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Upscaling of five commercial microalgae strains and harversting by induced flocculation and Salsnes Filter technology

Oppskalering av fem typer kommersielle mikroalgestammer og innhøsting ved indusert flokkulering og Salsnes Filter teknologi

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Acknowledgements

This study is composed of two parts: Upscaling and harvesting of commercial microalgae.

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Abstract

This study is composed of two parts. The first one is scaling up of the broth algae from the batch cultures and the second is the harvesting processes by using the commercial available Salsnes Filter technology in conjugation with flocculants. Each part is presented with their respective introduction, material and methods, results and conclusions, and at the end of the study, a finally summarized overall conclusion.

For the upscaling part, the experiments were performed first at plant laboratory for the batch phase of the microalgae cultivation and continuous illumination of 200µmol$m^{-2}s^{-1}$ subsequently in a greenhouse room deprived of artificial illumination and of heating devices, at SKP (Senter for klimaregulert planteforskning), at the northern geographical location (Ås, SKP, center for climate regulated plant environment, 59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level)

The five microalgae strains investigated were: *Chlorella vulgaris, Dunaliella salinas, Nannochloropsis oculata*, cultivated for a period of 63 days (20.06.12-23.08,12), and *Scenedesmus sp, Chlorella wild mix Årungen*, cultivated for a period of 42 days (20.07.12-23.08.12). The cultures were cultivated, in semicontinuous operation system, with enriched CO_2 air (3%) and prepared *in situ,* trifold nitrogen nutrition media (3N-BBM+Vit), and the volume was replenished when necessary. The cultivation occurred in polypropylene open tray photobioreactor (TPBR) in duplicate. The cultures were monitored daily and the following parameters were recorded: time of sampling, outside weather condition (cloudy, patchy-cloudy, sunny, rainy, unstable), outside temperature, culture temperature, irradiation on surface of culture, pH, dissolved oxygen (DO), conductivity, salinity, biomass concentration, i.e. total suspended solids (TSS).

The effects of irradiance and temperature on specific growth rate and daily growth were investigated, and was found that all the strains specific growth were affected positively by higher irradiation energy input in combination with a moderate culture temperature around 23°C. Among the freshwater microalgae strains, *Scenedesmus sp.* TPBR 7 had the highest specific growth rate with 0,05 h^{-1} and showed a fivefold increase when doubling the irradiation energy input and temperature kept at 23°C. Among the marine microalgae species, *Dunaliella salinas* TPBR 3 had the highest specific growth rate with 0,024h^{-1} and showed a 71,4% increase when doubling the irradiation energy input and temperature kept at 23°C.

The effects of outside weather conditions in conjugation with irradiation and temperature on oxygen evolution (dissolved, DO) were investigated and it was found that the sum of the five strains data (Table 3) for cloudy days gave a total of 70,71 mgL^{-1} of dissolved oxygen (DO) generated by a PAR irradiation sum of 298,62 MJ $m^{-2}d^{-1}$ mean while the sum for the sunny days gave 54,06 mgL^{-1} of dissolved oxygen (DO) mgL^{-1} of dissolved oxygen (DO) generated by a PAR irradiation sum of 787,24 MJ $m^{-2}d^{-1}$. By lowering the PAR irradiation 2,64 times it was generated 31% higher dissolved oxygen. The cloudy weather conditions in combination with lower PAR irradiance and lower culture temperature affected directly the algae cell´s O_2 evolution, which was emitted at higher volumes than when the sunny weather combined with higher irradiation and higher culture temperature.

The effects of dilution regime on biomass output per TPBR were investigated and fount that all data reported overstepped the recommended threshold of 0,20 of dilution rate. There were registered values of 0,353 and 0,733 d^{-1} for lowest and highest dilution rates, respectively. Among the freshwater microalgae strains, *Scenedesmus sp.* TPBR 7 had the highest biomass yield doubling its g DW when the dilution rate was 39% less harsh gave 200% higher algae output, than the dilution regime for the same algae strain in the respective TPBR 8. The benefits were of magnitude 1:5.

Among the marine microalgae species, *Dunaliella salinas* TPBR 3 had the highest biomass yield increased by 31% of it´s g DW when the dilution rate was 41% less harsh than the dilution regime for same algae strain in the respective TPBR 4. The effect of dilution on biomass output was 3% less harsh dilution and gave 31% higher algae output. The benefits were of magnitude 10,3. The cultures of *Chlorella wild mix Årungen*, freshwater strain, were washout due to the over dilution of almost the entire TPBR volumes were removed and replenished. The cultures were thrice inoculated with fresh broth from the backup batch cultures in the algae laboratory room.

The effects if irradiance on photosynthetic efficiency were investigated and found that among the values of the freshwater strains, a four times lower irradiance gave 4,6 times higher photosynthetic efficiency, and among the values of the saltwater strains a 61% lower irradiance gave 4 times higher photosynthetic efficiency. The level of irradiance had an inverted effect on the photosynthetic efficiency, i.e. lower irradiance absorbed by the high dilute algae culture gave higher photosynthetic efficiency of magnitude 4.

The effects of irradiance on areal CO_2 fixation rate were investigated and found after using in these experiments, the mean higher heating value (22,49 KJ g^{-1}) recorded by Hulatt et al., (2011), and comparing the results in this study, that the mean $C0_2$ fixation rate was 55,44 g CO_2 $m^{-2}d^{-1}$ (Table 6), which is 2,6 times higher values than found by Hulatt (2011).

The effects of mixing (turbulence) on biomass productivity (P) were investigated and found that according to Hu Q, et al., (1996) mixing is the factor, which enhances growth of algal culture and therefore its biomass productivity. In this study, was investigated the relationship between biomass productivity and the turbulence created by mixing the algae suspension by means of aquarium pump placed inside the TPBR 2 (*Chlorella vulgaris*), TPBR 4 (*Dunaliella salinas*), and TPBR 6 (*Nannochloropsis oculata*) and it was reported the following decrease/increase in biomass productivity: -11%, +62% and +57,14%, respectively. The benefits of a turbulence regime should have been higher if the dilution regime had been lower (approximately 0,20 d^{-1}) than the operated in this study, as well as the remained culture volume, which affected severely the biomass output. Keeping a semicontinuous cultures system in adequate volumes and at stable low dilution rate, will influence the biomass productivity yield increasing it multiple times.

The effects of outside weather conditions on TPBRs energetic efficiency were investigated and found that the overall irradiation utilization efficiency (*IUE*) provided by the TPBR on these experiments shows that the saltwater strain, *Nannochloropsis oculata,* TPBR 6, performed the highest values of all TPBRs, with 1,37 g M J^{-1} utilization efficiency of the irradiation that penetrated the walls and lid of the TPBR 6. This value is

too low compared to 5.1 and 6.4 g M J^{-1}, values recorded by Zhang (1999, 2000). TPBR3, saltwater strain *Dunaliella salinas,* reported the next highest efficiency with 0,923 g M $J^{-1}$was even lower. Comparing the results for performance by TPBR6 and TPBR 3, 1,36 and 0,923 g M J^{-1} with the average value of 5,75 g M J^{-1} (Zhang 1999, 2000), showed that Zhang´s flat PBR excelled in performance approximately five times better that the TPBR of this experiments. The differences could be explained in the optical pathway for the TPBR in this study and Zhang´s flat PBR, 0,09 and 0,01 cm, respectively.

These harvesting experiments were performed by use of Salsnes Water to Algae Treatment (SWAT), to harvest five microalgae strains by means of filtration after a flocculation process. The removal efficiency aim was ≥90% of the particles (algae) found in the water suspension. The method designed for wastewater treatment, is known as Salsnes Filter technology (SF), consist of commercial fine mesh sieve set. The five strains of microalgae were grown in TPBR in duplicate in TPBR in greenhouse conditions with no additional illumination. Two types of flocculators were used: bench scale jar tester and pilot scale 20L flocculator. An Instrument for size and morphology analysis of microalgae before and after flocculation was used. The poor removal efficiencies performed by gravity filtration corroborated the need of flocculants to harvest microalgae. PAX (inorganic chemical) and chitosan (organic powder of crustacean shell origin) are commercial flocculants with high removal efficiencies (≥95%), in some of the microalgae. Polycationic polymers were screened by means of test jar test flocculator aiming to find the optimal dosage, stirring speed, G-values, and removal efficiencies. Upscaling for rapid and slow mixing in 20L flocculator was found through G-values, calculated by using powers input and empirical equations. The conjugation of tested flocculators together with optimized polymer dosage achieved a harvested biomass in form of total suspended solids (TSS), with removal efficiencies ≥90% where reported for freshwater strains *Chlorella vulgaris* (98%, 55µm sieve cloth), and *Scenedesmus sp* (98%, 18µm sieve cloth), and for saltwater strain *Nannochloropsis oculata* (92%, 15 µm sieve cloth). Specific flow rate were recorded. No cationic polymer could achieve significant removal rate on saltwater strain, *Dunaliella salinas,* but the synergetic combination of PAX + anionic polymer produced flocs with poor compressibility factor when tested in pilot scale. The SWAT technology has to focus on G-value, polymer/chemical dosage and µm size mesh sieve specific for each microalgae strain, making it capable for using as a microalgae harvesting method

A protocol for preparation of microorganisms in microalgae suspension for study with SEM scanning electronic microscopy, was developed to analyse the effects of cryopreservation of algae suspension at 4°C for 100 days. It was found that the saltwater strains endured the storage in darkness and survived, but not the freshwater strains, which were decomposing (Appendix 8 –SEM protocol for microalgae suspensions)

Sammendrag

Dette studiet er todelt.
Del 1 handler om oppskalering av konsentrert algesuppe hentet fra batch-kulturer og del 2 om innhøstingsprosesser der kommersielt tilgjengelig filterteknologi fra Salsnes ble testet med og uten flokkulanter.
Hver del er presentert med introduksjon, materiale og metoder, resultater og diskusjoner. Til slutt følger en oppsummerende generell konklusjon.

Del 1: Oppskalering av konsentrert algesuppe
Forsøkene ble utført ved Senter for Klimaregulert Planteforskning (SKP), nordlig geografisk posisjon (Ås, SKP, 59° 40' 06.94'' N, 10 ° 46' 15,52'', 107 moh).
Batch-fasen ble gjennomført i plantelaboratoriets algerom med kontinuerlig bakgrunnslys på 200µmol$m^{-2}s^{-1}$. Videre dyrking fortsatte i drivhusrom uten kunstig belysning eller oppvarmingsutstyr.

Fem mikroalgestammer ble undersøkt: *Chlorella vulgaris, Dunaliella salinas og Nannochloropsis oculata* ble dyrket i 63 dager (20.06.12-23.08.12), mens *Scenedesmus sp og Chlorella wild mix Årungen* ble dyrket i 42 dager (20.07.12-23.08.12).
Kulturene ble dyrket med halvkontinuerlig drivsystem, med tilførsel av 3% CO_2-beriket luft. Trefoldig nitrogen næringsmedia (3N-BBM+Vit) ble tilberedt *in situ* og tilført fotobioreaktorene (TPBR) ved behov.
Dyrkingen ble gjennomført i åpne polypropylen-kar i duplikat.
Kulturene ble overvåket daglig og følgende parametre ble notert: opptakstid, utendørs værforhold (sol, delvis skyet, overskyet, nedbør, skiftende vær), utetemperatur, kulturtemperatur, lysinnstråling over algekulturens overflate, pH-verdi, mengde oppløst oksygen, konduktivitet, saltinnhold og biomassekonsentrasjon dvs. total suspendert tørrstoff.

Effektene av lysinnstråling og temperatur på spesifikk vekstrate og daglig vekst ble undersøkt. Det ble funnet at alle stammenes spesifikke vekstrate ble påvirket positivt ved høyere tilførsel av innstrålingsenergi i kombinasjon med moderat kulturtemperatur på ca. 23°C.
Av mikroalgestammene fra ferskvann, hadde *Scenedesmus sp.* TPBR 7 den høyeste spesifikke vekstraten på 0,05 h^{-1}. Vekstraten økte fem ganger når tilførselen av innstrålingsenergi ble doblet og temperatur var 23°C.
Av mikroalgestammer fra saltvann, hadde *Dunaliella salinas* TPBR 3 den høyeste spesifikke vekstrate på 0,024h^{-1}. Vekstraten økte 71,4% når tilførselen av innstrålingsenergi ble doblet og temperatur var 23°C.

Effektene som utendørs værforhold i kombinasjon med PAR-innstråling og temperatur ga på oksygenutvikling (oppløst, DO) ble undersøkt. Det ble funnet at summen av alle fem stammers data (tabell 3) for overskyede dager ga en total på 70,71 mgL^{-1} av utviklet oksygen (DO). Oksygenet ble generert ved PAR-innstråling som i sum for de overskyede dagene utgjorde 298,62 MJ $m^{-2}d^{-1}$.
Summen av de skyfrie dagene ga 54,06 mgL^{-1} utviklet oksygen (DO). Oksygenet ble generert ved PAR-innstråling som i sum for de skyfrie dagene utgjorde 787,24 MJ $m^{-2}d^{-1}$.
Ved å redusere PAR-innstrålingen 2,64 ganger ble det 31% høyere utvikling av oksygen.
Overskyet vær i kombinasjon med lavere PAR-innstråling og lavere kulturtemperatur

påvirket direkte algecellenes O_2-utvikling som ble utsondret i høyere volumer enn når det var skyfritt kombinert med høyere innstråling og høyere kulturtemperatur.

Effektene av fortynningsregime på biomasseutbytte per TPBR ble undersøkt. Det ble funnet at alle innsamlede data rapporterte overskridelse av den anbefalte terskelen på 0,20 d^{-1} fortynningsrate. Det ble registrert verdier på 0,353 og 0,733 d^{-1} for henholdsvis de laveste og høyeste fortynningsrate.
Av mikroalgestammene fra ferskvann, hadde *Scenedesmus sp.* TPBR 7 det høyeste biomasseutbyttet. Biomasseutbyttet ble fordoblet målt i g DW når fortynningsraten ble redusert med 39%. Redusert fortynningsrate i TPBR 7 ga 200% høyere biomasseutbytte enn det som ble registrert med samme algestammen i TPBR 8. Redusert fortynningsrate ga en effekt på 1:5 i biomasseutbytte.
Av mikroalgestammene fra saltvann hadde *Dunaliella salinas* TPBR 3 det høyeste biomasseutbyttet. I TPBR 3 var fortynningsraten redusert med 1,8%. Da økte biomasseutbyttet med 31% målt i g DW sammenlignet med samme algestamme i TPBR 4.
Redusert fortynningsrate i TPBR 3 var 1,8% og ga 31% høyere biomasseutbytte. Redusert fortynningsrate ga her en effekt på 1:17,2 i biomasseutbytte.

Ferskvannkulturen *Chlorella wild mix Årungen,* ble vasket ut på grunn av en overdreven fortynning, dvs. omtrent hele volumet ble fjernet og fylt opp på nytt. Disse kulturene ble inokulert tre ganger med fersk konsentrert algesuppe hentet fra backup av batch-kulturen fra laboratoriets algerom. Av denne grunn blir data fra fortynningen ikke analysert her.

Effektene av lysinnstråling på fotosyntetisk effektivitet ble undersøkt.
Blant ferskvannsstammer ga fire ganger lavere innstråling 4,6 ganger høyere fotosyntetisk effektivitet.
Blant saltvannsstammer ga 61% lavere innstrålingen 4 ganger høyere fotosyntetisk effektivitet.
Innstrålingsnivåene hadde en omvendt effekt på fotosyntetisk effektivitet, dvs. lavere absorbert innstråling i svært fortynnet algekultur ga høyere fotosyntetisk effektivitet.

