinese, who used *Nostoc* to survive during famine. However, microalgal biotechnology only really began to develop in the middle of the last century. Nowadays, microalgae are widely used in commerce (Spolaore, et al., 2006).

There are many advantages of microalgae compared with terrestrial plants. Firstly, microalgae have no direct competition with crops for agricultural land (Fu, et al., 2017); Secondly, they can utilize the energy of sunlight more efficiently compared with higher plants (Department of Biotechnology, 2012); Thirdly, it’s easy to produce microalgae, the only need is a simple culture medium with water, iron, magnesium, a source of nitrogen and phosphorus and some minor salts (de Jesus Raposo, et al., 2013a); Besides, it’s examined that for the majority of microalgae, the average quality of protein is equal and sometimes even superior than conventional plants (Becker, 2007); Microalgae also have 20–40 times more productivity of lipid than oil crops, which makes them have greater potential to be a major source of the production of renewable energy (Santhosh, et al., 2016).

Microalgae are easily digested overall (Spolaore, et al., 2006), so there is no limitation to use whole microalgae to feed animals. Whole microalgae can also be used as biofertilizers because of their high content of carbohydrates (Janssen & Lamers, 2014). Except for whole algae, the chemical compositions extracted from microalgae also make them valuable. For example, a protein-rich extract from *Arthrospira* has the function to prevent stria formation and repair the signs of early skin aging, thus can be applied for skin care products (Spolaore, et al., 2006). Therapeutic supplements from microalgae also takes a large market share mainly due to the compounds such as polyunsaturated fatty acid (PUFA) like DHA and EPA, β-carotene and astaxanthin (Department of Biotechnology, 2012).

## 1.1 Problem statement

For aquaculture industry, microalgae are mainly used as lives feeds for larvae of bivalves, crustaceans, marine fish and zooplankton used in maricultural food chains because they have high nutritional value (Brown, et al., 1997). The application for animal feed accounts for about 30% of the algal production in the word (Becker, 2007). While feed is the biggest cost in commercial aquatic product breeding, so high priority can be achieved through improving feed efficiency in industrial systems (Patil, et al., 2006).

Fry Marine(Vlissingen, NL) is a hatchery for marine feed and flatfish, they create a 'food-chain' for their flatfish and it starts from cultivating *Rhodomonas sp.* in four photobioreactors with LED lights, so the cultivation of *Rhodomonas sp.* with high productivity and good nutritional values is a main goal of Fry Marine, because they are the first trophic level as live feed and they can influence the whole process. However, the nutrients(mainly Nitrogen and Phosphorus) used for the algae cultivation are expensive chemicals and extra nutrients in the harvest/waste of algae cultivation can be harmful for fish/environment.

## 1.2 Research goal

Since the nutrients are artificially added into the medium in continuous systems, it is possible to find a cost-effective way to cultivate the algae by optimizing the nutrient application. By applying different concentrations of nitrogen and phosphorus in the cultivation medium, it could be found what combination of Nitrogen and Phosphorus concentrations leave the least amount of unused nutrients in the harvest and lead to a most cost-effective cultivation of *Rhodomonas sp.*, so the optimal combination can be applied for large scale production of *Rhodomonas sp.* with the advantages of both saving costs and reducing pollution. Besides, it’ll be tested what’s the effect of N and P starvations on the cell composition of *Rhodomonas sp.* in order to give a suggestion on if it’s better to apply a period of N or P starvation after the harvest of *Rhodomonas sp.*.

## 1.3 Research question

Main question

What is the effect of N and P concentrations on biomass productivity and cell composition of *Rhodomonas sp.*?

Sub-questions

(1) What is the effect of N on the productivity of *Rhodomonas sp.*?

(2) What is the effect of P on the productivity of *Rhodomonas sp.*?

(3) What concentrations of nutrients are optimal when wanting to achieve the highest productivity possible and leave the least amount of unused nutrients in the harvest?

(4) What is the effect of N starvation on cell composition of *Rhodomonas sp.* over time?

(5) What is the effect of P starvation on cell composition of *Rhodomonas sp.* over time?

# 2. Theoretical framework

## 2.1 Microalgae culture for aquaculture feed

It’s well known that microalgae are at the bottom of the food chain in all aquatic ecosystems, they are essential for aquaculture since they provide the main micronutrients such as pigments, nitrogen-containing compounds, sterols and specific fatty acids to most of the aquatic organisms such as bivalve mollusks (e.g. oysters, scallops, clams and mussels), crustaceans, some fish species and zooplankton used in aquaculture food chains (Sirakov, et al., 2015). For example, although fish oil seems to be a most conventional source of EPA, fish do not synthesize EPA themselves and these compounds are mostly derived from the microalgae they consume (Cardozo, et al., 2007).

Microalgae have to meet several criteria in order to be used in aquaculture. They need to be easily cultured, nontoxic, with the proper size and shape to be ingested, having a digestible cell wall to make nutrients available and a high nutritional quality (the nutritional value of microalgae is mainly determined by protein content) (Spolaore, et al., 2006).

Applying microalgae in aquaculture has many potential advantages: They can be easily produced with a simple culture medium; The conversion efficiency is high; they can grow in closed bioreactors with controlled growth conditions (de Jesus Raposo, et al., 2013b); In many cases microalgae can be directly used without needs for harvesting, processing or storage. For example, microalgae could be cultured directly in the production ponds of shrimp, so they can be used immediately after produced. While one problem needs to be improved is that the estimated costs of indoor production units and systems for microalgae are high, which range from US$ 200 to 1000 per kg of dry weight (Benemann, 1992).

## 2.2 Different phases of algal growth

The growth of microalgae in the way of monoculture generally contains four phases shown in Figure 1: the lag phase, the exponential phase, the stationary phase and the senescent phase.

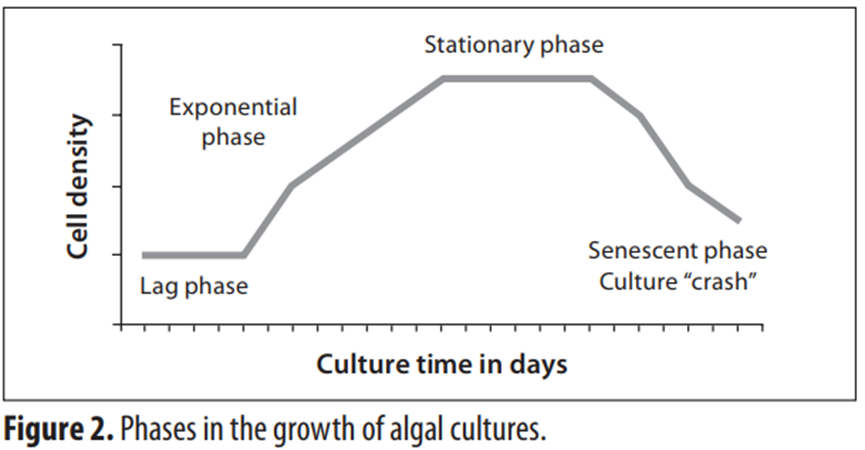
The first phase the algae will experience after being inoculated into a new medium is lag phase. The cells need to adapt to the new environment slowly to begin cell division. The length of lag phase is about 3 days while it varies depending on the inherent division rate of different algal species, the initial cell density, irradiance and temperature; Once acclimated, the algal cells begin cell division at an accelerating rate because there is sufficient nutrients and space, which means no competition between cells, so the population increases logarithmically as a result. The length of exponential growth phase is about 4 or more days. The cells from this phase are usually harvested for feeding; Then it’s the stationary phase, where cell division declines and there is no further increase in cell density. This is because the concentration changes of nutrients, self-shading of microalgae themselves(high cell density reduces the amount of light available to each algal cell), changes in the culture medium such as increasing pH and the accumulation of metabolic waste or secretions that inhibit self-growth. Algae at stationary phase should not be used for larvae culture because the line between feeding larvae and poisoning them can become blurry as algal cultures age. Although the algae may be nutritious, as they die the cells will rupture and bacteria can proliferate; At last it comes to the senescent phase where culture ages so the density of the culture will decline (Creswell, 2010).

Figure : Phases in the growth of algal culture (Creswell, 2010)

A series of reasons can cause the crash of algae culture in practice, the most common ones are lack of nutrients, high pH and contamination. Keep all algae cultures in the exponential phase is the key for success of algal production because the nutritional value of the algae gets worse once the culture reaches the stationary phase due to reduced digestibility, deficient composition, and possible production of toxic metabolites (Lavens & Sorgeloos, 1996).

## 2.3 Importance of Nitrogen and Phosphorus for algae cultivation

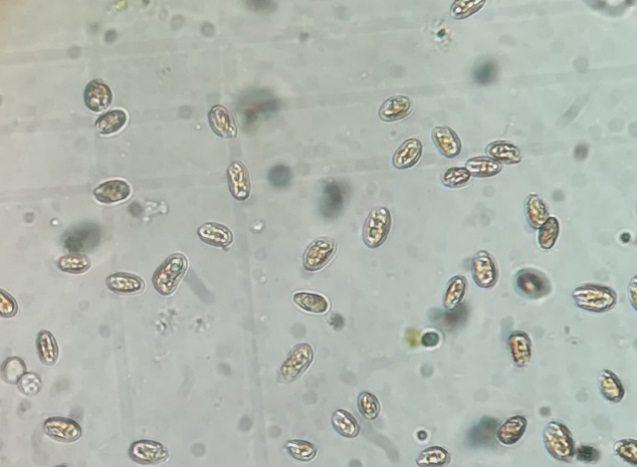
During the process of photosynthesis, microalgae need solar energy as well as several essential nutrients represented by C, N and P to synthesize their biomass compounds. The nutrients are also essential for algae to multiply their cells (Markou, et al., 2014). It has been found in many researches that the amount of Nitrogen and Phosphorus can influence the composition of microalgae cell, the affected components vary from species to species but are mainly about phycobilisome, lipid and chlorophyll (Collier & Grossman, 1992) (Carr & Whitton, 1982). Microalgae need specific amount of the essential nutrients and the deficiency of one nutrient can lead to growth reduction(Liebig's law of the minimum).

Living organisms absorb chemical elements from their environment in ratios as they occur in their tissues during growth (Spaargaren, 1996). Considering the universal Redfield ratio (The atomic ratio of carbon, nitrogen and phosphorus found in phytoplankton and throughout the deep oceans is 106:16:1), the essential nutrients have to be present in appropriate ratios so that the proportion of components in the medium will not change as the algae grow.

Microalgae have a high protein content about 30%-60% (Becker, 1994), and nitrogen is an essential constituent of all structural and functional proteins in algal cells such as amino acids, accounting for about 7–10% for dry weight (Hu, 2013), so the requirements of microalgae for nitrogen are high. Both nitrate (NO3-) and ammonia/ammonium (NH3/NH4+) can be used by microalgae as a source of nitrogen (Janssen & Lamers, 2014), and the most frequently used nitrate salt is NaNO3, followed by KNO3 (Grobbelaar, 2004). Specifically for cells of *Rhodomonas sp.*, N starvation causes a decrease in the content of phycoerythrin and a decline in the fluorescence capacity (da Silva, et al., 2009).

Phosphorus is necessary for the metabolism of microalgae because it’s a component of the important organic molecules such as nucleic acids, membrane phospholipids and ATP (Markou, et al., 2014). Phosphorus is taken up by the cells in the orthophosphate form, so inorganic phosphorus has to be converted to orthophosphate first in order to be used by microalgae. The most common used phosphorus source for microalgae cultivation is potassium phosphate, sodium phosphate, ammonium phosphate and superphosphate (Markou, et al., 2014).

## 2.4 *Rhodomonas sp.*



|  |  |
| --- | --- |
| Empire | Eukaryota |
| Kingdom | Chromista |
| Phylum | Cryptophyta |
| Class | Cryptophyceae |
| Order | Pyrenomonadales |
| Family | Pyrenomonadaceae |
| Genus | *Rhodomonas* |

Table : Taxonomic data of Rhodomonas sp.

Figure : Rhodomonas sp. under a light microscope with 100x magnification

As can be seen from table 1, the microalga *Rhodomonas sp.* is a member of the genus *Rhodomonas*. Generally *Rhodomonas sp.* are red to bright brown microalgae(other color will occur when the algae are not cultivated under a proper environment) and they are mainly marine species. Their size ranges from 7 to 14 µm and the optimal temperature for growth is between 19 to 26°C (Verbeeke, 2017). They have two flagella, the hair-like structures, which allow them to move with a “whip like” movement. The algae cells don’t have cell wall, they are covered by a periplast that forms a complex surface structure in combination with the cell membrane instead (Hausmann, 1979).

*Rhodomonas sp.* have high content of essential fatty acids(PUFAs) and nutrients, lipid accounts for about 20% and protein takes about 30% of the dry weight (Renaud, et al., 1999). So they can be excellent feed for aquaculture. It has been previously proved to constitute a high-quality diet to rear calanoid copepods and copepod Acartia sinjiensis (Seixas, et al., 2009).

# 

# 3. Material and Method

## 3.1 Overall arrangement of experiment

(1) Start *Rhodomonas sp.* stock cultures in the Orbital Incubator

(2) Inoculate the algae into photobioreactors(continuous system)

(3) Daily measurement of the algae biomass in the reactor

(4) Change medium after the culture can grow at steady state and keep daily measurement

(5) Cell composition measurement for N and P starvations

## 3.2 *Rhodomonas sp.* stock cultures

The microalgae strain *Rhodomonas sp.* for this research was obtained from Fry Marine (Vlissingen, NL). The medium that was used to maintain the algae was 20 times concentrated f/2 medium (see Appendix A for the protocol of f/2 medium), the salinity of the medium was created by using artificial seawater that was made from 30 g/L of salt. 0.7 g/L sodium bicarbonate was added to maintain the pH as a buffer around 8. To make a stock culture, 150 ml of medium was sterilized by filtering with a Sartorius liquid filter (0.2μm pore size) into a 300 ml Erlenmeyer flask (pre-autoclaved 20 minutes at 120 °C), then 15 ml of dense algae culture was inoculated into the flask. The filtration and inoculation were conducted in the laminar flow cabinet in order to keep the sterile condition. After the inoculation, the stock culture was labeled and put into an orbital incubator at around 20 °C with CO2 supply and a light intensity of around 120 µmol/m2/s. The stock cultures were renewed every two weeks and two stock cultures were made each time.

