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Use of selective cultivation regimes for microalgae to improve lipid and/or

Nannochloropsis

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Use of selective growth strategies to increase the specific lipid content in microalgae Nannochloropsis oculata for use in the production of biodiesel

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Abstract

Energy plays an essential role in the development of human society, but humans are facing an important issue about how to take advantage of energy eco-friendly. With the increasing depletion of fossil fuels resources and the progressive depravation of ecological environmental issue, the development of substitutes for fossil fuels resources is becoming urgent. The development of renewable, clean, pollution-free green alternative energy has received accelerating attention. While almost all the clean energy from water, sun light, geothermic energy, tidal energy and the wind are used to produce electricity, we still need a liquid fuel for the transportation in a long distance, such as the planes, cars, and the military transportations.

Microalgae biofuel can be used as biodiesel because of many advantages: microalgae have the higher photosynthetic efficiency than the other oil-producing plants. Microalgae can be grown all the year around and can be continuously provided as the raw material for biofuel.

The task for my master thesis is the continuation work from 2012 autumn project. *Nannochloropsis oculata* is one of the microalgae biodiesel sources, it is the experiment subject for the task. The main purpose of the task is to choose the growing conditions for *Nannochloropsis oculata*, the different growing medium effect on the growing parameter of *Nannochloropsis oculata*, such as the growing rate and lipid concentration, and use the Nile Red dye to detect the lipid concentration, in order to select an effective way to get high lipid content.

The result of the select methods in 2013 spring is not as good as expected. The highest lipid content in dry weight is approximately 0.025Lipid/dw[g/g]. It should mandate further study and research work, find out the better way to get high lipid content in *Nannochloropsis oculata* cells.

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1 Introduction

1.1 Background

Energy plays an essential role in the development of human society, but humans are facing an important issue about how to take advantage of energy eco-friendly. In industrialized countries, production and consumption of energy is indispensable for the economy. The development of energy resources is the prerequisite to become a developed society in many areas, such as agriculture, transport, and even information and communication technologies.

1.2 Conventional energy and renewable energy

In terms of energy categories, it can be mainly divided into conventional energy and renewable energy.

Conventional energy has mature technology and is commonly used, including primary energy from renewable water power resource and non-renewable resources, such as coal, petroleum and natural gas.

Renewable energy refers to the energy that is derived from natural resources such as water, wind, biomass, hydrogen and solar, geothermic, tidal energy. As most renewable-energy technologies are still in the stage of research and development, they have not economically competitive yet compared to the conventional energy. But the renewable energy is extremely abundant and extensively distributed in nature, thus would be the leading energy source in future.

Since the industrial revolution, the use of energy has increased drastically. Unfortunately, it poses a number of pressing problems, such as the global warming, which is the potential serious risk for the world. The atmospheric carbon dioxide concentrations have an increase of 26 per cent (from about 280 ppm to around 370 ppm, ppm: parts per million), because of the combustion of fossil fuels and deforestation. As the development trend of human economic activities, such as the huge growth of factories, manufacturing and transportation, the requirement of energy resources becomes increasingly higher, and the social

dependence and demands is also increasing rapidly. The price and the environmental safety of energy resources are now among the top concerns of the world.

With the increasing depletion of fossil fuels resources and the progressive depravation of ecological environmental issue, the development of substitutes for fossil fuels resources is becoming urgent. The development of renewable, clean, pollution-free green alternative energy has received accelerating attention, and the support from governments is also growing. As a result, the legislation process of increasing renewable energy research and industrialization is promoted significantly.[1]

1.3 Biofuel

While almost all the clean energy from water, sun light, geothermic energy, tidal energy and the wind are used to produce electricity, we still need a liquid fuel for the transportation in a long distance, such as the planes, cars, and the military transportations.

The biofuels are made from biomass (living organisms, such as plants and microalgae), extraction into solid, liquid or gaseous fuels. Biofuel has a high affinity with petroleum fuel in terms of energy density and incendiary. Biofuel can be divided into first generation biofuels and second generation biofuels.[2]

The first generation biofuels (also called agricultural biofuels), they are produced from food crop, such as soybean, corn, sugarcane and other kinds of vegetables. Such first generation biofuels need more fertile land for farming and large amounts of fresh water for irrigation. This situation competes with the land for food production, the consumption of the food sources would cause the economic problem for the increase price of the food, the risk for food crisis.

The second generation biofuels are nonfood crops biofuels, such as cellulosic ethanol and biodiesel. They are produced from cellulose, hemic celluloses and lignocellulose biomass. The raw materials are used from nonfood sources, Straw, withered grass, bagasse, rice husk, sawdust and other materials from waste crops. The animal fat and algae are used to make

biodiesel. The cost for second generation biofuels resources is very low and the source of raw material is wide. Recycling agricultural wastes ensure sustainable development of bioenergy, and resolve the issue of more energy and costly chemicals are used during the first generation biofuel production process, the treatment before it can be transformed into fuel.[3]

The second generation biofuels have much more advantages than the first generation biofuels in environmental protection and also in economics terms.

1.4 Microalgae biofuel

Microalgae biofuel can be used as biodiesel because of many advantages: microalgae have the higher photosynthetic efficiency than the other oil-producing plants. Microalgae can be grown all the year around and can be continuously provided as the raw material for biofuel. They can easily survive in seawater, reducing the waste of fresh water resource. Microalgae can use carbon dioxide to grow and produce the oil, industrial emissions of carbon dioxide can be used to cultivate microalgae so that the emission of carbon dioxide to the environment can be reduced. The production of microalgae bio biofuel has nontoxic and can be highly biodegradable. The waste land and the land cannot be farmed as well as sea beaches can be used to cultivate microalgae in biofuel industry, it does not need to take up the limited arable land resources. The fertilizer for cultivating microalgae, such as phosphorus and nitrogen, etc. can be obtained from the waste water, and the cultivation of microalgae does not need pesticides or herbicides. The residues of microalgae oil extraction also can be used as fertilizer, feed, or to produce ethanol and biogas by fermentation, it would be quite eco-friendly, as Figure 1 shows below.[4]

How can the algae oil be converted into biofuel/biodiesel? Photosynthesis converts light to chemical energy and ultimately responsible for supporting all the first step of the biofuel/biodiesel synthesis process. The integration of photosynthetic metabolic pathways is combined with kinds of complex mechanisms. This process transforms solar energy into biomass, carbon storage products, such as proteins, nucleic acids, carbohydrates, lipids, and hydrogen. (Figure2).[5]

Microalgae contain a lot of chlorophyll, and have strong photosynthetic capacity. The synthesis of microalgae oil comes from photosynthesis, solar power can be transferred into chemical energy through the processes PS I and PS II of microalgae photosynthetic system. The process is showed in figure 3.[6]

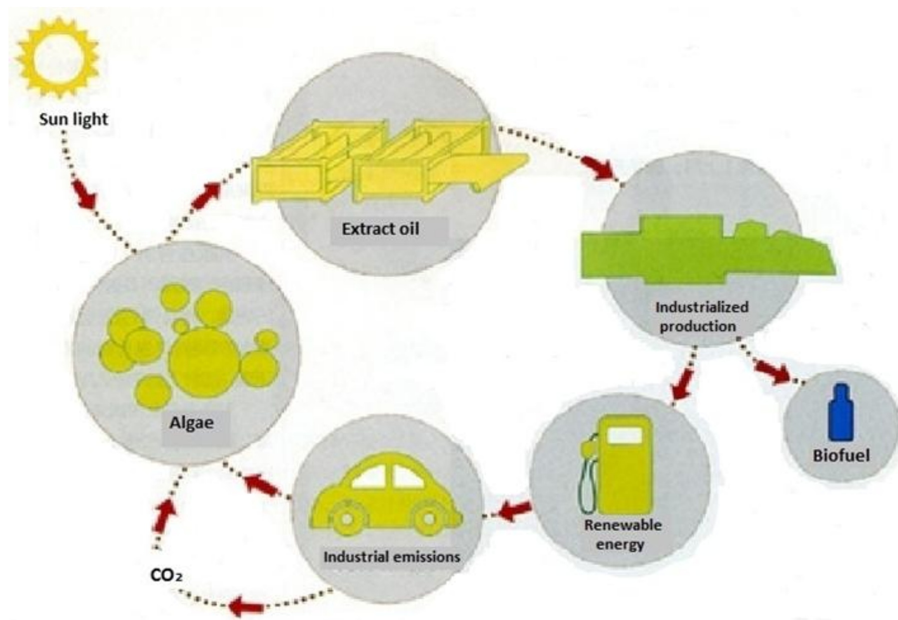


Figure 1. The cyclical model of eco-friendly microalgae oil production

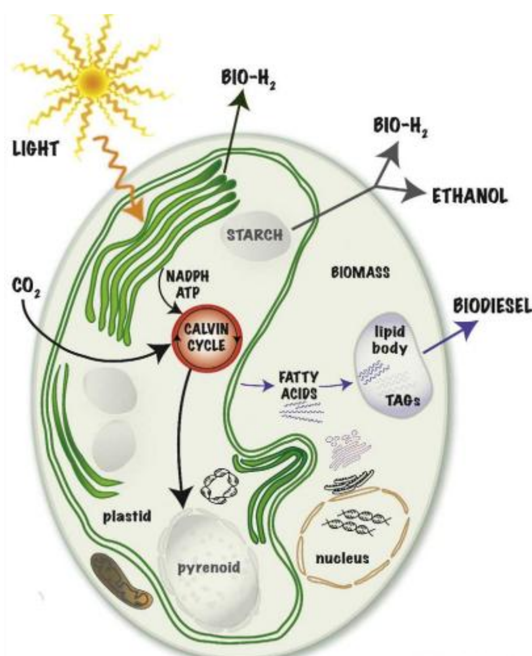


Figure 2. Metabolic pathways in microalgae related to biofuel production.

After a series of energy transfer, it enters in the Calvin cycle. In the process of Calvin cycle, the enzyme RuBisCO (Ribulose-1,5-bisphosphate carboxylase oxygenase) catalyzes the

reaction between RuBP (Ribulose-1,5-bisphosphate) with carbon dioxide, capturing the carbon dioxide, and then produce glycerate 3-phosphat. Glycerate 3-phosphat can be further produced to other oil. The enzymatic reaction is the key process to regulate the lipid synthesis rate and oxidation process. RuBisCO catalyzes the primary chemical reaction, by which inorganic carbon enters the biosphere, it is a quite important biologically enzyme.

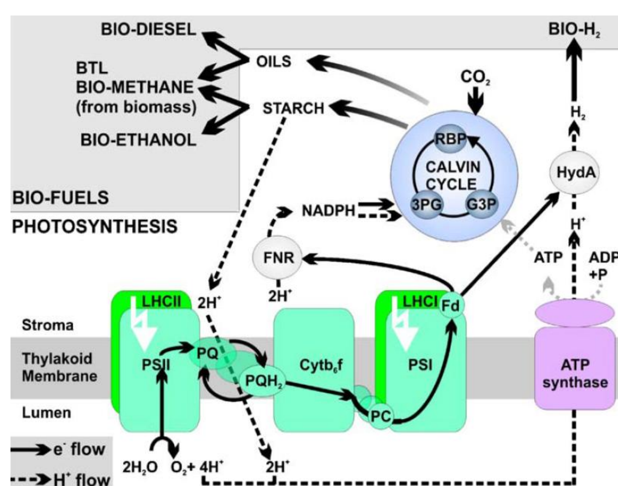


Figure 3. The process of photosynthesis coverts sun light energy into chemical energy and is key to all biofuel production systems in plants

1.5 Cultivation of microalgae

Most of algal species are photosynthetic organisms. They require light for their growth. The cultivation of microalgae for biofuel are commonly grown in open ponds systems and closed bioreactor systems.

1.5.1 Open ponds systems

Open ponds systems have lots of types for cultivate microalgae, the most commonly used design is the raceway pond. In raceway pond, the cultured algae, medium water and nutrients circulate around a race track. Providing the flow of feeding, algae keep on suspending in the medium water, and frequently circulated back to the surface. The shallow ponds continue to keep the algae exposed to the sun. Carbon dioxide and nutrients are constantly fed back into the pond, and at the other end, the water containing algae can be removed (Figure 1.5.1).[7] Culturing microalgae in large raceway ponds is the most cost-effective way. Even though the cost for the open bioreactor environment is very low, the

related technical parameters are hardly to be controlled, dependent on day-to-day weather conditions and the other conditions, such as nutrient concentration, PH values, carbon dioxide, temperature and light intensity. The microalgae grown in such a system often suffer from inconsistent growth rates and are more susceptible to local species invasion. As many microalgae cannot or has weak power to resist against other bacteria, the development of raceway ponds is limited.[8]

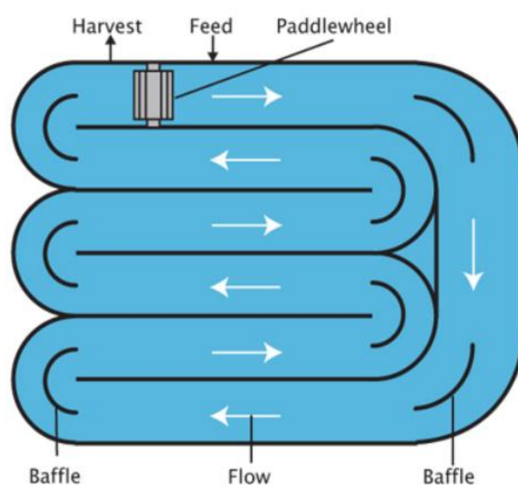


Figure 1.5.1 The first image is a large-scale Seambiotic Nannochloropsis sp. culture ponds. Image courtesy of Nature Beta Technologies Ltd, Eilat, Israel, su bsidiary of Nikken Sohonsha Co. Gifu, J apan. The second image shows the working process of the raceway pond.

1.5.2 Closed bioreactor systems

Closed bioreactor systems can provide tightly controlled cultivation conditions, and better protection against bacteria or other species invasion. Populations of microalgae can be doubling rapidly in a short time and achieving high cell densities. There are many advantages of closed bioreactor systems: saving water, energy, chemicals, pure culture environment and can be increasingly making the choice of reactor for biofuel production. Most closed bioreactors are designed as tubular reactors, plate reactors, or bubble column reactors. The tubular photo bioreactor is considered to be the most effective reactor for microalgae biomass producing (Figure 1.5.2). As figure 1.5.2 shows, the tubes are oriented from north to south, and arranged in a fence-like arrangement, in order to get the maximize sunlight capture. The ground under the tubes can be painted white or covered with white, so that it can increase reflectance and the total light received by microalgae reactor.

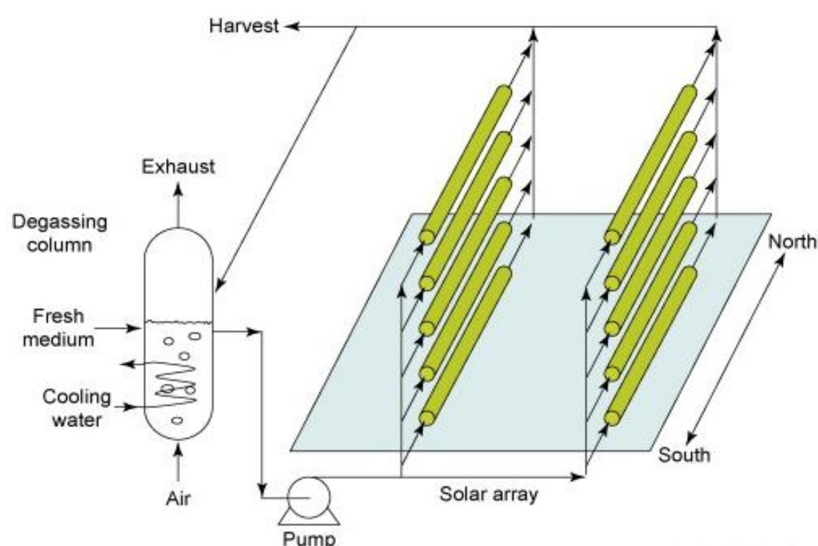


Figure 1.5.2 A tubular photobioreactor with fence-like solar collectors. Algal broth circlulates between the degassing column and the solar array with the propulsion of a pump, during which the sunlight is captured in solar array. Fresh culture medium is fed continuously to the degassing column during daylight while an equal volume of the circulating culture is removed from the stream before returning to the degassing column. Temperature is controlled by cooling water through coil inside the degassing colume. In addition, the oxygen produced from photosynthesis is extracted from the degassing column by continuously aerating the colume. [9]

1.6 Production of microalgae biodiesel

Figure 1.6.1 shows the downstream processing steps for produce biodiesel from microalgae biodiesel.[10]

The main methods to extract oil from algae are supercritical fluid (CO2) extraction, solvent extraction with hexane, and supersonic extraction. The commonly used organic solvents for extraction are either hexane or petroleum. Different extraction method has different effects. Using the same method but the different organic solvent, the result of extraction will be significantly different. In addition, lipid existing in microalgae cells, the “broken cell” improves the efficiency of oil extraction. Varying degrees of cell disruption lead to different effect. It is necessary to filter out the suitable method for particular microalgae from the numerous cell disruption methods.

During the downstream steps of microalgae biodiesel producing, transesterification is the main step. First, there is extraction of oil from microalgae (the oil is mainly triacylglyceride), and then after reaction with methanol, fatty acid methyl ester (the biodiesel) can be obtained. R1, R2, R3 are the fatty acid hydrocarbon chains. (Figure 1.6.2) After extracting triacylglyceride from microalgae, the remaining biomass fraction has the further value, which can be used as a high protein feed for livestock.[11]

1.7 The objective of the task

Nannochloropsis oculata is one of the microalgae biodiesel sources, it is the experiment subject for the task. The main purpose of the task is to choose the growing conditions for *Nannochloropsis oculata*, the different growing medium effect on the growing parameter of *Nannochloropsis oculata*, such as the growing rate and lipid concentration, and use the Nile Red dye to detect the lipid concentration, in order to select an effective way to get high lipid content. The task is the continuation work from 2012 autumn project.

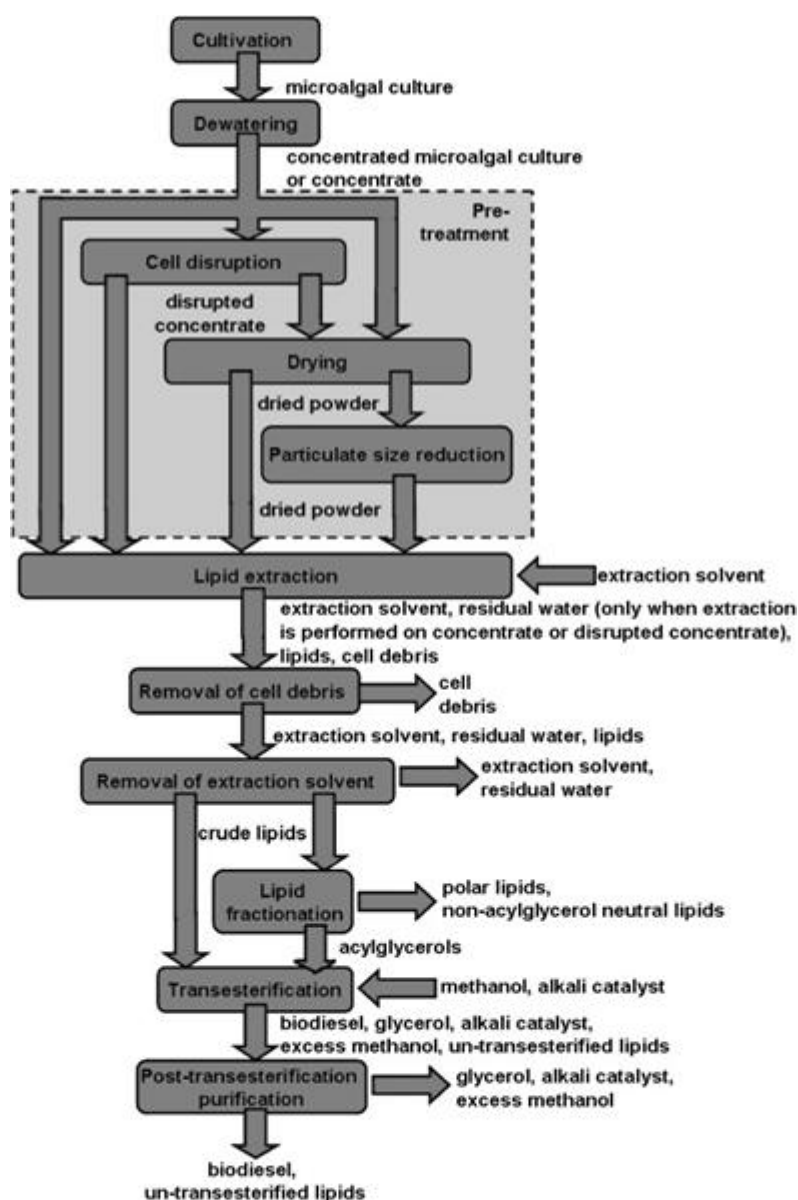


Figure 1.6.1 Process flow diagram showing the downstream processing steps needed to produce biodiesel from microalgae biodiesel.