Effektene av lysinnstråling på CO_2-fikseringsrate/areal ble undersøkt. Det ble funnet at middels CO_2-fikseringsrate var 55,44 g CO_2 $m^{-2}d^{-1}$ (Tabell 6), noe som er 2,6 ganger høyere enn verdiene funnet av Hulatt (2011). I forsøkene ble det holdt middels høye varmeverdier (22,49 KJ g^{-1}).

Effektene av miksing (turbulens) på biomasseproduktivitet (*P*) ble undersøkt. Ifølge Hu Q, et al., (1996) er miksing en av faktorene som fremmer vekst i algekulturer, ergo dens biomasseproduktivitet.
I dette studiet ble turbulens skapt ved miksing av algekulturen ved hjelp av en akvariepumpe plassert i TPBR 2 (*Chlorella vulgaris*), TPBR 4 (*Dunaliella salinas*) og TPBR 6 (*Nannochloropsis oculata*). Det ble registrert følgende økning/reduksjon av biomasseproduktivitet i disse kulturene - henholdsvis -11%, +62% og +57,14%.
Fordelen med turbulens skulle ha vært høyere hvis fortynningen hadde vært lavere (ca. 0, 20 d^{-1}). Det ble også funnet at det gjenværende kulturvolumet i karet påvirket biomasseutbyttet. Disse faktorene er vesentlige ved halvkontinuerlige kultursystemer.

Effektene av utendørs værforhold på TPBRs energetiske effektivitet ble undersøkt. Det ble funnet at lysinnstrålingens brukseffektivitet (*IUE*) tilført via polypropylen i TPBR-karene var positive.
Saltvannsstammen *Nannochloropsis oculata,* TPBR 6, fikk de høyeste verdiene av alle TPBRs, med 1,37 g M J^{-1} brukseffektivitet av lysinnstrålingen som trengte gjennom vegger og lokk. Denne verdien er lav sammenlignet med verdiene 5.1 and 6.4 g M J^{-1} som ble registrerte av Zhang (1999, 2000).
TPBR3, saltvannsstammen *Dunaliella salinas,* registrerte den nest høyest effektiviteten med 0,923 g M J^{-1}.
Resultatene for TPBR 6 og TPBR 3 i forhold til gjennomsnittsverdien 5,75 g M J^{-1} (Zhang 1999, 2000), viser at Zhangs flate fotobioreaktor har en ytelse omlag fem ganger bedre enn TPBR brukt i disse forsøkene. Forskjellene kan forklares ved hjelp av den optiske banen for TPBR (0,09 m dype) i dette studiet og Zhangs flate fotobioreaktor (0,01 m dype).

Del 2: Innhøstingsprosesser
Forsøkene ble gjennomført med Salsnes Water to Algae Treatment (SWAT) til å høste inn fem mikroalgestammer ved hjelp av filtrering etter flokkuleringsprosess. Målet for fjerningseffektiviteten ble satt til ≥90% av partikler (mikroalger) funnet i vannsuspensjonen .
Metoden som opprinnelig ble designet for avløpsvann - kjent som Salsnes Filter technology (SF) - består av kommersielle, finmaskede silsett.
De fem mikroalgestammene ble dyrket opp i TPBR i duplikat under drivhusforhold uten ekstra belysning.
To typer flokkulatorer ble brukt: Bench Scale Jar Tester og Pilot Scale 20L flokkulator. Et instrument for måling og morfologisk analyse av mikroalger før og etter flokkulering ble brukt.
Den lave fjerningseffektiviteten ga ved gravitasjonsfiltrering bekreftet behovet for flokkulanter for innhøsting av mikroalgene.
PAX (uorganisk kjemikalie) og chitosan (organisk pulver fremstilt av skalldyrskall) er kommersielle flokkulanter med høy fjerningseffektivitet (≥95%) på noen mikroalger. Polykationiske polymerer ble sortert ut ved hjelp av Jar Tester flokkulator for å finne den optimale doseringen, miksingshastighet, G-verdier og fjerningseffektivitet.

Oppskalering for rask og treg miksing i 20L flokkulator ble testet ved hjelp av G-verdiene, og G-verdiene ble regnet ut ved hjelp av kraftforbruk og empiriske likninger. Ved å teste flokkulator sammen med optimalisert polymer-dosering ble det innhøstet biomasse i form av total suspendert tørrstoff (TSS). Fjerningseffektiviteten på ≥90% ble rapportert for ferskvannstammene *Chlorella vulgaris* (98% i filter med mikroporer på 55µm) og *Scenedesmus sp* (98% i filter med mikroporer på 18µm), samt for saltvannsstammen *Nannochloropsis oculata* (92% i filter med mikroporer på 15 µm).

Spesifikk strømningshastighet ble registrert. Ingen kationiske polymerer kunne nå signifikant fjerningsrate i saltvannstammen *Dunaliella salinas,* men den synergiske kombinasjon av PAX og anioniske polymerer skapte flokker med lav kompressibilitetsfaktor når testet i Pilot Scale.
SWAT-teknologien må fokusere på G-verdien, polymer/kjemikaliedosering og mikrostørrelse på porene i filtrene som er spesifikk for hver mikroalgestamme for å være kapable til å bli brukt som innhøstningsmetode for mikroalger.

Det ble utviklet en protokoll for tilberedning og analyse av mikroalgesuspensjon ved hjelp av SEM-mikroskop. Effektene av kryopreservering på mikroalgecellene ble undersøkt og det ble funnet at saltvannsstammene overlevde 100 dagers lagring i mørket ved 4°C, mens ferskvannsstammene ikke gjorde det og var i forråtnelsestilstand (Appendix 8 –SEM protocol for microalgae suspensions)

Glossary

Anaplerotic processes - Replenishment reactions to restore the substrate removed from the Krebs cycle.

AP – Areal Productivity – productivity per unit of ground area occupied by the reactor (expressed as $gm^{-2}d^{-1}$) Areal Productivity (AP) was calculated according to *[(P/TSA)*V_o],* where *P* is daily productivity (g day^{-1}), *TSA* is the total surface area of the TPBR included the empty area over the algae suspension (0,913 m^2) and V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *AP* is expressed in (g m^{-2} d^{-1})

ABD - Area Based Diameter

Al – Aluminium compound of flocculant broad used in wastewater treatment industry.

Areal CO_2 Fixation rate (ACO_2Fr) was calculated according to (0,45 *IAP* * (44/12), where 0,45 the carbon content of dried cells (g carbon g $biomass^{-1}$) (Hulatt and Thomas, 2011), *P* is the IAP, the illuminated area productivity (g biomass m^{-2} d^{-1}), and 44 and 12 the molecular weights of carbon dioxide and carbon, respectively. (Zhang K et. al 2001) (ACO_2Fr) is expressed in (g CO_2 m^{-2} d^{-1})**.**

ATP, ADP – Adenosine triphosphate, Adenosine diphosphate

Autotrophic metabolism - implies generation of energy from absorbed light and used to reduce CO_2 by oxidation of water as substrate and evolution of O_2, as for example, microalgae requiring only inorganic mineral ions

1,3-BPGA: 1-3 bis-phosphoglycerate

Biochemical shift - the shift of the protein content, as the valuable bulk of the cell, together with the nucleic acids determine the potential of the growth rate. The shift implies the decrease of protein, lipid and carbohydrates as lipid content increases

Biomass output energy (E_{out}) was calculated according to *(HHV*IAP),* where *HHV* is the higher heating value registered by combusting approximately 1 g (dry weight) algae samples in a Parr 1341 oxygen bomb calorimeter, i.e. the higher heating value *(HHV)* produced by 1 g DW m^{-2} d^{-1} was 22,94 kJ g^{-1} and referred as biomass output energy (0,02294 MJ m^{-2} d^{-1}), (Hulatt and Thomas, 2011). *IAP* is the illuminated area productivity. E_{OUT} is expressed in (MJ/m2d1). (Hulatt and Thomas, 2010)

Biomass productivity rate (P) – expressed as g DW $L^{-1}d^{-1}$ or $gL^{-1}d^{-1}$

Betaine lipids – contain a betaine moiety as a polar group linked by an ether bond, at the sn-3 position of glycerol. They do not contain phosphorus nor carbohydrate groups, DGTS, DGTA, DGCC (Borowitzka and Moheimani, 2013).

Circadian rhythm - Is effectively a memory of a photoperiod, which in eukaryotic cells, are encoded genetically by a set of ubiquitous nuclear genes, *Per* (i.e., *per*iodicity) (Takahashi 1992), dictating the time of cell processes.

Clone – An isolate, which is propagated exclusively through asexual reproduction.

Compensation point - is the point at which the photosythesis and respiration curves intersect each other. The irradiance of compensation (E_c) at this point growth is possible only at higher irradiance levels.

Concentration factor *(CF)*- is the ration of the concentration of microalgae biomass in the final product to the initial concentration in culture (Eq. 19)(Lee et al. 2009)

$$Concentration\ factor\ (CF) = \frac{concentration\ of\ algae\ in\ final\ product}{initial\ concentration\ of\ algae\ in\ culture} \qquad \text{Eq. 19}$$

Cyt b6f - Cytochrome b6f complex

CW – Cellular wall

DGCC - 1,2-diacylglyceryl-3-*O*-carboxy- (hydroxymethyl)-choline, betaine lipid

DGTA - 1,2-diacylglyceryl-3-*O*-2´-(hydroxymethyl)-(N, N, N-trimethyl)-β-alanine, betaine lipid

DGTS - The main betaine lipid of microalgae, 1,2-diacylglyceryl-3-*O*-4´-(N, N, N-trimethyl)-homoserine

Dilution rate – The rate of flow of medium into a continuous culture system (Barsanti and Gualtieri, 2006) and is equal the rate of cell division in the culture, as the cells being removed by the outflow of medium are being replaced by an equal number through the cell division in the culture. Is expressed as the ratio of the rate of the new medium addition (F) to the total culture volume (V) (D=F/V).

Diffuse layer – is created when the charges achieved a dynamic equilibrium (co-ions and counter-ions) motion-free around the Stern layer and it extends from the edge of the Stern layer into the surrounding media until the concentration of counter-ions and co-ions equalizes producing a zero net charge.

Dissolved oxygen (DO) – expressed as mg L^{-1}

DNA, RNA – Deoxyribonucleic acid, Ribonucleic acid

DW – Dry weight, expressed as g dry biomass

EDTA - Ethylenediaminetetraacetic acid, chelating agent

Electrical conductivity (EC) - estimates the amount of total dissolved salts (TDS), or the total amount of dissolved ions in the water

Electrical double layer (EDL) – it forms around the microalgae cell, when the zero net charge result is produced and any difference in ion concentration create an electrical potential and it´s magnitude can be visualized as a function of distance

EPA - 20:5ω3, eicosapentanoic 3-omega acid, polyunsaturated

ETC – Electron transport chain

E_c – Irradiance of compensation

FAD – Flavin adenine dinucleotide, is a redox cofactor, a prosthetic group involved in metabolic reactions

GOGAT - Glutamine 2-oxoglutarate amino transferase

GS - Glutamine synthetase

Fd - ferredoxin

F_E - External force, gravitational force

F_B - Buoyance force, gravitational force

F_c - Drag force, gravitational force

FMN – Flavin mononucleotide

FNR - Ferredoxin-NASP reductase

Flashing light effect – Intermittent light (Emerson and Arnold, 1932) the algae cell is exposed to, and is beneficial for the biomass output by increasing the turbulence in TPBR, the production rates will have the potential for improvement when mixing frequency of 0,5 to 1 Hz will increase the areal production, exposing more cell surfaces to irradiation hence to flash periods (Laws et al., 1953)

F6P - Fructose-6-phosphate

G-value - Camp and Stein (1943), introduced the G-value as a measurement average value to replace the local velocity gradient during turbulent mixing, and its defined as the power requirements were calculated using the equation $G = \sqrt{p/uV}$, where P is the energy dissipation from mixing (W), u is the absolute viscosity of the liquid and V is the volume of the tank (m^3). P is defined as the power consumed by the mixer, and for a mechanical mixer, and is given by Bratby (2006). $P = \varphi \cdot \rho \cdot n3 \cdot D5$ (Nms^{-1}), Where φ is a dimensionless power number and ρ is the liquid density (kg m^3).

Glycolysis pathway – The pathway where hexose is oxidized to pyruvate, hence the reduction of NAD, providing substrates for further respiratory oxidative processes, occurring in the cytoplasm and in the chloroplast stroma.

GLs - Glycolipids or Glycosylglycerides – (glycolipids) are characterized by a 1,2 - diacylglycerol moiety with a mono-or oligosaccharide attached at the sn-3 position of the glycerol backbone, as is the case of galactose molecules, which compose the plastid lipid galactosylglycerides

G3P: glyceraldehyde 3-phosphate

G6P: glucose-6-phosphate

Harvest – in microalgae technology, it comply the several tailor-made processes and methods of collecting, dewatering, thickening, filtering and drying algae suspensions volume to obtain algae biomass as the main products with specific characteristics, and it´s choose is determined by the strain and final use of the algae biomass.

HHV – Higher Heating Value – the amount of energy release during fuel combustion once the products have returned to a temperature of 25 °C. This takes into account the latent heat of vaporization of water. (Borowitzka and Moheimani, 2013)

IGV - Institutt für Getreideverarbeitung GmbH, Potsdam, Germany

ISA – 0,56 m^{-2} of overall surface illuminated area of the suspension culture occupying the total TPBR area.

Isolate – A strains which arises from a single individual (cell)

ISP – Illuminated Surface Productivity – productivity per unit of reactor illuminated surface area (expressed as $gm^{-2}d^{-1}$). Illuminated area productivity (*IAP)* was calculated according to *(P/ISA*V_o),* where *P* is daily productivity (g day^{-1}), *ISA* is illuminated surface area of the algae culture in the TPBR (0,56 m^2) and V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *IAP* is expressed in (g m^{-2} d^{-1}).

IUE – The irradiation utilization efficiency was defined as the efficiency of solar irradiation conversion to biomass energy (Zhang et al., 2000), and was calculated to describe the impact of climatic conditions on the biomass productivity

J – Joule is a derived unit of energy, work, or amount of heat in the International System of Units. It is equal to the energy transferred (or work done) when applying a force of one newton through a distance of one meter (1 newton meter or N· m), or in passing an electric current of one ampere through a resistance of one ohm for one second. It is named after the English physicist James Prescott Joule (1818–1889)

In terms firstly of base SI units and then in terms of other SI units:

$$\mathrm{J} = \frac{\mathrm{kg} \cdot \mathrm{m}^2}{\mathrm{s}^2} = \mathrm{N} \cdot \mathrm{m} = \mathrm{Pa} \cdot \mathrm{m}^3 = \mathrm{W} \cdot \mathrm{s} = \mathrm{C} \cdot \mathrm{V}$$

Where kg is the kilogram, m is the meter, s is the second, N is the newton, Pa is the pascal, W is the watt, C is the coulomb, and V is the volt

One joule can also be defined as:

- The work required to move an electric charge of one coulomb through an electrical potential difference of one volt, or one '"coulomb volt" (C· V). This relationship can be used to define the volt

- The work required to produce one watt of power for one second, or one "watt second" (W· s) (compare kilowatt hour - 3.6 megajoules). This relationship can be used to define the watt (en.wikipedia.org)

KCS - β-ketoacyl-coenzyme A (CoA) synthase, catalyse the first steps of fatty acid elongation

kDa – KiloDalton. The **unified atomic mass unit** (symbol: **u**) or **dalton** (symbol: **Da**) is the standard unit that is used for indicating mass on an atomic or molecular scale (atomic mass). One unified atomic mass unit is approximately the mass of one nucleon (either a single proton or neutron) and is equivalent to 1 g/mol. It is defined as one twelfth of the mass of an unbound neutral atom of carbon-12 in its nuclear and electronic ground state, and has a value of $1.660538921(73)\times10^{-27}$kg.