## 3.3 Mediums tested in the experiment

Since previous research on *Rhodomonas Baltica* (Tigli, 2018) has shown that mediums with a nutritional combination that fits in the ratio of N:P=16:1 lead to higher growth rates as well as biomass productivities, the mediums used in this experiment are all based on the f/2 medium where the N:P ratio is extremely close to 16:1.

Besides, according to the result from the same research (Tigli, 2018) that a medium with 0.54 g/L Nitrate and 0.034 g/L Phosphate (10 times f/2 medium) is for growing *Rhodomonas* with the maximum productivity and the minimum waste of nutrients in a continuous system, this experiment is started with a 20x medium where the nutrients are ensured to be abundant. To get less and less wasted nutrients (unused nutrients left in the medium), other mediums that are less concentrated were tested after the 20x medium one by one in the order of concentration reduction. When the measurements for dilution rate, productivity and nutrient consumption of the algae biomass for the previous medium were stable, change to the next medium with less concentrations of Nitrogen and Phosphorus to see the effects. Mediums with different nutrient combinations that were applied are shown below in table 2.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Medium | 20X | 10X | 9X | Without N | Without P |
| PO43- (g/L) | 0.068 | 0.034 | 0.0306 | 0.034 | 0 |
| NO3- (g/L) | 1.08 | 0.54 | 0.486 | 0 | 0.54 |
| Nutrient Ratio (N:P) | 15.88:1 | 15.88:1 | 15.88:1 |  |  |

Table : Nutrient combinations of mediums tested in the experiment

## 3.4 Flat Panel Algaemist-S photobioreactor and its set up

### 3.4.1 Introduction

图片包含 室内, 电子产品, 地板

描述已自动生成The Flat Panel Algaemist-S photobioreactor used in this experiment is shown in figure 3. The reactor can be divided into 3 parts: the medium bottle, the part with several control panels and the flat panel part. By regulating the parameters on the control panels, abiotic factors such as temperature, pH, light intensity and CO2 can be controlled and kept constant at the optimal value. The flat panel part contains two transparent glass sheets, the one close to the light source is where algae culture grows and the other one is filled with water to regulate the temperature.

There are many advantages of the reactor’s design. For example, the air sparger in the flat panel is helpful for the mixing and circulating of algae culture and medium, which would potentially enhance the microalgae biomass productivity.

However, this design also possesses a few limitations such as the possibility of the biomass adhesion to the walls of the reactor (this disadvantage can be solved artificially by using a magnet to scratch the walls from time to time).

As a whole, this type of reactor is convenient and efficient enough for a small-scale experiment of algae cultivation.

Figure : Algaemist-S flat panel photobioreactor

### 3.4.2 Set up of the reactor

(1) Medium preparation: Medium (prepared following Appendix A) was pumped into the autoclaved medium bottle using a Jebao Auto Dosing Pump. Then the medium bottle was placed on top of the reactor and connected with the medium ingoing hose. At last, about 350ml of medium was released into the autoclaved reactor that has a volume of 400ml.

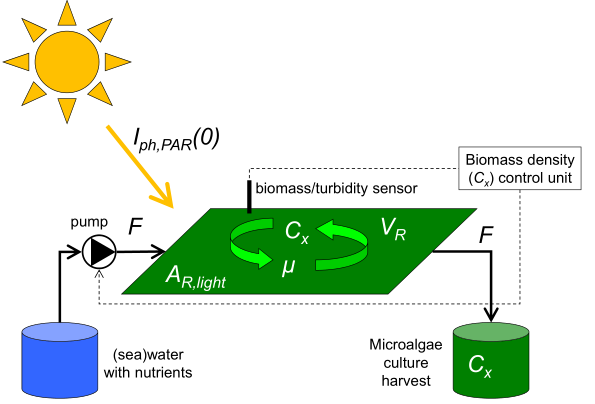
(2) pH: The pH was set at 7.6 on the control panel and kept constant by the reactor’s automatic adjustment on the amount of CO2 pumped in. There is a pH probe going into the algae culture to monitor the pH that was firstly calibrated with two buffer solutions at pH=7 and pH=4.

(3) Temperature: The water sheet of the reactor was filled with tap water and the water pump was turned on. The temperature was set on the control panel at 22°C and it was controlled constant by the cooler (Teco Tank Chiller Line TK2000). In the meantime, a thermometer was put into the algae culture to monitor the temperature.

(4) Light intensity: The primary light intensity was firstly set at around 100 µmol/m2/s after the algae were inoculated to resemble the environment of the orbital incubator where the algae grew at the beginning. Then the light intensity was raised gradually with the growth of the algae culture until reaching the optimal light intensity, which is around 300 µmol/m2/s for *Rhodomonas* (Tigli, 2018).

(5) Inoculate algae culture: The stock culture flask of *Rhodomonas sp.* was opened in the laminar flow cabinet and 50ml algae culture were extracted with a syringe that previously sterilized with ethanol, then the culture in the syringe was inoculated into the reactor that already has medium in.

## 3.5 Turbidostat mode of photobioreactor



Iph: light intensity

F: flow rate

AR: light area

CX: biomass concentration

µ: growth rate

VR: volume of the reactor

Figure : Schematic of turbidostat mode of operation of a microalgae cultivation system (Janssen & Lamers, 2014)

The 20x, 10x and 9x mediums were tested in photobioreactors in a continuous manner based on a turbidostat type of operation in this experiment. The operation of a turbidostat mode relies on the turbidity sensor that can measure the biomass concentration (CX) in the reactor. The medium pump will receive a signal whenever the biomass concentration changes, then the flow rate (F) of new medium coming into the reactor is automatically adjusted, which finally result in a constant biomass concentration at the pre-selected value. That’s to say, the algae culture will be diluted automatically with new medium every time when the measurement of turbidity sensor drops below the set point because of high culture density caused by a positive growth rate (µ). Once there is a dilution, a harvest is created because the volume of the reactor (VR) is fixed (400ml in this experiment). The higher the growth rate, the denser the biomass concentration, the faster the flow rate, then the more harvest is created with the same time.

In addition to the turbidostat mode, photobioreactors can also be operated in a continuous manner based on a chemostat mode, which is the operation mode of Fry Marine and most large-scale experiments because there is no turbidity sensor in their systems. These two types of operation modes can both lead to a steady state where growth rate is equal to dilution rate. The main difference between them is: In a chemostat mode, the dilution rate can be set manually at a selected value; In a turbidostat mode, the biomass concentration can be set at a selected value. Thus, the dilution rate for chemostat mode that can achieve a specific biomass concentration can be found through a small-scale experiment by operating the Flat Panel Algaemist-S photobioreactors in turbidostat mode.

## 3.6 Culture analysis

### 3.6.1 Growth analysis

Everyday samples were taken in duplicate from the harvest volume for several measurements shown below. From the results of daily measurement, the values for dry weight, growth rate, productivity and yield of biomass on light could also be known through calculations as described in 3.6.1.2.

#### 3.6.1.1 Daily measurement

(1) Harvest volume (ml)

The first thing to measure is always the volume of harvest culture in the collection bottle. It can be got by just measuring the weight of algae culture on the electronic balance because the density of algae culture can be approximately regarded as the density of water, which is 1. To be more precise, the harvest volume could be got through dividing harvest weight by 0.997 (the average density of algae culture).

(2) Cell density (cell/ml)

The cell density in a sample that was diluted 100 times with Isoton solution was measured with Coulter Counter (Backman Coulter, Z1 Coulter Particles Counter). At last the cell density in cell/mL of the algae culture can be found by multiplying the result of Coulter Counter with dilution times.

(3) OD750 (Abs)

To finally get dry weight by calculation, the absorption of the sample at 750 nm was measured with a spectrophotometer since there is a linear correlation between OD750 and dry weight of the algae culture (see Appendix C for the OD-DW curve). The samples were diluted with artificial seawater firstly in order to get results that fall in the reliable range, in this case the results read from spectrophotometer were multiplied by dilution times at last.

(4) Nitrate and Phosphate left in the medium (mg/L)

To measure the amount of unused Nitrate and Orthophosphate, firstly the culture was filtrated with KNFlab laboport and Whatman Glass Microfibre Filters GF/C ø 47mm filters. Then the protocols in Appendix D and Appendix E were followed to do the measurement. Dilutions are needed in order to fit in the reliable range before using the protocols to measure for both Nitrate and Phosphate.

(5) Secondary light intensity (μmol/m2/s)

The light intensity was measured with a photometer (Skye SKP 200) at 19 different places of the reactor in μmol/m2/s.

#### 3.6.1.2 Calculations

(1) Dry weight **·** DW (g/L)

In the formula, “X” represents OD750 and “Y” is dry weight (see Appendix C for the linear relation curve of OD750 and dry weight).

(2) Growth rate **·** μ ( /d)

When microalgae are grown continuously it means that the culture is harvested with a fixed rate and the harvest volume removed from the reactor is continuously replaced with new medium. Thus, a steady state can be reached finally where the biomass concentration will not change and become constant. Then the biomass balance can be written as follows:

Where: : Liquid volume of photobioreactor (i.e. reactor, ‘R’), ;

: biomass concentration in photobioreactor system, ;

F: Liquid flow rate through photobioreactor, .

It can be safely assumed that there are no microalgae in the inflow (medium), so: . Furthermore, the culture volume within the reactor is maintained constant, so: . Finally, assuming that the microalgae culture and medium in the reactor are ideally mixed, so: . Then it can be further simplified as follows:

So the growth rate can finally be calculated as follows in this experiment:

In the formula, the reactor volume is 400mL and dT is the time difference in days between two measurements.

(3) Productivity (g/L/d)

(4) Yield of biomass on light **·**  (g/mol)

In the formula, “d” represents the length of the light path for the Algaemist-S, and the value is 0,014m.

### 3.6.2 Biochemical analysis

In order to analyze the quality of the cultures under N or P starvation, several biochemical compositions including protein, phycobiliprotein and pigments were extracted and measured.

Firstly the algae were cultivated in two reactors using 10x medium with a continuous system under turbidostat mode. After around 4 days when the growth of the algae culture was stable under optimal conditions, cells were collected by centrifuging the culture in plastic vials. Cells from one reactor went to the group N- and cells from the other reactor went to the group P-. After the centrifuge, the supernatant was removed. The pellet of N starvation was washed with the special 10x medium without nitrate and pellet of P starvation was washed with the 10x medium without phosphate to rinse off residual nitrate or phosphate, then the mix was centrifuged again to only leave the pellet. Then the algae cells (the pellet) of the group N starvation and P starvation were suspended into the same volume of the 10x growth medium without nitrate or phosphate enrichment respectively, and the two reactors were set at the same conditions as the algae were cultivated under the continuous system.

A sample (sample volume depends on the culture density) was taken from each of the two reactors at different times (T= 0, 8, 15, 24, 32, 48, 74, 99, 120, 144, 168, 192h) and centrifuged. After the centrifuge, the pellet was washed with ammonium formate to clean the salt on the surface of cells so that the cell morphology can be maintained, then the mix was centrifuged again to get the pellet. The vials with pellet was kept in the fridge at -80°C. All the samples were taken out from the fridge after the last sampling was done, then cells were freeze-dried with the freeze dryer. Then each sample was divided into six samples (two samples for each analysis of protein, phycobiliprotein and pigments) with approximately equal dry weight on the analytical balance (Mettler Toledo ME104, Metler PM2000). At last, the measurements of protein, phycobiliprotein and pigments content were conducted according to the protocols shown in Appendix F, Appendix G and Appendix H.

# 4. Results

## 4.1 Effect of different N and P concentrations on the growth of *Rhodomonas sp.* in the continuous system

As it is described in the “Material and Method” part, three mediums were tested in the experiment, which is 20x, 10x and 9x in turn. When the growth of algae culture under 20x medium was stable with a constant dilution, it was found that the culture consumed 0.510 g/L nitrate and 0.027 g/L phosphate. 0.570 g/L nitrate and 0.041 g/L phosphate were found left in the medium, which is a great waste. To reduce the wasted amount of nutrients, 10x medium containing 0.54 g/L nitrate and 0.034 g/L phosphate was decided to be applied next, where the nutrients amount is a bit higher than the nutrients consumed under the 20x medium. Then the 9x medium was tested after a same analysis for the algae culture grown under 10x medium. The 9x medium lead to a result of no dilution and the color of algae culture turned yellow green over time, which suggested that the nutrients of this medium are not enough for the microalgae *Rhodomonas sp.* to grow up to the wanted density in continuous system. Thus, the comparison is focused on 20x and 10x mediums in terms of five aspects: dry weight, growth rate, productivity, biomass yield on light and nutrient consumption. Detailed data can be viewed in appendix L.

Graph : N and P consumption of Rhodomonas sp. in a continuous system with 10x and 20x medium

From graph 1 it can be seen that the amount of consumed nutrients is very close when applying 20x and 10x medium. The consumed nitrate is 0.448 g/L and the consumed phosphate is 0.025 g/L for 10x medium, which has a N:P ratio of 17.92 : 1. For 20x medium, it consumed 0.510 g/L nitrate and 0.027 g/L phosphate with a N : P ratio of 18.89 :1.

However, there are more nutrients left for the 20x medium since there are more nutrients from the beginning. The wasted nitrate and phosphate account for 17.04% and 26.47% respectively for 10x medium and it’s 52.78% and 60.29% for 20x medium correspondingly.