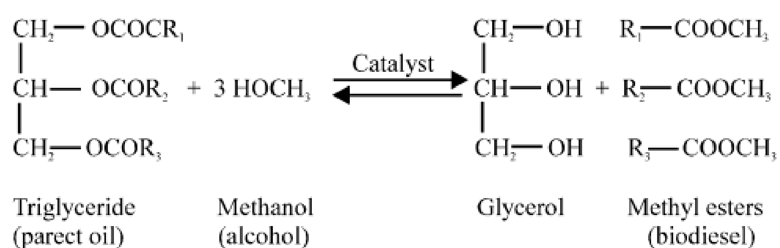


Figure 1.6.2 Transesterification of oil to biodiesel microalgae

2 Material and Method

2.1 Organisms and culture conditions

Microalgae *Nannochloropsis oculata* is the experimental subject which was obtained from NTNU Centre of Fisheries and Aquaculture. The work follows up the project 2012 autumn. The *Nannochloropsis oculata* was cultured in specific mediums and environment, in order to choose an effective way to get high lipid content. The algae was cultured in the light cabinet at the condition of 16 hours in light and 8 hours in dark, feed with air, the constant temperature is 20^0C , and the illumination intensity is 40% (Computer procedure controlling system).

Four different mediums were chosen to culture the microalgae: f/2 medium, 25% nitrogen f/2 medium, 10% nitrogen f/2 medium, and seawater medium. The medium recipes show in following table 2.1. f/2 medium is seawater medium designed for the growth of microalgae.[12]

Table 2.1 the medium recipes for culturing microalgae

Nutrition	F/2 medium	25% N f/2 medium	10% N f/2 medium	seawater medium
$NaNO_3$ (75.0 g/L dH_2O)	1.0 ml	0.25ml	0.1ml	0 ml
$NaH_2PO_4 \cdot H_2O$ (5.0 g/L dH_2O)	1.0 ml	1.0 ml	1.0 ml	0 ml
$Na_2SiO_3 \cdot 9H_2O$ (30.0 g/L dH_2O)	1.0 ml	1.0 ml	1.0 ml	0 ml
f/2 Trace Metal Solution	1.0 ml	1.0 ml	1.0 ml	0 ml
f/2 Vitamin Solution	0.5 ml	0.5 ml	0.5 ml	0 ml
Filtered seawater to	1.0 L	1.0 L	1.0 L	1.0 L

Before preparing the medium, seawater need to be filtered and then sterilizing with high temperature, in order to remove microorganisms and other impurities in seawater, keep the pure environment for objective culture.

2.2 Estimation of growth and physiology of cells

During the culturing steps, the necessary tools needed to be used to get specific parameters.

2.2.1 Parameters tested in the experiment

Ft: Instantaneous Chlorophyll Fluorescence. It was used as a biomass indicator.

QY: Quantum yield, measure the physiological state of cells.

OD: The optical density of culture measured at 750nm.

Nile red value: Indicate Nile red dye binds to the lipids and analyze the lipid content in living cells. In the appendix, the NRr means the Nile red reference value of the culture (without dye binds to intracellular lipids), the NR means the value of the culture with dye binds to intracellular lipids. The real Nile red value=NR-NRr.

dw: Try weight

Time (h): The time for culturing, and indicated by hours.

2.2.2 Equipment for parameters testing

Light cabinet: TERMAKS KB 8400L, 230V 50/56 Hz 1200W, cultured the *Nannochloropsis oculata* at the temperature is 20 ^{o}C, the light intensity is 100-150 µmol/sm^2 (16 hours light and the light turned off in 8 hours per day), and feed the culture with air 24 hours per day.

Spectrophotometer: HITACHI U-5100, measure the OD value at 750nm.

Fluormeter I: Aquapen Ap-100, make the quick measurement of Ft and QY.

Fluormeter II: Aquafluor 8000-010, make the quick measurement of Nile red value. Excitation wavelength is 525nm, emission wavelength is 575 nm.

Bürker counting chamber: Used for counting cell number of the culture in specific volume.

Electron microscope: Leica DM500, observing cells on counting chamber.

Fluorescence Spectrometer: Perkin Elmer LS 50B, observing the situation of Nile red dye binds to the lipids (time scan).

Bürker counting chamber and the electron microscope were used to count the cell number in the given volume, counting the number of monocytes in a total of 6 times E routers. The proximal average cell number (N) is N= (N1+N2+N3+N4+N5+N6)/6.
The formula for cell counting (E-rute): 1/5mm×1/5mm×1/10mm=1/250mm3=1/250 µL.
So that, the final cell concentration for the counting fluid (N/E-rute) can be get by calculate with the above formulas.

2.3 The main methods for lipid detection

Nile red (also called as Nile blue oxazone, 9-diethylamino-5-benzo[α]phenoxazinone) is a red phenoxazone dye and lipophilic stain. Nile red is produced by boiling Nile blue solution with sulfuric acid. Intracellular lipid droplets can be stained into fluorometric red by Nile red. Nile Red will not fluoresce in most polar solvents, however while in a lipid-rich environment, Nile red can be intensely fluorescent. It generally excites at 549nm, and emits at 628nm. The fluorescence of Nile red dye is dependent on the solvent used.[13] When Nile red combines with serum lipid proteins or proteins bearing hydrophobic domains, it is also fluoresces. [14]

The concentration of the Nile red (diluted with acetone) of the experiment was 250 mg/L. While detect the lipid concentration, put 50 µL Nile red solution into 250 µL measured culture. The Nile red is 5.0 µL /ml.

The earlier work in project 2012 has proved that DMSO can help the Nile red penetrate into the cell membranes of *Nannochloropsis oculata*, enhance Nile red straining. The DMSO solution can help the Nile red get into the cells of the *Nannochloropsis oculata,* and bind with the lipid droplets. As we can see the times scan was done by spectrofluorometry from

the figure 2.3.1 and figure 2.3.2, there is a big difference of Nile red value between treated without DMSO (low lipid concentration) and with DMSO (high lipid concentration) in *Nannochloropsis oculata* cells. After added DMSO solution, the Nile red value was stable after keep in dark for 15min. In this task, the concentration of DMSO is 10% (put 25 µL DMSO into 250 µL measured culture)[15,16]

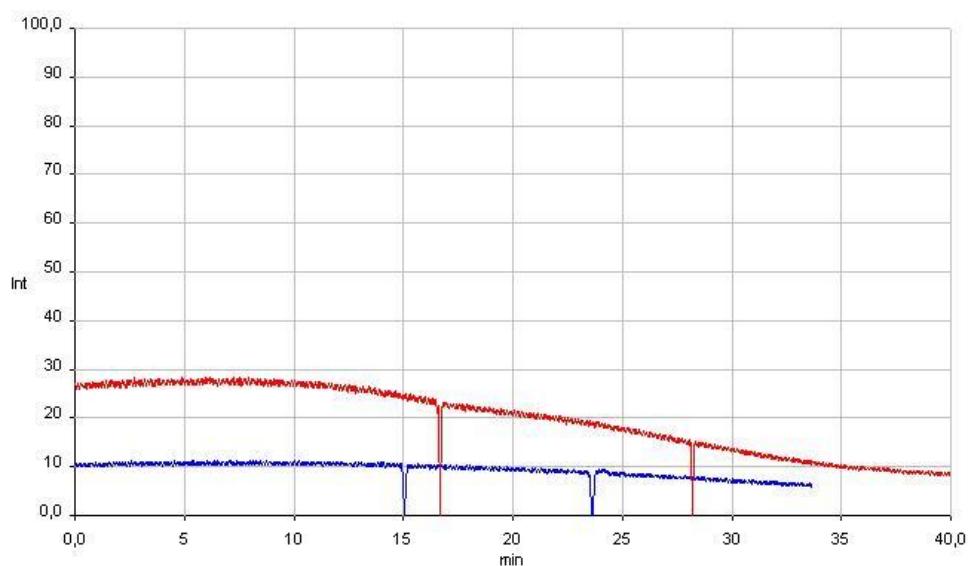


Figure 2.3.1 the spectrofluorometry time scan for the Nile red without treatment of DMSO. It was almost no changes during 15min, and after 15 min, it was reducing.

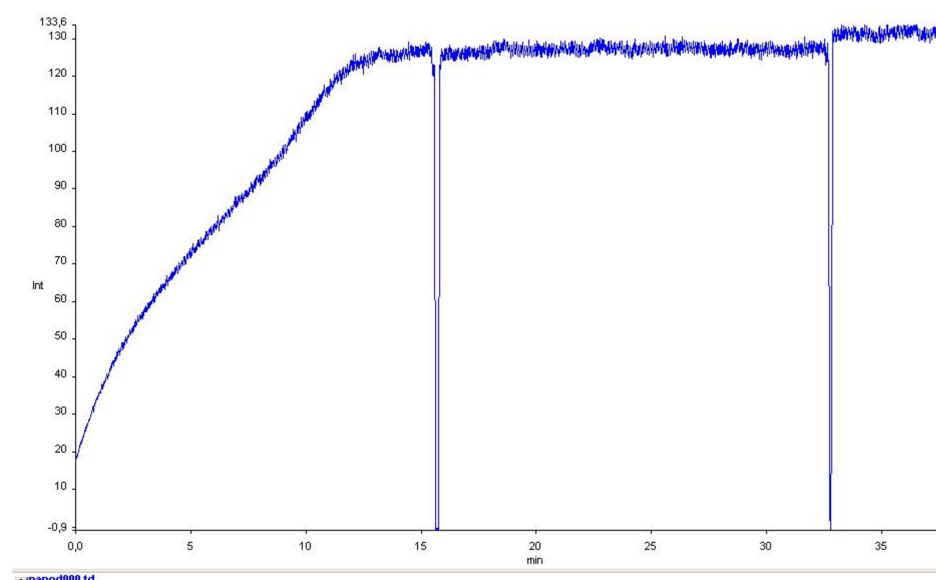


Figure 2.3.2 the spectrofluorometry time scan for the Nile red with DMSO solution.

2.4 The lipids content in dry weight Nannochloropsis

Factor between OD_{750} and carbon concentration in *Nannochloropsis* is Algal carbon concentration [ugC/ml]= 193×OD_{750} (Alice Mühlroth, personal communication), so that Algeabiomass[ug dw/ml]= 193×OD_{750}/0.46. The lipid equivalence is calculated from fluoresence 0.338.[17]

The formula for lipids content in dry weight *Nannochloropsis* [mg lipids/ mg dw]:

Nile red value×0.338/(193×OD_{750}/0.46)

3 Result

3.1 Initial growth experiments of reference cultures

As mentioned in chapter 2, some cultures from project 2012 were lost, because of evaporation by the air supply equipment during the Christmas holiday. Three ways were chosen to re-culture the cultures from project 2012. The low nitrogen (10% nitrogen) of f/2 medium was mainly used in project 2012. Culture the algae in high concentration of nitrogen, in order to increase the biomass.

The 10%, 25% and 100% concentration nitrogen of f/2 medium were chosen in separate cultivation of the algae. Two cultures of algae were chosen to continue the previous work: the original *Nannochloropsis* (O), and the starved culture that was re-cultured in light (S). Figure 3.2.1, figure3.2.2, and figure3.2.3 show the result of lipids content per dry weight *Nannochloropsis.*

The 25% concentration nitrogen medium (figure3.2.2) got the highest average lipid concentration. The lipid concentration increased to peak, with a concentration of around 0.033 (lipid/dw [g/g]), during 200th and 400th hour of cultivation. The lipid content is also stable during this time period. There is not much obvious difference of lipid concentration between O2 and S2. S2 contains only a little more lipid than O2. The growth of these two cultures was very similar.

As figure 3.2.1 and figure3.2.3 show, there was no obvious difference in lipid concentration between O1 and S1, so was O3 and S3. The average lipid concentration in 10% nitrogen medium (figure 3.2.1) was higher than in the f/2 medium (100% nitrogen, figure 3.2.3), although the nitrogen concentration in O3 and S3 was much higher than in O1 and S1. Possibly, the reason is that there is much more biomass population in f/2 medium than in 10% nitrogen medium. The higher biomass, the more nutrients are needed. Hence, there is more lipid consumed in f/2 medium (refer to the data in Appendix 6.1-6.6).

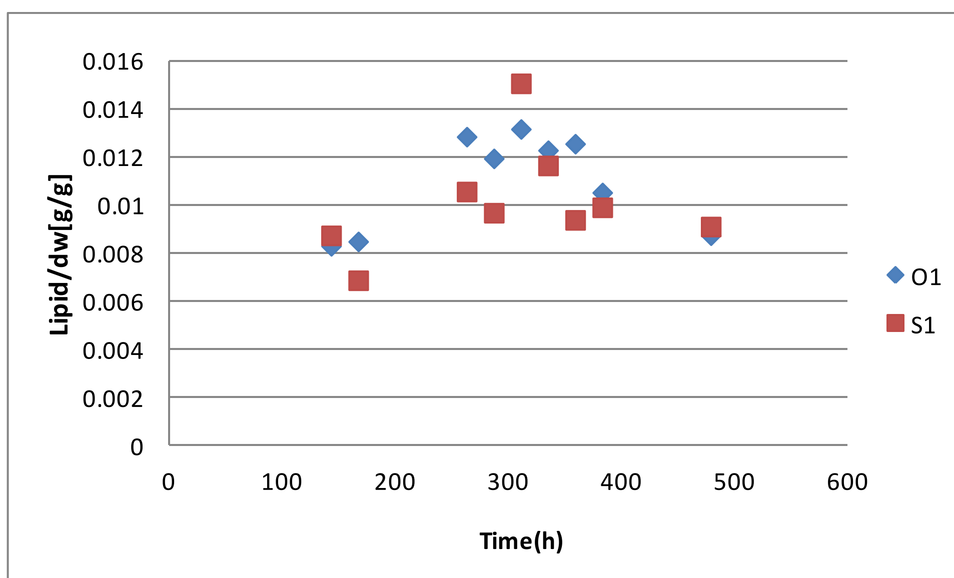


Figure 3.2.1 the development of specific lipid concentration in dry weight *Nannochloropsis* cultured in low nitrogen f/2 medium (10% nitrogen), the original culture (O) and starved culture (S).

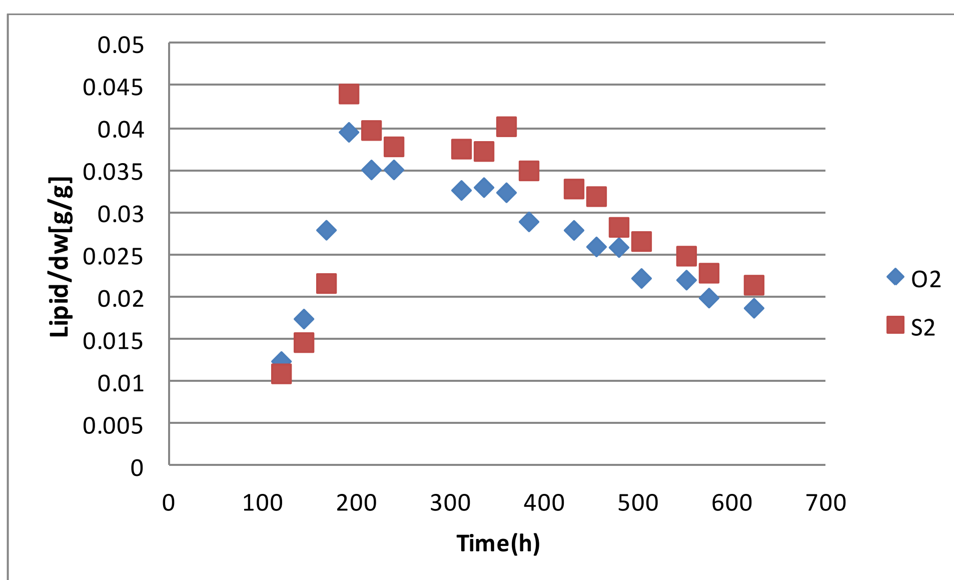


Figure 3.1.2 the development of specific lipid concentration in dry weight *Nannochloropsis* cultured in 25% nitrogen f/2 medium, the original culture (O2) and starved culture (S2).

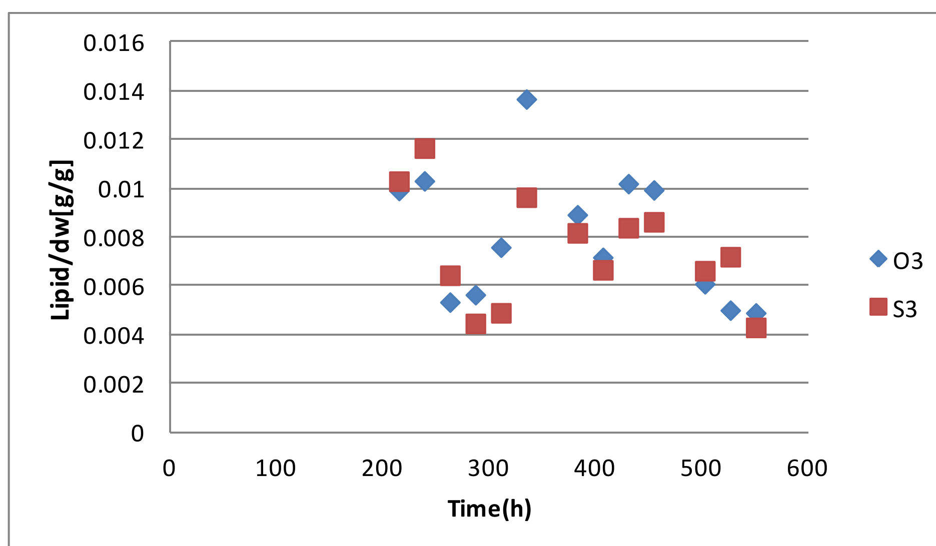


Figure 3.1.3 the development of specific lipid concentration in dry weight *Nannochloropsis* cultured in 100% nitrogen f/2 medium, the original culture (O3) and starved culture (S3).

3.2 Growth experiments for select high lipid method

After finish growth experiments in step 3.1, the high lipid concentration culture was chosen to continue work for high lipid selection. In consideration of low nitrogen cultivation, transferred culture O2 and S2 in seawater, kept these cultures cultivated in light model (16 hours light and 8 hours dark per 24 hours).

All the growth parameters results showed in figure 3.2.1. The growth rate was very low, after 150 hours, and Ft, QY and OD stopped increasing, even getting reduce. Therefore the consideration of keep on culturing O2 and S2 was given up. O3 and S3 were chosen to be cultivated in seawater, in the nitrogen concentration of 5%, 10% and 20%, respectively.