Krebs cycle (tricarboxylic acid or citric acid cycle) - These reactions occur in the mitochondrial matrix, and the produced pyruvate during glycolysis is anaerobically oxidized to CO_2 by means of NAS^+ and FAD. The intermediates from Krebs cycle are the backbone to amino acids, lipids, tetrapyrroles and other biosynthetic processes. Oxidation of pyruvate and hence NAD^+ reduction leads to O_2 evolution of NADH and H_2O.

LHC - A network of light harvesting complex (LHC) is composed by conglomerate of 2000 chlorophyll molecules with a reaction centre chlorophyll molecules.
Luxury uptake - is the term giving for the polyphosphates bodies where excess phosphorous (P) have been stored. There are LHC I and LHC II

μ - Specific growth rate – expressed as h^{-1}

μm or micrometer - also commonly known as a micron, is an SI derived unit of length equaling 1×10^{-6} of a meter (SI standard prefix "micro-" = 10^{-6}); that is, one millionth of a meter (or one thousandth of a millimeter, 0.001 mm, or about 0.000039 inch). The micrometer is a common unit of measurement for wavelengths of infrared radiation as well as sizes of biological cells and bacteria and is also commonly used in plastics manufacturing. Micrometers are the standard for grading wool (referring to the diameter of wool fibers). Any wool finer than 25 μm can be used for garments, while coarser grades are used for outerwear, rugs and carpets. A human hair is about 90 μm in diameter.

MGDG – Monogalctosyldiacylglycerol: *DGDG* - Digalotocyldiacylglycerol and *SQDG* - Sulfoquinovosyldiacylglycerol

mV – millivolt

η - is fluid dynamic viscosity

NAD^+ (NADH*) – Nicotinamide adenine dinucleotide (* oxidized form), a coenzyme and signalling molecule

$NADP^+$ (NADPH*) – The coenzyme nicotinamide adenine dinucleotide phosphate (*reduced form)

NLs - Neutral lipids

N_iR - Nitrite reductase, an enzyme that uses reduced ferredoxin

NR - Nitrate reductase, a cytoplasmic enzyme that uses either NADH or NADPH

OAP- Overall Area Productivity – the productivity obtained from the overall (including empty spaces) ground area occupied by the several reactors that constitute the plant. (Expressed as $gm^{-2}d^{-1}$)

OEC - Oxygen-evolving complex

Osmoticants – Microalgae cells are able to accumulate these osmoregulatory substances of molecular dimensions as cellular protectors in case of increasing salinity or environmental osmotic pressure, as for example, glycerol, mannitol, galactitol, sorbitol, glycerol galactoside, sucrose, and trehalose.

Oxidative pentose phosphate pathway (pentose phosphate shunt) - Further oxidation of hexose, producing NADPH, CO_2 and pentose phosphate, providing a nonphotosynthetic source of NADPH (via ferredoxin reduction), being essential in NO^{-}_2 reduction and lipid biosynthesis in the dark

ρ_s, ρ_l - are the solid and liquid densities

Pa – Pascal is the SI derived unit of pressure, internal pressure, stress, Young's modulus and ultimate tensile strength, defined as one newton per square meter. It is named after the French polymath Blaise Pascal

PAR - The photosynthetically active radiation is then 400-700 nm, which corresponds to 45-50% of total incoming radiation.

PAR irradiance input energy (E_{in}) was calculated according to *(I_o* CF),* where I_o is irradiation on registered above the surface of algae culture but under the lid of the TPBR *(*µmol $m^{-2}s^{-1}$) and *CF* is the conversion factor from PAR to $Jm^{-2}s^{-1}$, and is equal to 4,57 µmol m^{-2} s^{-1} per 1 J m^{-2} s^{-1} (Thimijan & Heins, 1983; Zhang et al., 2001), and expressed as the equivalent daily energy input MJ m^{-2} d^{-1}, [(4,57 * 10^{-6} MJ/86,4*10^{-6} d)= 0,395 MJ m^{-2} d^{-1}. (E_{in}) is expressed in (MJ/m2d1)

PAX - inorganic chemical used in wastewater treatment industry

PEPC – Phosphoenolpyruvate carboxylase, an enzyme

PFD – Photon Flux Density

PBR – Photobioreactor – a device growing plants or organisms (especially microalgae) that admits light, but otherwise operates with a system closed to the environment (no direct exchange of gases or water, generally)

PC – Plastocyanin

PE – Photosynthetic efficiency and is calculated by PE = 100(E_{out}/E_{in}), where E_{out} is the biomass output energy (MJ $m^{-2}d^{-1}$) and E_{in} is the PAR incident irradiance input energy (MJ $m^{-2}d^{-1}$)

PG – Phosphatidylglycerol

3-PGA: 3-phosphoglycerate

Phosphoglycerides: *PC:* phosphatidylcholine, *PE:* phosphatidylethanolamine, *PG:* phosphatidylglycerol

P_i – inorganic phosphorous

PQ, PQH_2 – oxidized Plastoquinone (Q_A, Q_B), fully oxidized plastoquinol

PLs – Phospholipids, Phosphoglycerides – (phospholipids) have glycerol as a basic structure, and it is backbone metabolically derived from glycerol-3-phosphate to which is linked at the 1- and 2-positions, esterified hydrophobic acyl groups, and phosphate at the sn-3 position, with further link to hydrophilic base group. (Borowitzka and Moheimani, 2013)

PM – Plasma membrane

P_{max} – Light saturated photosynthetic rate point

Ppm - Parts per million, measure of concentration in gases and liquids (mg L^{-1})

PSI, PSII - Photosystem I (λ =700nm; 1,6V), Photosystem II (λ=680nm; 1,7V)

PUFA – Polyunsaturated fatty acid

r - is cell radius

Respiration - The sum of metabolic processes that consume O_2 and evolve CO_2

Respiratory electron transport – They occurs in the inner mitochondrial membrane involves proton pumping, phosphorylation of ADP via a proton ATP synthase complex

Recovery efficiency *(RE)* - is defined as the ratio of the mass of cells recovered in the final product to the total mass of cells in the initial culture (Eq. 18)(Lee et al. 2009)

$$Recovery\ efficiency\ (RE) = \frac{mass\ of\ cells\ recovered}{mass\ of\ cells\ initial\ culture} \qquad \text{Eq. 18}$$

ROS - Reactive oxygen species, toxic to the cell

Rpm – revolutions per minute

RuBisCO - Ribulose bisphosphate carboxylase/oxygenase, a 536kDa heterodimeric protein complex

RuBP: ribulose-1-5-bisphosphate

SEM – Scanning electron microscopy

SF - Salsnes Filter technology

SKP – Centre for Climate Regulated Plant Research (Senter for Klimaregulert Planteforskning)

Specific flow rate – expressed as $m^3\ m^{-2}\ h^{-1}$

Species – The lowest taxonomic rank of algae having common characteristics and (usually) capable of mating with one another and named according to the International Code of Botanical Nomenclature (now named The International Code of Nomenclature for Algae, Fungi, and Plants) Knapp et al. (2011)

Stern Layer - The slightly negative surface charge exhibited by the microalgae cell repels negative ions (co-ions) in the solution and attracts positive ions (counter-ions) from the solution, hence creating a tightly bound layer of ions.

Strain – A unialgae culture of defined origin, which is kept as a distinct "lineage" by serial transfer or continuous culture

S/V – surface-to-volume ratio

SWAT - Salsnes Water to Algae Treatment.

Synchronous culture - In algae mass cultivation, the cultures in which the cells are at the same state of the cell cycle at a given time, the diel irradiance cycle is the natural synchronizing agent for unicellular algae, constraining growth rate and also applies to nutrient acquisition and this cycle is generally imposed by the transition from dark to light or by a stepwise temperature change

TAG – Triacylglycerols

TCA - Tricarboxylic acid cycle, a process in the mitochondria

Temperature coefficient (Q_{10}) – Defined as the two folding enzymatic reaction when the temperature raised 10°C, and in the mass culture of algae, this imply that the temperature in the algae suspension will take double amount of nutrients for each 10°C of temperature increase

Terminal velocity:

$$V = \frac{g(\rho_s - \rho_f)d^2}{18\mu} \qquad \text{Eq. 20}$$

Where V is the terminal velocity ($m\ s^{-1}$), g is the acceleration due to gravity ($m\ s^{-2}$), ρ_s is the density of the particle ($kg\ m^{-3}$), ρ_f is the density of the fluid ($kg\ m^{-3}$), d is the diameter of the spherical particle (m) and μ is the fluid viscosity ($kb\ m^{-1}\ s^{-1}$)

TrA - Transamination reactions, involved in nitrogen assimilation of eukaryotic

photoautotrophs

TSA - Total surface area of the TPBR, 0,913 m^2 of the upside-down truncated pyramid shaped TPBR

TSS – Total suspended solid, expressed as gL^{-1}

UV-B – Ultraviolet (UV) light is an electromagnetic radiation with a wavelength from 400 nm to 100 nm

V_p - Is the predicted volume for the illuminated area of 0,56 m^2, and equal to 31,314 L

V_o - Is the remaining algae culture volume in the TPBR at the moment of the sample (L) was collected

VP - Volumetric Productivity – productivity per unit reactor volume (expressed as $gL^{-1}d^{-1}$) Volumetric productivity (*VP)* was calculated according to $(P/V_o)*V_p)$, where *P* is daily productivity (g day^{-1}), V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L) and V_p is the predicted volume for the illuminated area of 0,56 m^2, and equal to 31,314 L. *VP* is expressed in (g L^{-1} d^{-1})

V_p - is the predicted volume for the illuminated area of 0,56 m^2, and equal to 31,314 L. *VP* is expressed in (g L^{-1} d^{-1})

Zeta potential – A special parameter of the colloids, which is the potential at the interface (share plane) of the Stern layer and diffuse layer, and it is negative in the range -10 to -35 mV (Henderson et al., 2008), and it´s dependent of pH and ionic strength of the culture media (Conductivity), and it´s reduced by salinity.

Introduction

Microalgae is one of the most ubiquitous organisms on the planet, found in most environments on earth, and has so far been estimated 35000 species, and the predominantly nourish form among them being the photoautotrophic, representing an exceptionally rich resource for bioprospecting for species with biochemistries and properties suitable for commercial exploit (Borowitzka, 1992a) bioactive compounds (Patterson et al. 1994), wastewater treatment (Oswald 1988), metabolic conversions (Pollio et al. 1994). At industrial scales, natural β-carotene is extracted from the halophile tolerant *Dunaliella salinas* (Borowitzka 2010b), astaxanthin from freshwater algae *Haematococcus pluvialis*, destined for nutraceutical and pharmaceutical purposes (Cysewki and Lorenz 2004), long-chain polyunsaturated fatty acids (Sukenik 1999), the fluorescent marker pigment phycobilin for research, medicine, cosmetic and cosmoceuticals (Kronich and Grossman 1983), even renewable biofuels like algae lipids biodiesel, hydrogen, methane from algae sugars and crude oils from algae biomass pyrolysis (Benemann 2000; Demirbas 2006; Christi 2007; Lee 2008; Torzillo et al. 2009; Borowitzka 2010a). Selection of the algae species and strain to be grown is the first and critical step in developing a reliable and viable process for algae biomass production, which was the main goal of this study, therefore four of the five algae strains cultivated were supplied by the IGV algae culture collection (IGV Potsdam, Germany), and the fifth freshwater algae strain, *Chlorella wild mix Årungen*, was kindly provided by PhD candidate Annette Åkerstrøm, and isolated from inland lake Årungen at the university campus at Ås, Norway (see Glossary for definitions).

Selecting a microalgae for the commercial-scale production is the first important process, due to the challenge and opportunity the diversity of the algae involves, thus the choice of species and strains in question depends of many specific criteria to yield efficiently high-productivity cultivation (i.e. thermic, un-optimal irradiation and salinity tolerance, efficiency and mechanisms of inorganic carbon uptake, photosynthetic capacity, rapid growth, shear tolerance, biochemical constituents´ quality and quantity), harvestability (i.e. cells size and morphology, high specific gravity) and extractability (i.e. wall cell covering or no, no production of autoinhibitors), and achieved after direct approach and screening.

1 Cultivation and upscaling (Literature study)

1.1 Microalgae and it´s biochemistry

The aspects of algae biology and biochemistry relevant for the economical exploitation is the composition of the algae biomass: lipids, carbohydrates and proteins.
Microalgae are unicellular organisms that can live individually or in colonies and are classified as eukaryotes. Their main characteristic is their ability to photosynthesize in different habitats: fresh, brackish, marine, hyper-saline environments. Considered by many authors as the cornerstone of oxygen producing organisms, their species are classified in 10 taxonomic groups: green algae (Chlorophyceae), diatoms (Bacillariophyceae), yellow-green (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae), dinoflagellates (Dinophyceae), Prasinophyceae and Eustigmatophyceae (Williams and Laurens, 2010).

Internal, subcellular structures, organelles (chloroplasts, mitochondria, nuclei), which are surrounded by lipid membranes composed by bilayer of polar molecules, like phospholipids and glycolipids are the mayor algal lipids components.

Microalgae metabolism is strongly influenced by their surface-to-volume ratio. Therefore it´s usually spherical morphology, benefits growth rate based on theirs cell diameters. (Williams and Laurens 2010)

1.2 Photosynthesis in aquatic environments

Biomass production in microalgae is direct proportional to the efficiency the atmospheric carbon is assimilated through the photosynthesis, which is the process driven by photons absorbed by chlorophyll molecules, hence starting the charge separation and ejections of electrons, which in turn drive the dissociation of water and generate protons and oxygen. This proton flow is used to trap the reduced CO_2 into organic material and evolving O_2.

1.2.1 Photosynthesis´ mechanics

The overall reaction of the photosynthesis present two phases:

1.2.1.1 The “light reaction”, with a sequence of photochemical and redox reactions

1.2.1.2 The “dark reactions”, with a set of enzymatic reactions, also known as “light-independent reactions”

$$Light\ reaction: 2H_2O \rightarrow 4[H*] + O_2 + energy \quad \text{Eq. 1}$$

$$Light - independent\ reactions: 4[H*] + CO_2 \rightarrow [CH_2O] + H_2O \quad \text{Eq. 2}$$

(Notation [H*] corresponds to the combination of reduced coenzyme nicotinamide adenine dinucleotide phosphate (NADPH) and an electron)

There are big differences in timescales of operation between the to reactions set: from femtoseconds to milliseconds for the light reactions, and from seconds to hours for the light-independent reactions, which create a temporal mismatch and lead to

inefficiencies in adaptation to the changing environmental conditions (temperature and irradiance). This is a factor to consider when maximising yields of algae biomass.