Graph : Dry weight, growth rate, productivity, biomass yield on light of Rhodomonas sp. in a continuous system with 10x and 20x medium

From graph 2, it is possible to see several parameters of the *Rhodomonas sp.* cultures grown in photobioreactors. The cultures grown in 10x and 20x mediums didn’t differ so much from each other on dry weight, growth rate, productivity and biomass yield on light in general.

The algae grew in 10x medium achieved a dry weight of 0.956 g/L, which is 0.125 g/L higher than the 0.831 g/L for 20x medium; For growth rate, there is a difference of 0.156 day-1 between the 0.879 day-1 for 10x medium and 1.035 day-1 for 20x medium; For biomass yield on light, the value of 20x medium is 0.057 g/mol higher than that for 10x medium, which are 0.566 and 0.509 g/mol respectively. Especially, there is only a slight difference of 0.014 g/L/d regarding the productivity, which are 0.837 and 0.851 g/L/d for 10x and 20x mediums respectively.

## 4.2 Effect of N/P starvation on the growth and cell composition of *Rhodomonas sp.*

Samples were taken at T= 0, 8, 15, 24, 32, 48, 74, 99, 120, 144, 168, 192h after the start of starvation phase for N and P starvations. Pictures of the samples were taken, cell density and OD750 were measured for each sample to see the growth condition of the algae culture. Besides, to analyze the quality of the cultures, protein, phycobiliprotein and pigments contents were extracted and measured. Results about the growth and cell composition of *Rhodomonas sp.* under N and P starvationsare shown in the following paragraphs.

### 4.2.1 Growth of *Rhodomonas sp.* under N starvation

The growth situation of *Rhodomonas sp.* under N- is shown and analyzed mainly from three aspects: the color of algae culture, cell density and cell size.

图片包含 室内, 排列

描述已自动生成

Figure : Sample photos of N starvation from T1 to T11

Great changes in color of the *Rhodomonas sp.* culture under N starvation were observed overtime as shown in figure 5. On one hand, the gradual fading of color demonstrates a decrease in cell density as time passed. On the other hand, the shift from reddish brown to yellowish green indicates the decrease of pigment, which can be confirmed by the significant decline of phycobiliprotein content for N- from T1 to T4 as shown in Graph 8.

Graph : Size and density of Rhodomonas sp. overtime under N starvation

Graph : Cell density of Rhodomonas sp. under N starvation

As is shown in graph 3 and graph 4, there is a decreasing trend of the cell density of *Rhodomonas sp.* culture on the whole, and the cell size was shrinking. To find out if the reason for the decrease of cell density is nitrogen starvation or the removal of harvest volume (sample volume), the results of cell density under two cases (the blue one is the change pattern of cell density when only considering the removal of harvest volume, the orange one is the changes in reality) are shown in graph 4, and the difference between orange and blue is the change of cell density under nitrogen starvation excluding the influence of harvest volume.

Through analyzing the graph, it can be found that the cell density of actual situation is always greater than or approximately equal to that when only considering the removal of harvest volume, which means the decrease of cell density is caused by the removal of harvest volume. The cell density was actually increasing within the first 48 hours and then remained stable under N starvation when excluding the influence of harvest volume.

### 4.2.2 Growth of *Rhodomonas sp.* under P starvation

The growth situation of *Rhodomonas sp.* under P- is also shown and analyzed mainly from three aspects: the color of algae culture, cell density and cell size.

Error: an unexpected dilution happened at T7 (99h) because of unclosed clump (amount is about 1 L).

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Figure : Sample photos of P starvation from T1 to T11

As can be seen from figure 6, the color of the culture under P starvation did not change significantly. The only change can be found in the picture is a sudden fading of color between T7 and T8 due to an error of the experiment.

Graph : Size and density of Rhodomonas sp. overtime under P starvation

Graph : Cell density of Rhodomonas sp. under P starvation

As it is shown in graph 5 and graph 6, there is not a significant increasing or decreasing trend of the cell density of *Rhodomonas sp.* culture, and the cell size was not shrinking on the whole. Through the same analysis as N starvation on the reason for cell density change, conclusion can be drawn from graph 6 that the cell density was actually increasing all the time within the 192 hours under P starvation when excluding the influence of harvest volume removal.

### 4.2.3 Changes of *Rhodomonas sp.* on cell composition under N or P starvation

For both N- and P-, samples were set in duplicate every time to measure the contents of protein, phycobiliprotein and pigments. And the results are shown below.

Graph : Protein content of Rhodomonas sp. cells under N or P starvations (mg protein/mg DW)

Graph : Phycobiliprotein content of Rhodomonas sp. cells under N/P starvations (mg phycobiliprotein/mg DW)

It’s possible to conclude from graph 7 and graph 8 that the cells have a tendency of losing protein and phycobiliprotein all the way after 8 hours in N starvation medium. But the protein and phycobiliprotein content of cells grown in the P starvation medium did not change significantly during the entire experiment on the whole.

Graph : Chlorophyll content of Rhodomonas sp. cells under N or P starvations (mg chlorophyll/mg DW)

Graph : Chl a content under N and P starvations

Overall, it can be concluded from graph 9 that the total chlorophyll decreased with stressed N and there was no obvious characteristic of the change under P starvation. Besides, combining with graph 10, it can be found that the fluctuations of Chltot and Chl a are almost the same for both N- and P-. In terms of the numerical value, the majority of total chlorophyll is composed of chlorophyll a (e.g. The contents of Chltot and Chl a are 0.0071 and 0.0062 mg/mg DW respectively for N- at T=0 (h), Chl a accounts for 87% of the total chlorophyll content).

Thus, it can be safely assumed that the total chlorophyll content in *Rhodomonas sp.* cells is mainly composed of chlorophyll a and the change of total chlorophyll content is mainly affected by the content of chlorophyll a. (the graph for Chlb can be found in appendix K).

Graph : Carotenoids content of Rhodomonas sp. cells under N or P starvations (mg carotenoids/mg DW)

From graph 11 it’s found that the content of carotenoids had no obvious change trend for both N and P starvations.

# 5. Discussion

## 5.1 Effect of different N and P concentrations on the growth of *Rhodomonas sp.* in the continuous system

The experimental results of this part are of high reliability by comparing it to the previous research on *Rhodomonas Baltica* (Tigli, 2018). It can be seen that the amount of consumed nutrients is very close when applying 20x and 10x medium, which is the same result as is found for *Rhodomonas Baltica*. Furthermore, the consumed amount of nutrients when applying 10x medium is also close for this two algae: the consumed nitrate is 0.448 g/L and the consumed phosphate is 0.025 g/L for *Rhodomonas sp.* culture, which are 0.49 g/L and 0.03 g/L respectively for *Rhodomonas Baltica*.

Besides, the difference between 10x and 20x mediums regarding productivity is also similar for the two algae strains: a difference of 0.014 g/L/d for *Rhodomonas sp.* and 0.026 g/L/d for *Rhodomonas Baltica*. However, the productivity of *Rhodomonas sp.* (0.837 g/L/d ) is significantly higher than that of *Rhodomonas Baltica* (0.486 g/L/d) when comparing the results of 10x medium, the former is about 1.7 times higher than the latter. Furthermore, it’s reported that *Rhodomonas salina* could achieve a maximal growth rate of 0.84 ± 0.17 d-1 when they’re cultivated in photobioreactors (Vu & Jepsen, 2015), which is even a bit less than the growth rate of 0.88 d-1 when *Rhodomonas sp.* is cultivated in the 10x medium. Therefore, it can be safely assumed that cultivating *Rhodomonas sp.* in photobioreactors with 10x medium can achieve the highest growth rate and productivity compared with other species of *Rhodomonas*.

## 5.2 Effect of N/P starvation on the growth and cell composition of *Rhodomonas sp.*

Firstly, it can be concluded that for both N and P starvations, the algae culture of *Rhodomonas sp.* didn’t decrease in cell density in a short time, at least within 192 hours as it has been tested in the experiment.

Previous research on *Rhodomonas sp.* (da Silva & Lourenc¸o, 2009)showed that transferring to N starvation medium resulted in a 3.2-fold increase of the cell volume, a decrease of chlorophyll and a massive loss of phycoerythrin during the acclimation process. It has also been demonstrated in other researches that cells of *Rhodomonas sp.*, as also occurs in some other cryptomonads, witnesses a drastic decrease of the phycoerythrin content upon N starvation (Bartual & Lubián, 2002) (Sciandra & Lazzara, 2000). The result of this experiment shows the same change for chlorophyll and phycoerythrin under N starvation, but the continuous shrinking of the cell size is quiet contrary to the increased cell size as found in previous studies. The shrinking of the cell size might be caused by a total biomass loss due to more decrease in protein than the increase in lipids, it may also be the result of errors of the measurements.

A summary can be drawn to P starvation that no noteworthy variation has been found for the contents of protein, phycobiliprotein, chlorophyll and carotenoids, so cells could survive in the case of P- without changing their composition. In addition, due to the lack of relevant researches on P starvation for *Rhodomonas sp.*, it is not possible to verify the accuracy and reliability of the experimental results compared with previous findings for the time being.

## 5.3 Experiment defects

There are some errors in this experiment, which might have affected the accuracy of the experimental results in different degrees. Firstly, the optimal growth conditions of temperature, pH and light intensity applied in this experiment were the test results on *Rhodomonas Salina* obtained from a previous report. However, these values might vary slightly for *Rhodomonas sp.*. Thus, researches on optimal growth conditions of temperature, light intensity and pH for *Rhodomonas sp.* should be carried out for more accurate result of similar experiment in the future.

Besides, all the nutrient combinations in this experiment (20x, 10x, 9x, N- and P-) were tested only once in one reactor each because of limited time, which means that more accurate results could be obtained if they could be tested in duplicate in two reactors at the same time or be repeated for a second time.

In addition, for the algae culture grown in the reactor of P-, an unexpected dilution happened at T7 (99h) because of unclosed clump (dilution amount was about 1 L), which has caused a huge fluctuation in the measurement results of *Rhodomonas sp.*’growth situation between T7 and T8. Thus, the analysis of growth situation under P- was mainly based on the results before T7.

Last but not least, it is worth noting that because the dry weight of each sample used for the measurements for protein, phycobiliprotein and pigments is about 1 mg, it is difficult to guarantee the accuracy in the process of manual weighing, which might have seriously affected the accuracy of the results. Thus, for later similar experiments, it is suggested to increase the dry weight of samples used for the measurement for biochemical compositions of algae cells as much as possible, which is helpful to improve the accuracy of the experimental results.

# 6. Conclusion

In order to grow *Rhodomonas sp.* in photobioreactors based on a turbidostat mode with a biomass of 0.956 g/L, a minimum of 0,54 g/L of Nitrate and 0,034 g/L of Phosphate are needed in the medium.

The medium with 0,54 g/L of Nitrate and 0,034 g/L Phosphate (10x medium) is optimal when wanting to achieve the highest productivity possible and leave the least amount of unused nutrients in the harvest, which can lead to a productivity of 0.837 g/L/d and the proportions of wasted nutrients are 17.04% and 26.47% respectively for nitrate and phosphate. Less content of nutrients (9x medium in this research) would result in no dilution of the algae culture in the end because of a not enough high cell density. More nutrients (20x medium in this research) would lead to a similar productivity but with more nutrients wasted.

Besides, comparing with the previous research on *Rhodomonas Baltica*, it can be found that the productivity of *Rhodomonas sp.* (0.837 g/L/d ) is significantly higher than that of *Rhodomonas Baltica* (0.486 g/L/d) when applying 10x medium, the former is about 1.7 times higher than the latter.

The cell density didn’t decrease under both N and P starvations. Applying a N starvation medium after the biomass production phase of *Rhodomonas sp.* culture would result in decreases on the contents of protein, phycobiliprotein and chlorophyll, the carotenoids content was not affected significantly. The application of P starvation didn’t affect the content of protein, phycobiliprotein, chlorophyll and carotenoids as a whole during the experiment period. Thus, N starvation is not recommended to be applied after the harvest of *Rhodomonas sp.* culture. And if there is a need, P starvation medium can be used to store the algae culture after the harvest because algae cells could survive in the case of P- without changing their composition.

In short, if Fry Marine use the optimal 10 times concentrated f/2 medium to grow *Rhodomonas sp.* in photobioreactors and set the dilution rate of chemostat mode at 0.879 day-1, they can expect a productivity of 0.837 g/L/d and the waste of nutrients will be around 17.04% of nitrate and 26.47% of phosphate. N starvation is not recommended to be applied after the final harvest and P starvation medium can be used to store the algae culture after the harvest.

Last but not least, repeated experiments on the effect of N or P starvation on the cell composition of *Rhodomonas sp.* should be carried out in the future to verify the reliability of the experimental results.

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# 

# Appendix A: Protocol for f/2 medium preparation

|  |  |  |
| --- | --- | --- |
| Stocks | per liter | |
| (1) NaNO3 | 75g | |
| (2) NaH2PO4·2H2O | 5.65g | |
| (3) Trace elements (chelated) | Na2EDTA | 4.16g |
| FeCl3·6H2O | 3.15g |
| CuSO4·5H2O | 0.01g |
| ZnSO4·7H2O | 0.022g |
| CoCl2·6H2O | 0.01g |
| MnCl2·4H2O | 0.18g |
| Na2MoO4·2H2O | 0.006g |
| (4) Vitamin mix | Cyanocobalamin (Vitamin B12) | 0.0005g |
| Thiamine HCl (Vitamin B1) | 0.1g |
| Biotin | 0.0005g |

*Note: Because of the lack of FeCl3·6H2O in the lab, the 3.15g FeCl3·6H2O is replaced with 0.51g NaFeEDTA; Already have Vitamin mix stock in the lab before the start of experiment.*

|  |  |
| --- | --- |
| Medium | per liter |
| NaNO3 | 1.0 ml |
| NaH2PO4·2H2O | 1.0 ml |
| Trace elements stock solution (1) | 1.0 ml |
| Vitamin mix stock solution (2) | 1.0 ml |

*\* Add while stirring*

*Make up to 1 liter with filtered natural seawater. Adjust pH to 8.0 with 1M NaOH or HCl. For agar add 15g per liter Bacteriological Agar. Sterilize by autoclaving for 15 minutes at 15 psi and use when cooled to room temperature.*

*Reference:*

*\* Guillard RRL & Ryther JH (1962) Studies of marine planktonic diatoms. I. Cyclotellanana Hustedt and Detonula confervaceae (Cleve) Gran. Can. J. Microbiol. 8: 229-239.*

# Appendix B: Procedure of making stock culture

1. Make artificial seawater with 30 grams of salt in one liter of dH2O.

500mL medium will be made, so first add some dH2O into the flask and then put into 15 grams of salt.