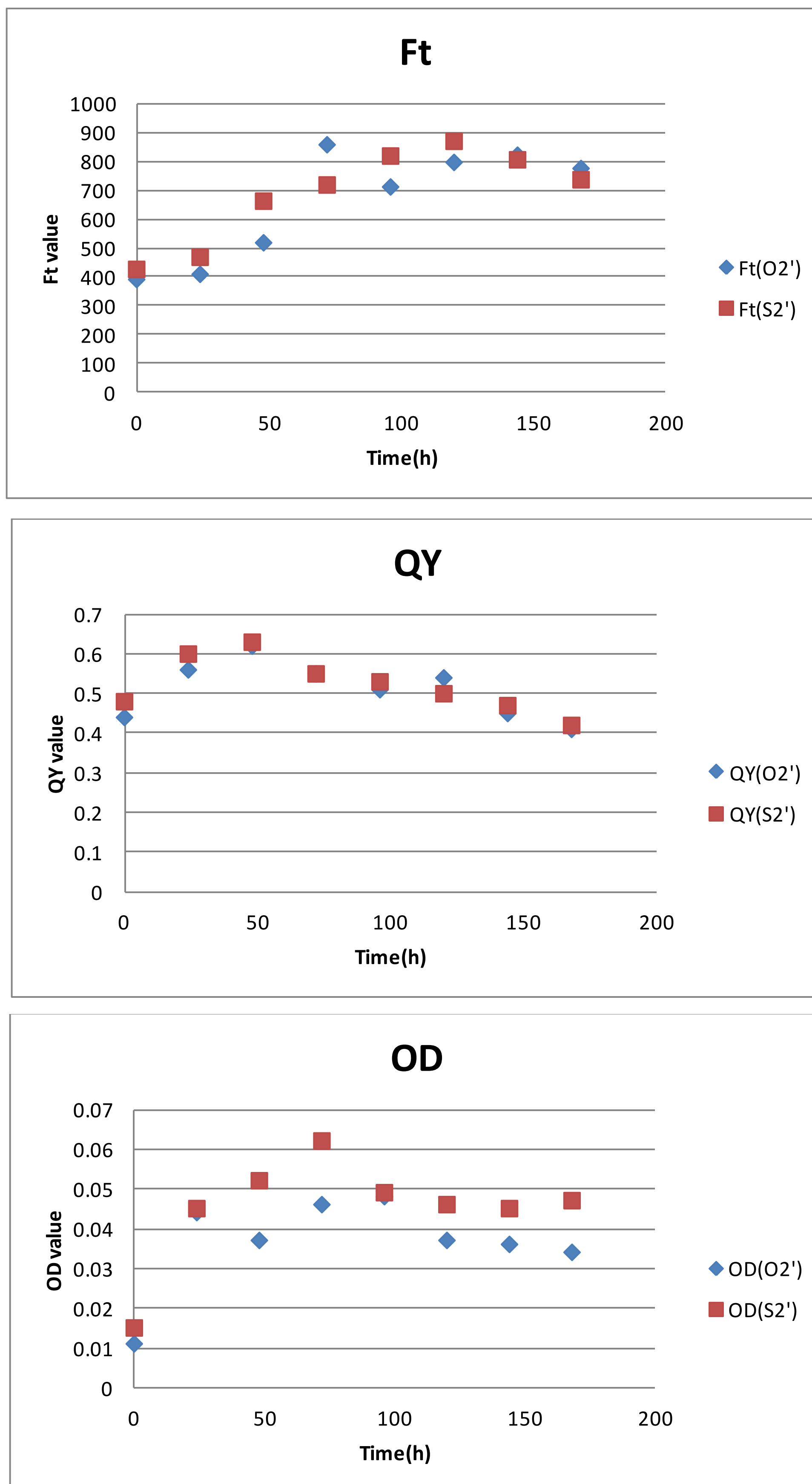


Figure 3.2.1 the growth parameter for transferred culture O2 and S2 in seawater (5% concentration in seawater): Ft, QY, and OD, the growth rate for these parameters was very low.

The estimated lipids content per dry weight of *Nannochloropsis* for transferring culture O3 and S3 in seawater are shown in figures below. As it can be observed from figure 3.2.2, figure3.2.3, and figure 3.2.4, by comparison the 20% nitrogen culture in seawater, the lipid concentration increased to peak during 200th and 300th hour. The average lipid content for control groups is very similar. The lipid content is around 0.015 Lipid/dw[g/g].

Figure 3.2.5, figureb3.2.6, figure 3.2.7 indicate that despite these three cultures had different nitrogen concentration cultures, the growth rates of cultures were all very stable. The estimated lipids content per dry weight of these groups had no significant difference.

Concerning the similar lipid contents among these three groups, possible reason is that the nile red value and the OD value have grown proportionately, so look at the formula for lipids content in dry weight=**Nile red value×0.338/(193×OD_{750}/0.46)**, while the biomass population grows, the lipid content also increase proportionately. It also means that the lipid content in alga cells did not really increased, unfortunately.

Therefore, to transfer the high nitrogen cultivated culture into seawater is not a good method to cultivate and select the high lipid populations. But it might be a good way to cultivate the high lipid culture for store the lipid concentration steadily.

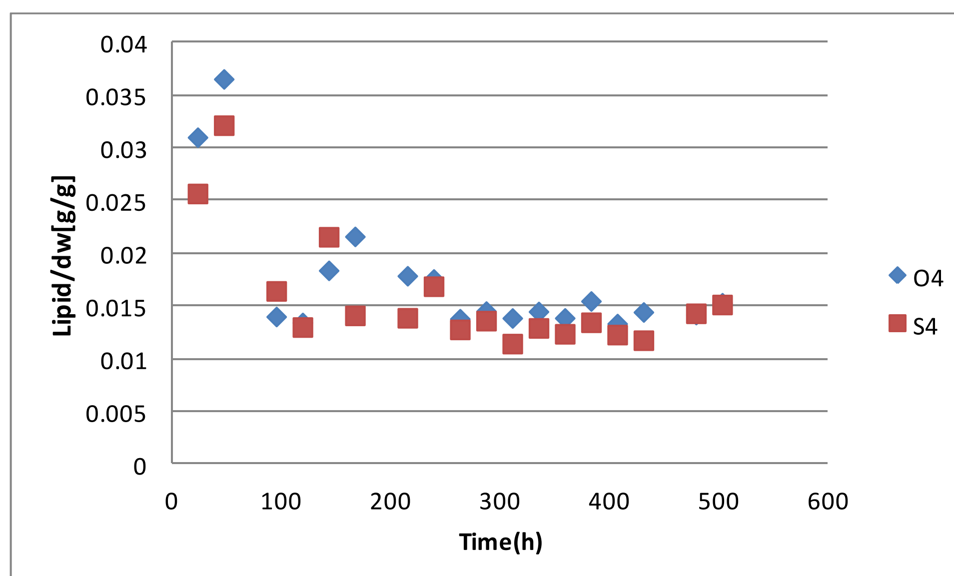


Figure 3.2.2 the development of specific lipid concentration in dry weight *Nannochloropsis* cultured 5% culture in seawater medium, compared with the original culture (O4) and the starved culture (S4).

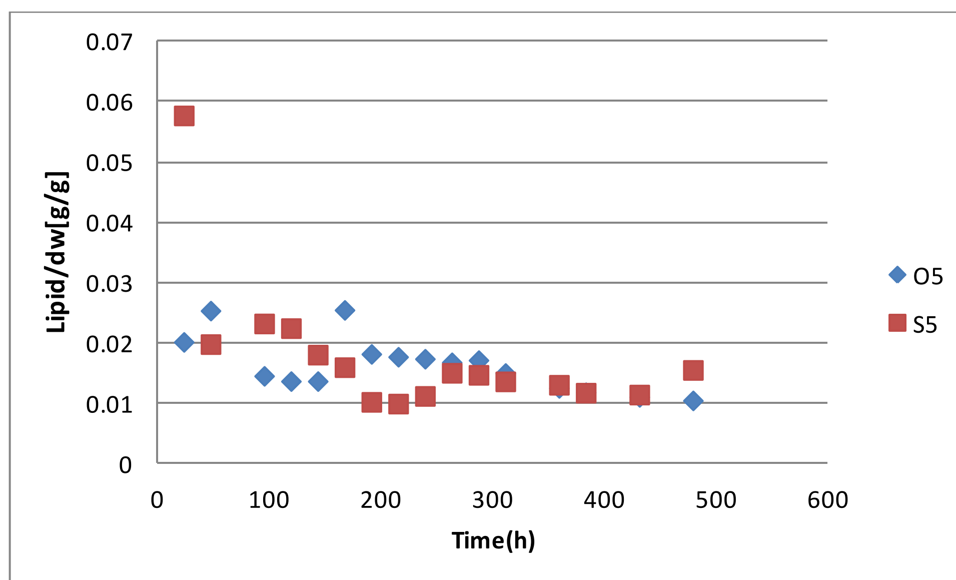


Figure 3.2.3 the development of specific lipid concentration in dry weight *Nannochloropsis* cultured 10% culture in seawater medium, compared with the original culture (O5) and the starved culture (S5).

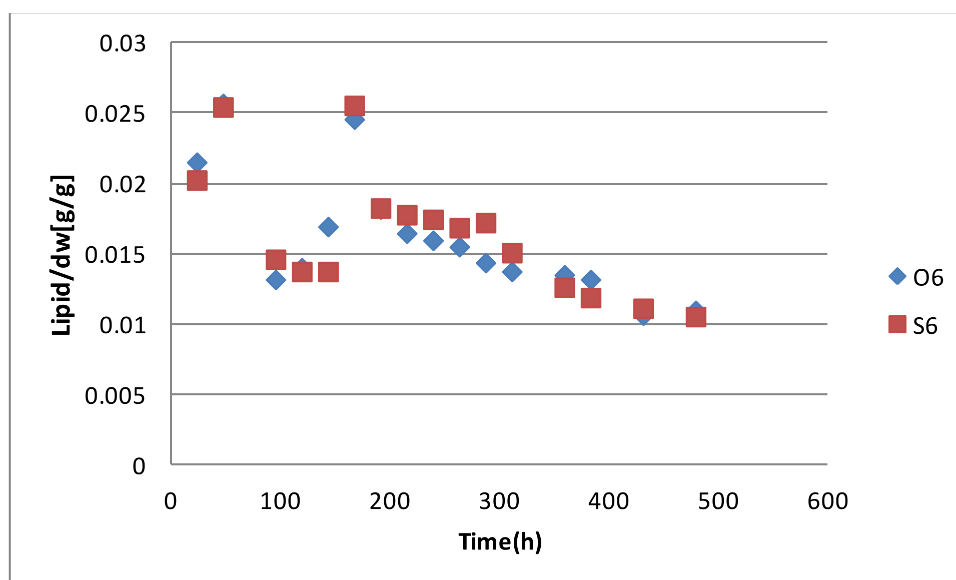


Figure 3.2.4 the development of specific lipid concentration in dry weight *Nannochloropsis* cultured 20% culture in seawater medium, compared with the original culture (O6) and the starved culture (S6).

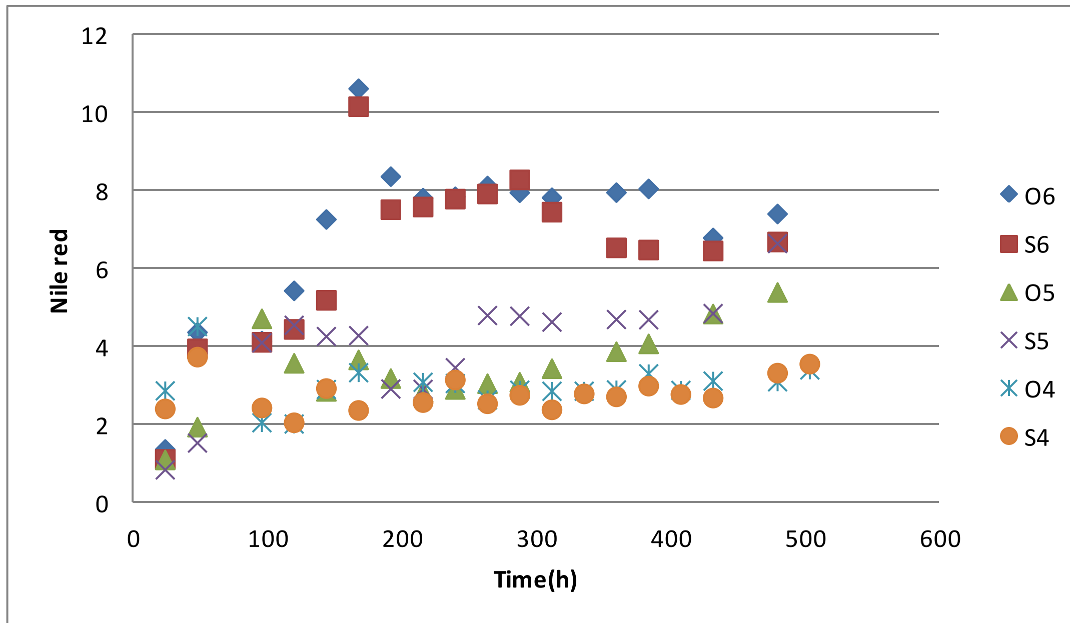


Figure 3.2.5 the development of Nile red in algal cultures with different seawater concentration medium, the controlled experiment groups are O6&S6 (20% in seawater), O5&S5 (10% in seawater), O4&S4 (5% in seawater)

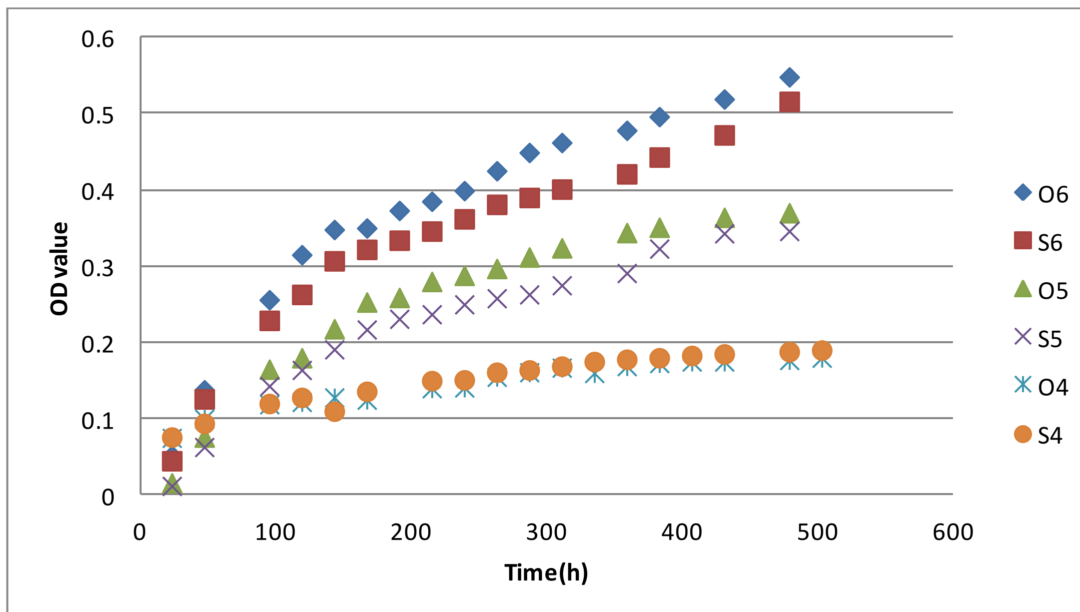


Figure 3.2.6 the development of OD in algal cultures with different seawater concentration medium, the controlled experiment groups are O6&S6 (20% in seawater), O5&S5 (10% in seawater), O4&S4 (5% in seawater)

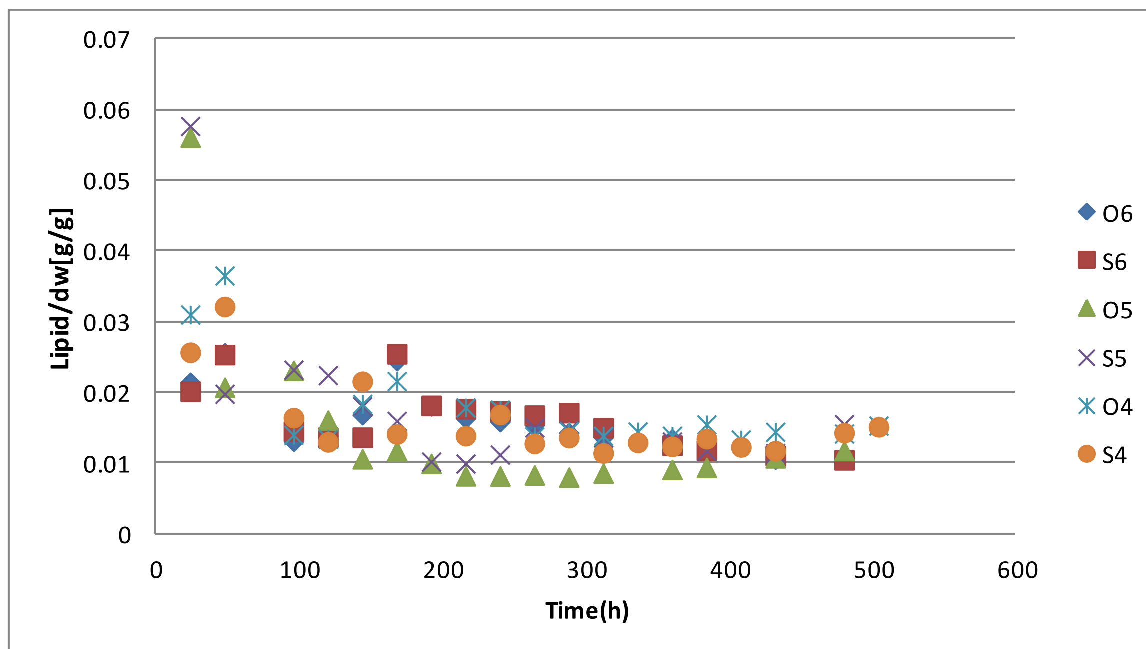


Figure 3.2.7 the development of dry weight ipid content in algal cultures with different seawater concentration medium, the controlled experiment groups are O6&S6 (20% in seawater), O5&S5 (10% in seawater), O4&S4 (5% in seawater)

3.3 Cultured the highest lipid concentration culture in low N medium

From step 3.2, the cultures O6 and S6 had highest lipid content and highest biomass, in order to get higher lipid concentration, culture O6 and S6 were transferred into the low nitrogen medium (10% nitrogen f/2 medium) for continuing cultivation.

As figure 3.3 shows, there is no obvious growing difference in lipid content between O7 and S7. The lipid content kept on increasing during 200 hours, and then got decrease until 300th hour, after 300 hours, it star to increase again. Because of the limit lab work time, there is no time to continue to further observation.

From current situation, the lipid content has not increase obviously compare to the result in step 3.2. The lipid concentration per dry weight in *Nannochloropsis* of culture O7 and S7 is around 0.015 Lipid/dw[g/g].

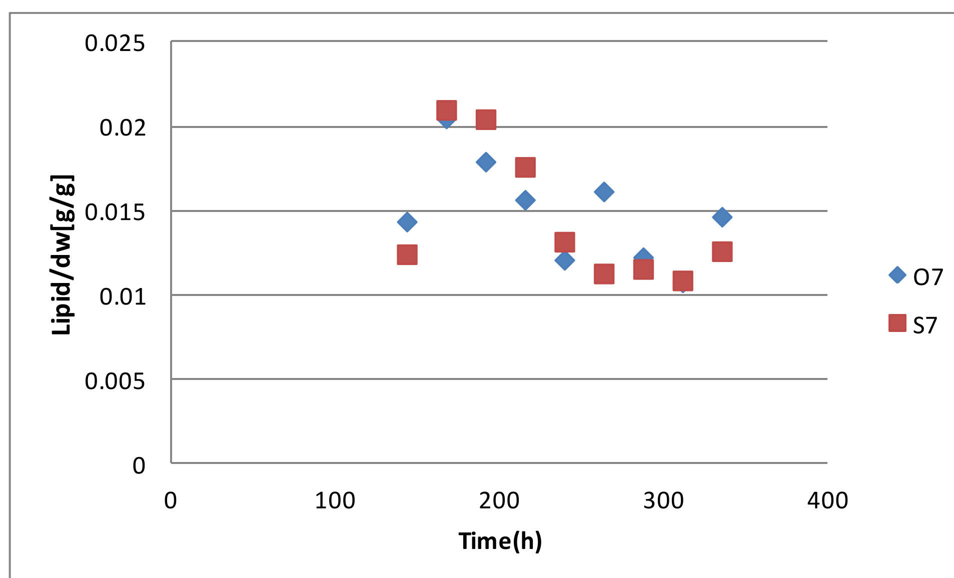


Figure 3.3 the development of specific lipid concentration in dry weight *Nannochloropsis* cultured in low nitrogen medium (10% nitrogen f/2 medium) from 20% culture in seawater medium, compared with the original culture (O7) and the starved culture (S7)

3.4 The data of dry weight lipid content from project 2012

Lipid concentration per dry weight in *Nannochloropsis* of the original culture and starved culture (kept on cultivating in dark) was calculated, which was obtained from project 2012 autumn. As figure 3.4.1 shows, the lipid content of starved culture was higher than the original culture. The lipid concentration of starved culture was almost 0.075 Lipid/dw[g/g], and the lipid concentration of original culture was around 0.055 Lipid/dw[g/g].