1.2.1.1 Light absorption and reducing capacity

The incoming solar irradiation is attenuated due to light scattering and cloud absorption by the atmosphere´s gases, and reach the earth surface by some 30%. The chlorophyll a molecule has strong absorption band in the region 400-450 and 650-700 nm. The photosynthetically active radiation (PAR) is then 400-700 nm, which corresponds to 45-50% of total incoming radiation. Chlorophyll a captures some 30-40% of PAR. B-carotene is one the auxiliary light capturing pigments used by the algae cell to increase the range of spectrum usable for photosynthetic reactions. The jump of electrons from higher to lower wavelengths, represent a loss of energy calculated to be of 21% of the original irradiation (fig. 1)

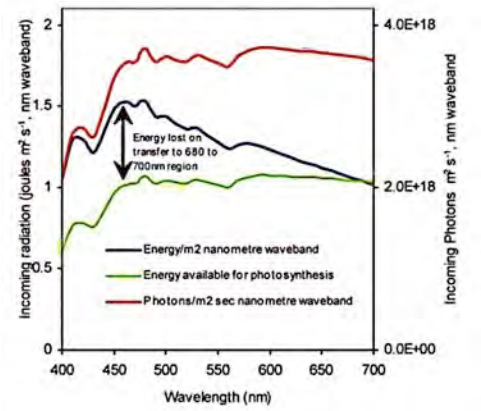

Figure 2 – The distribution of energy (blue line) and photons flux (red line) in incoming radiation. The lower green line shows the residual energy after it has been transferred to a frequency equivalent to that of chlorophyll a at 680700 nm absorption bands. (Illustration from Williams and Laurens, 2010)

The sites of photon absorption are functionally separated but by a chain of redox carrier molecules which coupled in both in tandem. The energy required is 164 and 154 kJ/Einstein for the Photosystem II PSII (λ=680nm; 1,7V) and for the photosystem I (λ =700nm; 1,6V), respectively. The "Z-scheme" of photosynthesis showed in fig. 2 how the one-photon/electron rule where the water is dissociated accumulation of four electrons to evolve one molecule of oxygen. The accumulator is a tetra-manganese complex, oxidised stepwise four times, and when the oxidised form is complete, it draws an oxygen molecule plus four protons. In the lipid membrane of flattened sac-like structures located in the chloroplast, thylakoids, occurs these complicated reactions: capturing mechanisms of photons, separation of charge, metabolic energy generation and reducing capability, and splitting system of water molecule.

Two molecules of NADPH, the reducing agent, rise by the pumping of four electrons in the PSII and PSI, transported with a free energy gain of 220 kJ mol^{-1} NADPH, is used in the carbon assimilation process. When the proton pump is activated, a charge gradient is created in the lumen (thylakoid inner chamber), and when the proton is returning, a molecular rotor promote the synthesis of ATP (adenosine triphosphate), the biological coin. Totally, twelve protons produce three ATP molecules, and irradiate 50 $kJmol^{-1}$ of free energy and two NADPH molecules that equal to a yield of 590 kJ/4 moles electrons of potential energy. 8-10 photons are needed to transport four electrons.

A network of light harvesting complex (LHC) is composed by conglomerate of 2000 chlorophyll molecules with a reaction centre chlorophyll molecules (fig. 2)

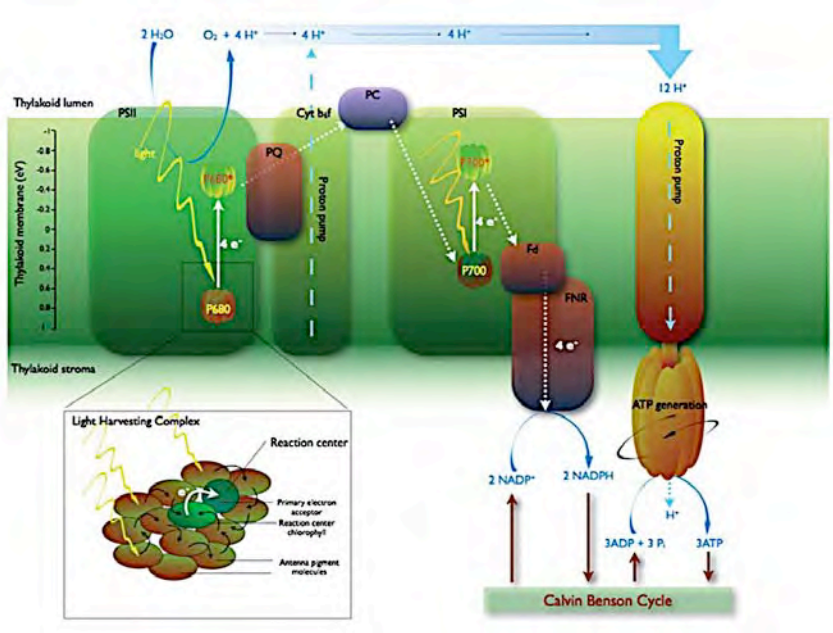

Figure 2 – Illustration of the light reaction of photosynthesis (Z-scheme). The major functional units are represented as oval shapes; photosystem II (PSII), plastoquinone (PQ), plastocyanin (PC), cytochrome b6f complex (Cyt b6f), photosystem I (PSI), ferredoxin (Fd), ferredoxin-NASP reductase (FNR) (in order of electon transport chain) and ATP synthase. P680 and P700, refer to the reaction centres of PSII and PSI respectively, the asterisk (*) indicates the excited state. The inset shows a schematic close-up of the light harversting complex (LHC) (Illustration from Williams and Laurens, 2010).

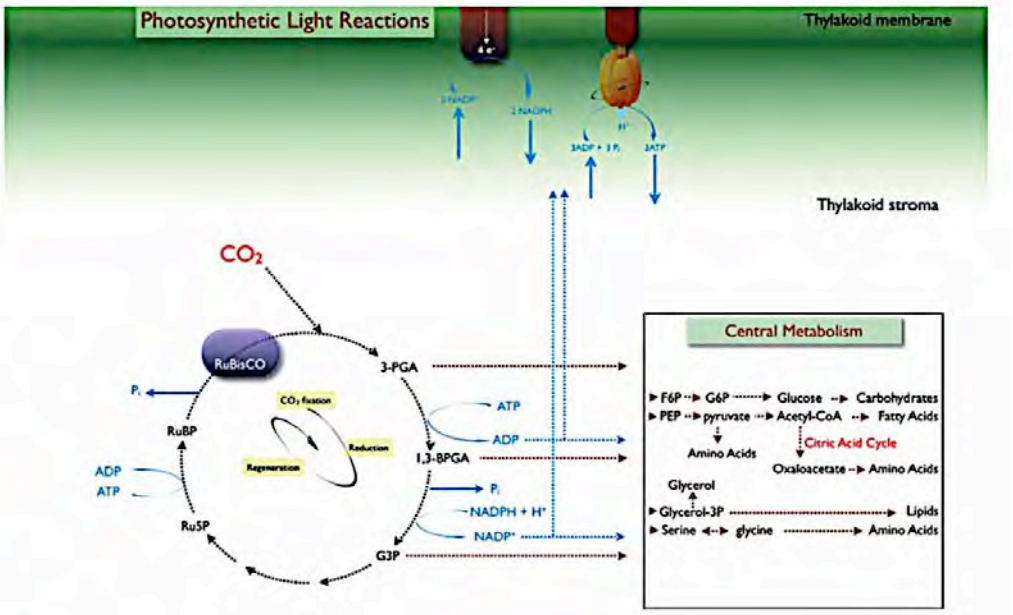

Figure 3 – Schematic simplified representation of the Calvin-Benson cycle in three parts, (i) CO2 fixation, (ii) reduction and (iii) regeneration. The average cycling time of one "round" of CO_2 assimilation is 100 to 500 ms. The necessary energy (ATP) and reductant (NADPH + H^+) (not shown) stoichiometrically) are originating from the photosynthetic light reactions. RuBisCO_ ribulose bisphosphate carboxylase/oxygenase_ RuBP: ribulose-1-5-bisphosphate, 3-PGA: 3-phosphoglycerate; 1,3-BPGA: 1-3 bis-phosphoglycerate; G3P: glyceraldehyde 3-phosphate; F6P: fructose-6-phosphate; G6P: glucose-6-phosphate; PEP: phosphoenolpyruvate. (Illustration from Williams and Laurens, 2010)

1.2.1.2 The "light-independent reactions"

The products of the light reactions ATP (energy) and NADPH + H^+ (the reducing power), are used in the CO_2 sequestration into organic material (reduction), during photosynthesis by the enzymatic part of the "light-independent reaction" (fig. 3). It has been estimated approximately 469 kJ mol^{-1} C to be the product´s calorific value. It starts as follows: carboxylation of the sugar ribulose 1:5 biphosphate (Ru5BP) by the specific enzyme ribulose bisphosphate carboxylase oxygenase (RuBisCO, a 536 kDa heterodimeric protein complex), that catalyse both the oxidation and carboxylation of its substrate, fixing 2-10 molecules of CO_2 per active site per second. Marine algae species actively pump in CO_2 increasing its concentration and encapsulating with it RuBisCO, to overcome the overflow of oxygen over carbon dioxide (Williams et Laurens 2010).
The 3-carbon organic acids are the first stable products of the reactions that become precursors for fats, fatty acids, proteins and sugars (fig 3). As shown in the insert in fig.3, the central metabolism, the biosynthesis of amino acids and lipids increase the demands of acetyl-Co-A, hence carbon and oxygen get loss, biomass loss in form of CO_2.

1.2.2 Respiration

Respiration is the sum of metabolic processes that consume O_2 and evolve CO_2. When the cell provides substrates for cell growth, it has to oxidize organic carbon. During the light-independent production of CO_2, the photosynthetic organism is taken from light to darkness and in doing so it occurs a net consumption of O_2, the photosynthesis ceases (dark respiration) (Raven and Beardall 1981, 2003), and photorespiration. It is distinguished four components or reactions sequences of the dark respiration.

1.2.2.1 Dark respiration

1.2.1.1.1 Glycolysis pathway, where hexose is oxidized to pyruvate, hence the reduction of NAD, providing substrates for further respiratory oxidative processes, occurring in the cytoplasm and in the chloroplast stroma (Raven and Beardall 1981, 2003)

1.2.1.1.2 Oxidative pentose phosphate pathway (pentose phosphate shunt). Further oxidation of hexose producing NADPH, CO_2 and pentose phosphate, providing a nonphotosynthetic source of NADPH (via ferredoxin reduction), being essential in NO^-_2 reduction and lipid biosynthesis in the dark.

1.2.1.1.3 Krebs cycle (tricarboxylic acid or citric acid cycle). These reactions occur in the mitochondrial matrix, and the produced pyruvate during glycolysis is anaerobically oxidized to CO_2 by means of NAS^+ and FAD. The intermediates from Krebs cycle are the backbone to amino acids, lipids, tetrapyrroles and other biosynthetic processes. Oxidation of pyruvate and hence NAD^+ reduction leads to O_2 evolution of NADH and H_2O. Replenishment reactions to restore the substrate removed from the Krebs cycle are known as the anaplerotic processes.

1.2.1.1.4 Respiratory electron transport occurs in the inner mitochondrial membrane involves proton pumping, phosphorylation of ADP via a proton ATP synthase complex.

1.2.2.2 Photorespiration in microalgae, the ratio of oxygenase to carboxylase activity is less that found at air equilibrium due to CO_2 enrichment of the medium or a CO_2

concentration mechanism (Raven 1984b; Yokota et al. 1987), and the overproduction of phosphoglycolate becomes a sink for phosphorus, hence the necessity of recycling phosphorus avoiding phosphoglycolate accumulation, thus leading to photosynthesis inhibition (fig. 4). By hydrolysing phosphoglycolate, inorganic phosphate and glycolate are produced, and glycolate excretion is a reflection of oxygenase activity of Rubisco and hence of photorespiration. In microalgae some 0,25 to 1,0 carbon atoms as CO_2 per carbon from glycolate is metabolized and 0 to 0,75 of the remaining carbon of the glycolate can be used in biosynthesis (the pathway of glycolate metabolism) (Raven et al 2000; Beardall et al. 2003). Raven suggested that a purely diffusive exchange of CO_2 between the medium, Rubisco, and the sites of O_2 evolution would give competitive kinetics for the effects of varied external O_2 and CO_2 concentrations on the rate of CO_2 fixation (Raven 1984b, 1993).

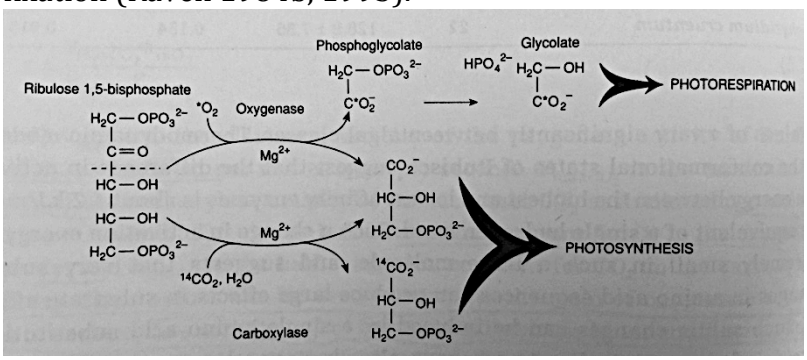

Figure 4 – The photorespiratory pathway. If Rubisco oxygenates rather than carboxylates RuBP, a primary product is 2-phosphoglycolate. The product can be dephosphorylated, leading to the formation of glycolate, and subsequently, via amination, to produce glycine and serine (Illustration from Falkowski and Raven, 2007)

Microalgae need a supplementary process for energy generation independent of light to support metabolism during dark periods and to balance the temporal mismatch between the light and light-independent reactions during photosynthesis, which can saturates the flow of electrons at the electron transport chains (ETC), leading to wastage of captured electrons, and the excess of captured electrons have to be disposed as fluorescence or other mechanism, resulting in very low photosynthesis rate, in addition to mitochondrial respiration, the maintenance of basic energy level, the reparation or replacement of damaged light gathering components, all of them represent energy consumption. The compensation point is the point at which the photosythesis and respiration curves intersect each other. The irradiance of compensation (E_c) at this point growth is possible only at higher irradiance levels (fig. 5).

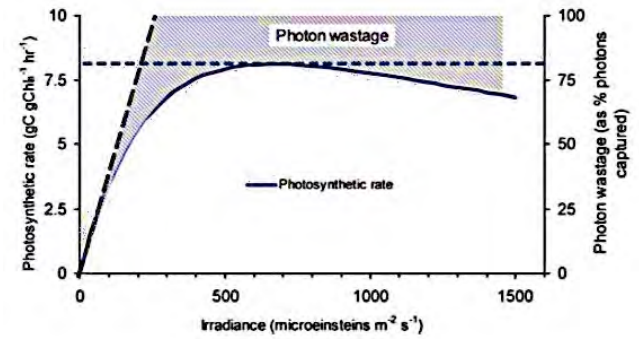

Figure 5 – The photosynthesis versus irradiance (PvE) relationship. The projected line of the initial rate (dashed black line), and the hatched area between this and the blue curved line for photosynthesis is an indication of photon wastage. The zone of unexpressed enzyme activity is the area between the blue dashed line and the solid blue line for photosynthesis (Illustration from Williams and Laurens, 2010)

The algae cell can adapt to the increased irradiance if it is sustained for one hour approximately, or by increasing the capacity for enzymatic activity or just by reducing the size of the antennae (light-collecting). Hence the yield of optically dense cultures depends directly from the light adaptation the algae cell is capable of, switching between to physiological states: Low light adapted with high chlorophyll content and high light adapted with low chlorophyll content (Richardson et al. 1983).

1.3. Metabolites production

Most authors agreed in the four principal biochemical classes of molecules appreciated in algae: carbohydrates, proteins, lipids and nucleic acids.

1.3.1 Carbohydrates

Both monomers and polymers are present in algae structure with their metabolic functions: they are the primary products of photosynthesis and precursors in the biosynthesis of other biochemicals. Algae species store their starch production as amylose and amylopectin (green algae), while other algae store it as chrysolaminarin, polysaccharide, linear polymer of β (1→3) and β (1→6) linked glucose units (Roessler, 1987). This compounds is often accumulated in pyrenoids, organelles in the chloroplast with high activity of carbon assimilation.