2. Accelerate the dissolution by placing the flask on a concussion table.

Put a magnetic stirrer into the flask and it needs to be removed before final volume is set.

3. Add the stocks of nutrients, trace elements and Vitamin mix into the flask, and continue to dissolve.

For the stock culture a 20x medium is needed, so 10ml solution was added into the 500ml conical flask for NaNO3, NaH2PO4·2H2O and Trace elements stock solution (1); 1ml Vitamin mix stock solution (2) was added into the flask.

4. Add sodium bicarbonate (0.7g in one-liter artificial seawater) to maintain the balance of pH as a buffer.

0.35g sodium bicarbonate was added in the experiment since the final volume is 500ml.

5. Add dH2O to make a total volume of 500 ml so the medium is done.

6. Filtrate the medium in the flow cabinet into two 300ml flasks, each flask should have more than 150ml medium (the two 300ml flasks have been autoclaved).

7. Add 10ml algae into each of the two 300ml flasks in the flow cabinet.

8. Finally, write down algae name, date and operator name on two flasks, then the cultures were placed in the Orbital Shaker Incubator where there is light, temperature and CO2 supply.

# Appendix C: Linear relation curve of OD750 and dry weight

图片包含 文字, 地图, 天空

描述已自动生成

Graph : Linear relation curve of OD750 and dry weight (obtained from Christos)

# Appendix D: Protocol for Nitrate measurement

**Materials, chemicals and solution**

Spectrophotometer DR5000, Quarts cuvettes and cuvette paper, KNO3 , 100 ppm NO3- stock solution (weigh 163,03 mg KNO3 and add to 1 L dH2O), Calibration series NO3 0,1 ppm – 2,5 ppm (by diluting the 100-ppm stock solution), Artificial seawater with same salinity as the samples, Dissolve 30 grams salt in 1 L dH2O, Whatman GF/C filter.

This method is based on analysis in the ultraviolet range. The absorbance is measured at 220 nm and 275nm.

The nitrate concentration is calculated as follows: Factor = Abs. (220 nm) – 2\*Abs. (275 nm)

For calibration: first make a range of solutions of known nitrate concentrations in artificial seawater with the same salinity as the samples.

The result is a calibration series for nitrate:

图片包含 屏幕截图

描述已自动生成

Graph : Calibration curve of nitrate

Using the formula, the unknown concentration of NO3- in the sample can be determined.

**Procedure measurements**

1. Filter all samples with a .45 filter or Whatman GF/C filter.

2. Set the Perkin Elmer spectrophotometer at “ultraviolet visible” at wavelength 275 nm and 220 nm.

3. Enter the number of samples and name them if necessary.

4. Measure the blank (artificial seawater): one cuvette filled with artificial seawater.

5. After measuring the blank, remove the cuvette and fill with sample.

6. Measure the samples and make sure there are no air bubbles on the inside or water on the outside of the cuvette.

7. Determine with help of the measured absorbance’s the factor (Factor = Abs. (220 nm) – 2\*Abs. (275 nm)).

8. Then, determine with help of the formula from the calibration series the unknown concentration of NO 3 - in the sample.

9. When diluted: keep in mind the dilution factor while calculating! Diluting is necessary when the factor is higher than the factor on de X-axis of the calibration series.

# Appendix E: Protocol for Phosphate measurement

USEPA1 PhosVer 3 (Ascorbic Acid) Method2 (Method 8048): 0.02 to 2.50 mg/L PO43–

Scope and Application: For water, wastewater and seawater.

Before starting the test: For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Materials needed for the Powder Pillow Test:

|  |  |
| --- | --- |
| Description | Quantity |
| PhosVer® 3 Phosphate Reagent powder pillow | 1 |
| Sample Cells, 1-inch, 10-mL | 2 |
| Stopper for 18 mm Tube | 1 |

Method for power pillows:

图片包含 屏幕截图

描述已自动生成

# Appendix F: Protocol for chlorophyll and total carotenoids determination

**Procedure**

1. Take 1ml of culture (the volume of sample depends on the density) and put it into a 15ml plastic vial.

2. Centrifuge at 4400rpm for 8 min at 4°C.

3. Add 5ml of methanol 100% to the pellet.

4. Put 5 min in ultrasound bath to disregard the pellet with methanol.

5. Incubate the cell suspension at 60°C for 50min.

6. Incubate the cell suspension at 0°C for 15 min.

7. Centrifuge the suspension at 4400rpm for 8 min.

8. Add more methanol if the pellet is not white after centrifugation and repeat the extraction.

9. Measure chlorophyll and carotenoid content in a spectrophotometer at 470nm, 652nm and 665nm in a quartz cuvette (blank done with methanol).

**Calculation**

Use Arnon’s equations to determine the chlorophyll and carotenoid content:

*References:*

*\* Arnon’s equation based on: Liechtenthaler: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes (1987). Methods on enzymology 148: 350-382*

*\* Temperature shock based on: Leu, K.L., Hsu, B.D.: A programmed cell disintegration of Chlorella after heat stress. (2005). Plant Science, 168: 145-152*

# Appendix G: Protocol for the estimation of phycobiliproteins

**Procedure**

1. 0.05M Phosphate buffer (containing equal volumes of 0.1 M K2HPO4 and KH2PO4) is prepared. To prepare 0.1M K2HPO4, 1.741 g K2HPO4 is added into 100 ml dH2O. For 0.1 M KH2PO4, 1.36 g KH2PO4 is added into 100 ml dH2O.

2. Equal volumes of K2HPO4 and KH2PO4 are mixed, the pH is set to 6.7 by adding KH2PO4 to K2HPO4.

3. The sample is centrifuged at 3000-4000 rpm for 10-15 mins.

4. Pellet is suspended into 5 ml newly prepared 0.05M Phosphate buffer and kept in -80 °C for 24-48 h in plastic screw-capped tubes (Avoid use of glassware during freezing).

5. Then it is kept at below 5 °C for 24-36 h for thawing.

6. The sample is centrifuged and supernatant is taken up to measure the absorbance at 545 nm (blank done with 0.05M Phosphate buffer).

**Calculation**

Where: C = phycobilin concentration

A = Absorbance

ε = Molar extinction coefficient (Phycoerythrin: 2.41\*106 L·mol-1·cm-1)

d = Path length of the cuvette (1 cm)

MW = Molecular weight of phycobilin (Phycoerythrin: 240.000 g/mol)

*Reference:*

*\* Bennett, A., & Bogorad, L. (1973). COMPLEMENTARY CHROMATIC ADAPTATION IN A FILAMENTOUSBLUE GREEN ALGA. The Journal of Cell Biology, 58(2), 419-435.*

*\* Lawrenz, E., Fedewa, E. J., & Richardson, T. L. (2011). Extraction protocols for the quantification of phycobilins in aqueous phytoplankton extracts. Journal of Applied Phycology, 23(5), 865-871. https://doi.org/10.1007/s10811-010-9600-0.*

# Appendix H: Protocol for the protein determination

**Reagents**

|  |  |  |
| --- | --- | --- |
| Solution | Preparation method | Conservation period |
| A | 2% of Na2CO3 in 0.1 N NaOH(4g/L) | 15 days |
| B | 0.5% of CuSO4·5H2O in dH2O | 1 month |
| C | Tartrate of K or Na 1% | 1 month |
| D | 50ml of A + 1ml of B + 1ml of C | 1 day |
| E | Folin reagent diluted 2 times with dH2O | 1 day |

**Procedure**

1. Put the sample in a numbered tube.

2. Add 5mL of Solution D and homogenize.

3. Let the tubes rest for 10 minutes at room temperature.

4. Add 0.5mL of Solution E and homogenize the sample.

5. Let the tubes rest for 30 minutes at room temperature.

6. Centrifuge the samples at 3000r/min for 10 minutes.

7. Transfer the supernatant into a new tube and read the absorbance at 750nm (blank done with the mix of 5mL Solution D and 0.5mL Solution E).

**Calculation**

Standard curve from Chris:

Where: y = Absorbance

x = Protein concentration (µg/mL)

*Reference:*

*\* Lowry, o.H., Rosebrough, N.J., Farr, A.L, Randall, R.J., 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193, 265-275.*