Lipid concentration per dry weight in *Nannochloropsis* of cell sorting in project 2012 also had higher lipid content than the cultures cultivated in 2013. The peak of the lipid content was about 0.055 Lipid/dw[g/g].(figure 3.4.2)

Comparing the results from step 3.2, 3.3 with the data from project 2012, the lipid concentration of the culture from project 2012 is much higher than the culture from step 3.2 and 3.3. The evaporation problem of cultures from project 2012 may cause this situation, some cells may die because of the evaporation and the lipid concentration also loose for this reason.[18]

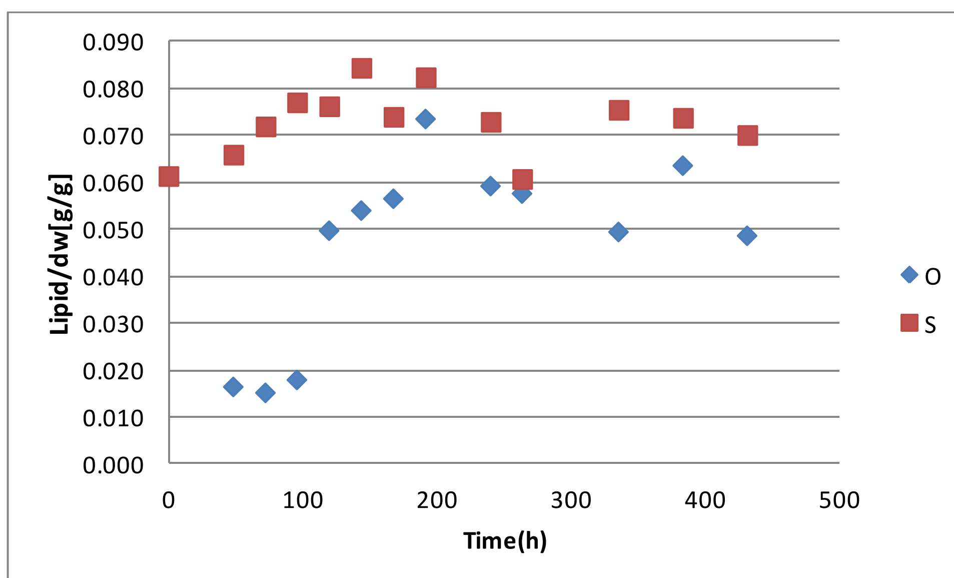


Figure 3.4.1 the development of specific lipid concentration per dry weight in *Nannochloropsis* cultured in low nitrogen medium (10% nitrogen f/2 medium)

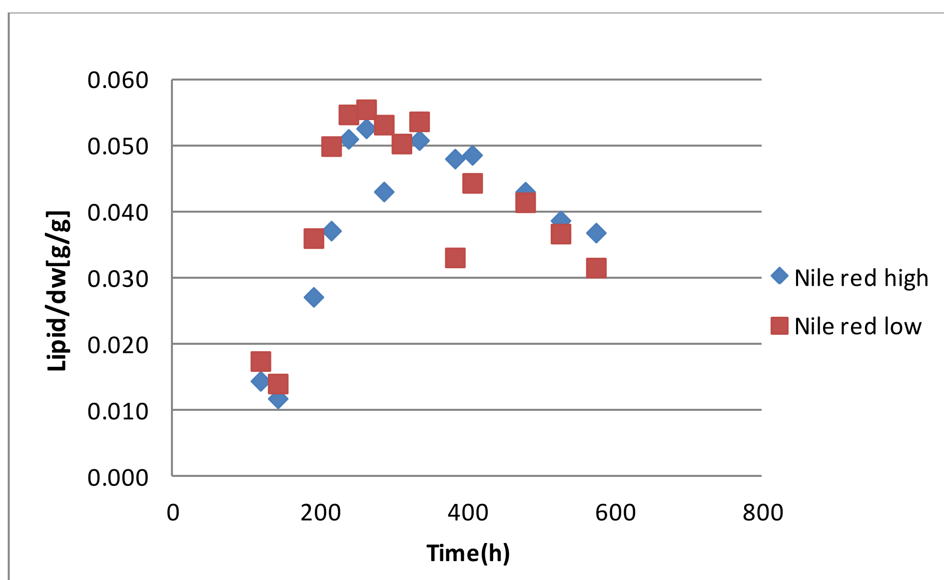


Figure 3.4.2 the development of specific lipid concentration per dry weight in *Nannochloropsis* cultured in low nitrogen medium (10% nitrogen f/2 medium) after cell sorting.

3.5 Cell counting

Table 3.5.1 Cell numbers of *Nannochloropsis oculata* original culture

5% in seawater(O4)		10% in seawater(O5)		20% in seawater(O6)	
Area (E)	**Cell No.**	**Area (E)**	**Cell No.**	**Area (E)**	**Cell No.**
1	20	**1**	32	**1**	38
2	18	**2**	38	**2**	34
3	13	**3**	31	**3**	40
4	11	**4**	37	**4**	42
5	12	**5**	33	**5**	36
6	18	**6**	32	**6**	34
Average(/ μL)	3.83×10^{3}	**Average(/ μL)**	8.46×10^{3}	**Average(/ μL)**	9.33×10^{3}

Table 3.5.2 Cell numbers of *Nannochloropsis oculata* starved culture (re-cultured in light during 2013 spring)

5% in seawater(S4)		10% in seawater(S5)		20% in seawater(S6)	
Area (E)	**Cell No.**	**Area (E)**	**Cell No.**	**Area (E)**	**Cell No.**
1	11	**1**	27	**1**	44
2	17	**2**	26	**2**	50
3	14	**3**	30	**3**	46
4	20	**4**	41	**4**	43
5	19	**5**	39	**5**	51
6	22	**6**	31	**6**	58
Average(/ μL)	4.29×10^{3}	**Average(/ μL)**	9.08×10^{3}	**Average(/ μL)**	1.22×10^{4}

After counted the cell numbers in cultures O4&S4, O5&S5, O6& S6 by Bürker counting chamber, the result is listed in table 3.5.1 and table 3.5.2. It can be observed that the culture has higher lipid content, also has more cells (biomass).

4 Discussion

Comparing the result from my project with Ruben Mathias Sæther 2013 (chose partial data from both projects). In Ruben Mathias Sæther's project, his experimental subject was *Isochrysis T-iso*, cultured through low nitrogen medium. He got a high capacity for lipid storage through light starvation, the maximum achieved lipid content for Ruben's project was 0.408±0.015 lipid/dw [g/g]. As figure 4.2 shows, the lipid yield increased obviously after two cycles of light starvation under nitrogen limitation (D,1 & D,2). The lipid content increased from about 0.104 lipid/dw [g/g] to 0.170 lipid/dw [g/g].

In the other hand, in my project 2012, the lipid content per dry weight of starved culture (S) was 0.015 lipid/dw [g/g] more than original culture (O). While the cultures were cultivated in light model in 2013 (both O and S), one representative group (O7 & S7) showed that the lipid content decreased, much less than O&S and the cell sorting cultures (NRL&NRH). In my project 2012, I did the starved process, and got satisfactory result (lipid content of culture S was around 0.085 lipid/dw [g/g]). I did not continue starving circle with the culture during my work in 2013, the lipid content in *Nannochloropsis oculata* reduced much.

Comparing the cell counting result in project 2013 with 2012, the maximum cell number in project 2013 was 1.22×10^4/ µL (Table 3.5.2), and the cell number after cell sorting in project 2012 is 1.5×10^4/ µL approximately. The cell numbers of in specific volume of S6 and NRH were proximate. But lipid content of NRH was almost twice as much as S6 has, this shows the intracllular lipid content in *Nannochloropsis oculata* reduced, and part of lipid was lost.

After cell sorting in 2012, the lipid content per dry weight of Nile red high culture just had about 0.002 lipid/dw [g/g] more than the Nile red low culture. It should note that while I was doing the cell sorting step, I did not add the DMSO solution into the culture (I had not got the DMSO solution yet at that time), and *Nannochloropsis oculata* has thick cell wall, DMSO can be used as an enhancer method for Nile red straining, and help the Nile red penetrate into the cell walls of *Nannochloropsis oculata*. Cell sorting without DSMO may not really select out the high lipid culture, which is one of the reasons that I did not get the desired lipid content.

The research work from Thi-Thai Yen Doan,etc. 2011 illustrated that after initial cell selection of *Nannochloropsis* strains, intracellular lipid accumulation was improved effectively. It is desirable to enhance biodiesel resource production. They enhanced the use of flow cytometry combined with cell sorting. The total lipid content of *Nannochloropsis* (strain 47) was double in biomass dry weight after consecutive three times of cell sorting. The cell sorting should be effectual for selecting high lipid culture, and provide help for further algal cultivation to get the high lipid content.

To sum up about my lab work result, I ignored two mainly effective steps for cultivation. One is the culturing in cycles of light starvation, the other one is rounds of cell sorting by flow cytometry. These two ways can combine together in further research work for algal high lipid content selecting. Thi-Thai Yen Doan,etc. 2011 also motioned that "cell lipid enhancement is most readily achieved when parent cells are in mid-stationary phase and the proportion of dead cells does not exceed 6% of the cell population." [19] Related to this situation, cultures were lost by evaporation might the third reason for lipid content reducing in my project work 2013.

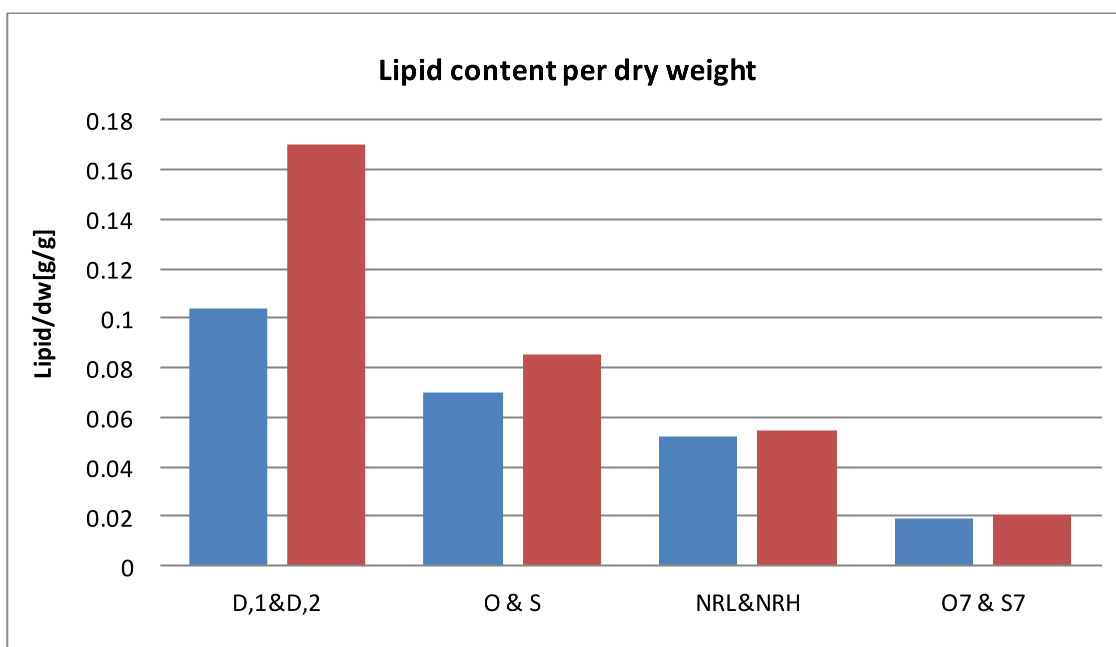


Figure 4.2 comparing the lipid content per dry weight from Jin Su 2013 with Ruben Mathias Sæther 2013. In group 1, D,1 is the first stage of starved culture, D,2 is the second stage of starved culture (Ruben Mathias Sæther 2013). In group 2, O is the original culture, S is the starved culture (Jin Su 2012). In group 3, the cultures were done by cell sorting, NRL is Nile red low culture, NRH is Nile red high culture (Jin Su 2012). In group 4, O7 is the original culture cultivated in Low nitrogen f/2 medium, S7 is the starved culture from project 2012, but re-cultured in light model in 2013 (Jin Su 2013).

5 Conclusion

The use of microalgae to product diesel has economic and ecological significance. Microalgae has high growth rate, using sea water as a natural medium for saving agricultural resources. Microalgae can produce much more oil than terrestrial plant. Production of biodiesel contains no sulphur, and does not emit toxic gases while burning. The exhaust released into the environment can also be micro-biologically degradable, without polluting the environment. Therefore, there is a promising future for the oil-rich algae to be used for producing biodiesel.

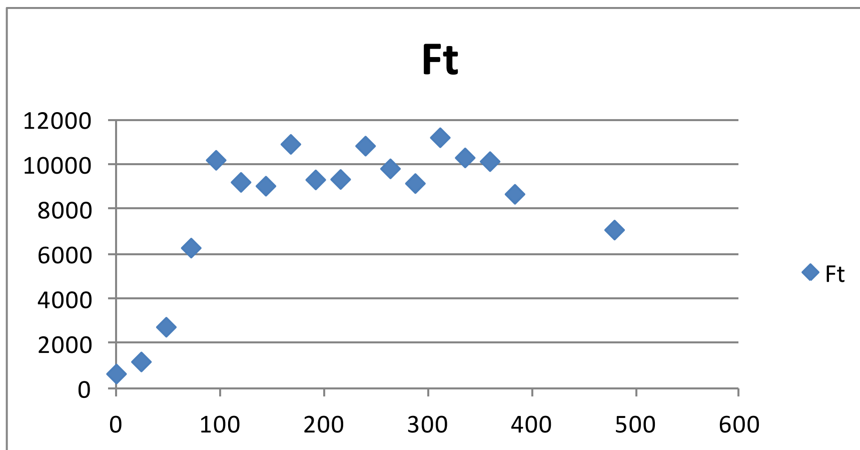
Although I did not get the expect lipid content for my culture, there are some methodologies, in brief, for improving oil production from microalgae: accelerate the growth rate of microalgae, improve the photosynthetic efficiency, and increase the oil content of algae. Furthermore, the genetic engineering approach can be used to improve the lipid content of microalgae and reduce the growing period as well. To be more specific, manipulate the relevant genes in the lipid synthesis pathway in microalgae. For instance, overexpress the pivotal enzymes utilized in lipid synthesis. Alternatively, inactivate the enzymes that compete for the intermediate components in the lipid producing pathway, which can combat the problem in the genetic level, ameliorating the oil production.[20]

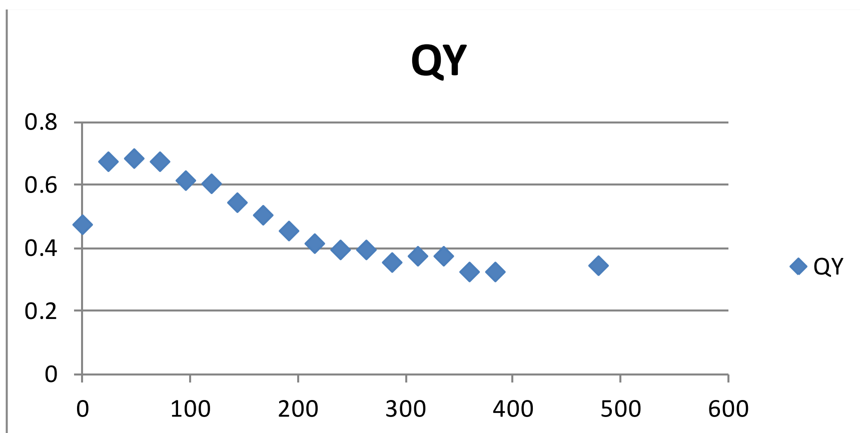
In conclusion, with the advancement in the selection of oil-rich microalgae species, the progress of downstream technology, e.g. the culture conditions, culture methods, bioreactor, and the improvement in the techniques of extraction, separation and transformation, the microalgae-biodiesel industry would, definitely, become more economically and prosperous.

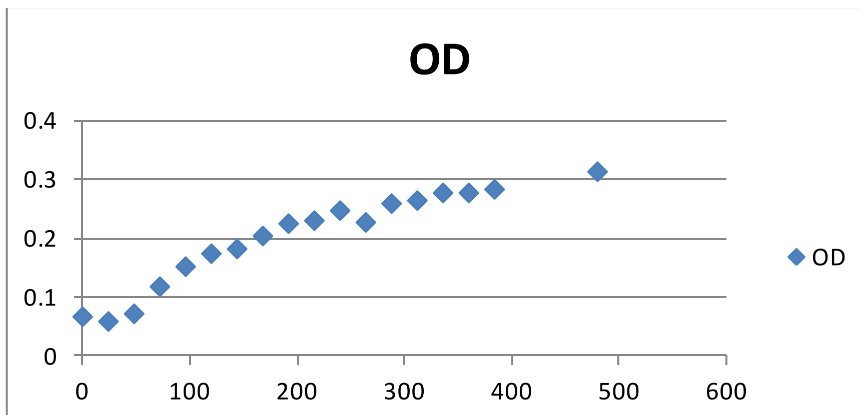
6 Appendix

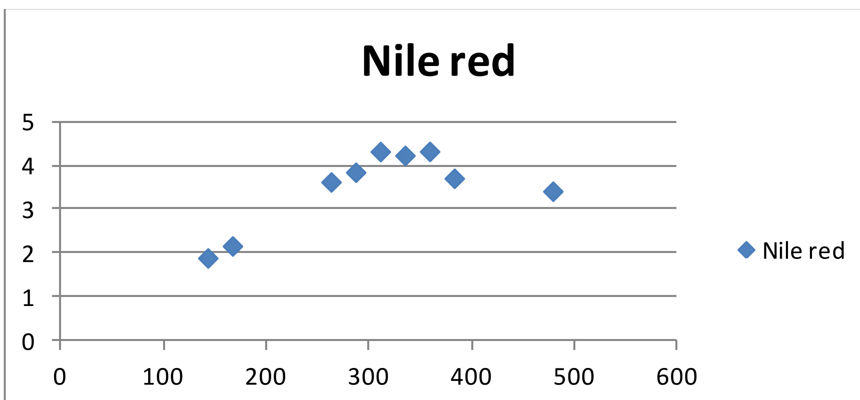
6.1 Nannochloropsis (original) 10%N f/2 medium

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
667	0.47	0.065				0
1206	0.67	0.057				24
2757	0.68	0.070				48
6291	0.67	0.116				72
10211	0.61	0.150				96
9225	0.60	0.172				120
9056	0.54	0.180	0.110	1.966	1.856	144
10923	0.5	0.202	0.112	2.241	2.129	168
9336	0.45	0.223				192
9355	0.41	0.228				216
10846	0.39	0.245				240
9831	0.39	0.225	0.126	3.713	3.587	264
9173	0.35	0.257	0.119	3.928	3.809	288
11220	0.37	0.262	0.110	4.390	4.280	312
10319	0.37	0.275	0.121	4.313	4.192	336
10153	0.32	0.275	0.108	4.392	4.284	360
8693	0.32	0.281	0.122	3.793	3.671	384
7096	0.34	0.311	0.124	3.501	3.377	480