1.3.2 Proteins

The role as structural and metabolic compounds is fundamental as enzymes, catalysing cell metabolism for growth. In the chloroplast, proteins assemble the chlorophyll molecules to form light harvesting complexes, and embed the lipid membranes. In some algae, the cellular walls could consist of cross-linked hydroksy-proline-rich glycoproteins (*Chlamydomonas reinhardtii*) (Roberts et al., 1972). Fundamental in the composition of essential amino acids, that animal are unable to synthesise for them selves, make them valuable in animal feed dietary with their high nutritional quality.

1.3.3 Lipids

Considered as energy reserves and structural components cellular membranes. The simplest of them all are triglycerides. The more complex, phospholipids and glycolipids, with their hydrophilic polar phosphate or sugar moieties, determines the fluidity of the cellular membranes with their level of saturation (amount of C=C double CC bindings present) of the fatty acyl chains. In case of rapid changes in temperatures, the algae cell has to endure a critic situation and its cellular membranes are able to recycle, reshaping of the fatty acids (*de novo* synthesis). The phosphate fraction present in phospholipids, are associated with the internal chloroplast membranes where the photosynthetic activities happen, i.e. thylakoids. And in conjunction with sulphur lipids (sulfoquinovosyldiacyl glycerol), they represent a proportion of the overall lipid fraction changes as the metabolic rate slows down. Among the published analysis, the minimum cellular lipid content is not lower than 15%. The high occurrence of unsaturated and polyunsaturated fatty acids maintains the fluidity of the membrane and the algae acclimation ability to changing weather conditions (for detailed description see 1.7).

1.3.4 Nucleic acids

In conjunctions with proteins and their monomers, the nucleic acids (RNA and DNA) provide the basis for algae division and growth. The major fraction of cellular phosphate and nitrogen content are enclosed in nuclei acids.
Among valuable co- and by-products, the microalgae cell is able to produce: carotenoids (β-carotene, lutein, astaxanthin and cashaxanthin), pigments like (phycocyanin, phycoerythrin), ω-3 fatty acids (eicosapentanoic and docohexaenoic acids), vitamins (tocopherols, vitamin B_{12} and provitamin A), polysaccharides and proteins.

1.4. Biochemical shift

The shift of the protein content, as the valuable bulk of the cell, together with the nucleic acids determine the potential of the growth rate. The shift implies the decrease of protein, lipid and carbohydrates as lipid content increases.

1.5 Physiology

The response the cell undergoes as a result of the sense change (limiting- or stress factor) is an inherent characteristic of any living organism. (Vonshak and Torzillo, 2010). A limiting factor determines the rate of growth or biochemical reaction affecting the rate without any requirement for acclimation process. Stress factor, is an environmental condition resulting in metabolic imbalance that requires biochemical and metabolic adjustments before a new steady state of growth can be established. In a green house (indoor) the changes occurred normally in outdoors algae cultures, in two different timescales, are not present: circadian cycle, variation in light and temperature in a 24 h cycle. Seasonal cycle varies according to climatic and geographical location, i.e., particular habitat of the algae strain. The imposition of a third cycle related by intensive mixing system of the biotechnology of algae cultivation. This light-dark cycle fluctuates in accordance to the mixing rate and can be seen as an anomaly to the two other cycles. These alterations of light quality and intensity (irradiance) force acclimation responses to compensate the disturbances in the light-harvesting complex synthesis and degradation. The alterations in physiology are aimed to balance the efficiency in absorption of excitation energy and production of NADPH (reducing power) and ATP (chemical energetic coin) destined to cellular maintain and growth. A result produce of the overexcited photosynthetic apparatus are ROS (reactive oxygen species), toxic to the cell. The role of polar glycerolipids and fatty acids in photosynthesis is of great importance to understand the metabolic pathways leading to new biomass production.

The chloroplast membranes have a high level of fatty acid unsaturation (PG-16: 1(3t) and galactosylglycerides) are important in the normal photosynthetic processes (Murat and Siegenthaler 1998). PG function in the LHCII trimerization process is vital, interacting with specific sites in the chlorophyll-protein complex (Dubertret et al. 2002). Lowered unsaturation of chloroplast lipids contributes to tolerance and adaptation of photosynthesis to high temperature (Borowitzka and Moheimani, 2013). During the light period, TAG synthesis is promoted and stored in cytosolic lipid bodies for further polar lipid synthesis (Thompson 1996)

1.6 Cell walls and plasma membrane structure

The particularities of the biomass from microalgae are the chemistry of cell wall and plasma membrane, the high presence of water and the small cell size. The distribution of lipids within the algae cell and their association to non-lipid molecules have been classified (Pohl and Zurheide 1982) (fig. 6):

1.6.1 Neutral lipids (NLs): (triacylglycerols, TAGs, wax esters, hydrocarbons, fatty acids, Fas, and sterols) (see Glossary for definition)

1.6.2 Phospholipids (PLs): (phosphatidycholine, PC, phosphatidylethanolamine, PEA, phosphatidylglycerol (PG), phosphatidylserine (PS), diphosphatidylglycerol (DPG) (or cardiolipin) and phosphatidiylinositol, (PI); and

1.6.3 Glycolipids or Glycosylglycerides (GLs): (sulfoquinovosyldiacylglycerol, SQDG, monogalactosyldiacylglycerol, MGDG, and digalactosyldiacylglycerol, DGDG) (see Glossary for definition).

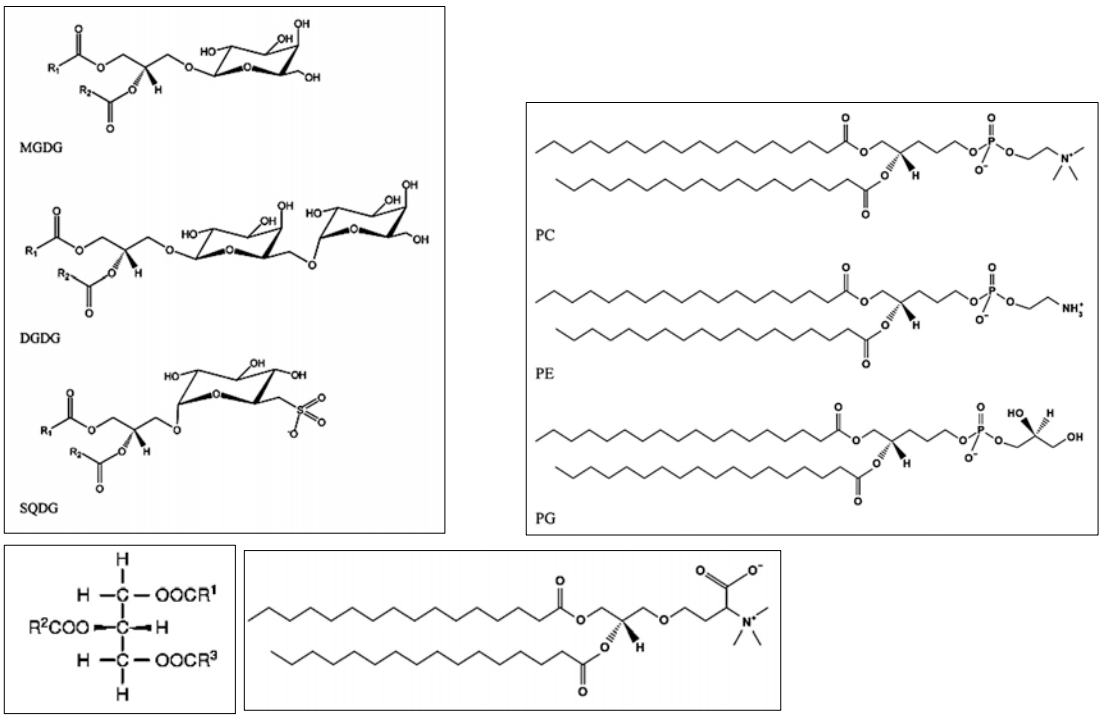

Figure 6 – Biochemistry in microalgae. Upper left: main glycosylglyserides R_1 and R_2 are the two fatty acyl chains. *MGDG* monogalctosyldiacylglycerol: *DGDG* digalotocyldiacylglycerol_ *SQDG* sulfoquinovosyldiacylglycerol. Upper right: mayor phosphoglycerides. *PC:* phosphatidylcholine, *PE:* phosphatidylethanolamine, *PG:* phosphatidylglycerol. Down left: *TAG*– Triacylglycerol structure. R_1, R_2 and R_3 are (usually different) fatty acid chains. Down right: the main lipid of algae, 1,2-diacylglyceryl-3-*O*-4´-(N, N, N- trimethyl)-homoserine (DGTS)

TAGs are abundant storage products, catabolised in case of energetic deficit wax esters have thermo-isolating function for cold waters algae species (Gushina and Harwood 2008, 2009a, 2009b).

The functions of some PL are key intermediate (or precursor of intermediates) in cell signalling pathways (e.g. inositol lipids, oxidative products of PUFAs, sphingolipids). GL are mostly located in photosynthetic membranes.

The plasma membrane (PM) and cell wall (CW) are of highly importance in the extraction process and hence the harvesting methods.

Marine algae are rich in long chains (> 18 carbons), to them belongs the polyunsaturated fatty acids (PUFAs) among them eicosapentanoic acid (EPA, C20: 5n3), and docohexanoic acid (DHA. C22: 6n3), as shown in table 1

Table 8 – Fatty acids in selected microalgae groups (Modified from Ackman and Tocher 1968; Kayama et al. 1989)

Division/Classes	Monoun-/Saturated	Polyunsaturated
Chlorophyta/Chlorophyceae (*Dunaliella salinas) (Chlorella vulgaris, Scenedesmus sp)*	C16:0; C16: 1n7	C18: 2n6; C18: 3n3
Eustigmatophyta/Eustigmatophyceae (Nannochloropsis oculata)	C16:0; C16: 1n7; C18: 1n9	C18: 2n6;C18: 3n3; C20: 4n6; C20: 5n3

The plasma membrane (PM) and cell wall (CW) determine the pre-treatment procedures during harvesting. CW gives rigidity, strength and protection again mechanical stress, and its composed by extracellular polymeric polysaccharides, proteoglycans, peptides, proteins and associated inorganic elements, and the marine species are often sulphated and rich in negatively charged non-crystallines polysaccharides (Tomaselli 2004). CW is structurally organized in (1) fibrillar embedded and (2) continuous matrix. The first one is an interwoven network of cellulose (sometimes glucose, mannans or xylans) acting like a skeleton. The second one is the substance soluble in dilute sodium hydroxide, made up of amorphous mucilaginous (lipids), protein, amino sugars, and hemicellulose (Lee 1989). *Dunaliella salinas* lack CW and is classified as naked. *Nannochloropsis* contain the insoluble non-hydrolizable biopolymers (algaenan) in the outher CW and hence very resistant to non-oxidative chemical treatment hence to harvest (Allan and Templier 2000; Gelin et al. 1997; Mendes Pinto et al 2001).

PM contains the cytoplasm and is in direct contact with the CW, and it is in contact with the interconnecting network of concentric shells of the thylakoid membrane, merging with the PM´s inner surface. Phospholipids (PLs) are the main constituent of the PM and glycolipids (GLs) of the eukaryotic algae chloroplasts. The CW variation among algae strains is huge and is necessary to characterize them before pre-treatment and harvesting procedures of biomass.

1.6.4 Betaine lipids

Betaine lipids are components of algae, and other lower organisms like bryophytes, ferns, fungi, lichens and protozoans, but not found in gymnosperms nor angiosperms (higher plants) (Dembitsky 1996). (See Glossary for definition). There are three identified betain lipids in algae:

(i) 1,2-diacylglyceryl-3-*O*-4´-(N, N, N-trimethyl)-homoserine (DGTS),

(ii) 1,2-diacylglyceryl-3-*O*-2´-(hydroxymethyl)-(N, N, N-trimethyl)-β-alanine (DGTA), and

(iii) 1,2-diacylglyceryl-3-*O*-carboxy- (hydroxymethyl)-choline (DGCC).

The importance of this lipids consist in the zwitterionic nature at neutral pH being equally positive and negative charged (thrimethylammonium group contra carboxyl group) (fig. 6)

In freshwater algae, betaine lipids are found in saturated fatty acids (C14:0 and C16:0), at the sn-1 position of the glycerol backbone and at the sn-2 position in C18 acids (C18: 2n-6 and C18: 3n-3).

1.7 Parameters of influence in microalgae cultures

1.7.1 Temperature

The effect of temperature on membrane lipids composition and content has been paid much attention among algae researchers. They found that decreasing the growth temperature affected directly the degree of unsaturation of lipids in the membrane systems. Infra-optimal temperatures increased the fluidity of cellular membranes in special the thylakoid membranes, enhancing their stability protecting the photosynthetic apparatus from damages under low temperatures (Nishida and Murata, 1996). Thompson et al., 1992 found that algae cells as an adaptive mechanism for maintaining rates of photosynthesis and respiration, were able to increase enzymatic production as a protective reaction to decreasing growth temperature to infra-optimal levels. This could explain the overwintering capacities microalgae physiology have to endure chilling by actively accumulate polyols, amino acids or amino acid derivatives as compatible solutes.

Growth temperature influences not only the pattern quota of carbon and nitrogen in the cell but the cellular volume as well (Goldman and Mann, 1980). The optimal temperature level produces cells of minimum size, carbon and nitrogen contents. Disturbances in growth temperature, produces changes in those cellular parameters. Darley, 1982, summarized that to produce cells at the same growth rate at non-optimal temperature requires more carbon and nutrients.

1.7.2 Light intensity (Irradiation)

Dubinsky et al., 1995, published that photoacclimation or photoadaptation refers to the effects of light on biochemical composition of photosynthetic algae. The algae undergo dynamic changes in cellular composition, altering their biophysical, physiological properties (ultrastructure) to augment the photosynthesis and hence algal growth. In environment with decreasing light intensity the algae cell answer increasing the content of chlorophyll a and other light-harvesting pigments (chlorophylls b, c, phycobilliproteins and primary carotenoids). Under high light intensity the pigments involved in the photosynthesis decrease and the photoprotective pigments (secondary carotenoids like astaxanthin, β-carotene, zeaxanthin) increase, accumulating in special organells, cytoplasmic lipid bodies or plastoglobuli of plastids (Vetchel et al. 1992; Ben-Amotz et al., 1982). Stress temperature conditions force the cell to accumulate carotenoids from the flow of carbon and nitrogen levels. Tredici et al. (1991) showed that cyanobacteria *Spirulina platensis*, grown outdoors, had higher carbohydrate synthesis on sunny days than on cloudy days. This means that light irradiation (intensity) directly affected the biochemical constituents of the cell.

The major constituents of thylakoid membranes, are directly affected by the irradiance of the light under growing conditions, hence the increased in total thylakoid membranes in cells are related with the low light-enhanced production of PUFA (Burner et al., 1989)

Cohen (1999) showed that polyunsaturated fatty acids or PUFAs (one of them EPA, 20:5ω3, eicosapentanoic 3-omega acid) and cellular content of lipid are inversely related to growth light intensity. Means while Sukenik et al. (1989) wrote about *Nannochloropsis* strains, which accumulated high levels of both lipids and EPA content under light-limiting conditions.