# Appendix I: Raw data of *Rhodomonas sp.*’s cell density under N starvation

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Diameter(um) | T0 | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 |
| 5.00231 | 208 | 170 | 303 | 87 | 87 | 51 | 47 | 22 | 30 | 29 | 42 | 18 |
| 5.05935 | 210 | 164 | 294 | 85 | 88 | 62 | 41 | 37 | 24 | 25 | 35 | 16 |
| 5.11703 | 223 | 168 | 256 | 72 | 80 | 46 | 44 | 33 | 16 | 18 | 25 | 12 |
| 5.17538 | 196 | 153 | 253 | 76 | 68 | 48 | 35 | 30 | 18 | 23 | 23 | 22 |
| 5.23439 | 209 | 144 | 232 | 86 | 79 | 30 | 40 | 26 | 20 | 14 | 21 | 15 |
| 5.29407 | 183 | 150 | 251 | 62 | 89 | 46 | 30 | 22 | 21 | 18 | 17 | 16 |
| 5.35443 | 200 | 139 | 238 | 69 | 81 | 41 | 42 | 36 | 14 | 16 | 16 | 16 |
| 5.41548 | 186 | 130 | 233 | 62 | 48 | 43 | 29 | 20 | 16 | 22 | 22 | 7 |
| 5.47723 | 200 | 117 | 210 | 70 | 87 | 36 | 23 | 19 | 14 | 17 | 20 | 10 |
| 5.53968 | 160 | 155 | 226 | 71 | 69 | 33 | 16 | 23 | 17 | 15 | 18 | 21 |
| 5.60284 | 172 | 120 | 185 | 58 | 41 | 35 | 32 | 19 | 20 | 7 | 21 | 5 |
| 5.66672 | 145 | 125 | 188 | 55 | 47 | 39 | 26 | 22 | 14 | 17 | 19 | 15 |
| 5.73133 | 148 | 114 | 204 | 64 | 51 | 29 | 30 | 20 | 12 | 13 | 9 | 9 |
| 5.79668 | 140 | 106 | 177 | 57 | 51 | 17 | 28 | 18 | 13 | 15 | 17 | 12 |
| 5.86277 | 134 | 78 | 172 | 49 | 43 | 21 | 30 | 29 | 16 | 11 | 12 | 11 |
| 5.92962 | 119 | 105 | 158 | 51 | 45 | 25 | 14 | 18 | 16 | 19 | 12 | 13 |
| 5.99723 | 139 | 110 | 153 | 43 | 55 | 30 | 19 | 19 | 17 | 12 | 15 | 3 |
| 6.06561 | 123 | 86 | 187 | 39 | 43 | 31 | 20 | 17 | 13 | 11 | 14 | 12 |
| 6.13477 | 132 | 108 | 124 | 53 | 42 | 42 | 20 | 10 | 8 | 8 | 13 | 10 |
| 6.20471 | 85 | 97 | 122 | 56 | 23 | 17 | 19 | 15 | 9 | 4 | 9 | 5 |
| 6.27546 | 122 | 101 | 189 | 45 | 45 | 29 | 13 | 16 | 6 | 9 | 21 | 3 |
| 6.34701 | 134 | 97 | 166 | 47 | 42 | 21 | 24 | 17 | 7 | 11 | 6 | 13 |
| 6.41938 | 145 | 108 | 148 | 48 | 44 | 25 | 25 | 14 | 11 | 11 | 8 | 2 |
| 6.49257 | 109 | 86 | 125 | 44 | 19 | 23 | 13 | 17 | 16 | 12 | 12 | 8 |
| 6.5666 | 125 | 88 | 150 | 40 | 20 | 17 | 16 | 18 | 10 | 13 | 9 | 9 |
| 6.64147 | 104 | 87 | 156 | 40 | 40 | 28 | 17 | 16 | 18 | 11 | 7 | 7 |
| 6.71719 | 105 | 96 | 139 | 27 | 35 | 15 | 24 | 30 | 13 | 21 | 13 | 11 |
| 6.79378 | 126 | 89 | 148 | 44 | 42 | 20 | 29 | 37 | 14 | 19 | 14 | 14 |
| 6.87124 | 112 | 66 | 146 | 38 | 34 | 25 | 28 | 46 | 23 | 32 | 11 | 19 |
| 6.94959 | 114 | 84 | 134 | 39 | 46 | 29 | 35 | 60 | 33 | 17 | 17 | 24 |
| 7.02883 | 105 | 90 | 154 | 47 | 34 | 27 | 64 | 79 | 63 | 20 | 25 | 37 |
| 7.10897 | 95 | 87 | 122 | 45 | 35 | 33 | 86 | 133 | 73 | 53 | 15 | 50 |
| 7.19002 | 142 | 92 | 133 | 40 | 58 | 24 | 90 | 189 | 124 | 66 | 41 | 62 |
| 7.272 | 124 | 86 | 150 | 45 | 70 | 54 | 130 | 230 | 147 | 111 | 48 | 112 |
| 7.35492 | 129 | 87 | 137 | 64 | 82 | 51 | 225 | 295 | 204 | 140 | 58 | 119 |
| 7.43878 | 101 | 79 | 117 | 61 | 122 | 77 | 282 | 357 | 250 | 171 | 75 | 144 |
| 7.52359 | 109 | 89 | 123 | 72 | 129 | 105 | 342 | 373 | 315 | 177 | 106 | 159 |
| 7.60938 | 105 | 83 | 150 | 101 | 196 | 168 | 434 | 498 | 330 | 262 | 120 | 222 |
| 7.69614 | 113 | 73 | 171 | 123 | 225 | 246 | 474 | 526 | 432 | 318 | 146 | 228 |
| 7.78389 | 121 | 84 | 134 | 179 | 315 | 303 | 511 | 558 | 482 | 346 | 206 | 297 |
| 7.87264 | 109 | 90 | 165 | 263 | 408 | 371 | 641 | 649 | 507 | 375 | 203 | 294 |
| 7.9624 | 100 | 103 | 155 | 348 | 459 | 471 | 676 | 643 | 519 | 405 | 232 | 317 |
| 8.05319 | 115 | 88 | 198 | 469 | 521 | 545 | 698 | 739 | 639 | 476 | 268 | 328 |
| 8.14501 | 146 | 107 | 200 | 573 | 651 | 581 | 726 | 724 | 607 | 482 | 321 | 371 |
| 8.23787 | 139 | 113 | 242 | 749 | 701 | 739 | 797 | 776 | 639 | 566 | 337 | 442 |
| 8.3318 | 111 | 133 | 284 | 798 | 804 | 845 | 853 | 796 | 672 | 585 | 348 | 471 |
| 8.4268 | 137 | 159 | 279 | 1003 | 922 | 903 | 877 | 862 | 668 | 607 | 434 | 367 |
| 8.52288 | 150 | 198 | 356 | 1083 | 946 | 1025 | 899 | 898 | 672 | 649 | 470 | 461 |
| 8.62006 | 159 | 203 | 468 | 1270 | 1093 | 1139 | 967 | 857 | 704 | 772 | 517 | 465 |
| 8.71834 | 199 | 261 | 487 | 1363 | 1242 | 1241 | 1002 | 837 | 775 | 725 | 519 | 474 |
| 8.81775 | 233 | 304 | 634 | 1492 | 1249 | 1318 | 977 | 844 | 796 | 761 | 510 | 486 |
| 8.91828 | 251 | 357 | 711 | 1614 | 1354 | 1269 | 945 | 884 | 727 | 733 | 522 | 490 |
| 9.01997 | 315 | 440 | 830 | 1774 | 1413 | 1387 | 1005 | 841 | 643 | 739 | 536 | 473 |
| 9.12281 | 346 | 472 | 962 | 1882 | 1461 | 1399 | 985 | 774 | 703 | 730 | 549 | 438 |
| 9.22683 | 450 | 568 | 1112 | 1896 | 1507 | 1400 | 902 | 746 | 672 | 711 | 584 | 447 |
| 9.33203 | 527 | 676 | 1239 | 2032 | 1538 | 1379 | 841 | 663 | 619 | 664 | 576 | 378 |
| 9.43844 | 659 | 792 | 1281 | 1992 | 1673 | 1390 | 824 | 653 | 564 | 638 | 575 | 438 |
| 9.54605 | 671 | 879 | 1384 | 1981 | 1608 | 1325 | 722 | 598 | 548 | 580 | 565 | 331 |
| 9.65489 | 836 | 1054 | 1516 | 1967 | 1677 | 1270 | 735 | 554 | 553 | 555 | 532 | 322 |
| 9.76498 | 979 | 1127 | 1617 | 1944 | 1684 | 1191 | 725 | 532 | 466 | 462 | 500 | 325 |
| 9.87632 | 1137 | 1246 | 1819 | 1990 | 1789 | 1182 | 643 | 469 | 444 | 490 | 514 | 259 |
| 9.98892 | 1192 | 1299 | 1813 | 1998 | 1759 | 1093 | 594 | 400 | 378 | 420 | 463 | 244 |
| 10.1028 | 1299 | 1355 | 1876 | 1904 | 1770 | 1191 | 567 | 387 | 321 | 383 | 440 | 184 |
| 10.218 | 1363 | 1408 | 1934 | 1945 | 1710 | 1057 | 553 | 329 | 303 | 331 | 367 | 195 |
| 10.3345 | 1406 | 1501 | 1989 | 1924 | 1554 | 1108 | 478 | 302 | 310 | 302 | 379 | 178 |
| 10.4523 | 1525 | 1532 | 1861 | 1888 | 1636 | 1016 | 455 | 245 | 224 | 288 | 359 | 144 |
| 10.5715 | 1705 | 1591 | 1833 | 1914 | 1525 | 983 | 406 | 236 | 200 | 243 | 310 | 149 |
| 10.6921 | 1661 | 1578 | 1840 | 1770 | 1504 | 982 | 337 | 189 | 166 | 156 | 272 | 118 |
| 10.814 | 1774 | 1720 | 1614 | 1669 | 1337 | 794 | 288 | 156 | 138 | 146 | 225 | 106 |
| 10.9373 | 1914 | 1691 | 1671 | 1550 | 1188 | 743 | 235 | 106 | 122 | 123 | 224 | 67 |
| 11.062 | 1920 | 1724 | 1636 | 1502 | 1047 | 729 | 227 | 102 | 94 | 98 | 191 | 44 |
| 11.1881 | 1808 | 1743 | 1618 | 1392 | 931 | 633 | 158 | 85 | 73 | 92 | 173 | 49 |
| 11.3157 | 1966 | 1762 | 1480 | 1229 | 760 | 545 | 147 | 49 | 51 | 57 | 120 | 36 |
| 11.4447 | 2020 | 1789 | 1450 | 1067 | 641 | 426 | 120 | 27 | 38 | 48 | 112 | 20 |
| 11.5752 | 1900 | 1828 | 1395 | 1058 | 512 | 361 | 123 | 39 | 37 | 28 | 63 | 11 |
| 11.7071 | 1911 | 1753 | 1325 | 866 | 420 | 335 | 82 | 28 | 25 | 26 | 69 | 15 |
| 11.8406 | 1854 | 1716 | 1193 | 809 | 310 | 245 | 59 | 17 | 18 | 28 | 58 | 13 |
| 11.9756 | 1715 | 1695 | 1122 | 677 | 233 | 188 | 64 | 12 | 18 | 10 | 59 | 2 |
| 12.1122 | 1620 | 1608 | 1041 | 531 | 208 | 144 | 36 | 11 | 17 | 10 | 36 | 4 |
| 12.2503 | 1467 | 1536 | 936 | 509 | 179 | 138 | 30 | 17 | 13 | 8 | 36 | 4 |
| 12.39 | 1347 | 1430 | 884 | 425 | 146 | 105 | 27 | 14 | 6 | 6 | 25 | 5 |
| 12.5312 | 1134 | 1206 | 760 | 351 | 128 | 73 | 28 | 13 | 7 | 3 | 25 | 4 |
| 12.6741 | 1036 | 1136 | 623 | 233 | 89 | 81 | 22 | 6 | 5 | 5 | 29 | 2 |
| 12.8186 | 868 | 919 | 583 | 223 | 106 | 56 | 14 | 10 | 3 | 5 | 17 | 2 |
| 12.9648 | 701 | 756 | 478 | 200 | 69 | 34 | 13 | 2 | 2 | 6 | 21 | 0 |
| 13.1126 | 549 | 615 | 383 | 161 | 77 | 31 | 11 | 6 | 4 | 2 | 18 | 0 |
| 13.2621 | 431 | 489 | 315 | 156 | 51 | 32 | 10 | 9 | 3 | 4 | 18 | 3 |
| 13.4133 | 329 | 375 | 256 | 119 | 48 | 30 | 6 | 4 | 2 | 3 | 10 | 1 |
| 13.5662 | 262 | 290 | 225 | 99 | 41 | 23 | 9 | 2 | 1 | 4 | 18 | 0 |
| 13.7209 | 211 | 218 | 179 | 85 | 36 | 16 | 9 | 9 | 4 | 2 | 12 | 2 |
| 13.8774 | 165 | 170 | 146 | 61 | 24 | 13 | 6 | 4 | 3 | 2 | 19 | 0 |
| 14.0356 | 162 | 143 | 111 | 59 | 19 | 9 | 2 | 4 | 2 | 3 | 9 | 0 |
| 14.1956 | 111 | 112 | 124 | 42 | 12 | 10 | 6 | 5 | 0 | 2 | 4 | 2 |
| 14.3575 | 118 | 138 | 85 | 31 | 12 | 14 | 5 | 5 | 0 | 1 | 5 | 3 |
| 14.5212 | 74 | 104 | 101 | 25 | 11 | 5 | 2 | 2 | 1 | 1 | 5 | 1 |
| 14.6868 | 65 | 82 | 71 | 29 | 8 | 5 | 5 | 4 | 1 | 2 | 4 | 1 |
| 14.8542 | 48 | 69 | 57 | 22 | 3 | 6 | 5 | 2 | 0 | 0 | 7 | 1 |
| 15.0236 | 52 | 51 | 58 | 12 | 1 | 4 | 5 | 2 | 2 | 3 | 5 | 0 |
| 15.1949 | 38 | 62 | 35 | 13 | 3 | 2 | 3 | 2 | 2 | 1 | 1 | 1 |
| 15.3681 | 31 | 30 | 13 | 9 | 4 | 4 | 3 | 6 | 1 | 1 | 1 | 3 |
| 15.5433 | 24 | 36 | 23 | 10 | 3 | 1 | 3 | 0 | 0 | 1 | 3 | 0 |
| 15.7206 | 12 | 35 | 20 | 7 | 3 | 1 | 6 | 2 | 2 | 0 | 0 | 0 |
| 15.8998 | 16 | 23 | 21 | 10 | 1 | 0 | 2 | 6 | 0 | 2 | 3 | 0 |
| 16.0811 | 12 | 22 | 17 | 4 | 1 | 2 | 3 | 3 | 0 | 1 | 0 | 0 |
| 16.2645 | 12 | 18 | 16 | 9 | 1 | 0 | 6 | 6 | 0 | 0 | 2 | 0 |
| 16.4499 | 4 | 11 | 16 | 7 | 1 | 0 | 3 | 2 | 0 | 1 | 1 | 0 |
| 16.6375 | 9 | 10 | 15 | 3 | 1 | 2 | 3 | 3 | 0 | 1 | 1 | 1 |
| 16.8272 | 5 | 9 | 9 | 3 | 3 | 1 | 4 | 1 | 0 | 0 | 3 | 1 |
| 17.019 | 1 | 9 | 8 | 1 | 2 | 0 | 2 | 6 | 3 | 0 | 1 | 0 |