Ft
12000
10000
8000
6000
4000
2000
0
0
100
200
300
400
500
600
Ft

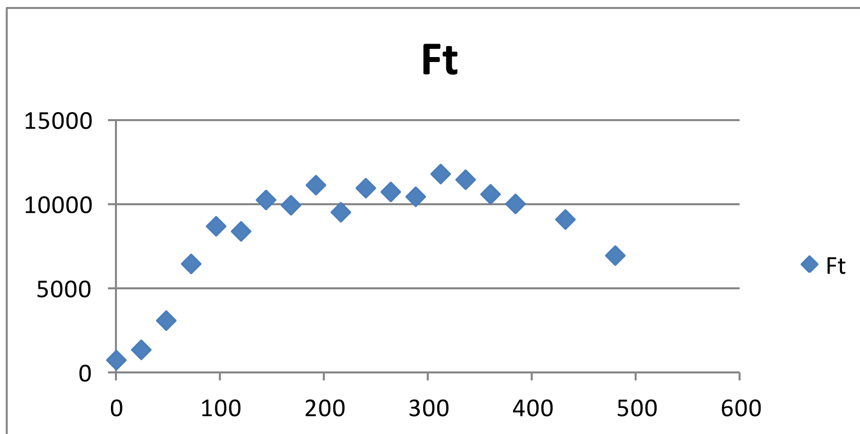

QY
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
QY

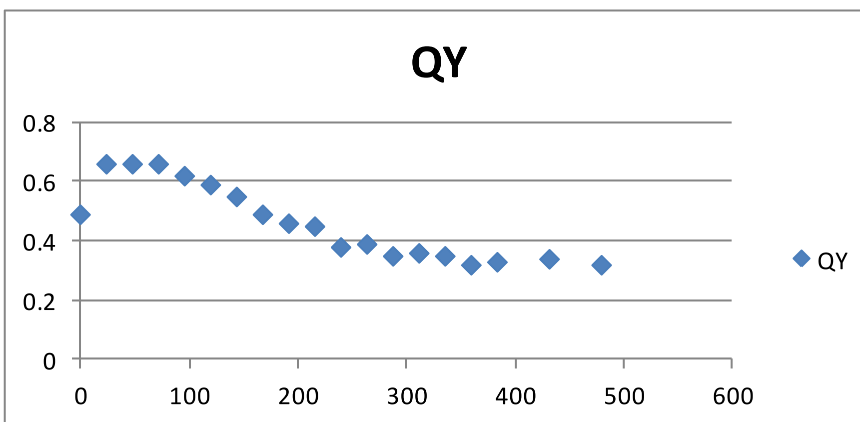

OD
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
OD

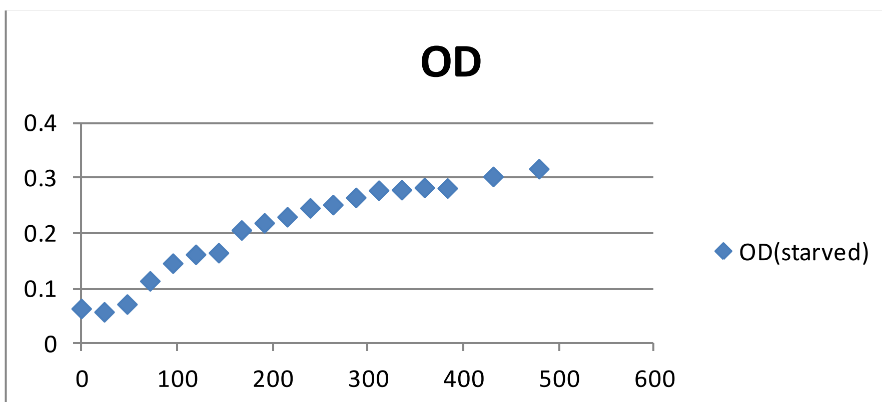

Nile red
5
4
3
2
1
0
0
100
200
300
400
500
600
Nile red

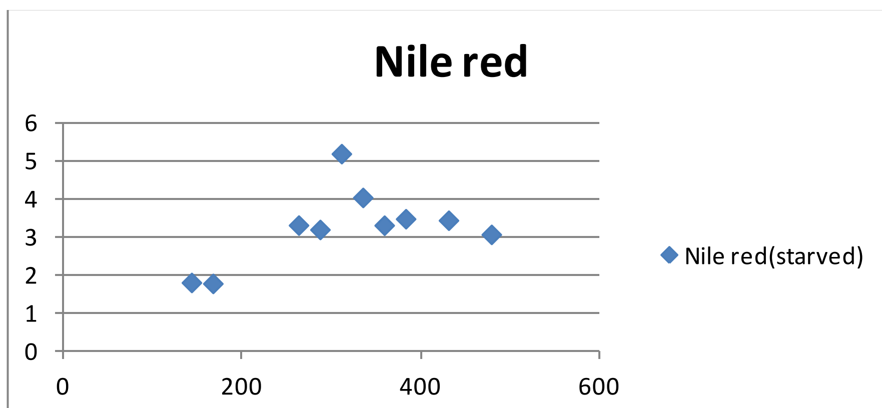
6.2 Nannochloropsis (starved culture in 2012project, cultured in light again) 10%N f/2 medium

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
743	0.49	0.061				0
1357	0.66	0.055				24
3092	0.66	0.069				48
6463	0.66	0.111				72
8698	0.62	0.143				96
8395	0.59	0.159				120
10263	0.55	0.162	0.109	1.867	1.758	144
9949	0.49	0.203	0.120	1.853	1.733	168
11144	0.46	0.216				192
9532	0.45	0.227				216
10965	0.38	0.243				240
10741	0.39	0.249	0.117	3.382	3.265	264
10454	0.35	0.262	0.109	3.258	3.149	288
11805	0.36	0.275	0.129	5.269	5.14	312
11466	0.35	0.276	0.117	4.107	3.99	336
10601	0.32	0.280	0.128	3.390	3.262	360
10029	0.33	0.279	0.120	3.551	3.431	384
9101	0.34	0.300	0.117	3.509	3.392	432
6956	0.32	0.314	0.119	3.139	3.02	480


Ft
15000
10000
5000
0
0
100
200
300
400
500
600
Ft

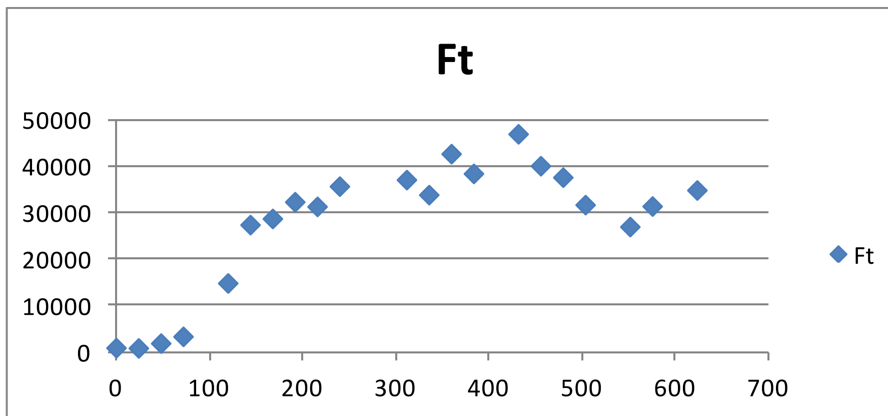

QY
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
QY

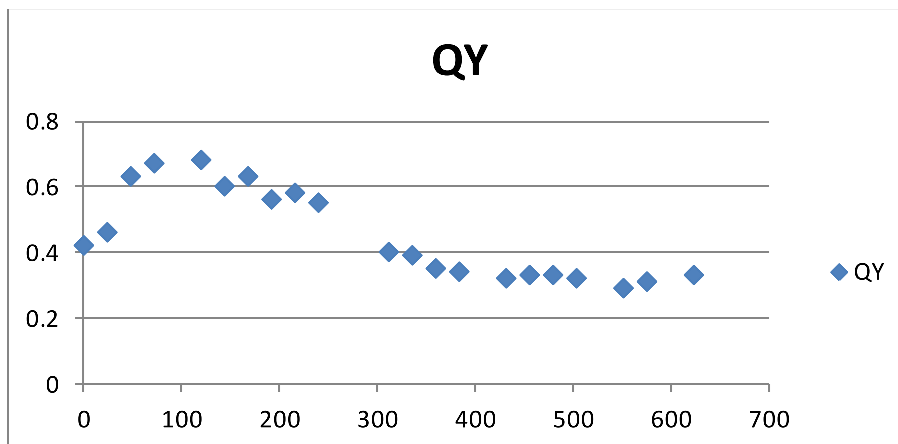

OD
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
OD(starved)

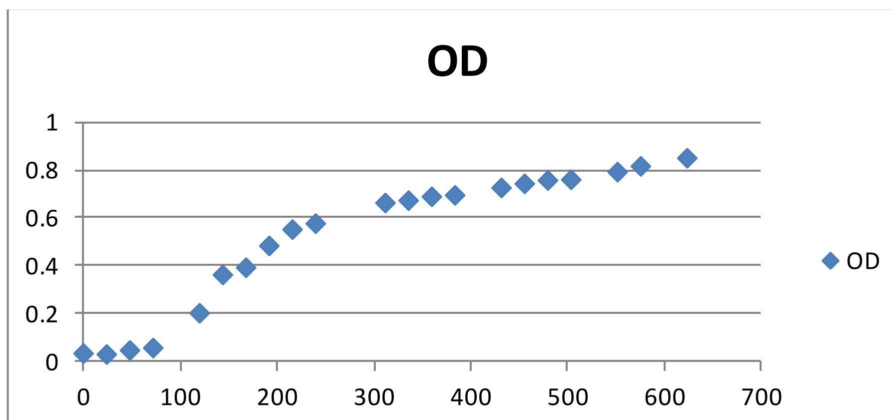

Nile red
6
5
4
3
2
1
0
0
200
400
600
Nile red(starved)

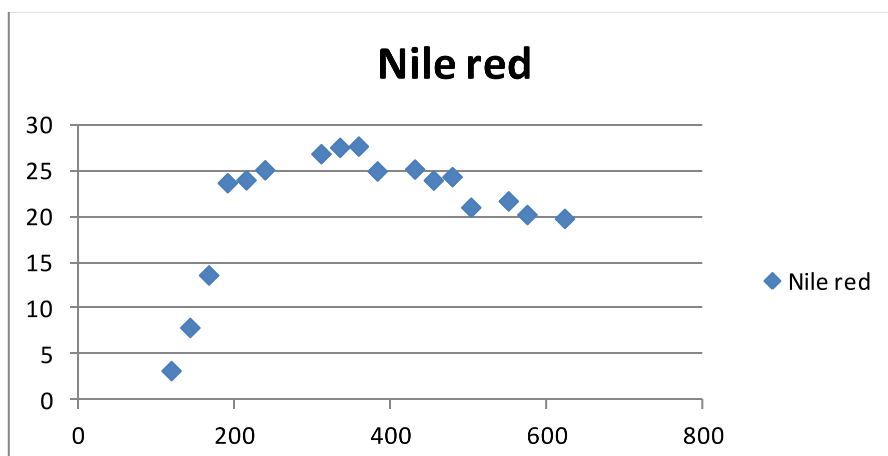
6.3 Nannochloropsis (original) 25%N in medium

QY	OD	NRr	NR	Nile red	Time(hours)
0.42	0.03				0
0.46	0.026				24
0.63	0.043				48
0.67	0.053				72
0.68	0.198	0.083	3.13	3.047	120
0.6	0.358	0.138	7.88	7.742	144
0.63	0.388	0.168	13.62	13.452	168
0.56	0.479	0.195	23.69	23.495	192
0.58	0.547	0.22	24.03	23.81	216
0.55	0.572	0.227	25.13	24.903	240
0.4	0.658	0.253	26.91	26.657	312
0.39	0.668	0.257	27.6	27.343	336
0.35	0.684	0.248	27.73	27.482	360
0.34	0.69	0.249	25.02	24.771	384
0.32	0.721	0.259	25.24	24.981	432
0.33	0.738	0.28	24.06	23.78	456
0.33	0.752	0.272	24.43	24.158	480
0.32	0.755	0.263	21.09	20.827	504
0.29	0.787	0.263	21.78	21.517	552
0.31	0.811	0.265	20.3	20.035	576
0.33	0.845	0.286	19.9	19.614	624


Ft
50000
40000
30000
20000
10000
0
0
100
200
300
400
500
600
700
Ft

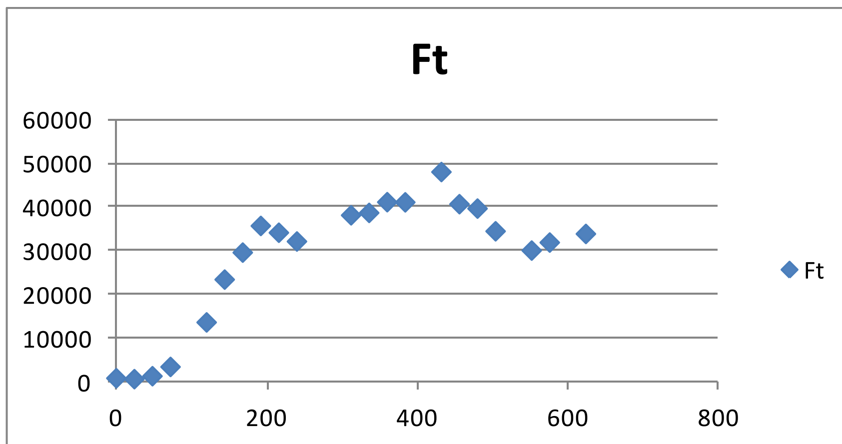

QY
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
700
QY

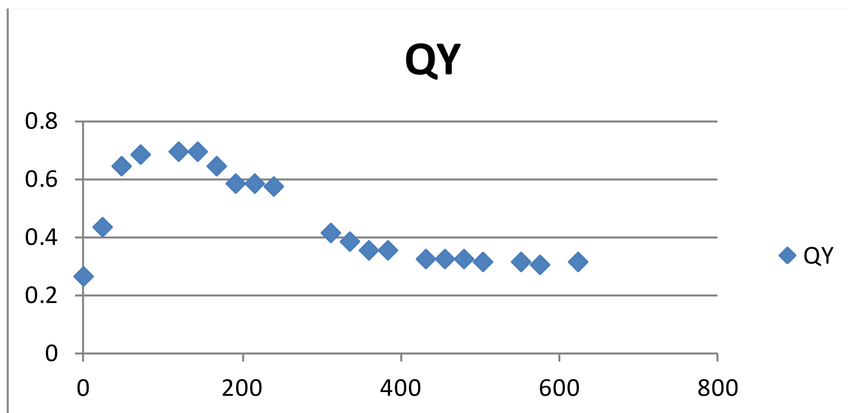

OD
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
700
OD

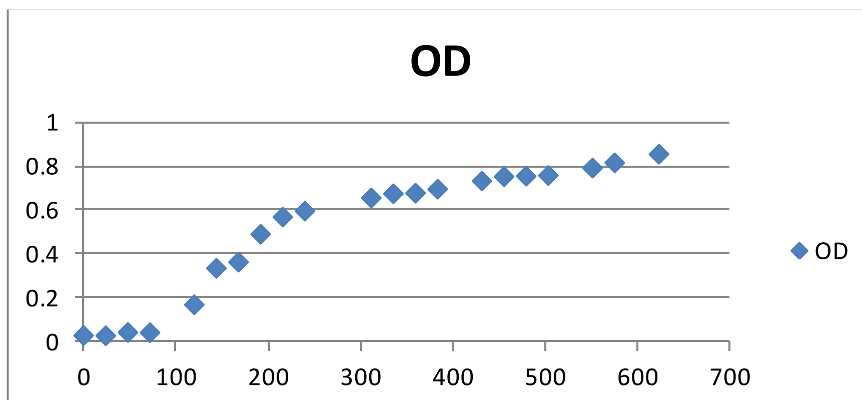

Nile red
30
25
20
15
10
5
0
0
200
400
600
800
Nile red

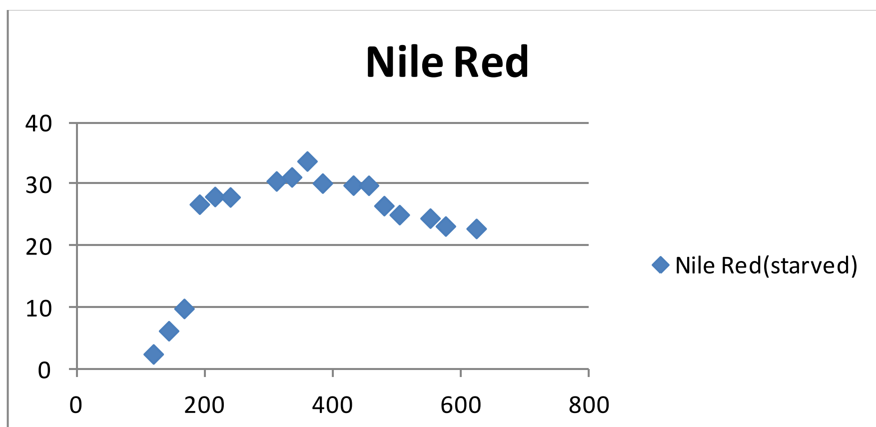
6.4 Nannochloropsis (starved culture cultured in light again) 25%N in medium

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
1002	0.26	0.024				0
801	0.43	0.023				24
1475	0.64	0.038				48
3594	0.68	0.037				72
13759	0.69	0.164	0.104	2.331	2.227	120
23539	0.69	0.330	0.119	6.114	5.995	144
29711	0.64	0.358	0.144	9.754	9.61	168
35802	0.58	0.485	0.201	26.72	26.519	192
34247	0.58	0.563	0.205	27.97	27.765	216
32243	0.57	0.590	0.228	27.91	27.682	240
38225	0.41	0.650	0.239	30.52	30.281	312
38783	0.38	0.669	0.256	31.19	30.934	336
41224	0.35	0.672	0.245	33.76	33.515	360
41191	0.35	0.690	0.262	30.2	29.938	384
48116	0.32	0.727	0.273	29.88	29.607	432
40768	0.32	0.747	0.284	29.87	29.586	456
39761	0.32	0.749	0.277	26.56	26.283	480
34576	0.31	0.752	0.267	25.09	24.823	504
30169	0.31	0.786	0.283	24.54	24.257	552
32004	0.30	0.810	0.268	23.24	22.972	576
33974	0.31	0.849	0.285	22.87	22.585	624


Ft
60000
50000
40000
30000
20000
10000
0
0
200
400
600
800
Ft

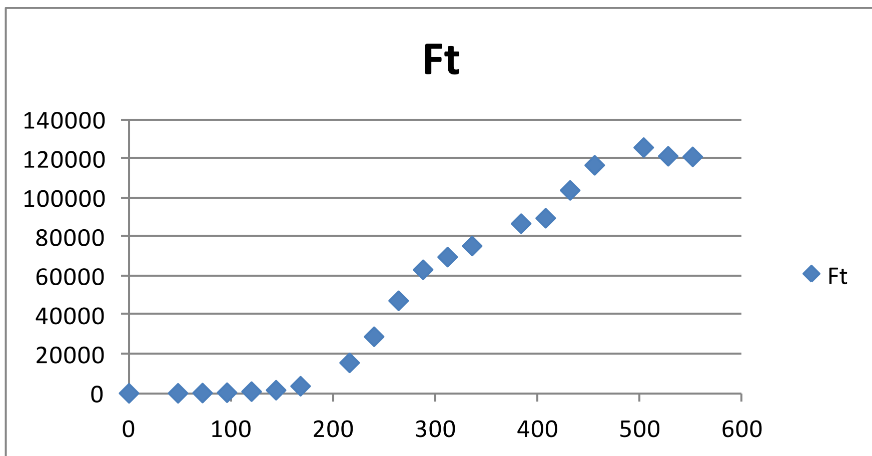

QY
0.8
0.6
0.4
0.2
0
0
200
400
600
800
QY

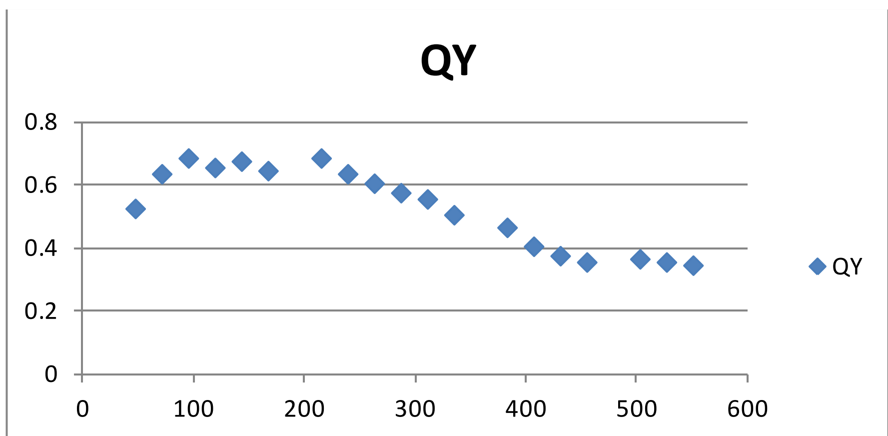

OD
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
700
OD

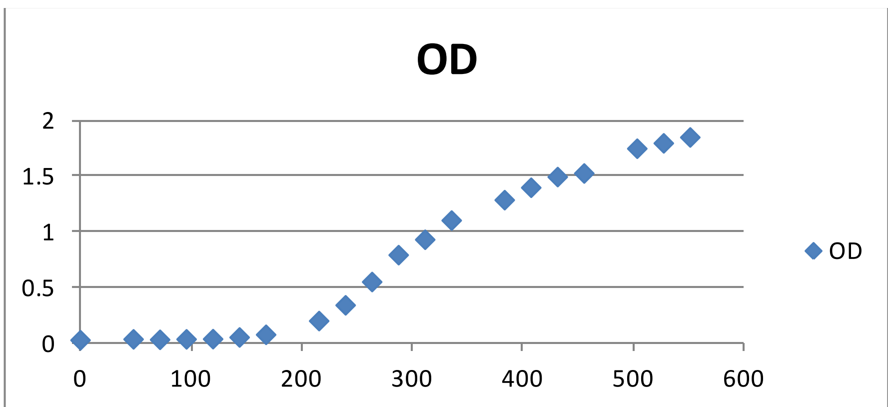

Nile Red
40
30
20
10
0
0
200
400
600
800
Nile Red(starved)