1.7.2.1 Diel cycles and circadian rhythms

The diel variation in irradiance is one of the mayor determinants of variability in photosynthetic response, because it promoted a diel variation in cellular chlorophyll content and the rates of light-saturated and light-limited photosynthesis normalize as an effect of the increased reaction centers during the photoperiod, and this appear to be related to diel variations in the ratio of RuBisCO to reaction centers (Harding et al. 1981) A circadian rhythm is effectively a memory of a photoperiod, which in eukaryotic cells, are encoded genetically by a set of ubiquitous nuclear genes, *Per* (i.e., *per*iodicity) (Takahashi 1992), dictating the time of cell processes. Protein kinase-phosphatase pathway mediates the signal transduction in processes such, cell division, photosynthetic and respiratory rates plastid orientation and migration, bioluminescence and chlorophyll biosynthesis (Comolli et al. 1996; Puiseus-Dao 1981). In algae mass cultivation, the cultures in which the cells are at the same state of the cell cycle at a given time (i.e., synchronous culture), the diel irradiance cycle is the natural synchronizing agent for unicellular algae, constraining growth rate and also applies to nutrient acquisition. The synchronous cycle is generally imposed by the transition from dark to light or by a stepwise temperature change. During the light period, nuclear DNA replication, storage of carbon reserves in the form of polysaccharides or lipids occurs, while cell function maintenance, protein synthesis, cell growth, mitosis and cell division occurrs in the dark period. (Falkowski and Raven 2007)

1.7.3 CO_2 and DO (dissolved oxygen)

All oxygenic photoautotrophs form carbohydrates by adding four protons and four electrons to the carbon atom thus incorporating CO_2 into organic matter. This is the first step in the photosynthetic assimilation of carbon. The net reaction for carbon fixation may be explicitly summarized as

$$CO_2 + 2NADPH + 2H^+ + 3ATP \rightarrow (CH_2O) + H_2O + 2NADP^+ + 3ADP + 3P_i \qquad \text{Eq. 3}$$

Stoichiometrically the ATP and NADPH were generated by photosynthetic electron transport chain (ETC). The photosynthetic carbon reduction cycle reactions (also known as Calvin-Benson-Bassham cycle or C3 pathway) are responsible of the primary metabolic pathway and occur in the water-soluble phase of the chloroplast stroma in eukaryotic microalgae (Benson 2002; Bassham 2003).

$$2H_2O + 2NADP^+ + 3ADP + 3P_i + \geq 8\, photons \rightarrow O_2 + 2NADPH + 2H^+ + 3ATP \qquad \text{Eq. 4}$$

It is in the photosystem II (PSII) where the H_2O is oxidized to O_2. The overall reaction is

$$2H_2O + 2PQ \rightarrow O_2 + 2PQH_2 \qquad \text{Eq. 5}$$

Where PQ refers to the oxidized plastoquinone, and PQH_2, is the fully oxidized plastoquinol. The part of PSII reaction center is the oxygen-evolving complex (OEC) (Debus, 1992;Yachandra et al., 1993; Brick and Ghanotakis, 1996), and is composed by a tetranuclear Mn cluster, that accumulates four oxidizing equivalents from four photochemical turnovers of the photoactive pigments. PSII is located in the thylakoid membrane, with a molecular mass of 300 kDa; the reaction center contains the D1 and

D2 proteins and the α and β subunits for cyt b_{559}. D1 and D2 carry all essential prosthetic groups vital for the charge separation and its stabilisation, tyrosine Z (primary electron donor), P680, pheophytin and Q_A, Q_B (primary and secondary quinone acceptors) (fig. 7) The excitation energy is transferred from the outer antennae to reaction centre by the inner core antennae formed by the intrinsic Chl a-proteins CP43 and CP47 (Masojídek et al., 1999) (fig. 7)

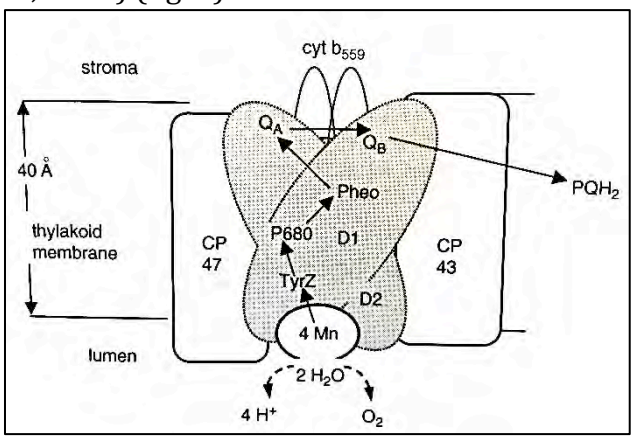

Figure 7 – Schematic diagram of molecular organization of the PSII core. The major protein subunits CP43, CP47, cyt b_{559} and the D1 and D2 proteins are labelled with bold letters. The two shaded proteins subunits D1 and D2 are known to bind most of the electron carriers (a manganese cluster – 4Mn, a tyrosine molecule Tyr Z, the special part of chlorophyll a molecules P680, pheophytin Pheo, the plastoquinones Q_A and Q_B, and the plastoquinone pool PQH_2). The water splitting complex represented by four manganese atoms is located in the thylakoid lumen. Arrows indicate principal electron transport pathways. (Illustration from Richmond Amos, 2007)

1.7.4 pH

It has been reported that alkaline pH stress increased the accumulation of TAG and decreasing the relative composition of membrane lipids in *Chlorella sp.* (Guckert and Cookser 1990). Tatsuzawa proposed that *Chlamydomonas* reacted to low pH in order to decrease membrane lipid fluidity, increasing the saturation of fatty acids in membrane lipids (Tatsuzawa et al. 1996).

1.7.5 Cultivation modes

Microalgae are able to nourish (trophy) themselves by means of autotrophy (phototrophy) and heterotrophy (phagothrophy). Autotrophic metabolism implies generation of energy from absorbed light and used to reduce CO_2 by oxidation of water as substrate and evolution of O_2. Microalgae requiring only inorganic mineral ions are defined as photoautrophic, and obligate photoautotrophic if they only grow in the light. Most species of algae belongs to the last category.

Heterotrophic nourish themselves from organic substances produced by other organisms. Photoheterotrophic requires light to metabolize organic compounds. When the algae needs small amounts of essential organic compounds (amino acids, vitamins) is defined as auxotrophic. When the microalgae needs both organic compounds and CO_2 to growth, they are classified as mixotrophic or amphitrophic. Interchanges between the trophics levels are possible because they are dependent from the growth conditions. The obligate trophic types are the exception. The excreted substances from microalgae are considered sometimes beneficial for the cultures, for example, alkaline phosphatases expelled by the algae cells under P-depravations levels (Grobelaar, 1983). In other cases, the exudants are used to inhibit other algae strains or as anti-predatory activities (Richmond, 2000).

1.7.6 Nutrition media requirements

Vonshak (1987) summarized the requirements for nutrition media for algae cultivations in salt contents, major ionic components such as K^+, Mg^{2+}, Na^+, Ca^{2+}, SO^-_4 and Cl^-, nitrogen and carbon sources, pH, trace elements, chelating agents (EDTA, ethylenediaminetetraacetic acid), vitamins. (Appendix 1. Nutrition media 3N-BBM + vitamins)

For autotrophic growth the formulation of the nutrient recipes are presented as follows: macronutrients are supplied at concentrations of gL^{-1} and micronutrients in mgL^{-1}. These experiments highest end is the high biomass yields per reactor volume or illuminated surface area. Grobelaar (2000) elucidated the physiological and technological considerations necessary for achieving high yields.

1.7.7 Nutrition assimilation

1.7.7.1 Carbon

High yield of autotrophic algae production system require supplies of CO_2. Atmospheric CO_2 is not able to diffuse at rates that support productivities around 10 g DW $m^{-2}d^{-1}$ (Grobelaar, 2007). The same author reported that the system CO_2-H_2CO_3-HCO^-_3-CO^{2-}_3 works at a buffer in freshwaters and its control allows keeping pH level stable for optimal mass-cultivation of algae.

Further, the buffer system provides CO_2 for photosynthetic activities:

$$2HCO_3^- \leftrightarrow CO_3^{2-} + H_2O + CO_2 \quad \text{Eq. 6}$$

$$HCO_3^- \leftrightarrow CO_2 + OH^- \quad \text{Eq. 7}$$

$$CO_3^{2-} + H_2O \leftrightarrow CO_2 + 2OH^- \quad \text{Eq. 8}$$

The level of OH^- gradually accumulates during photosynthesis and the pH of the growth solutions arise along as the CO_2 fixation. The logic of the system tells that supplying CO_2 into the culture, keep the pH under control for obtain high algae biomass yield. On the contrary when the algae actively photosynthesize, pH increases and release of CO_2 occurs during respiration. Grobelaar and Soeder (1985) wrote about the general rule dark respiration of about 10% of the total photosynthesis production.

1.7.7.2 Nitrogen

Nitrate (NO^-_3), nitrite (NO^-_2) and ammonium (NH^+_4) are the only forms of inorganic nitrogen that are assimilated directly, and in the aquatic environment, nitrate being the more highly oxidized form is the most thermodynamically stable form, and its transport across the plasmalemma is an energy-dependent process, consuming directly or indirectly ATP. In microalgae, nitrate´s secondary active influx is powered by an H^+ or Na^+ gradient, being the ATP used to generate the gradient (Raven 1984b). Inside the cell the chemical reduction of NH^+_4 allows the NO^-_2 assimilation, by means of nitrate reductase and nitrite reductase enzymatic activities (Falkowski 1983a). The nitrate reductase localized in the cytosol, catalyse the electron pair by means of NAD (P) H.

Nitrite reductase, localized in the chloroplast, reducing ferredoxin from the photosynthetic flow (Raven 1976a) (fig 8).
The overall stoichiometry for the reduction of nitrate to ammonium is:

$$NO_3^- + 8e^- + 10H^+ \rightarrow NH_4^+ + 3H_2O \qquad \text{Eq. 9}$$

During the amino acids synthesis, the ammonium is incorporated by enzymatic action of glutamine synthetase (GS) and glutamine 2 - oxoglutarate amino transferase (GOGAT) (Falkowski and Rivkin 1976; Zher and Falkowski 1988.). Glutamate is involved as substrate to irreversible form glutamine:

$$glutamate + NH_4^+ + ATP \rightarrow glutamine + ADP + P_i \qquad \text{Eq.10}$$

Glutamate again reduces by 2-oxoglutarate (from the Krebs cycle), producing two molecules of glutamate:

$$2 - oxoglutarate + glutamine + NADPH \rightarrow 2(glutamate) + NAPD \qquad \text{Eq. 11}$$

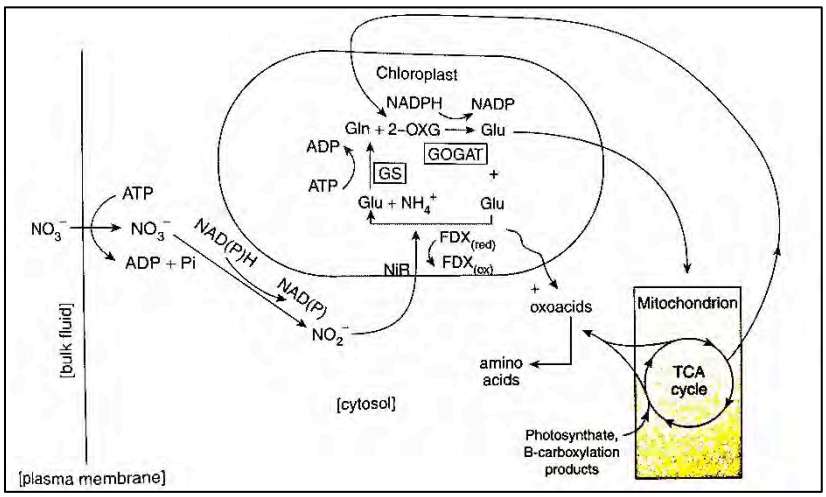

Figure 8 – Pathway of nitrogen assimilation in aquatic, eukaryotic photoautotrophs. NO^-_3 and NH^+_4 are actively transported from the environment across the plasma membrane into the cytoplasm and can be stored within the vacuole of the cell. When amino acid synthesis is required, NO^-_3 is reduced to NO^-_2 by nitrate reductase (NR), a cytoplasmic enzyme that uses either NADH or NADPH. NO^-_2 is reduced to NH^+_4 in the chloroplast by nitrite reductase (NiR); an enzyme that uses reduced ferredoxin. NH^+_4 is incorporated into glutamic acid to form glutamine via the action o glutamine synthetase (GS); this process leads to ATP hydrolysis to ADP. The finally yield of two molecules of glutamate is obtained when the amido nitrogen of glutamate is transferred to 2-oxoglutarate (GOGAT). The second glutamate molecule is exported to the cytoplasm where it undergoes transamination reactions (TrA) with α-keto acids produced in tricarboxylic acid cycle (TCA) in the mitochondria. The first glutamate molecule reenters the assimilation pathway as a substrate for GS, hence forming ac cycle (Illustration from Falkowski and Raven 2007).

The second important nutrient to biomass production is nitrogen. Its limitation gives discolouration caused by the chlorophyll decrease and carotenoids increase, yet the accumulation of polysaccharides and oils (PUFA) occurs. Supplemented as nitrate (NO^-_3) or ammonia (NH^+_4). The volatility of ammonia, when the pH increases, makes the decision to fall on nitrate. The supply of nitrate or ammonia gives similar energetic benefits for mass algae production (Grobelaar, 2007).
According to analysis published, nitrogen accounts for 7-10% of algae cell dry weight. This macro-element constitutes an essential structural and functional role in protein algae cells. Algae cell grown in nitrogen-deficit medium, actively and specifically degrade its phycobilisomes using them as nitrogen-source. Thompson (1996) showed that neutral lipids in the form of triacylglycerols are a direct product of nitrogen-depleted

cells. Not polar lipids as in the nitrogen-sufficient cells. *Dunaliella* strains yet, are able to synthesize more carbohydrates than lipids accumulating glycerol and polysaccharides as well. Richmond (1986) found than some *Chlorella* strains accumulate more starch while other accumulate neutral lipids under nitrogen depravation conditions. Ben-Amotz et al. (1982) showed that *Dunaliella* carotenogenesis increased under nitrogen-limited conditions while the chlorophyll content of the cells decreased.

1.7.7.3 Phosphor

Soluble inorganic phosphorus as orthophosphate anions, $H_2PO^{2-}_4$, are present in freshwater, while HPO^{2-}_4, and PO^{3-}_4, in marine aquatic environments (Stumm and Morgan 1981). The uptake of phosphate is similar to the nitrate uptake (Raven 1984b; Reid et al. 2000; Weiss et al. 2001).

Yet in the cytosol, phosphate can direct be incorporated into nucleic acid precursors, hydrocarbon and carbohydrate skeletons without electron transfer reactions (hydrolytic and dehydration chemistry). Ribosomal RNA is the main site where the phosphorus is allocated. Photosynthesis can be limited by phosphorus limitation in two ways:

(i) Reduction in the rate of synthesis of and regeneration of substrates in the Calvin-Benson cycle, hence reducing the rate of light utilization by carbon fixation, and

(ii) Limitation of nucleic acid synthesis at genome replication level or at RNA synthesis level (i.e., form for transcriptional control), and by reducing the rate of synthesis of proteins in the photosynthetic apparatus, affecting the photosynthesis energy conversion (effectively negative feedback) (Woodrow and Berry 1988).

Orthophosphate (PO^{2-}_4) is the preferred form supplied to the mass culture of algae cell. Its uptake is energetic dependent. Phosphorous is essential in many energy transfer processes like DNA biosynthesis. In spite of it's low contain in algae biomass, less than 1%, it's the most crucial growth limiting factor in algae cultivation. The reason is found in its loosely bound to other ions and it´s precipitation makes it unable for cellular uptake. Luxury uptake is the term giving for the polyphosphates bodies where excess P have been stored (Borowitzka, 1988). Biosynthesis of carbohydrates and lipids are dependent of the P supplies. The ratio N: P in the formulae of growth media is essential to maintain the survival and dominance of the chosen algae strain in the culture, hence it´s biomass productivity.