# Appendix J: Raw data of *Rhodomonas sp.*’s cell density under P starvation

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Diameter(um) | T0 | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 |
| 5.00231 | 317 | 192 | 117 | 48 | 24 | 29 | 24 | 36 | 13 | 8 | 22 | 14 |
| 5.05935 | 291 | 202 | 122 | 48 | 25 | 25 | 29 | 33 | 14 | 14 | 23 | 11 |
| 5.11703 | 331 | 182 | 125 | 30 | 14 | 30 | 31 | 27 | 18 | 17 | 14 | 16 |
| 5.17538 | 265 | 169 | 111 | 33 | 23 | 18 | 30 | 23 | 14 | 24 | 19 | 16 |
| 5.23439 | 302 | 173 | 107 | 35 | 19 | 33 | 25 | 19 | 15 | 9 | 17 | 12 |
| 5.29407 | 283 | 167 | 118 | 30 | 16 | 28 | 24 | 26 | 7 | 11 | 16 | 10 |
| 5.35443 | 275 | 144 | 115 | 34 | 17 | 25 | 21 | 32 | 7 | 9 | 13 | 11 |
| 5.41548 | 289 | 177 | 91 | 35 | 9 | 16 | 23 | 15 | 11 | 11 | 20 | 17 |
| 5.47723 | 282 | 163 | 123 | 31 | 9 | 9 | 21 | 27 | 8 | 9 | 13 | 13 |
| 5.53968 | 280 | 159 | 90 | 31 | 15 | 17 | 32 | 22 | 8 | 7 | 14 | 13 |
| 5.60284 | 259 | 149 | 91 | 35 | 17 | 19 | 16 | 18 | 15 | 6 | 12 | 9 |
| 5.66672 | 241 | 158 | 98 | 29 | 11 | 24 | 8 | 25 | 10 | 18 | 17 | 10 |
| 5.73133 | 280 | 128 | 98 | 23 | 14 | 23 | 24 | 20 | 9 | 5 | 15 | 11 |
| 5.79668 | 248 | 143 | 109 | 25 | 11 | 12 | 10 | 21 | 6 | 15 | 19 | 13 |
| 5.86277 | 224 | 138 | 97 | 30 | 11 | 11 | 24 | 25 | 13 | 10 | 16 | 13 |
| 5.92962 | 221 | 145 | 94 | 18 | 13 | 10 | 19 | 15 | 6 | 6 | 10 | 11 |
| 5.99723 | 210 | 134 | 97 | 15 | 14 | 8 | 13 | 16 | 2 | 10 | 11 | 2 |
| 6.06561 | 219 | 146 | 87 | 29 | 15 | 10 | 16 | 22 | 5 | 6 | 8 | 13 |
| 6.13477 | 223 | 121 | 70 | 26 | 11 | 11 | 12 | 12 | 6 | 5 | 12 | 5 |
| 6.20471 | 195 | 123 | 74 | 28 | 7 | 9 | 22 | 20 | 6 | 7 | 7 | 5 |
| 6.27546 | 194 | 151 | 86 | 25 | 7 | 10 | 9 | 22 | 8 | 8 | 10 | 6 |
| 6.34701 | 193 | 134 | 71 | 18 | 12 | 9 | 13 | 14 | 9 | 5 | 12 | 6 |
| 6.41938 | 194 | 134 | 67 | 27 | 18 | 5 | 12 | 20 | 10 | 3 | 15 | 3 |
| 6.49257 | 186 | 136 | 65 | 23 | 7 | 7 | 22 | 11 | 5 | 4 | 11 | 5 |
| 6.5666 | 158 | 149 | 60 | 21 | 12 | 7 | 10 | 11 | 12 | 2 | 11 | 11 |
| 6.64147 | 177 | 102 | 63 | 22 | 13 | 16 | 14 | 16 | 10 | 5 | 5 | 7 |
| 6.71719 | 172 | 124 | 65 | 20 | 12 | 11 | 15 | 16 | 9 | 10 | 8 | 6 |
| 6.79378 | 162 | 115 | 76 | 25 | 10 | 13 | 18 | 8 | 9 | 8 | 5 | 10 |
| 6.87124 | 143 | 111 | 57 | 17 | 7 | 11 | 14 | 6 | 4 | 4 | 6 | 6 |
| 6.94959 | 166 | 128 | 83 | 17 | 7 | 8 | 14 | 14 | 8 | 8 | 10 | 4 |
| 7.02883 | 168 | 127 | 71 | 17 | 7 | 13 | 12 | 19 | 3 | 6 | 8 | 4 |
| 7.10897 | 164 | 109 | 59 | 8 | 16 | 8 | 16 | 9 | 3 | 17 | 8 | 7 |
| 7.19002 | 155 | 105 | 58 | 13 | 3 | 10 | 12 | 11 | 4 | 7 | 4 | 5 |
| 7.272 | 151 | 129 | 65 | 20 | 14 | 17 | 18 | 20 | 7 | 7 | 6 | 6 |
| 7.35492 | 136 | 108 | 62 | 12 | 14 | 14 | 16 | 13 | 10 | 12 | 12 | 7 |
| 7.43878 | 155 | 101 | 73 | 18 | 13 | 13 | 20 | 11 | 2 | 8 | 8 | 11 |
| 7.52359 | 130 | 104 | 58 | 18 | 12 | 10 | 17 | 13 | 6 | 6 | 10 | 12 |
| 7.60938 | 141 | 114 | 60 | 17 | 13 | 11 | 20 | 22 | 7 | 14 | 16 | 9 |
| 7.69614 | 140 | 96 | 67 | 20 | 11 | 8 | 25 | 24 | 3 | 14 | 19 | 17 |
| 7.78389 | 147 | 84 | 66 | 18 | 13 | 15 | 42 | 29 | 17 | 31 | 20 | 27 |
| 7.87264 | 149 | 96 | 61 | 27 | 20 | 5 | 38 | 39 | 12 | 29 | 30 | 32 |
| 7.9624 | 138 | 91 | 55 | 19 | 23 | 12 | 58 | 48 | 27 | 47 | 33 | 42 |
| 8.05319 | 130 | 106 | 66 | 29 | 39 | 15 | 77 | 74 | 30 | 43 | 51 | 70 |
| 8.14501 | 145 | 84 | 76 | 34 | 37 | 19 | 79 | 72 | 21 | 73 | 80 | 68 |
| 8.23787 | 166 | 87 | 89 | 31 | 63 | 28 | 131 | 109 | 30 | 90 | 113 | 103 |
| 8.3318 | 187 | 77 | 118 | 51 | 69 | 41 | 154 | 137 | 61 | 115 | 115 | 131 |
| 8.4268 | 192 | 60 | 98 | 52 | 76 | 54 | 176 | 154 | 54 | 159 | 144 | 145 |
| 8.52288 | 218 | 68 | 150 | 61 | 124 | 52 | 215 | 187 | 80 | 159 | 158 | 160 |
| 8.62006 | 235 | 72 | 154 | 78 | 133 | 66 | 276 | 266 | 80 | 182 | 166 | 163 |
| 8.71834 | 238 | 83 | 154 | 76 | 174 | 97 | 280 | 249 | 99 | 174 | 174 | 209 |
| 8.81775 | 273 | 76 | 187 | 125 | 176 | 117 | 327 | 299 | 102 | 198 | 219 | 192 |
| 8.91828 | 292 | 65 | 187 | 145 | 191 | 123 | 334 | 326 | 115 | 199 | 221 | 218 |
| 9.01997 | 366 | 52 | 233 | 182 | 224 | 181 | 405 | 369 | 109 | 249 | 243 | 237 |
| 9.12281 | 500 | 64 | 263 | 202 | 269 | 195 | 400 | 409 | 152 | 247 | 218 | 226 |
| 9.22683 | 552 | 84 | 292 | 250 | 275 | 194 | 432 | 437 | 156 | 244 | 263 | 207 |
| 9.33203 | 601 | 97 | 297 | 226 | 339 | 247 | 465 | 431 | 173 | 254 | 251 | 273 |
| 9.43844 | 648 | 99 | 372 | 313 | 331 | 246 | 446 | 464 | 161 | 270 | 257 | 235 |
| 9.54605 | 744 | 81 | 387 | 354 | 326 | 287 | 524 | 476 | 182 | 244 | 263 | 250 |
| 9.65489 | 822 | 131 | 384 | 360 | 376 | 290 | 564 | 512 | 202 | 263 | 301 | 280 |
| 9.76498 | 911 | 116 | 464 | 416 | 397 | 332 | 512 | 512 | 220 | 277 | 269 | 307 |
| 9.87632 | 1009 | 162 | 493 | 398 | 413 | 340 | 488 | 601 | 204 | 247 | 298 | 271 |
| 9.98892 | 1067 | 158 | 521 | 365 | 424 | 330 | 576 | 565 | 205 | 303 | 304 | 225 |
| 10.1028 | 1141 | 199 | 486 | 384 | 391 | 371 | 521 | 536 | 190 | 238 | 261 | 275 |
| 10.218 | 1260 | 232 | 552 | 393 | 389 | 355 | 555 | 547 | 189 | 249 | 293 | 256 |
| 10.3345 | 1298 | 214 | 588 | 356 | 358 | 304 | 513 | 533 | 199 | 257 | 275 | 249 |
| 10.4523 | 1388 | 272 | 544 | 385 | 372 | 378 | 479 | 477 | 199 | 235 | 253 | 249 |
| 10.5715 | 1370 | 280 | 586 | 393 | 375 | 363 | 443 | 481 | 192 | 213 | 243 | 199 |
| 10.6921 | 1487 | 336 | 616 | 355 | 353 | 356 | 445 | 462 | 207 | 197 | 235 | 212 |
| 10.814 | 1483 | 310 | 587 | 359 | 313 | 349 | 433 | 433 | 193 | 179 | 210 | 201 |
| 10.9373 | 1561 | 308 | 499 | 351 | 293 | 340 | 448 | 436 | 172 | 190 | 203 | 176 |
| 11.062 | 1548 | 358 | 533 | 366 | 257 | 313 | 481 | 430 | 146 | 167 | 191 | 151 |
| 11.1881 | 1592 | 349 | 549 | 345 | 251 | 284 | 384 | 398 | 153 | 155 | 148 | 116 |
| 11.3157 | 1554 | 381 | 502 | 364 | 233 | 296 | 369 | 387 | 166 | 149 | 145 | 121 |
| 11.4447 | 1514 | 383 | 494 | 342 | 236 | 271 | 341 | 352 | 136 | 142 | 134 | 116 |
| 11.5752 | 1399 | 373 | 442 | 329 | 193 | 255 | 325 | 339 | 102 | 116 | 156 | 113 |
| 11.7071 | 1410 | 427 | 433 | 290 | 168 | 232 | 328 | 281 | 129 | 125 | 119 | 105 |
| 11.8406 | 1265 | 453 | 385 | 279 | 176 | 225 | 273 | 254 | 100 | 104 | 116 | 85 |
| 11.9756 | 1157 | 451 | 351 | 292 | 139 | 227 | 282 | 246 | 87 | 90 | 96 | 63 |
| 12.1122 | 992 | 446 | 331 | 265 | 114 | 202 | 236 | 189 | 70 | 64 | 70 | 70 |
| 12.2503 | 915 | 417 | 285 | 262 | 106 | 198 | 197 | 168 | 66 | 76 | 60 | 52 |
| 12.39 | 852 | 442 | 277 | 208 | 88 | 159 | 159 | 112 | 42 | 47 | 44 | 43 |
| 12.5312 | 720 | 457 | 221 | 170 | 64 | 121 | 129 | 99 | 42 | 40 | 47 | 29 |
| 12.6741 | 606 | 409 | 206 | 178 | 62 | 109 | 115 | 84 | 41 | 22 | 30 | 22 |
| 12.8186 | 470 | 367 | 230 | 143 | 37 | 78 | 69 | 68 | 27 | 27 | 24 | 20 |
| 12.9648 | 346 | 294 | 188 | 134 | 25 | 54 | 64 | 52 | 23 | 15 | 17 | 14 |
| 13.1126 | 281 | 300 | 118 | 98 | 25 | 41 | 45 | 30 | 9 | 8 | 20 | 10 |
| 13.2621 | 215 | 216 | 118 | 82 | 15 | 42 | 31 | 23 | 6 | 6 | 8 | 4 |
| 13.4133 | 204 | 203 | 92 | 59 | 18 | 31 | 19 | 17 | 5 | 4 | 8 | 5 |
| 13.5662 | 143 | 149 | 102 | 39 | 11 | 25 | 19 | 13 | 2 | 6 | 2 | 1 |
| 13.7209 | 114 | 124 | 66 | 38 | 4 | 10 | 8 | 7 | 4 | 5 | 5 | 2 |
| 13.8774 | 98 | 99 | 67 | 30 | 3 | 14 | 6 | 14 | 3 | 4 | 3 | 1 |
| 14.0356 | 90 | 60 | 36 | 19 | 1 | 10 | 13 | 11 | 3 | 1 | 3 | 2 |
| 14.1956 | 65 | 44 | 34 | 10 | 1 | 6 | 4 | 6 | 0 | 1 | 1 | 3 |
| 14.3575 | 61 | 25 | 20 | 8 | 1 | 5 | 9 | 8 | 2 | 0 | 2 | 0 |
| 14.5212 | 50 | 22 | 21 | 4 | 1 | 7 | 3 | 8 | 0 | 1 | 3 | 1 |
| 14.6868 | 38 | 13 | 24 | 10 | 1 | 4 | 2 | 0 | 1 | 0 | 2 | 2 |
| 14.8542 | 33 | 13 | 15 | 5 | 3 | 2 | 6 | 5 | 1 | 1 | 1 | 0 |
| 15.0236 | 33 | 5 | 12 | 4 | 3 | 2 | 3 | 3 | 1 | 1 | 1 | 0 |
| 15.1949 | 17 | 10 | 14 | 1 | 0 | 0 | 4 | 2 | 0 | 1 | 1 | 0 |
| 15.3681 | 20 | 8 | 8 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 |
| 15.5433 | 13 | 7 | 6 | 3 | 1 | 2 | 2 | 4 | 0 | 0 | 1 | 0 |
| 15.7206 | 15 | 5 | 4 | 1 | 0 | 0 | 1 | 2 | 0 | 1 | 1 | 0 |
| 15.8998 | 8 | 3 | 7 | 4 | 0 | 2 | 1 | 3 | 0 | 0 | 2 | 0 |
| 16.0811 | 11 | 1 | 3 | 1 | 0 | 0 | 3 | 2 | 0 | 1 | 4 | 0 |
| 16.2645 | 9 | 4 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 3 | 0 |
| 16.4499 | 5 | 3 | 0 | 0 | 0 | 2 | 2 | 1 | 0 | 0 | 0 | 0 |
| 16.6375 | 2 | 3 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 0 |
| 16.8272 | 4 | 2 | 3 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 0 | 0 |
| 17.019 | 4 | 3 | 4 | 0 | 1 | 2 | 1 | 2 | 0 | 0 | 1 | 0 |

# Appendix K: Chlb content under N and P starvations

Graph : Chl b content under N and P starvations

# Appendix L: Result of daily measurement and calculations for 10x and 20x medium

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 10x medium (Result of calculations) | | | | | | | | | |
| Date | Cell density | Dry weight | Growth rate | Productivity | Biomass yield on light | NO3- left | NO3- consumed | PO43- left | PO43- consumed |
| 2019.3.29 | 7092900 | 0.834562 | 0.957433775 | 0.79903785 | 0.486743328 | 0.0935175 | 0.4464825 | 0.009 | 0.025 |
| 2019.3.30 | 7581250 | 0.887023 | 0.948649282 | 0.84147373 | 0.509148283 | 0.1215605 | 0.4184395 | 0.0085 | 0.0255 |
| 2019.3.31 | 8023550 | 0.977473 | 1.038375013 | 1.01498354 | 0.61482215 | 0.123351 | 0.416649 | 0.0085 | 0.0255 |
| 2019.4.1 | 7591600 | 0.972046 | 0.977121326 | 0.94980688 | 0.583853915 | 0.1072365 | 0.4327635 | 0.009 | 0.025 |
| 2019.4.2 | 6409350 | 0.845416 | 0.711381794 | 0.60141355 | 0.366909901 | 0.073217 | 0.466783 | 0.0085 | 0.0255 |
| 2019.4.3 | 9358050 | 1.214452 | 0.769648257 | 0.93470087 | 0.567464064 | 0.0535215 | 0.4864785 | 0.009 | 0.025 |
| 2019.4.4 | 7446850 | 0.957574 | 0.747105883 | 0.71540917 | 0.433032432 | 0.0750075 | 0.4649925 | 0.0085 | 0.0255 |
| Average | 7643364.29 | 0.95550657 | 0.878530761 | 0.83668937 | 0.508853439 | 0.09248736 | 0.447512643 | 0.008714286 | 0.025285714 |
| Standard deviation | 908812.967 | 0.12861481 | 0.13132609 | 0.14473813 | 0.088199945 | 0.02649415 | 0.026494152 | 0.000267261 | 0.000267261 |