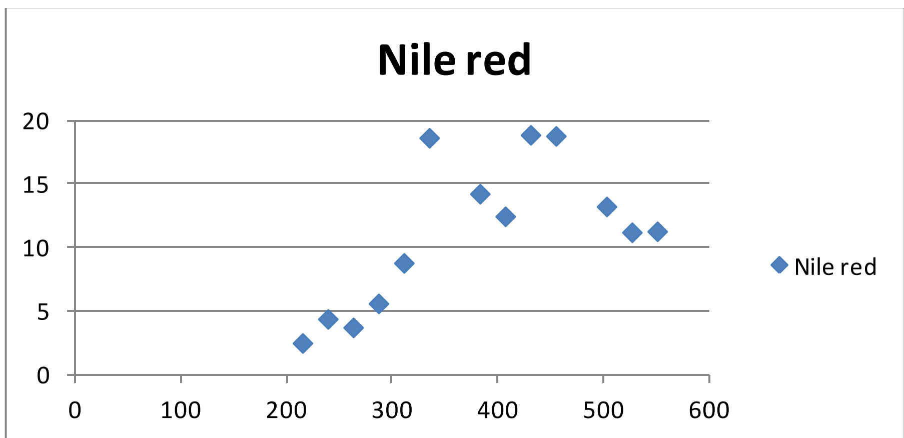
6.5 Nannochloropsis (original) f/2 medium

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
94		0.019				0
						24
109	0.52	0.027				48
246	0.63	0.023				72
419	0.68	0.027				96
974	0.65	0.029				120
1697	0.67	0.044				144
3741	0.64	0.068				168
15703	0.68	0.191	0.080	2.429	2.349	216
29049	0.63	0.332	0.120	4.366	4.246	240
47466	0.60	0.541	0.191	3.771	3.58	264
63331	0.57	0.782	0.209	5.678	5.469	288
69834	0.55	0.920	0.145	8.803	8.658	312
75491	0.50	1.092	0.335	18.85	18.515	336
86936	0.46	1.275	0.431	14.54	14.109	384
89730	0.40	1.386	0.488	12.82	12.332	408
103970	0.37	1.482	0.515	19.26	18.745	432
116812	0.35	1.514	0.547	19.21	18.663	456
125882	0.36	1.736	0.576	13.68	13.104	504
121446	0.35	1.784	0.606	11.68	11.074	528
121127	0.34	1.837	0.612	11.76	11.148	552


Ft
140000
120000
100000
80000
60000
40000
20000
0
0
100
200
300
400
500
600
Ft

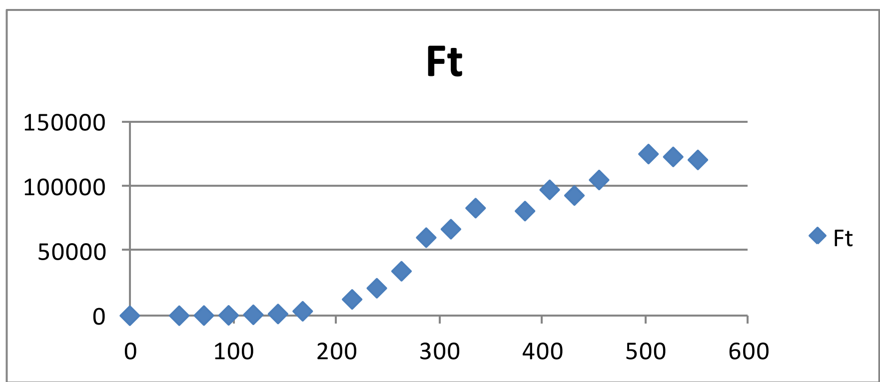

QY
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
QY

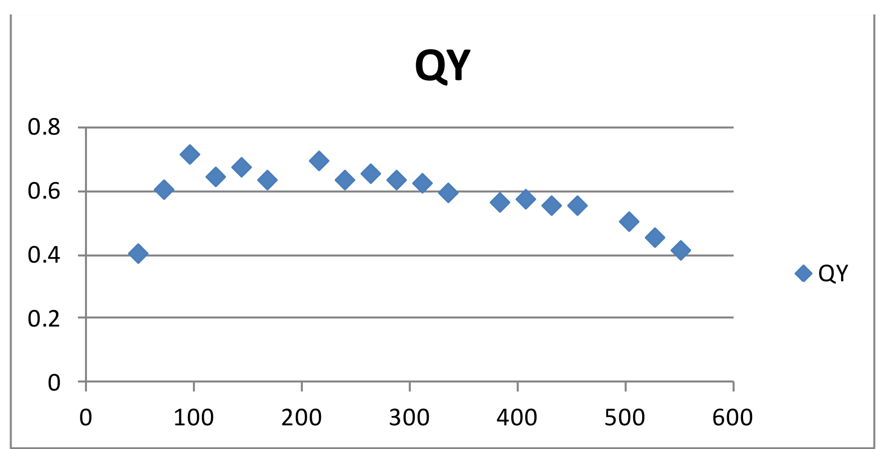

OD
2
1.5
1
0.5
0
0
100
200
300
400
500
600
OD

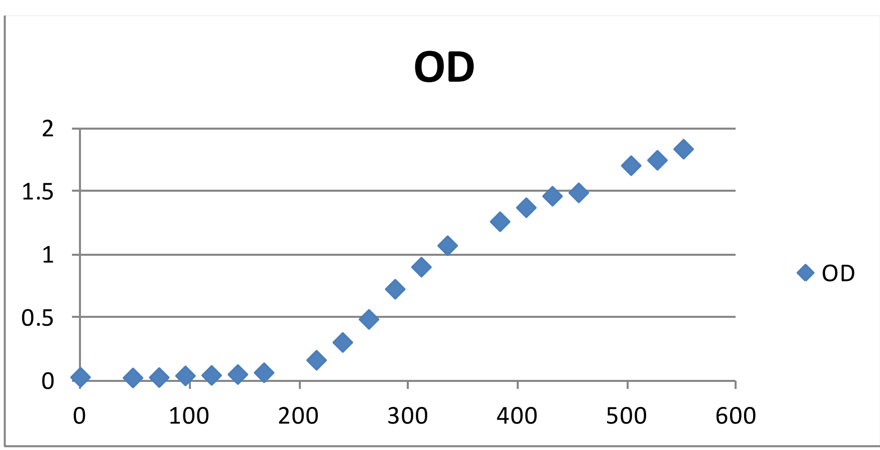

Nile red
20
15
10
5
0
0
100
200
300
400
500
600
Nile red

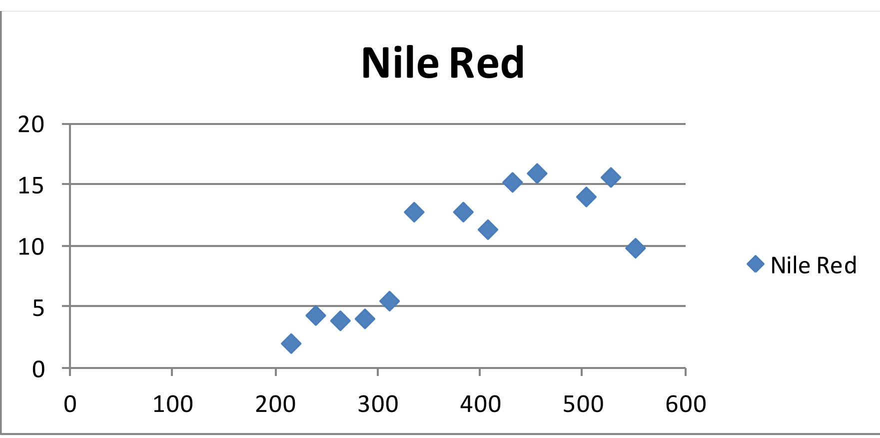
6.6 Nannochloropsis (starved culture, cultured in light again) f/2 medium

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
102		0.016				0
						24
128	0.40	0.011				48
226	0.60	0.014				72
380	0.71	0.027				96
818	0.64	0.031				120
1432	0.67	0.038				144
3533	0.63	0.052				168
12702	0.69	0.151	0.069	1.998	1.929	216
21353	0.63	0.292	0.109	4.331	4.222	240
34498	0.65	0.475	0.148	3.941	3.793	264
60503	0.63	0.714	0.187	4.137	3.950	288
67037	0.62	0.889	0.131	5.529	5.398	312
83326	0.59	1.059	0.326	12.99	12.664	336
81049	0.56	1.249	0.400	13.07	12.67	384
97501	0.57	1.36	0.455	11.69	11.235	408
93079	0.55	1.451	0.493	15.59	15.097	432
105108	0.55	1.478	0.545	16.37	15.825	456
125190	0.50	1.693	0.570	14.48	13.91	504
122991	0.45	1.736	0.590	16.09	15.50	528
120648	0.41	1.824	0.65	10.37	9.720	552


Ft
150000
100000
50000
0
0
100
200
300
400
500
600
Ft

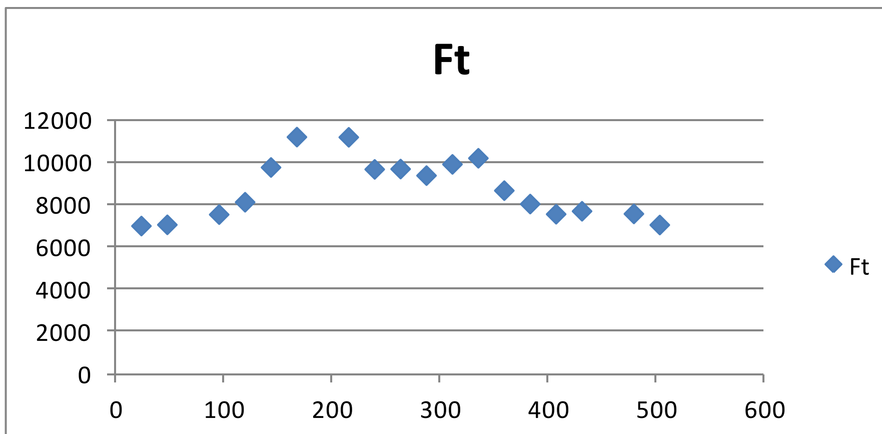

QY
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
QY

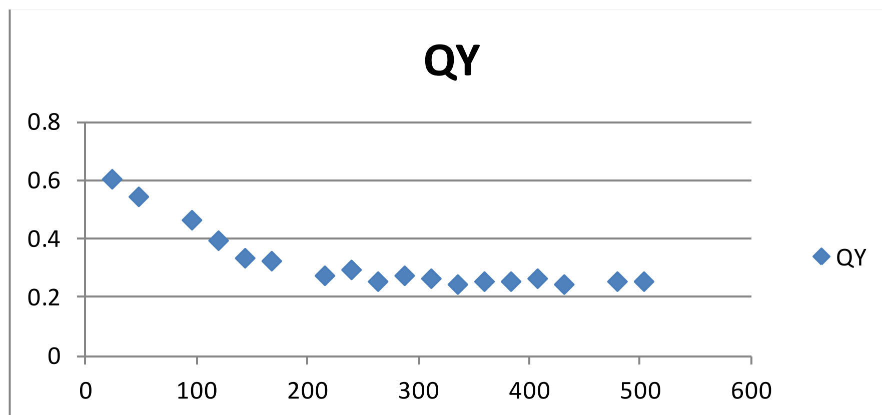

OD
2
1.5
1
0.5
0
0
100
200
300
400
500
600
OD

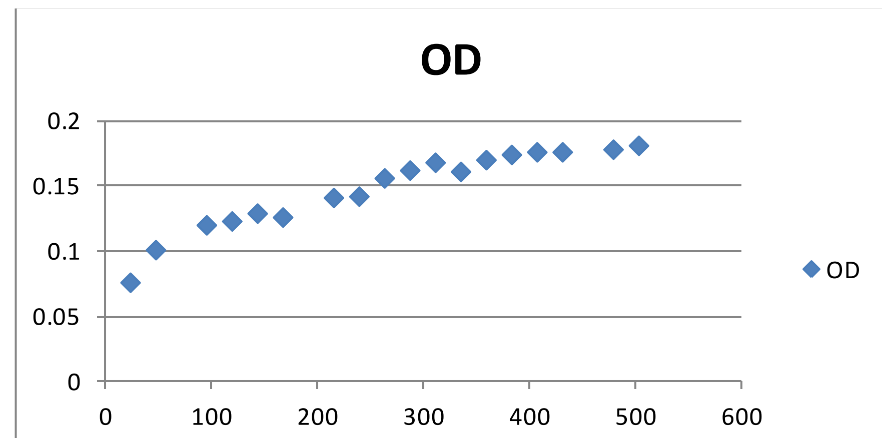

Nile Red
20
15
10
5
0
0
100
200
300
400
500
600
Nile Red

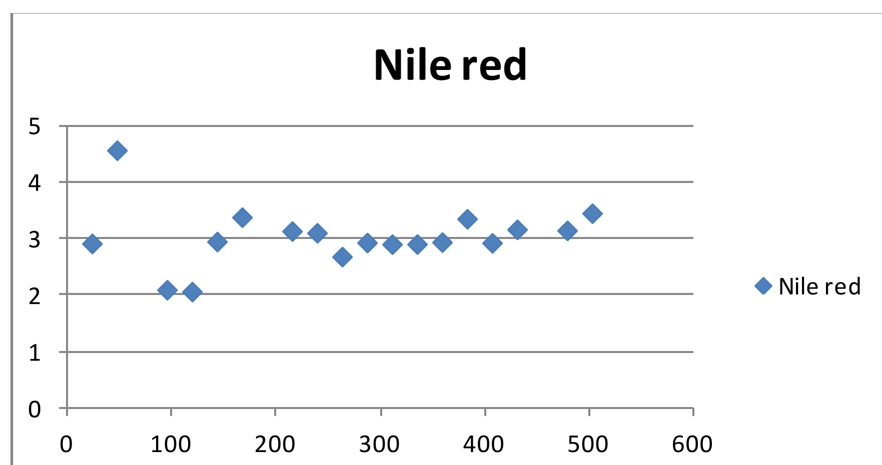
6.7 Nannochloropsis (original) f/2 medium 5% in seawater

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
7006	0.6	0.075	0.064	2.951	2.887	24
7064	0.54	0.1	0.064	4.599	4.535	48
7546	0.46	0.119	0.077	2.146	2.069	96
8130	0.39	0.122	0.078	2.114	2.036	120
9771	0.33	0.128	0.103	3.024	2.921	144
11206	0.32	0.125	0.084	3.436	3.352	168
11196	0.27	0.14	0.07	3.176	3.106	216
9681	0.29	0.141	0.059	3.134	3.075	240
9697	0.25	0.155	0.078	2.733	2.655	264
9387	0.27	0.161	0.066	2.969	2.903	288
9918	0.26	0.167	0.078	2.953	2.875	312
10206	0.24	0.16	0.056	2.933	2.877	336
8677	0.25	0.169	0.06	2.971	2.911	360
8045	0.25	0.173	0.065	3.389	3.324	384
7564	0.26	0.175	0.07	2.968	2.898	408
7704	0.24	0.175	0.069	3.205	3.136	432
7579	0.25	0.177	0.077	3.195	3.118	480
7055	0.25	0.18	0.075	3.497	3.422	504


Ft
12000
10000
8000
6000
4000
2000
0
0
100
200
300
400
500
600
Ft

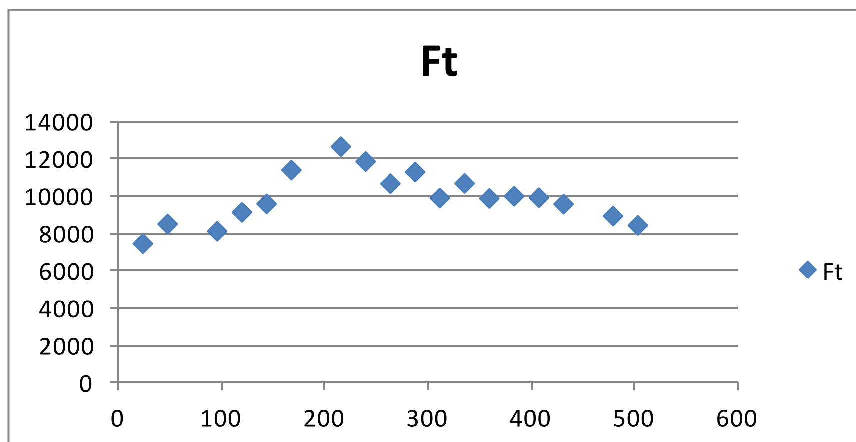

QY
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
QY

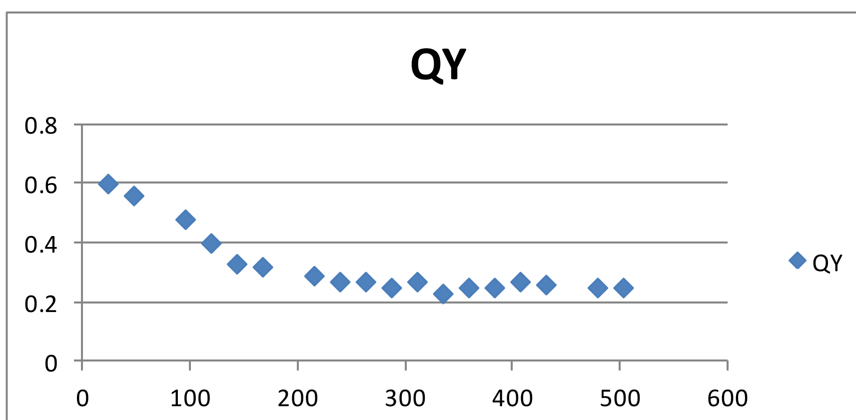

OD
0.2
0.15
0.1
0.05
0
0
100
200
300
400
500
600
OD

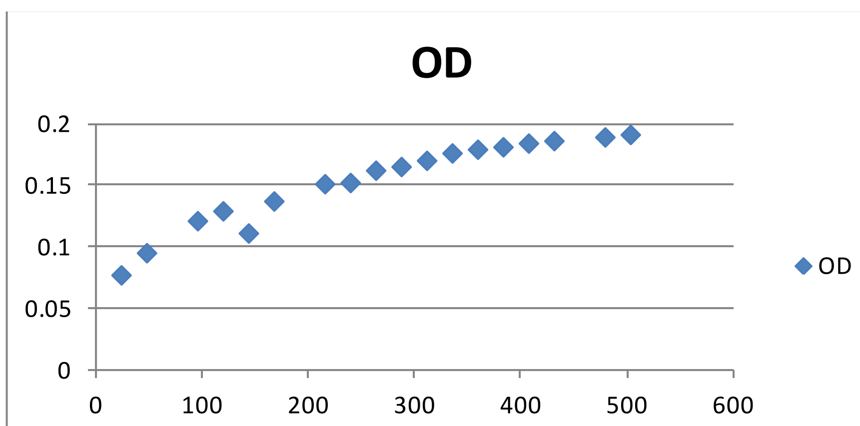

Nile red
5
4
3
2
1
0
0
100
200
300
400
500
600
Nile red

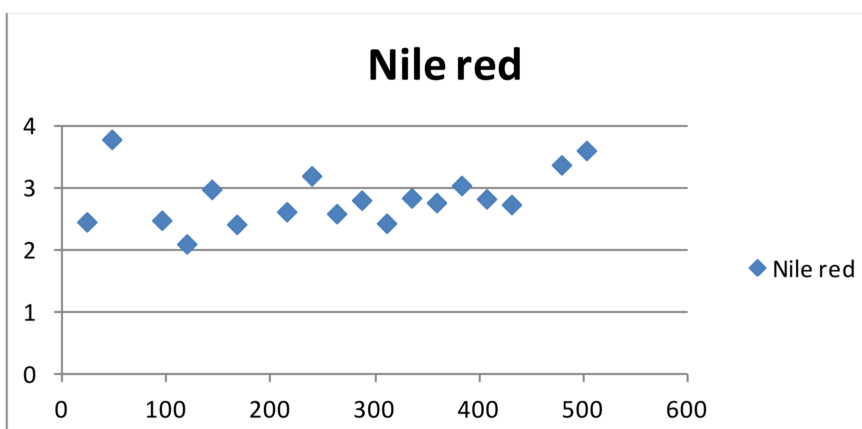
6.8 Nannochloropsis (starved) f/2 medium 5% in seawater