This macronutrient plays main functions in algae cellular metabolic processes. When the growth conditions are growth enhancing, the algae cell accumulates the inorganic phosphate in polyphosphate granules. The algae cell grown under deficit conditions, direct affects the chlorophyll a content that tends to decrease, while the carbohydrate content increase. Some degradation of the phycobilisome occurs, because cell division depletes the old ones and biosynthesis of new ones stops. Ben-Amotz et al. registered that Dunaliella algae accumulated β-carotene (Ben-Amotz et al., 1982).

1.7.7.4 Sulphate

Sulphur (S), is the third macronutrient is required for the cysteine and methionine amino acid synthesis, as well as for sulphur-containing lipids synthesis (10% of the thylakoid lipids). Uptake of sulphate is ATP-dependent process with a secondary active transport energized by ion gradients (Ritchie 1988; Matsuda and Colman 1995; Raven 1984b; Weiss et al. 2001). NADH and NADPH are the reduction agents of sulphate

molecule to sulphite, activated by ATP (Schmidt and Jager 1992), and it´s reduction accounts for 1% of the total phosynthetic electron flow, hence not a sink for reductant in algae (Raven 1993). In marine waters, sulphate is second to chloride in abundance. In freshwater sulphate may become limiting to protein synthesis, inducing in freshwater autotrophs, a high-affinity transport system, for acquiring sulphate at deficient external concentrations (Falkowski and Raven 2007). Studies shown that sulphate limitation in *Dunaliella salinas* leads to a decreased content of photosynthetic pigments and Rubisco and PEPC enzymes, lowering the accumulation of molecular mass in nitrogen compounds, hence reducing the photosynthetic activity (Giordano et al. 2000).

The assimilation of the three macronutrients (N, P, S) requires carbon skeletons, which are generated in the chloroplast, and mainly in the mitochondria by respiratory pathways.

1.7.7.5 Iron

This essential trace mineral element with its redox properties participates in photosynthesis, respiration, nitrogen fixation and DNA synthesis. Hardie et al., (1983) showed that Iron deficiency induces metabolic changes like degradation of chlorophyll a and c-phycocyanin, Ferredoxin, component of the photosynthetic electron transport chain, dropped when the no-containing Fe electron carrier, flavodoxin, increased under Fe-deficit (McKay et al., 1999). On the other hand, the excess of iron in algae cell, induce oxidative stress. The production of hydroxyl radicals (OH*) after the cellular level of Fe^{2+} with H_2O_2, by the Fenton reaction:

$$Fe(III) + O_2^- \rightarrow Fe(II) + O_2^- \qquad \text{Eq. 12}$$

$$H_2O_2 + FE(II) \rightarrow OH^o + OH^- + Fe(III) \qquad \text{Eq. 13}$$

It has been reported by Kobayashi et al., (1993) that Fe^{2+} could enhance the production of antioxidants (carotene molecules) in the algae cells, by generating OH^- and other active species ($^1O_2$, O^-_2, H_2O_2, or AO_2).
No less important are S, K, Na, Fe, Mg, Ca, and the micronutrients B, Co, Cu, Mn, Mo, Se, V and Zn (see appendix 1, tables). Many of these elements play key roles in the enzymatic reactions, and participate in B_{12} vitamin biosynthesis (Co). These trace elements have a tendency to bind easily to another ions and leading to precipitation hence unavailability. Chelators like EDTA to the solution are added to secure stability in Ferric compounds.
The Redfield ratio of 106C: 16N: 1P is widely used as a reference to keep and avoid possible nutrition limitation (Grobelaar, 2007).

1.7.8 Salinity tolerance and stress

Microalgae are able to support normal biological activity when the natural salinity level they evolved to, are stable. The normal range of salinity tolerance is 8 ppm, for freshwater species and 32-35 ppm for marine species. Hu (2007) refers to the ability of microalgae of accumulation of osmoticants, osmoregulatory substances of molecular dimensions that protect the cell in case of increasing salinity or environmental osmotic pressure. Among these osmoticants, polyols are the most important: glycerol, mannitol, galactitol, sorbitol, glycerol galactoside, sucrose, and trehalose. In *Dunaliella* strains, glycerol accounted for almost 50% of the DW. Borowitzka, (1979), proposed that the starch content breakdown accounted for the glycerol biosynthesis. The same author in

1988 have shown that the total lipid content increased in *Dunaliella sp* as a follow in salinity level increase, while *Nannochloropsis oculata* EPA (eicosapentanoic, unsaturated, fatty acid) decreased. Increased salinity directly increased the carotenoid concentration in *Dunaliella* strains.
Since Vonshak and Richmond, (1981), focused on the interactions of salinity stress with the photosynthetic apparatus, reported analysis of the inhibition in the PSII activity lead to photosynthesis decrease. It has been reported how in *Dunaliella* strains, the flow of electrons in the cyclic and non-cyclic electron transport chain became disturbed under osmotic stress (Gilmour et al., 1984). The ability of the marine algae species to respond immediately to osmotic change in the environment and subsequently to adapt and establish a new steady state for growth is a prerequisite for survival.
Baker (1991) reported that the environmental stress could afflict damage to the photosynthetic apparatus located in PS II. Lu and Vonshak (2002) did suggest that during adaptation to salt stress, the damaged occurred at the acceptor side of PS II and/or in the PS II reaction center led to decrease in PS II activity and increase in PS I activity. This protected PS II from excessive excitation energy under salt stress and increased the respiratory rate of salt-adapted cells might provide more energy destined to the synthesis of organic osmolytes and at the same time allowing cellular extruding of ionic Na^+ to maintain osmotic balance (Hibino et al., 1996).
It has been shown in *Dunaliella salinas* that transference from 0,5 to 3,5 M Nacl induced the expression of β-ketoacyl-coenzyme A (CoA) synthase (KCS) (catalyse the first steps of fatty acid elongation) (Azachi et al. 2002).

1.7.9 Conductivity

Electrical conductivity (EC) estimates the amount of total dissolved salts (TDS), or the total amount of dissolved ions in the water. In the microalgae mass culture, untreated tap water was used as part of the nutrition media, containing a high amount of microorganisms. Bacterial metabolism releases the potential energy stored in the chemical bonds of the organic carbon compounds, consumes oxygen in oxidizing these compounds, and releases carbon dioxide (CO_2) after the energy has been liberated (burned). This CO_2 rapidly dissolves in water to form carbonic acid (H_2CO_3), bicarbonate ions (HCO^-_3) and carbonate ions (CO^-_3) the relative amounts depending on the pH of the water. This newly created acid gradually decreases the pH of the water and the "new" ions increase the TDS, and therefore the EC, of the cultivation media. Essentially, the microorganisms are "eating" organic matter and releasing CO_2, oxidizing organic carbon using O_2 that they breathe out of the air as an oxidant, using the energy to drive their metabolism and exhale the oxidized carbon as CO_2. The oxygen is simultaneously chemically reduced and exhaled as water vapour (H_2O; the oxidation state of gaseous molecular oxygen is reduced from 0 to -2 in the process) (Michaud 1991; Moore 1989).
The ionic salt concentration (electric conductivity) in freshwater for *Chlorella vulgaris* was in the range 1,32 to 1,71 $mScm^{-1}$, *Scenedesmus* sp was in the range 1,19 to 1,78 $mScm^{-1}$, and *Chlorella wild mix Årungen* was in the range 1,47 to 1,63 $mScm^{-1}$; and the conductivity in saltwater for *Dunaliella salinas* was in the range 29,9 to 39,1 $mScm^{-1}$, *Nannochloropsis oculata* was in the range 28,6 to 40,1$mScm^{-1}$.

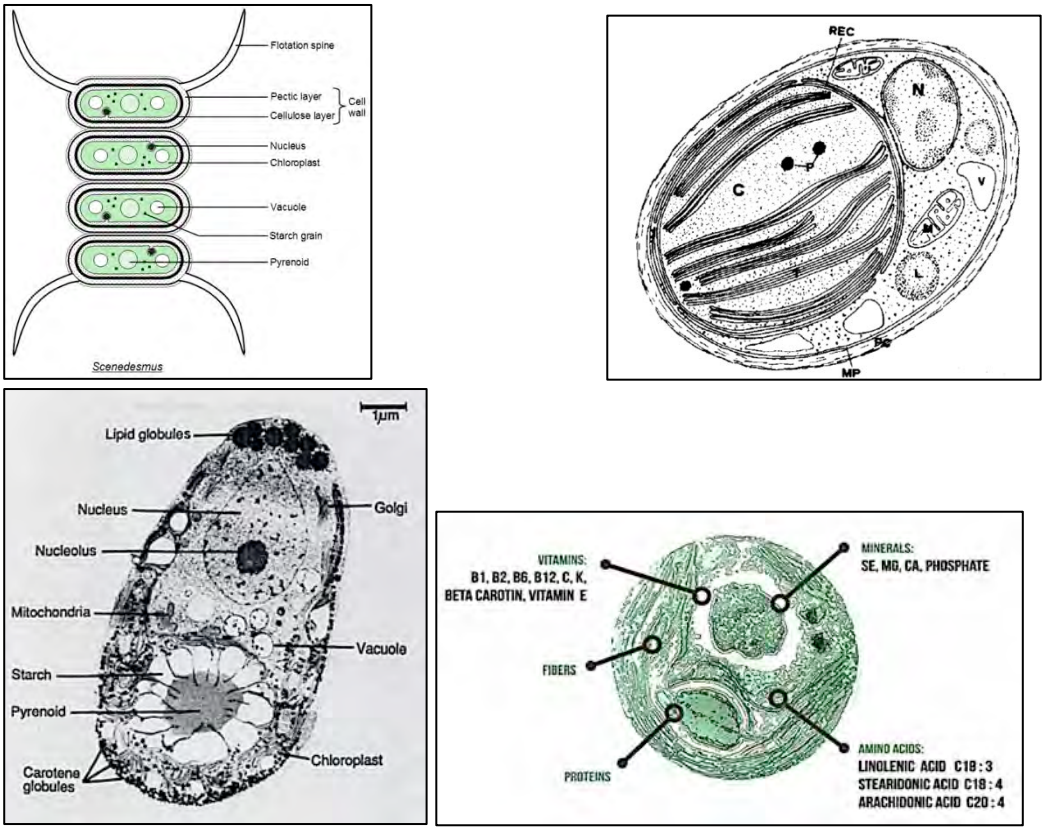

Figure 9 – Representative scheme of the important ultra-structural characteristics of *Scenedesmus sp.* (Upper left). *Nannochloropsis gaditana* CCMP526. C: chloroplast; MP: plasmatic membrane; N: nuclei; M: mitochondria; REC: endoplasmic reticulum; T; thylakoid; PC: cellular wall; V: vacuole; L: lipids; P: plastoglobuli (Upper right) (illustration from Lubián Luis Maria 1982.); Electron micrograph of a section through β-carotene-rich *Dunaliella bardawii*, thin cellular membrane (Be-Amotz, A. and Avron, M., 1990) (Down left); *Chlorella vulgaris* (Chlorella's Composition | Chlorella Echlorial at http://science-img.com/chlorella-cell) (Down right).

As shown in fig 9, the illustrations represent the principal characteristics of four different algae strains related the species selected and provided by IGV, Potsdam, Germany.

1.7.10 Turbulence

When the nutritional requirements and the environmental conditions are not growing-limiting, mixing is necessary to create and maintain turbulent flow to achieve high biomass yields of algae (Richmond and Becker, 1986). Turbulence enhance exchange rates of nutrients and metabolites between the cells and the growth media in the culture, as well as increasing the light-dark frequencies the cell is exposed to increasing the photosynthetic efficiency hence productivity (Grobelaar, 1994). The boundary layer is affected and the diffusion gradient for nutrients and metabolites.

Uptake of nutrients depends on physical factors like light, temperature and turbulence. Harris (1978) showed that the photosynthetic irradiance (P/I) relationship direct affected the rate of nutrients uptake, which increased when the light energy was available until it got constant at saturating irradiation levels.
Goldmann and Carpenter (1974) established the temperature coefficient (Q_{10}) for the two folding enzymatic reaction when the temperature raised 10°C. in the mass culture of algae, this imply that the temperature in the algae suspension will take double amount of nutrients for each 10°C of temperature increase. Grobelaar et al., (1990), showed that

there is a synergetic relationship between light energy and temperature which influence the nutrition uptake.
Fauchot et al., (2000) went further in their studies and showed that UV-B radiation affected directly the nitrogen uptake in phytoplankton, and when this radiation is blocked out from the algae culture, the NO^{-}_{3}, NH^{-}_{4} and urea rate uptake increased.

1.8 Harvest

In microalgae technology the most challenging process is the efficiency of the harvesting processes. How to handle the small size and negativity of surface charge of the microalgae, the similarity of the density algae cell-growth media and the algae growth rate which impose a regularly harvest. Methods and protocols are many: sedimentation, flocculation, centrifugation, filtration or a combination of these methods. There is no universal harvest solution for them all. This influences the design and operation of upstream and downstream processes and of the biochemistry and properties of the end product.

1.8.1 Introduction

The quality and quantity of the energy rich algae biomass yield diluted in the suspension determine if its viable energetic balance to put in the harvest process. Microalgae size below 25 μm, its density almost similar to the growth medium, its negative charged surface maintain the colloid nature of the suspension, together to the algae growth rate challenge a whole spectrum of harvesting techniques (Moraine et al., 1979; Reynolds 1984; Edzwald, 1993; Molina Grima et al., 2003; Packer, 2009). A key factor limiting the commercial use of microalgae has always been the cost effective harvesting. Mata et al., (2010), Verma et al., (2010) suggested an average cost of 20-30% due to harvesting, whilst Greenwell et al., (2010) estimated 50%. The frequent harvesting due to the high growth rate of the algae cell, increment the cost of dewatering and harvesting of the algae suspension (Benemann et al., 1977). The energy requirements reported for separation by settlement and centrifugation, 1 MJ Kg^{-1} of dry biomass, surpasses the 0,7-09 MJ Kg^{-1} energetic cost of harvesting wood (Sawayama et al., 1999). As Molina Grima et al, (2003) pointed out, the important criterion in selection of the harvesting method is the final moisture content of the harvested algae biomass. The moisture in the final algae product should not exceed 85% otherwise the cost and methods of further processing and energy extraction from the biomass will have substantial increase. Table 2 shows the pros and cons of the different harvesting methods in microalgae technology (tab 2).