Table : Result of calculations for dry weight, growth rate, productivity, biomass yield on light and nutrients consumption of 10x medium

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 20x medium (Result of calculations) | | | | | | | | | |
| Date | Cell density | Dry weight | Growth rate | Productivity | Biomass yield on light | NO3- left | NO3- consumed | PO43- left | PO43- consumed |
| 2019.2.23 | 6411900 | 0.738685 | 1.127989813 | 0.83322916 | 0.547988617 | 0.56304 | 0.51696 | 0.0405 | 0.0275 |
| 2019.2.24 | 6303800 | 0.789337 | 1.132176616 | 0.89366889 | 0.595471931 | 0.580945 | 0.499055 | 0.042 | 0.026 |
| 2019.2.27 | 6764400 | 0.82009 | 1.020910885 | 0.83723881 | 0.560869583 | 0.5719925 | 0.5080075 | 0.043 | 0.025 |
| 2019.2.28 | 6667100 | 0.977473 | 0.857622712 | 0.83830305 | 0.558810197 | 0.56304 | 0.51696 | 0.0375 | 0.0305 |
| Average | 6536800 | 0.83139625 | 1.034675007 | 0.85060998 | 0.565785082 | 0.56975438 | 0.510245625 | 0.04075 | 0.02725 |
| Standard deviation | 214995.24 | 0.10300583 | 0.128777823 | 0.02878896 | 0.020581823 | 0.00857137 | 0.008571366 | 0.002397916 | 0.002397916 |

Table : Result of calculations for dry weight, growth rate, productivity, biomass yield on light and nutrients consumption of 20x medium

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 10x medium (Daily measurement) | | | | | | | | | | | | | |
| Date | Time | Time  Difference | Harvest weight | Secondary light intensity | Absorbed light intensity | Cell density (cell/mL) | | OD750(Abs)  (diluted by 5 times) | | NO3- left (mg/L) | | PO43- left (mg/L) | |
|  |  | (day) | (g) | (μmol/m2/sec)  ingoing light intensity: 300.8μmol/m2/sec | | sample 1 | sample 2 | sample 1 | sample 2 | factor 1 (1000 times diluted) | factor 2 (1000 times diluted) | sample 1 | sample 2 |
| 2019.3.29 | 9:30 | 0.9931 | 379.19 | 34.8 | 266 | 6872100 | 7313700 | 0.258 | 0.26 | 0.006 | 0.008 | 10 | 8 |
| 2019.3.30 | 9:50 | 1.0139 | 383.58 | 33 | 267.8 | 7691300 | 7471200 | 0.27 | 0.277 | 0.03 | 0.031 | 9 | 8 |
| 2019.3.31 | 11:10 | 1.0139 | 419.86 | 33.3 | 267.5 | 8451500 | 7595600 | 0.295 | 0.302 | 0.027 | 0.035 | 8 | 9 |
| 2019.4.1 | 9:50 | 0.9444 | 368.01 | 37.2 | 263.6 | 7398800 | 7784400 | 0.299 | 0.295 | 0.027 | 0.026 | 9 | 9 |
| 2019.4.2 | 10:20 | 1.0208 | 289.6 | 35.2 | 265.6 | 6253400 | 6565300 | 0.259 | 0.265 | 0.018 | 0.016 | 8 | 9 |
| 2019.4.3 | 10:25 | 1.0035 | 308.01 | 33.9 | 266.9 | 9526200 | 9189900 | 0.357 | 0.371 | 0.01 | 0.013 | 8 | 10 |
| 2019.4.4 | 10:15 | 0.9931 | 295.89 | 33.1 | 267.7 | 7615000 | 7278700 | 0.292 | 0.294 | 0.018 | 0.017 | 9 | 8 |

Table : Result of daily measurement for 10x medium

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 20x medium (Daily measurement) | | | | | | | | | | | | | |
| Date | Time | Time  Difference | Harvest weight | Secondary light intensity | Absorbed light intensity | Cell density (cell/mL) | | OD750(Abs)  (diluted by 5 times) | | NO3- left (mg/L) | | PO43- left (mg/L) | |
|  |  | (day) | (g) | (μmol/m2/sec)  ingoing light intensity: 271.981μmol/m2/sec | | sample 1 | sample 2 | sample 1 | sample 2 | factor 1 (1000 times diluted) | factor 2 (1000 times diluted) | sample 1 | sample 2 |
| 2019.2.23 | 12:20 | 0.9514 | 427.98 | 25.6 | 246.381 | 6202900 | 6620900 | 0.199 | 0.266 | 0.027 | 0.029 | 41 | 40 |
| 2019.2.24 | 11:40 | 0.9722 | 438.96 | 28.8 | 243.181 | 6001800 | 6605800 | 0.253 | 0.24 | 0.027 | 0.031 | 42 | 42 |
| 2019.2.27 | 13:40 | 0.9931 | 404.33 | 30.1 | 241.881 | 6436600 | 7092200 | 0.252 | 0.258 | 0.026 | 0.031 | 43 | 43 |
| 2019.2.28 | 13:30 | 0.9931 | 339.66 | 28.9 | 243.081 | 6926100 | 6408100 | 0.302 | 0.295 | 0.027 | 0.029 | 38 | 37 |

Table : Result of daily measurement for 20x medium

# Appendix M: Result of daily measurement for *Rhodomonas sp.*’ growth under N and P starvations

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| N Starvation | | | | | | | | | | | | |
| T | Date & Time | | T (h) | Time difference (day) | Harvest volume (mL) | Cell density (cell/mL) | | | OD750(Abs) (diluted by 5 times) | | | Dry weight (g/L) |
|  |  |  |  |  |  | sample 1 | sample 2 | average | sample 1 | sample 2 | average |  |
| T0 | 2019/4/24 | 9:00 | 0 |  | 20 | 4872900 | 4844900 | 4858900 | 0.282 | 0.283 | 0.2825 | 0.919585 |
| T1 | 17:00 | 8 | 0.25 | 20 | 4956000 | 5006600 | 4981300 | 0.275 | 0.274 | 0.2745 | 0.890641 |
| T2 | 2019/4/25 | 0:00 | 15 | 0.292 | 40 | 5248700 | 4854600 | 5051650 | 0.237 | 0.237 | 0.237 | 0.754966 |
| T3 | 9:00 | 24 | 0.375 | 40 | 5785700 | 5225600 | 5505650 | 0.245 | 0.24 | 0.2425 | 0.774865 |
| T4 | 17:00 | 32 | 0.333 | 40 | 4607800 | 4441100 | 4524450 | 0.198 | 0.192 | 0.195 | 0.60301 |
| T5 | 2019/4/26 | 9:00 | 48 | 0.667 | 50 | 4072400 | 4166200 | 4119300 | 0.153 | 0.154 | 0.1535 | 0.452863 |
| T6 | 2019/4/27 | 11:00 | 74 | 1.083 | 50 | 2522800 | 2119400 | 2321100 | 0.093 | 0.092 | 0.0925 | 0.232165 |
| T7 | 2019/4/28 | 12:00 | 99 | 1.042 | 50 | 2168800 | 2154600 | 2161700 | 0.081 | 0.081 | 0.081 | 0.190558 |
| T8 | 2019/4/29 | 9:00 | 120 | 0.875 | 50 | 1829600 | 1748000 | 1788800 | 0.073 | 0.072 | 0.0725 | 0.159805 |
| T9 | 2019/4/30 | 9:00 | 144 | 1 | 50 | 1737400 | 1712000 | 1724700 | 0.064 | 0.063 | 0.0635 | 0.127243 |
| T10 | 2019/5/1 | 9:00 | 168 | 1 | 50 | 1468400 | 1311200 | 1389800 | 0.051 | 0.052 | 0.0515 | 0.083827 |
| T11 | 2019/5/2 | 9:00 | 192 | 1 | 50 | 1150100 | 1143000 | 1146550 | 0.042 | 0.043 | 0.0425 | 0.051265 |

Table : Result of daily measurement for Rhodomonas sp.’ growth under N starvation

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| P Starvation | | | | | | | | | | | | |
| T | Date & Time | | T (h) | Time difference (day) | Harvest volume (mL) | Cell density (cell/mL) | | | OD750(Abs) (diluted by 5 times) | | | Dry weight (g/L) |
|  |  |  |  |  |  | sample 1 | sample 2 | average | sample 1 | sample 2 | average |  |
| T0 | 2019/4/24 | 9:00 | 0 |  | 20 | 3992100 | 4153900 | 4073000 | 0.229 | 0.232 | 0.2305 | 0.731449 |
| T1 | 17:00 | 8 | 0.25 | 40 | 1190000 | 1285600 | 1237800 | 0.096 | 0.095 | 0.0955 | 0.243019 |
| T2 | 2019/4/25 | 0:00 | 15 | 0.292 | 40 | 1647200 | 1521400 | 1584300 | 0.097 | 0.096 | 0.0965 | 0.246637 |
| T3 | 9:00 | 24 | 0.375 | 40 | 1131400 | 1008900 | 1070150 | 0.059 | 0.06 | 0.0595 | 0.112771 |
| T4 | 17:00 | 32 | 0.333 | 50 | 969700 | 942700 | 956200 | 0.055 | 0.05 | 0.0525 | 0.087445 |
| T5 | 2019/4/26 | 9:00 | 48 | 0.667 | 40 | 943400 | 1022300 | 982850 | 0.051 | 0.052 | 0.0515 | 0.083827 |
| T6 | 2019/4/27 | 11:00 | 74 | 1.083 | 50 | 1492400 | 1475700 | 1484050 | 0.075 | 0.075 | 0.075 | 0.16885 |
| T7 | 2019/4/28 | 12:00 | 99 | 1.042 | 50 | 1444400 | 1364200 | 1404300 | 0.077 | 0.08 | 0.0785 | 0.181513 |
| T8 | 2019/4/29 | 9:00 | 120 | 0.875 | 50 | 502700 | 545300 | 524000 | 0.032 | 0.035 | 0.0335 | 0.018703 |
| T9 | 2019/4/30 | 9:00 | 144 | 1 | 50 | 733000 | 733000 | 733000 | 0.04 | 0.04 | 0.04 | 0.04222 |
| T10 | 2019/5/1 | 9:00 | 168 | 1 | 50 | 773000 | 713700 | 743350 | 0.04 | 0.04 | 0.04 | 0.04222 |
| T11 | 2019/5/2 | 9:00 | 192 | 1 | 50 | 711700 | 713000 | 712350 | 0.038 | 0.038 | 0.038 | 0.034984 |

Table : Result of daily measurement for Rhodomonas sp.’ growth under P starvation

# Appendix N: Cell composition measurements under N and P starvations

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Protein (mg/mg) | | | | | | | | | | | | | | | |
| T | Time (h) | Dry weight of sample (mg) | | | | λ750 | | | | Protein | | | | | |
| N- | | P- | | N- | | P- | | N- | | | P- | | |
| Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Average | Sample 1 | Sample 2 | Average |
| T0 | 0 | 1.1 | 1.4 | 1.1 | 1.3 | 0.477 | 0.9 | 0.957 | 1.318 | 0.332867 | 0.493956 | 0.413412 | 0.668531 | 0.77929 | 0.723911 |
| T1 | 8 | 1.3 | 1 | 1.2 | 1.2 | 1.011 | 1.045 | 1.003 | 1.018 | 0.597633 | 0.803077 | 0.700355 | 0.642308 | 0.651923 | 0.647115 |
| T2 | 15 | 1.6 | 1.4 | 1.6 | 1.6 | 1.108 | 1.22 | 1.405 | 1.502 | 0.532212 | 0.66978 | 0.600996 | 0.675 | 0.721635 | 0.698317 |
| T3 | 24 | 1.7 | 1.6 | 0.9 | 0.9 | 1.15 | 1.261 | 0.795 | 0.47 | 0.51991 | 0.605769 | 0.562839 | 0.678632 | 0.400855 | 0.539744 |
| T4 | 32 | 1.2 | 1.2 | 1 | 0.9 | 0.651 | 0.839 | 0.931 | 0.876 | 0.416667 | 0.537179 | 0.476923 | 0.715385 | 0.747863 | 0.731624 |
| T5 | 48 | 1.6 | 1.7 | 0.9 | 0.7 | 0.784 | 0.883 | 0.828 | 0.641 | 0.376442 | 0.399095 | 0.387769 | 0.706838 | 0.703297 | 0.705067 |
| T6 | 74 | 1.8 | 1.9 | 1.2 | 1.7 | 1.188 | 0.985 | 1.32 | 1.392 | 0.507265 | 0.398381 | 0.452823 | 0.845513 | 0.629412 | 0.737462 |
| T7 | 99 | 1.1 | 1.6 | 1.1 | 1.2 | 0.59 | 1.255 | 0.875 | 0.974 | 0.411888 | 0.602885 | 0.507386 | 0.611189 | 0.623718 | 0.617453 |
| T8 | 120 | 1.1 | 0.9 | 0.5 | 0.4 | 0.592 | 0.689 | 0.623 | 0.569 | 0.413287 | 0.588034 | 0.50066 | 0.956923 | 1.092308 | 1.024615 |
| T9 | 144 | 1 | 1.3 | 0.3 | 0.3 | 0.679 | 0.761 | 0.672 | 0.763 | 0.521538 | 0.449704 | 0.485621 | 1.720513 | 1.953846 | 1.837179 |
| T10 | 168 | 0.3 | 0.3 | 0.6 | 0.2 | 0.247 | 0.614 | 0.449 | 0.394 | 0.630769 | 1.571795 | 1.101282 | 0.574359 | 1.511538 | 1.042949 |
| T11 | 192 | 0.3 | 0.7 | 0.4 | 0.3 | 0.397 | 0.506 | 0.534 | 0.8 | 1.015385 | 0.554945 | 0.785165 | 1.025 | 2.048718 | 1.536859 |