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
7472	0.60	0.076	0.08	2.502	2.422	24
8523	0.56	0.094	0.065	3.816	3.751	48
8137	0.48	0.120	0.073	2.521	2.448	96
9151	0.40	0.128	0.077	2.144	2.067	120
9605	0.33	0.110	0.071	3.016	2.945	144
11416	0.32	0.136	0.078	2.461	2.383	168
12672	0.29	0.150	0.082	2.668	2.586	216
11875	0.27	0.151	0.073	3.237	3.164	240
10690	0.27	0.161	0.060	2.615	2.555	264
11310	0.25	0.164	0.075	2.847	2.772	288
9921	0.27	0.169	0.066	2.466	2.400	312
10699	0.23	0.175	0.076	2.884	2.808	336
9897	0.25	0.178	0.079	2.812	2.733	360
10015	0.25	0.180	0.07	3.078	3.008	384
9937	0.27	0.183	0.069	2.862	2.793	408
9590	0.26	0.185	0.078	2.779	2.701	432
8953	0.25	0.188	0.081	3.421	3.34	480
8453	0.25	0.190	0.08	3.652	3.572	504


Ft
14000
12000
10000
8000
6000
4000
2000
0
0
100
200
300
400
500
600
Ft

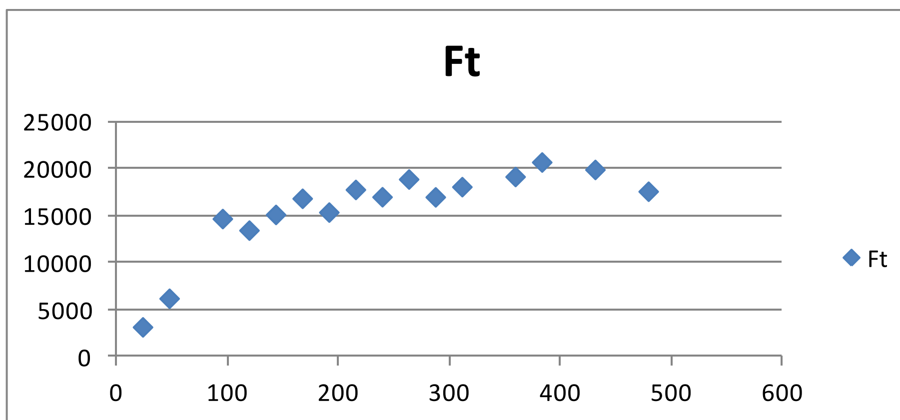

QY
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
QY

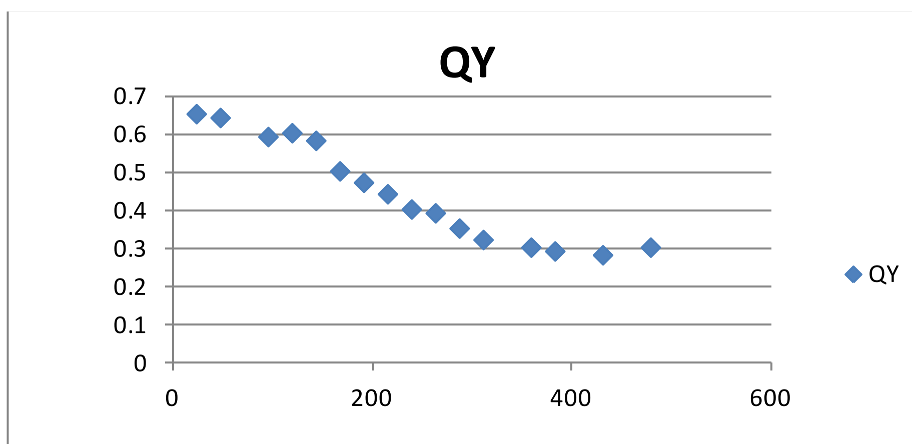

OD
0.2
0.15
0.1
0.05
0
0
100
200
300
400
500
600
OD

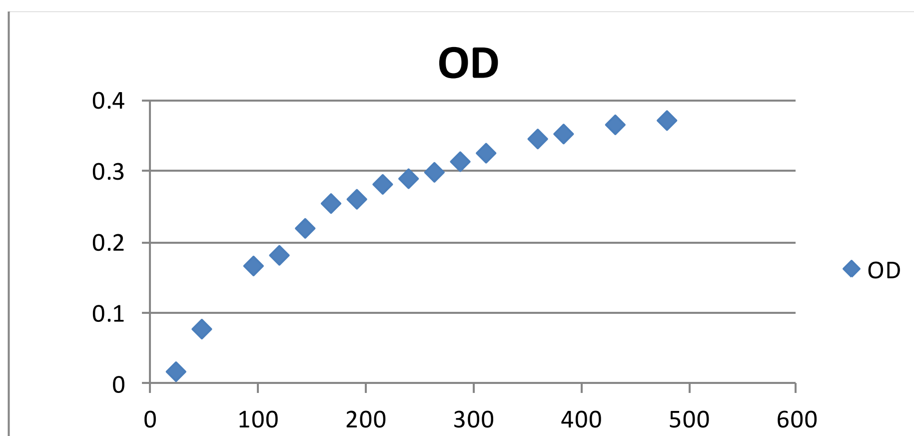

Nile red
4
3
2
1
0
0
100
200
300
400
500
600
Nile red

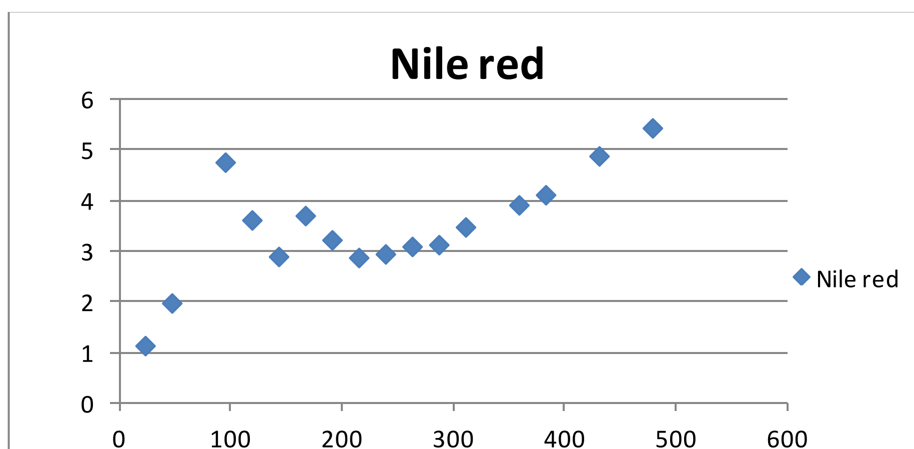
6.9 Nannochloropsis (original) f/2 medium 10% in seawater

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
						0
3197	0.65	0.016	0.050	1.164	1.114	24
6234	0.64	0.076	0.054	2.009	1.955	48
14696	0.59	0.165	0.074	4.808	4.734	96
13470	0.60	0.180	0.085	3.678	3.593	120
15135	0.58	0.218	0.109	2.982	2.873	144
16849	0.50	0.253	0.121	3.801	3.680	168
15384	0.47	0.259	0.088	3.289	3.201	192
17787	0.44	0.280	0.123	2.975	2.852	216
17013	0.40	0.288	0.113	3.036	2.923	240
18891	0.39	0.297	0.093	3.164	3.071	264
16998	0.35	0.312	0.105	3.211	3.106	288
18083	0.32	0.324	0.123	3.578	3.455	312
19160	0.30	0.344	0.120	4.011	3.891	360
20698	0.29	0.351	0.117	4.208	4.091	384
19911	0.28	0.364	0.117	4.974	4.857	432
17597	0.30	0.370	0.105	5.515	5.410	480


Ft
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
600
Ft

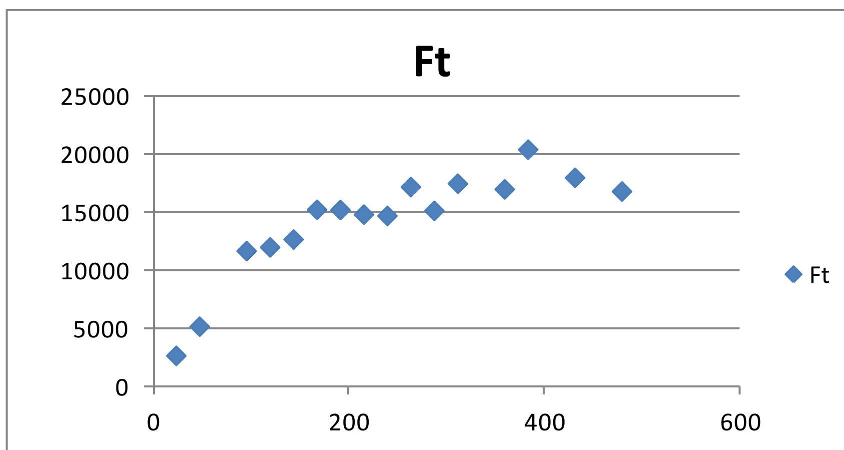

QY
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
QY

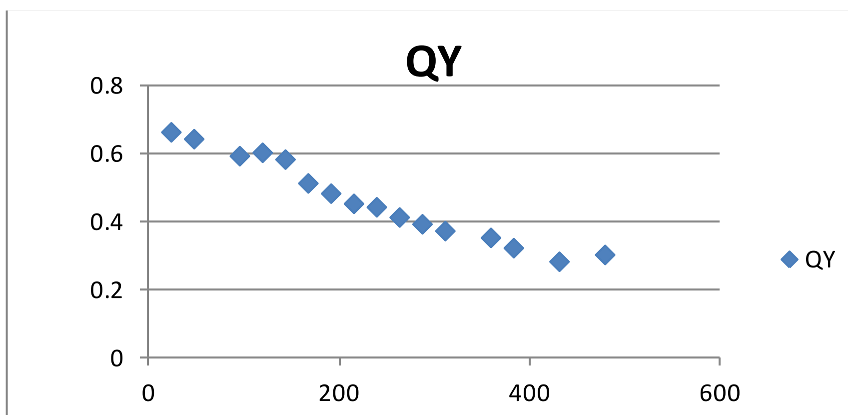

OD
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
OD

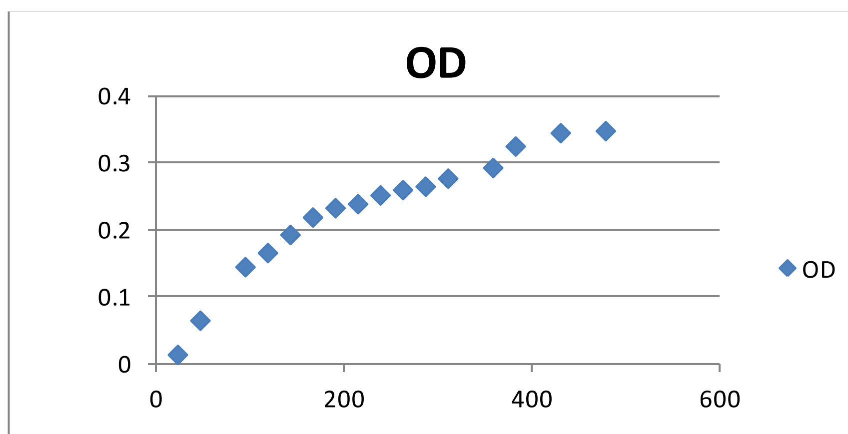

Nile red
6
5
4
3
2
1
0
0
100
200
300
400
500
600
Nile red

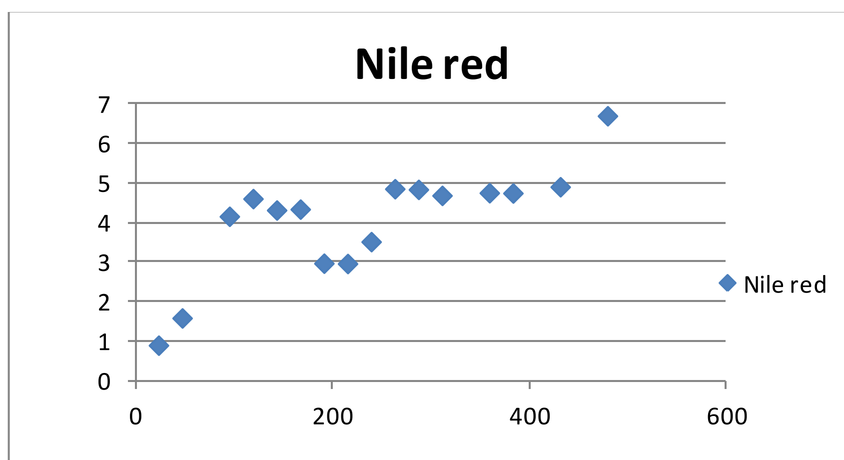
6.10 Nannochloropsis (starved) f/2 medium 10% in seawater

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
						0
2663	0.66	0.012	0.048	0.907	0.859	24
5178	0.64	0.063	0.055	1.603	1.548	48
11682	0.59	0.143	0.068	4.188	4.12	96
12004	0.6	0.164	0.074	4.642	4.568	120
12672	0.58	0.191	0.09	4.369	4.279	144
15234	0.51	0.217	0.098	4.4	4.302	168
15215	0.48	0.231	0.08	3.013	2.933	192
14816	0.45	0.237	0.09	3.015	2.925	216
14707	0.44	0.25	0.093	3.572	3.479	240
17193	0.41	0.258	0.101	4.919	4.818	264
15142	0.39	0.263	0.098	4.899	4.801	288
17481	0.37	0.275	0.095	4.744	4.649	312
16993	0.35	0.291	0.101	4.817	4.716	360
20409	0.32	0.323	0.115	4.824	4.709	384
17986	0.28	0.343	0.106	4.974	4.868	432
16809	0.3	0.346	0.111	6.775	6.664	480


Ft
25000
20000
15000
10000
5000
0
0
200
400
600
Ft

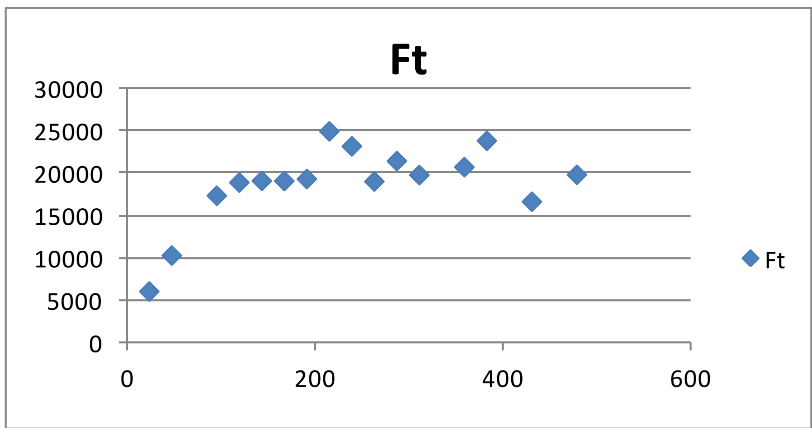

QY
0.8
0.6
0.4
0.2
0
0
200
400
600
QY

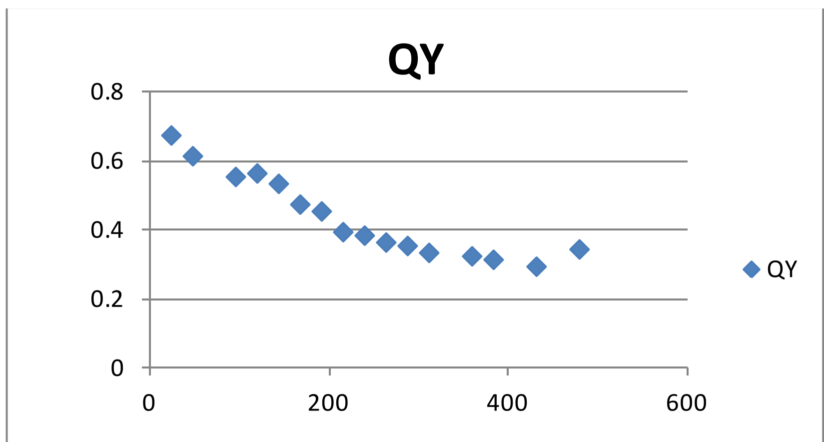

OD
0.4
0.3
0.2
0.1
0
0
200
400
600
OD

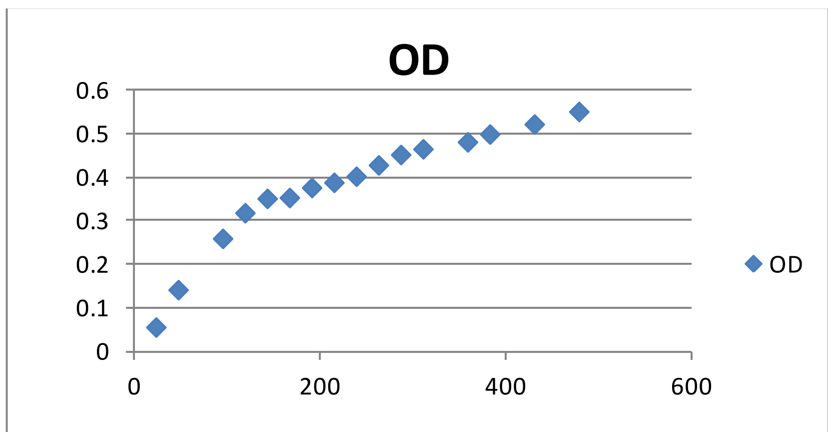

Nile red
7
6
5
4
3
2
1
0
0
200
400
600
Nile red

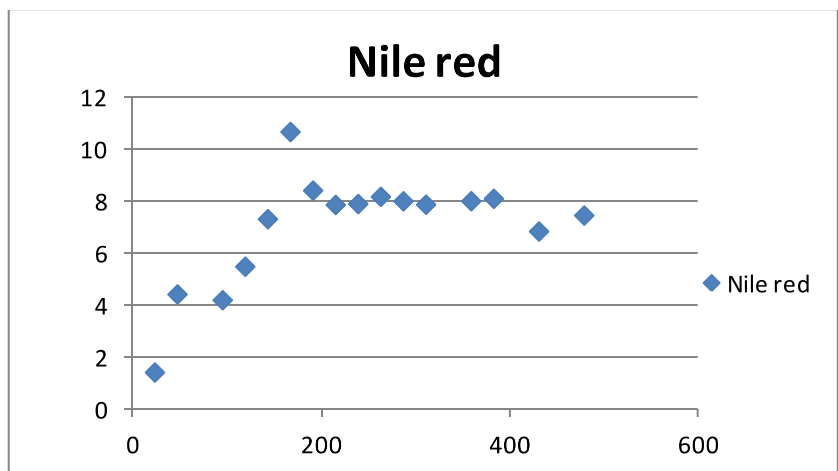
6.11 Nannochloropsis (original) f/2 medium 20% in seawater

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
						0
6129	0.67	0.052	0.054	1.437	1.383	24
10374	0.61	0.138	0.065	4.45	4.385	48
17438	0.55	0.256	0.121	4.284	4.163	96
18993	0.56	0.315	0.154	5.605	5.451	120
19192	0.53	0.348	0.138	7.419	7.281	144
19173	0.47	0.35	0.125	10.76	10.635	168
19412	0.45	0.373	0.144	8.524	8.38	192
25035	0.39	0.385	0.139	7.968	7.829	216
23274	0.38	0.399	0.147	8.011	7.864	240
19116	0.36	0.425	0.161	8.301	8.14	264
21531	0.35	0.449	0.163	8.131	7.968	288
19879	0.33	0.462	0.154	7.993	7.839	312
20813	0.32	0.478	0.16	8.131	7.971	360
23918	0.31	0.496	0.167	8.233	8.066	384
16730	0.29	0.519	0.162	6.969	6.807	432
19910	0.34	0.548	0.165	7.587	7.422	480