Table : Measurement of protein content under N and P starvations

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phycobiliproteins (mg/mg) | | | | | | | | | | | | | | | |
| T | T (h) | Dry weight of sample (mg) | | | | λ545 | | | | Phycobiliproteins | | | | | |
| N- | | P- | | N- | | P- | | N- | | | P- | | |
| Sample1 | Sample2 | Sample1 | Sample2 | Sample1 | Sample2 | Sample1 | Sample2 | Sample1 | Sample2 | Average | Sample1 | Sample2 | Average |
| T0 | 0 | 1.4 | 1.2 | 1.3 | 1.3 | 0.032 | 0.026 | 0.066 | 0.099 | 1.13811E-05 | 1.08E-05 | 1.10848E-05 | 2.52793E-05 | 3.79E-05 | 3.15991E-05 |
| T1 | 8 | 1 | 1 | 1.1 | 1.2 | 0.037 | 0.041 | 0.031 | 0.031 | 1.84232E-05 | 2.04E-05 | 1.94191E-05 | 1.40324E-05 | 1.29E-05 | 1.34478E-05 |
| T2 | 15 | 1.7 | 1.4 | 1.4 | 1.5 | 0.042 | 0.058 | 0.042 | 0.057 | 1.23017E-05 | 2.06E-05 | 1.6465E-05 | 1.49378E-05 | 1.89E-05 | 1.69295E-05 |
| T3 | 24 | 1.6 | 1.7 | 1 | 1.1 | 0.026 | 0.034 | 0.035 | 0.035 | 8.09129E-06 | 9.96E-06 | 9.0249E-06 | 1.74274E-05 | 1.58E-05 | 1.66352E-05 |
| T4 | 32 | 1.2 | 1.3 | 1.5 | 1.1 | 0.016 | 0.006 | 0.092 | 0.049 | 6.639E-06 | 2.3E-06 | 4.46856E-06 | 3.05394E-05 | 2.22E-05 | 2.63599E-05 |
| T5 | 48 | 1.6 | 1.6 | 0.9 | 1.2 | 0.012 | 0.009 | 0.036 | 0.054 | 3.73444E-06 | 2.8E-06 | 3.26763E-06 | 1.9917E-05 | 2.24E-05 | 2.11618E-05 |
| T6 | 74 | 1.5 | 1.6 | 1.4 | 1.6 | 0.016 | 0.012 | 0.065 | 0.083 | 5.3112E-06 | 3.73E-06 | 4.52282E-06 | 2.3118E-05 | 2.58E-05 | 2.44739E-05 |
| T7 | 99 | 1.1 | 1.5 | 1 | 1 | 0.004 | 0.003 | 0.063 | 0.044 | 1.81064E-06 | 9.96E-07 | 1.40324E-06 | 3.13693E-05 | 2.19E-05 | 2.6639E-05 |
| T8 | 120 | 0.9 | 1.3 | 0.5 | 0.6 | 0.001 | 0.003 | 0.026 | 0.008 | 5.5325E-07 | 1.15E-06 | 8.51154E-07 | 2.58921E-05 | 6.64E-06 | 1.62656E-05 |
| T9 | 144 | 1 | 1.2 | 0.3 | 0.2 | 0.008 | 0.02 | 0.025 | 0.019 | 3.9834E-06 | 8.3E-06 | 6.14108E-06 | 4.14938E-05 | 4.73E-05 | 4.43983E-05 |
| T10 | 168 | 0.6 | 1 | 0.7 | 0.4 | 0.04 | 0.005 | 0.038 | 0.014 | 3.3195E-05 | 2.49E-06 | 1.78423E-05 | 2.70302E-05 | 1.74E-05 | 2.22288E-05 |
| T11 | 192 | 0.5 | 0.7 | 0.4 | 0.3 | 0.004 | 0.005 | 0.017 | 0.01 | 3.9834E-06 | 3.56E-06 | 3.77001E-06 | 2.11618E-05 | 1.66E-05 | 1.88797E-05 |

Table : Measurement of phycobiliprotein content under N and P starvations

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Chlorophyll & Total carotenoids (mg/mg) (1) | | | | | | | | | | | | | | | | | |
| T | T (h) | Dry weight of sample (mg) | | | | λ470 | | | | λ652 | | | | λ665 | | | |
| N- | | P- | | N- | | P- | | N- | | P- | | N- | | P- | |
| Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 |
| T0 | 0 | 1.1 | 1.2 | 1.1 | 1.1 | 0.127 | 0.145 | 0.158 | 0.133 | 0.039 | 0.045 | 0.059 | 0.047 | 0.076 | 0.093 | 0.112 | 0.09 |
| T1 | 8 | 1.1 | 1.3 | 1 | 1.3 | 0.144 | 0.204 | 0.191 | 0.231 | 0.043 | 0.062 | 0.057 | 0.07 | 0.09 | 0.129 | 0.118 | 0.142 |
| T2 | 15 | 1.5 | 1.4 | 1.5 | 1.5 | 0.188 | 0.219 | 0.236 | 0.155 | 0.057 | 0.065 | 0.07 | 0.054 | 0.113 | 0.132 | 0.141 | 0.103 |
| T3 | 24 | 1.5 | 1.9 | 1 | 1 | 0.208 | 0.224 | 0.14 | 0.111 | 0.058 | 0.063 | 0.047 | 0.034 | 0.115 | 0.122 | 0.096 | 0.071 |
| T4 | 32 | 1 | 1.2 | 1.1 | 0.9 | 0.126 | 0.207 | 0.133 | 0.078 | 0.033 | 0.048 | 0.047 | 0.026 | 0.057 | 0.099 | 0.092 | 0.052 |
| T5 | 48 | 1.4 | 1.2 | 1.2 | 1.2 | 0.219 | 0.163 | 0.111 | 0.112 | 0.049 | 0.037 | 0.032 | 0.03 | 0.095 | 0.071 | 0.067 | 0.059 |
| T6 | 74 | 1.5 | 1.4 | 1.4 | 1.5 | 0.137 | 0.157 | 0.153 | 0.215 | 0.027 | 0.028 | 0.036 | 0.05 | 0.053 | 0.056 | 0.075 | 0.107 |
| T7 | 99 | 1.4 | 1.5 | 0.9 | 1 | 0.161 | 0.067 | 0.133 | 0.18 | 0.032 | 0.011 | 0.027 | 0.038 | 0.059 | 0.023 | 0.057 | 0.077 |
| T8 | 120 | 1.1 | 0.9 | 0.4 | 0.4 | 0.111 | 0.07 | 0.05 | 0.085 | 0.02 | 0.015 | 0.014 | 0.018 | 0.037 | 0.025 | 0.024 | 0.037 |
| T9 | 144 | 0.8 | 0.9 | 0.3 | 0.3 | 0.051 | 0.105 | 0.029 | 0.064 | 0.009 | 0.018 | 0.009 | 0.01 | 0.016 | 0.034 | 0.015 | 0.019 |
| T10 | 168 | 0.5 | 0.5 | 0.6 | 0.5 | 0.052 | 0.086 | 0.151 | 0.062 | 0.009 | 0.013 | 0.031 | 0.016 | 0.018 | 0.026 | 0.056 | 0.03 |
| T11 | 192 | 0.4 | 1.1 | 0.3 | 0.4 | 0.048 | 0.142 | 0.056 | 0.092 | 0.002 | 0.03 | 0.015 | 0.021 | 0.009 | 0.062 | 0.024 | 0.038 |

Table : Measurement of pigments content under N and P starvations (part 1)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Chlorophyll & Total carotenoids (mg/mg) (2) | | | | | | | | | | | |
| Chl a | | | | | | Chl b | | | | | |
| N- | | | P- | | | N- | | | P- | | |
| Sample 1 | Sample 2 | Average | Sample 1 | Sample 2 | Average | Sample 1 | Sample 2 | Average | Sample 1 | Sample 2 | Average |
| 0.005813 | 0.006666 | 0.00624 | 0.008478 | 0.006836 | 0.007657 | 0.001071 | 0.000659 | 0.000865 | 0.001909 | 0.001445 | 0.001677 |
| 0.007069 | 0.008556 | 0.007813 | 0.010156 | 0.009332 | 0.009744 | 0.000577 | 0.000767 | 0.000672 | 0.000981 | 0.001166 | 0.001073 |
| 0.00638 | 0.008058 | 0.007219 | 0.008009 | 0.005728 | 0.006869 | 0.00101 | 0.000994 | 0.001002 | 0.001082 | 0.001246 | 0.001164 |
| 0.006494 | 0.005389 | 0.005941 | 0.008222 | 0.00613 | 0.007176 | 0.001027 | 0.001045 | 0.001036 | 0.000947 | 0.000519 | 0.000733 |
| 0.004555 | 0.007091 | 0.005823 | 0.007049 | 0.00491 | 0.00598 | 0.001778 | 0.000721 | 0.00125 | 0.00125 | 0.000714 | 0.000982 |
| 0.005698 | 0.004948 | 0.005323 | 0.004825 | 0.004151 | 0.004488 | 0.001094 | 0.001029 | 0.001062 | 0.000392 | 0.000707 | 0.000549 |
| 0.002981 | 0.003399 | 0.00319 | 0.004621 | 0.006212 | 0.005416 | 0.000516 | 0.000494 | 0.000505 | 0.000406 | 0.000325 | 0.000365 |
| 0.003467 | 0.001324 | 0.002396 | 0.005489 | 0.006576 | 0.006032 | 0.000947 | 0.00011 | 0.000528 | 0.000385 | 0.000832 | 0.000608 |
| 0.002771 | 0.002182 | 0.002477 | 0.004778 | 0.007941 | 0.00636 | 0.000741 | 0.001006 | 0.000874 | 0.001934 | 0.000845 | 0.00139 |
| 0.001619 | 0.003139 | 0.002379 | 0.003928 | 0.005275 | 0.004602 | 0.000545 | 0.000732 | 0.000639 | 0.001811 | 0.00118 | 0.001496 |
| 0.003059 | 0.004419 | 0.003739 | 0.007611 | 0.004971 | 0.006291 | 0.000445 | 0.000642 | 0.000544 | 0.002346 | 0.001219 | 0.001782 |
| 0.002313 | 0.004848 | 0.00358 | 0.006157 | 0.007753 | 0.006955 | -0.00121 | 0.000479 | -0.00037 | 0.003375 | 0.002367 | 0.002871 |

Table : Measurement of pigments content under N and P starvations (part 2)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Chlorophyll & Total carotenoids (mg/mg) (3) | | | | | | | | | | | |
| Chltot | | | | | | Carottot | | | | | |
| N- | | | P- | | | N- | | | P- | | |
| Sample 1 | Sample 2 | Average | Sample 1 | Sample 2 | Average | Sample 1 | Sample 2 | Average | Sample 1 | Sample 2 | Average |
| 0.006883609 | 0.007325 | 0.007104 | 0.010386 | 0.008281 | 0.009334 | 0.003156 | 0.003525 | 0.003341 | 0.003669 | 0.00316 | 0.003414 |
| 0.007646482 | 0.009323 | 0.008485 | 0.011137 | 0.010498 | 0.010817 | 0.00385 | 0.004642 | 0.004246 | 0.005509 | 0.005149 | 0.005329 |
| 0.00739074 | 0.009053 | 0.008222 | 0.009091 | 0.006975 | 0.008033 | 0.003619 | 0.004575 | 0.004097 | 0.004601 | 0.00285 | 0.003726 |
| 0.00752052 | 0.006434 | 0.006977 | 0.00917 | 0.006649 | 0.007909 | 0.004035 | 0.003452 | 0.003744 | 0.003924 | 0.003224 | 0.003574 |
| 0.00633339 | 0.007812 | 0.007073 | 0.008299 | 0.005624 | 0.006962 | 0.003113 | 0.005135 | 0.004124 | 0.003243 | 0.002328 | 0.002785 |
| 0.00679185 | 0.005977 | 0.006384 | 0.005216 | 0.004858 | 0.005037 | 0.004554 | 0.003865 | 0.004209 | 0.002745 | 0.002651 | 0.002698 |
| 0.00349734 | 0.003893 | 0.003695 | 0.005027 | 0.006536 | 0.005782 | 0.002715 | 0.003366 | 0.003041 | 0.003299 | 0.004407 | 0.003853 |
| 0.0044136 | 0.001434 | 0.002924 | 0.005874 | 0.007408 | 0.006641 | 0.003303 | 0.001373 | 0.002338 | 0.004433 | 0.005258 | 0.004845 |
| 0.003511964 | 0.003189 | 0.00335 | 0.006713 | 0.008785 | 0.007749 | 0.002858 | 0.001915 | 0.002386 | 0.001574 | 0.005582 | 0.003578 |
| 0.002164838 | 0.003871 | 0.003018 | 0.005739 | 0.006455 | 0.006097 | 0.001681 | 0.003283 | 0.002482 | 9.84E-05 | 0.004759 | 0.002429 |
| 0.00350406 | 0.005061 | 0.004283 | 0.009957 | 0.006189 | 0.008073 | 0.002827 | 0.004773 | 0.0038 | 0.006021 | 0.002697 | 0.004359 |
| 0.00109935 | 0.005328 | 0.003213 | 0.009532 | 0.010119 | 0.009826 | 0.005199 | 0.003849 | 0.004524 | 0.000419 | 0.004332 | 0.002375 |

Table : Measurement of pigments content under N and P starvations (part 3)