Ft
30000
25000
20000
15000
10000
5000
0
0
200
400
600
Ft

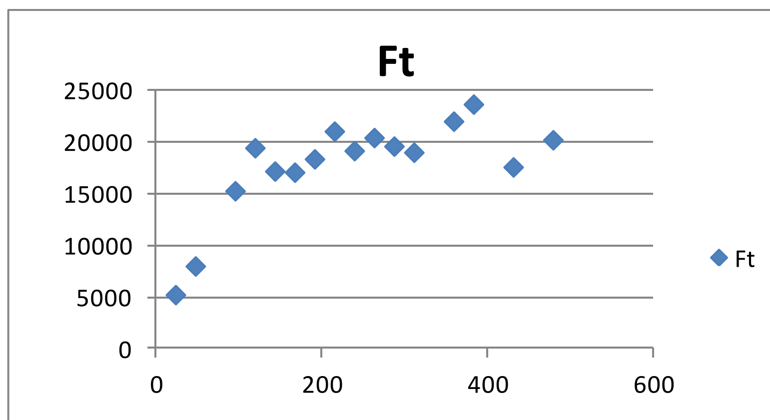

QY
0.8
0.6
0.4
0.2
0
0
200
400
600
QY

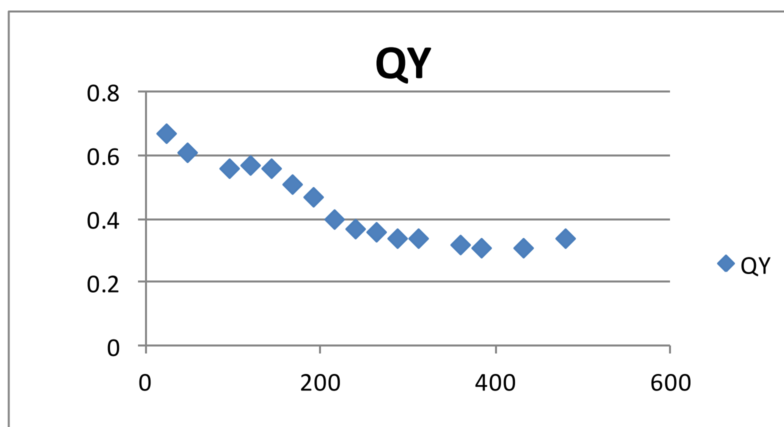

OD
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
OD

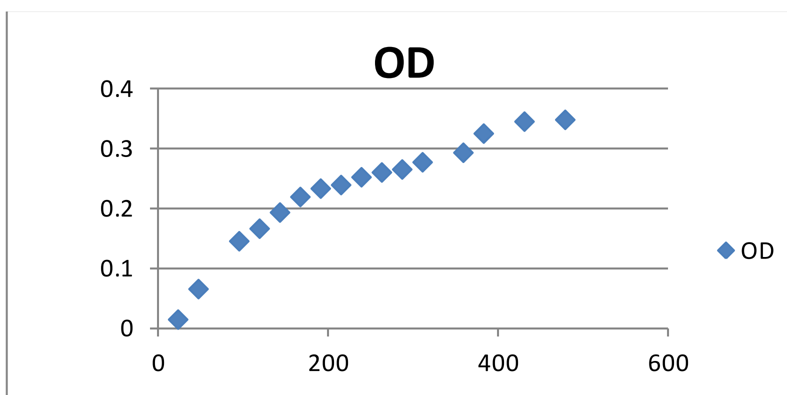

Nile red
12
10
8
6
4
2
0
0
200
400
600
Nile red

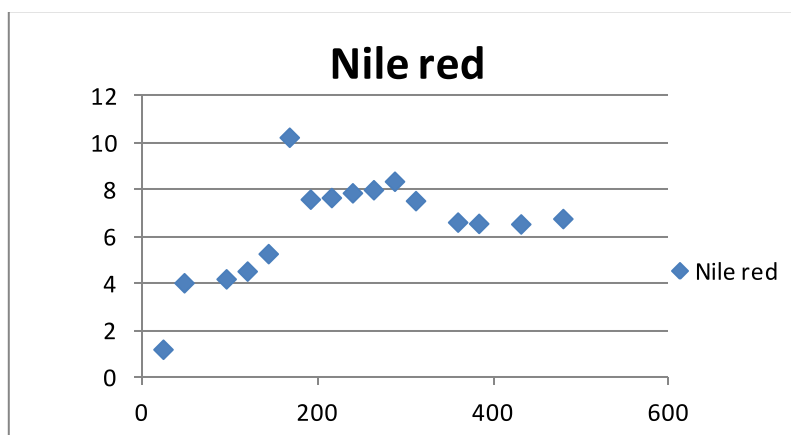
6.12 Nannochloropsis (starved) f/2 medium 20% in seawater

Ft	QY	OD	NRr	NR	Nile red	Time(h)
						0
5238	0.67	0.045	0.06	1.186	1.126	24
8005	0.61	0.126	0.091	4.055	3.964	48
15274	0.56	0.229	0.102	4.233	4.131	96
19432	0.57	0.263	0.123	4.585	4.462	120
17179	0.56	0.307	0.168	5.376	5.208	144
17069	0.51	0.322	0.124	10.30	10.176	168
18355	0.47	0.334	0.116	7.649	7.533	192
21037	0.40	0.346	0.117	7.719	7.602	216
19152	0.37	0.362	0.121	7.925	7.804	240
20417	0.36	0.381	0.135	8.071	7.936	264
19590	0.34	0.390	0.141	8.439	8.298	288
18997	0.34	0.401	0.137	7.607	7.470	312
22001	0.32	0.421	0.149	6.703	6.554	360
23649	0.31	0.443	0.153	6.652	6.499	384
17577	0.31	0.472	0.164	6.639	6.475	432
20199	0.34	0.516	0.171	6.876	6.705	480


Ft
25000
20000
15000
10000
5000
0
0
200
400
600
Ft

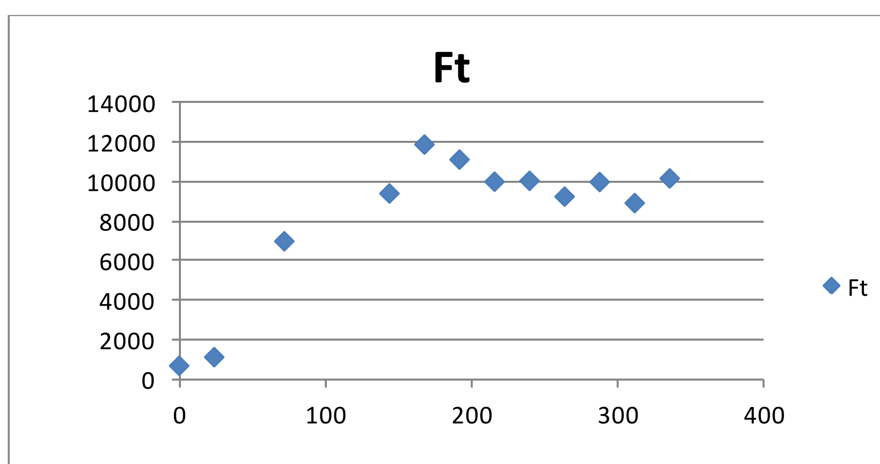

QY
0.8
0.6
0.4
0.2
0
0
200
400
600
QY

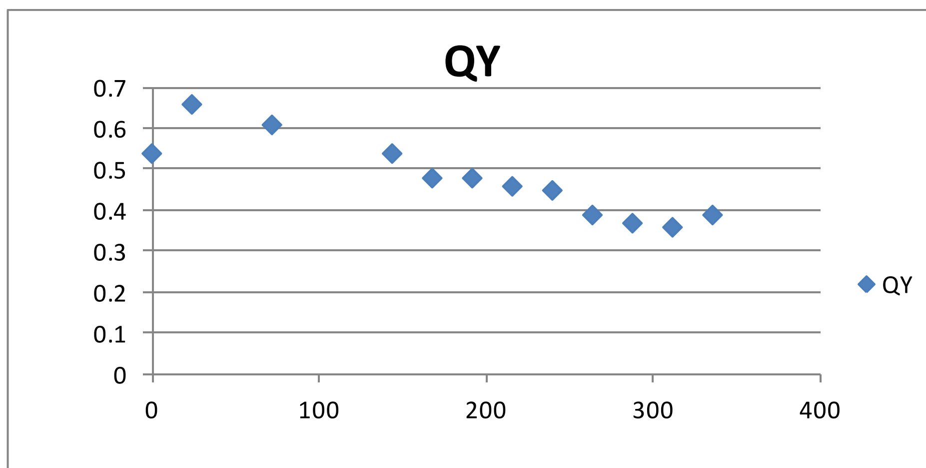

OD
0.4
0.3
0.2
0.1
0
0
200
400
600
OD

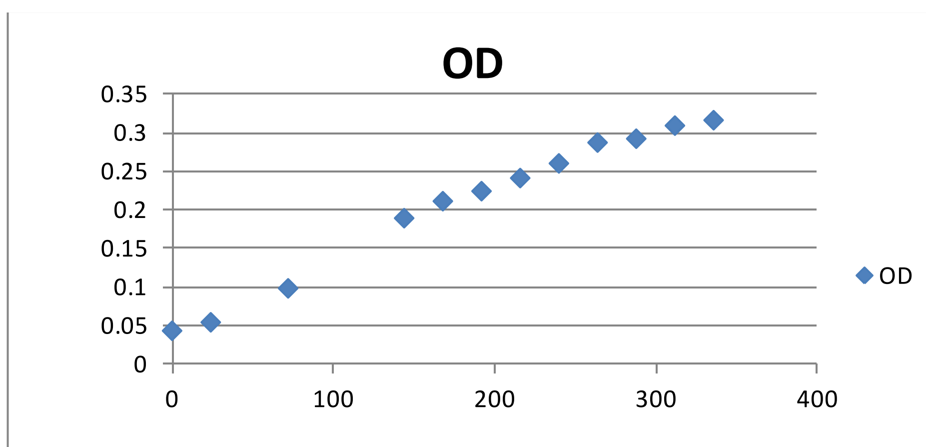

Nile red
12
10
8
6
4
2
0
0
200
400
600
Nile red

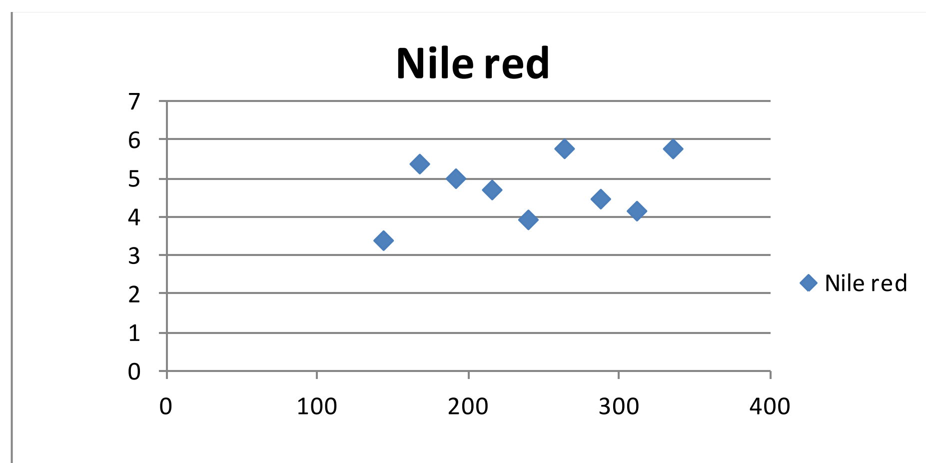
6.13 Nannochloropsis culture from high N (original) cultured in low N f/2 medium 5%

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
667	0.54	0.042				0
1104	0.66	0.053				24
6971	0.61	0.097				72
9391	0.54	0.188	0.112	3.465	3.353	144
11868	0.48	0.210	0.118	5.456	5.338	168
11107	0.48	0.223	0.118	5.078	4.960	192
9987	0.46	0.240	0.120	4.786	4.666	216
10032	0.45	0.259	0.108	3.999	3.891	240
9231	0.39	0.286	0.127	5.862	5.735	264
9972	0.37	0.291	0.127	4.556	4.429	288
8905	0.36	0.308	0.129	4.247	4.118	312
10154	0.39	0.315	0.127	5.858	5.731	336



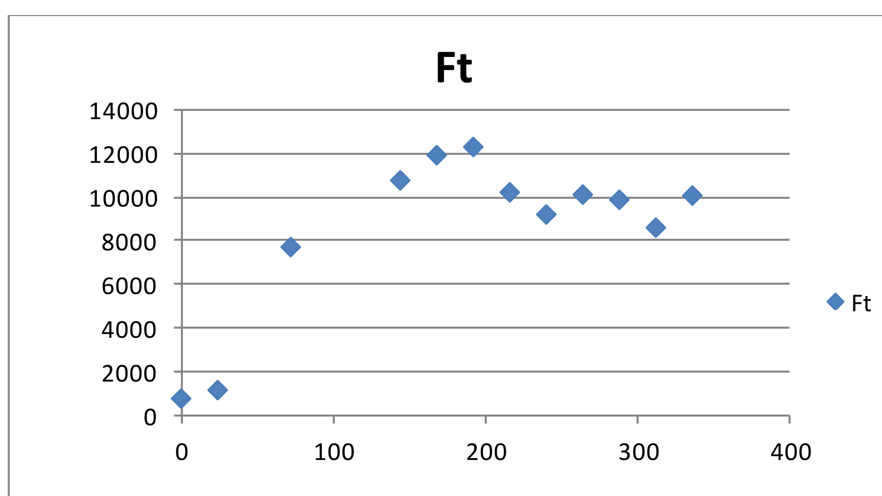

QY
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
QY

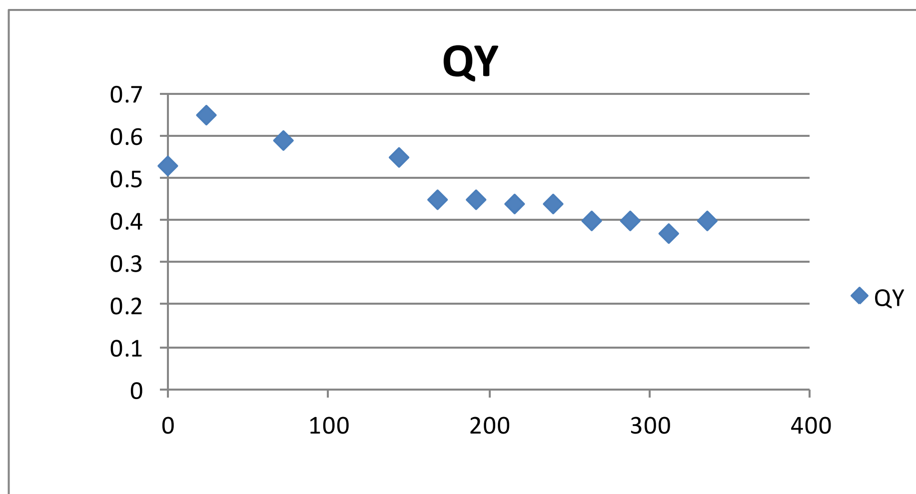

OD
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
100
200
300
400
OD

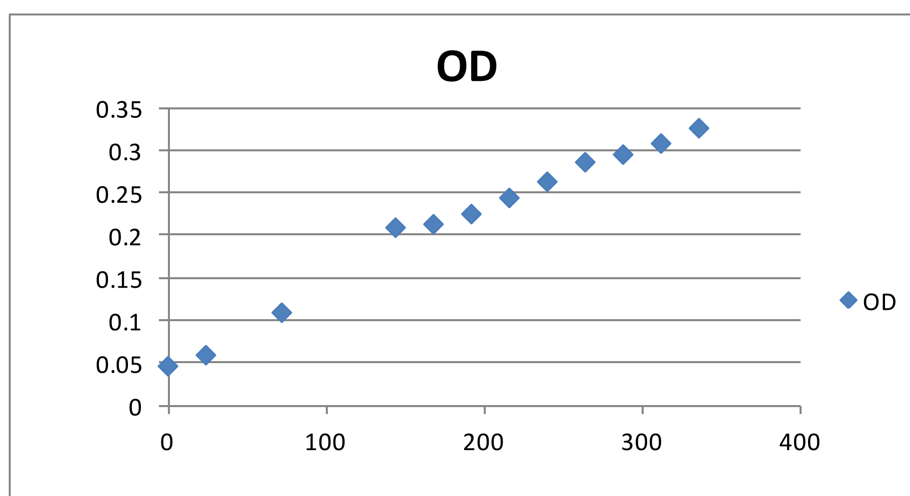

Nile red
7
6
5
4
3
2
1
0
0
100
200
300
400
Nile red

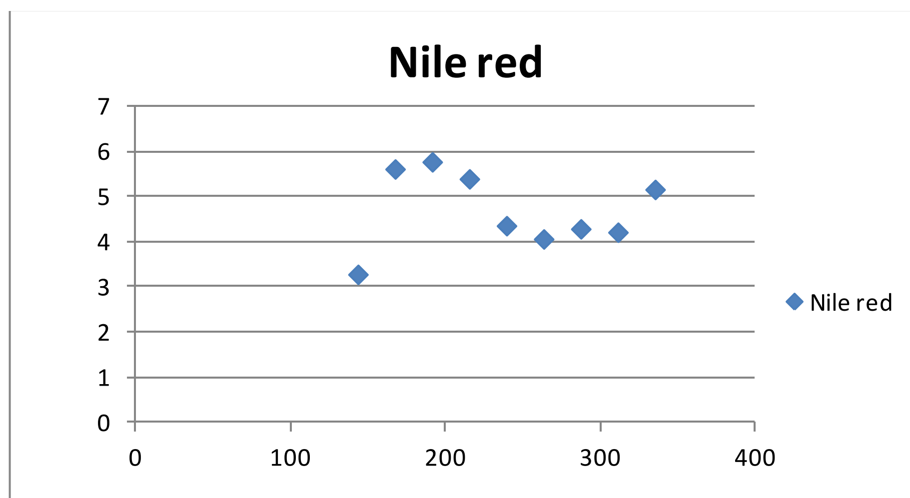
6.14 Nannochloropsis culture from high N (starved) cultured in low N f/2medium 5%

Ft	QY	OD	NRr	NR	Nile red	Time(hours)
773	0.53	0.047				0
1161	0.65	0.060				24
7708	0.59	0.110				72
10760	0.55	0.210	0.130	3.372	3.242	144
11914	0.45	0.214	0.122	5.696	5.574	168
12289	0.45	0.226	0.110	5.842	5.732	192
10213	0.44	0.245	0.124	5.479	5.355	216
9200	0.44	0.264	0.137	4.456	4.319	240
10110	0.40	0.287	0.129	4.154	4.025	264
9873	0.40	0.296	0.117	4.366	4.249	288
8596	0.37	0.309	0.124	4.299	4.175	312
10065	0.40	0.327	0.136	5.257	5.121	336




QY
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
QY


OD
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
100
200
300
400
OD


Nile red
7
6
5
4
3
2
1
0
0
100
200
300
400
Nile red

6.15 Cell number counting

Nannochloropsis original culture

5% in seawater		10% in seawater		20% in seawater	
Area (E)	**Cell No.**	**Area (E)**	**Cell No.**	**Area (E)**	**Cell No.**
1	20	**1**	32	**1**	38
2	18	**2**	38	**2**	34
3	13	**3**	31	**3**	40
4	11	**4**	37	**4**	42
5	12	**5**	33	**5**	36
6	18	**6**	32	**6**	34
Average(/ μL)	3.83×10^3	**Average(/ μL)**	8.46×103	**Average(/ μL)**	9.33×103

Nannochloropsis starved culture

5% in seawater		10% in seawater		20% in seawater	
Area (E)	**Cell No.**	**Area (E)**	**Cell No.**	**Area (E)**	**Cell No.**
1	11	**1**	27	**1**	44
2	17	**2**	26	**2**	50
3	14	**3**	30	**3**	46
4	20	**4**	41	**4**	43
5	19	**5**	39	**5**	51
6	22	**6**	31	**6**	58
Average(/ μL)	4.29×103	**Average(/ μL)**	9.08×103	**Average(/ μL)**	1.22×104

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