Table 9 – Comparison of microalgae harvesting methods (Mohn 1988; Molina Grima et al. 2004; Shen et al. 2009) (Illustration from Milledge and Heaven 2013)

	Advantages	Disadvantages	Dry solids output conc' (%)
Centrifugation	Can handle most algal types with rapid efficient cell harvesting	High capital and operational costs	10–22
Filtration	Wide variety of filter and membrane types available	Highly dependent on algal species; best suited to large algal cells. Clogging or fouling an issue	2–27
Ultrafiltration	Can handle delicate cells	High capital and operational costs	1.5–4
Sedimentation	Low cost, potential for use as a first stage to reduce energy input and cost of subsequent stages	Algal species specific, best suited to dense non-motile cells. Separation can be slow. Low final concentration	0.5–3
Chemical flocculation	Wide range of flocculants available, price varies although can be low cost	Removal of flocculants, chemical contamination	3–8
Flotation	Can be more rapid than sedimentation. Possibility to combine with gaseous transfer	Algal species specific. High capital and operational cost	7

1.9 Sedimentation

The gravitational forces cause liquid or solid particles to separate from the liquid with different densities. This is a slow process. In term of Stokes´ Law, sedimentation velocity is proportional to the share square of the (Stokes´) radius of the cells and the difference in density existing between microalgae cell and the medium around it.

$$Setting\ velocity = \frac{2}{9}\, g\, \frac{r^2}{\eta}\, (\rho_s - \rho_l) \qquad \text{Eq. 14}$$

Where r is cell radius, η is fluid dynamic viscosity and ρ_s and ρ_l are the solid and liquid densities. Millero and Lepple (1973) reported about how close the densities of microalgae and of freshwater and salt water were at 1,025 Kgm^{-3}. This value compared to the cytoplasm of marine microalgae is close (1,030-1,1 Kg m^{-3}), density of freshwater green microalgae (1,040-1,14 Kg m^{-3}). It has been found that spherical shaped like fresh water *Chlorella* strains (density 1,070 Kg m^{-3}, average cell diameter 5 µm at 20 °C registered a density of 998 Kg m^{-3} and a viscosity of $1x10^{-3}$ Pa s^{-1}, (Edzwald 1993) ha), reported a settlement velocity of 0,1 m day^{-1}. Stokes´ Law holds for spheroidal shapes and is not always the case of the morphology of the algae cell. Smayda (1970), Sournia (1978) proposed a theory about how evolution developed different algae shapes to avoid settling from the euphotic zone. Colonial algae like *Scenedesmus* sp.; with a cluster diameter of approx. 60µm have been shown that settlement is a mode of harvesting. Chlorella is not the case. Settlement of algae cell is not only species dependent but is direct affected by light intensity, nutrient deficiency and age of culture, specially in senescent cells (last stage after dividing-level and before death) and spore-producing cells (Smayda, 1970; Bienfang, 1981; Waine et al. 1992). The levels of biochemical constituents in the microalgae determine the density of the cell. Carbohydrate is 1,500 Kg m^{-3}, lipid 860 Kg m^{-3} and protein 1,300 Kg m^{-3} (Reynolds 1984). Collet et al, 2011 has reported cell recoveries of 60-65% and 1,5% of solid concentrations (Uduman et al. 2010), which are low and is only used for colonial and larger algae as a pre-concentration step for use in combination with other harvesting techniques.

1.10 Flocculation

Brenna and Owende (2010) recommended the use of flocculation in conjunction with other harvesting techniques. The rate of settling or flotation can be increased by increasing the size of particles by the aggregation of algae cells by means of flocculation (Mata et al. 2010). The wide range of microalgae and the large quantities of suspension flocculation allows, make it the predilection method of harvesting. Auto-flocculation in some algae is the possible and is a response to environmental stress, nitrogen changes, and pH and dissolved oxygen (Schenk et al.2008). Flocculation can be induced chemically (inorganic or organic) or biologically (microorganisms) and the flocculant substance is species-specific, because its interaction with the algae cell affects directly the shape, size and composition of the flocs, hence the recovery of the yielded biomass. The elected flocculant have not only to be non-fossil fuel derived, sustainable, renewable, but also inexpensive, nontoxic and effective in low concentrations (Molina Grima et al. 2003).

Since the 1920 wastewater treatments calcium has been used hydroxide (lime) to remove suspended solids and microalgae (Oswald 1988). Aluminium chloride (alum), ferric sulphate, ferric chloride and multivalent metal salts are the usually flocculants used in wastewater treatments. Molina Grima et, al. (2003) reported that alum is high effective in harvesting *Chlorella* and *Scenedesmus* by flocculation. In respect of optimal dose, pH, quality of the resultant water and slurry, aluminium salts have been found to be superior to ferric salts (Shelef et al., 1984; Papazi et al. 2010). Its has been found that inorganic flocculants also have negative effects on algae cell viability, modifications of the growth media, impeding recycling and reuse (Molina Grima et al., 2003). In spite of the low prices of inorganic flocculants comparing to synthetic organic flocculants, the higher dosage rates required needed, can result in expensive cost per unit algae cell flocculated (Mohn 1988).

Polycationic electrolytes have proved to be the most effective flocculants for harvest microalgae (Uduma et al 2010). Granados et al. (2010), reported from Almeria University that cationic polyelectrolytes have excelled metal salts in effectiveness in flocculating freshwater algae, yielding biomass concentrations at lower dosage rates of 2-25 mgL^{-2}. In conjunction with pH adjustment (10-10,6), low dosages (0,5 mgL^{-1}) of Magnafloc LT25, non-ionic polymer (BASF), have been found effective in flocculation producing settled floc 200-800 times higher than in the start suspension (Knuckey et al. 2006). The derived crustacean shells, Chitosan (cationic inorganic polymer) has been used in the food industry wastewater and has been found effective in freshwater microalgae too. The disadvantages are the high costs for the high dosage required 20-150 mgL^{-1} and reports of from the oyster industry of the reduced survival of oyster larvae fed chitosan treated microalgae (Molina Grima et al. 2003; Harith et al. 2009).

Mohn (1988) reported of the settle effect on microalgae of starch and modified starch, and its use in treatment of drinking water, apparently not affect the viability of microalgae. Vandame (2010) wrote about cationic starch effectiveness in wastewater and paper mill industry and flocculation of *Scenedesmus* and found that the large degree of variation in effectiveness and high dosage of the tested cationic starches were disadvantageous.

Vandamme et al (2010) wrote that the flocculants used by the wastewater industry has not been designed for microalgae industry, and its modification to improve performance of microalgae yielded biomass will increase the cost further.

Salinity levels about 5 gL^{-1} has reported inhibition of flocculation (Molina Grima et al. 2003; Knuckey et al. 2006) meanwhile the typically sea waters´ salinity level ~35 gL^{-1} Polyelectrolytes tend to fold tightly and fail to bridge between the algae cells to form a floc, due to the high ionic strengths, and in marine systems the flocculation effectiveness of polyelectrolytes have been proved only when they are used in conjunction with ferric salts (inorganic flocculant)(Fig. 10)(Molina Grima et al. 2003; Knuckey et al 2006). For the marine microalgae, the dosage is 5-10 times higher than for the freshwater algae. To achieve a 90% removal of algae from suspensions, the amount of flocculant in the dosage increase linearly with salinity as expressed in ionic strength (Shelef et al. 1984: Sukenik et al. 1988)(fig 10).

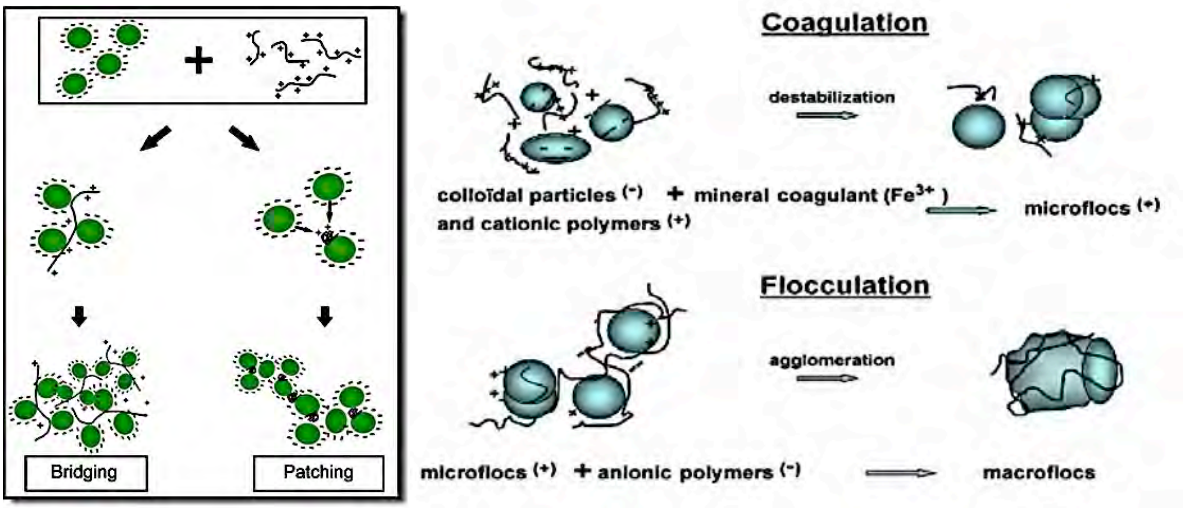

Figure 10 – Schematic view of possible mechanisms involved in polymer-induced flocculation bridging and patching (left – from Salime et al., 2010) and coagulation vs. flocculation processes (right – from http://www.aquasure.fr/spip.php?article74).

Increments of pH levels up to 11-12 has been proved effective to induce flocculation *Chlorella*, but extreme pH is a disadvantage because it may cause damage to the algae cell and lead to death (Lee et al. 2009). The overall reported conclusion was that prior to flocculation a low energy pre-concentration settlement technology was required, but the great disadvantage was the extra cost of modifications and amount of alkali required (Schlesinger et al. 2012).

It has been found that bacteria metabolites produced from bacteria strains living in the photic zone of open waters, which can make up to 30% of the biomass (Sournia 1978), can be used as bio-flocculant with some efficiency in the flocculation of *Chlorella* (Molina Grima et al. 2003).

At bench scale of 1 L, *Chlorella* has been harvest by using sacrificial aluminium or iron anodes. The method was effective and is named as electro-coagulation-flocculation. Vandamme et al. (2011) reported the efficiency of aluminium anodes was superior contra iron anodes. In comparison to centrifugation, the consumption of power was lower, in the range of 0,3 and 2 $kWhkg^{-1}$, for saltwater and freshwater, respectively. Electro-coagulation is a good option for harvesting marine algae strains.

$$Anode\ halfreaction\ I: M_{(s)} \rightarrow M^{n+}_{(aq)} + ne^- \quad \text{Eq. 15}$$

$$Anode\ halfreaction\ II: 2H_2O_{(l)} \rightarrow O_{2(g)} + 4H^+_{(aq)} + 4e^- \quad \text{Eq. 16}$$

$$Cathode\ halfreaction\ I: 2H_2O_{(l)} + 2e^- \rightarrow H_{2(g)} + 2OH^- \quad \text{Eq. 17}$$

Half-reactions occurs at each electrode and the dominated anode and cathode half reactions are shown in Eq. 15, 16 and 17, where M is the metal anode, n is the charge of the metal ion, generating hydrogen gas at the cathode and realising cations at the anode, reducing and neutralising the negative surface charge of the destabilised microalgae cell forcing it to flocculate. The flocs can sink or being attached to the hydrogen bubbles and float to the surface. No anions are introduced to the growth media, and it´s the main advantage over inorganic (chemical) flocculation (Vandamme et al. 2001). Voltage, current, residence time, electrode material and system design are the major factors affecting the electroflocculation of algae cell. The main disadvantageous issue is the contamination of the recovered biomass and growth media with metal salts from the sacrificial anode, the formation of oxide layer on the cathode and the high cost of anode replacement, being the operational cost depending of the initial biomass concentration, biomass recovery mode (flotation or sedimentation) and percentage, and salinity and conductivity level of the media (Uduman et al. 2011). Based on the applied current, voltage, residence time and growth medium volume, can the electroflocculation energy consumption be calculated, and it´s system design and operational mode dependent. The marine algae strains are less biomass recovery energy demanding as the electrical resistance in freshwater systems is higher thus in order to achieve the same current, higher voltage is required (Borowitzka and Moheimani, 2013).

Poelman et al. (1997) experimented with electrolytic flocculation non-sacrificial anodes. The negatively charged microalgae flew towards the anode loosing its negativity and able to form flocs. In spite of the fouling of the anode, the effective levels of removing algae biomass from suspension was 95% and required just 0,2 $kWhkg^{-1}$. This process is called electrolytic flocculation (Poelman et al. 1997; Uduman et al. 2010).

Flocculation by means of ultra-sound has been investigated by Bosma et al. (2003) but the concentration factors reported was lower than the other mentioned methods.
Disadvantages with electro-coagulation-flocculation, electrolytic flocculation and ultrasonic flocculation are complex and the direct application in commercial or industrial scale needs further investigation. The lowest energy input required for sedimentation and flocculation present it self as a promissory harvest method (Danquah et al. 2009).

Harvesting using microalgae predators has been considered but the conversion to animal biomass from plant biomass is inefficient, most due to respiration and metabolic processes.

1.11 Flotation

Flotation is an ability of some algae strains and can be promoted by air bubbles addition (Singh et al., 2011). The flotation of microalgae flocs is due to its reduced density and can be used as a method of separating flocculated microalgae (Mohn 1988)

According to the method of bubble production, Flotation processes are classified as dissolved air flotation, electrolytic flotation and dispersed air flotation (Shelef et al. 1984) In the wastewater industry, flocculation combined with flotation by means of fine air bubbles, with gas pressure of 3 atmospheres, has been found effective in removal of algae cells (Moraine et al. 1979)

The process where the generated bubbles have a mean size of 40 μm (10-100 μm) is known as dissolved air flotation (DAF) (Edzwald 1993). In spite of its effectiveness, the input of energy in form of pressure required is high (Hanotu et al. 2012)

Another method of high efficiency in algae cell removal and equal energy demanding is the electro-flotation method (Shelef et al. 1984). Quantum Fracturing™ developed by Origin Oil is a process based on electromagnetic fields pulsed in conjunction with modifications in pH separates the lipid from algae cell walls by fracturing and floating and settling out the biomass of the remains (Gouveia 2011)

One interesting method, demanding less energy, is based on generation of micro-bubbles by fluidic oscillation, and has been found of high efficiency in removal of algae cell from suspensions (Zimmerman et al. 2009; Hanotu et al. 2012).

1.12 Filtration

The main disadvantage of filters is to be hampered by low throughput and rapid clogging (Mohn 1998). According to the pore or membrane size, filters are classified in: macro filtration (>10 μm), micro filtration (0,1-10 μm), ultra filtration (0,02-0,2 μm), and reverse osmosis (<0,001 μm)

The increments of the energy inputs as pressure to force the fluid through the membrane, increases with the reduction of membrane pore size. Microalgae typically size ranges 2-30 μm (Molina Grima et al. 2003), which fit the size to the micro filtration as most appropriate for algae cells harvesting: *Chlorella* at 5-6μm, *Dunaliella* at 7-8 μm and *Nannochloropsis* at 4-6 μm (Edzwald 1993). Flocculated cells and larger algae cells like *Scenedesmus* at 20-22 μm require the use of macro filtration. Naked cells are fragile and ultra filtration is a good alternative for harvesting but the costs of operation and maintenance are very high (Mata et al. 2010). Gouveia (2011) suggested that ultra filtration of algae cells using the same procedures as for seawater desalination could be an option, thus the energy input required could be 3 $kWhm^{-3}$, equivalent to the energetic demands by reverse osmosis desalination.

Vibrating screens has been proved to be high energetic saving at 0,4 $kWhm^{-3}$, producing 6% dry weight of *Spirulina* (Van den Hende et al. 2011; Mohn 1988). The simplicity and flexibility in design and capability to treat wide range of microalgae slurries gives filter presses a high utilization rage (Richardson et al. 2002). The disadvantage is the unpredictable cake washing yields can increases the demand for labour operators and their costs (Brennan et al. 1969). Mohn (1988) removed effectively the microalgae *Scenedesmus* from filter membrane with a modified filter press with plastic diaphragms, which inflate to harvest the algae cell.

Small colonies microalgae has been filtered to form a cake of dry weight of 18% solids by means of rotary vacuum filters (Brennam et al. 1969; Richardson et al. 2002). Larger or colonial microalgae has been harvested by means of Vacuum belt filters, but opposed disadvantages because of the high cost of investments and energy (Mohn 1988). *Chlorella* species did filter poorly even with 5 μm pore size (Goh 1984).

In water treatment industry belt filters are widely used and could be applied to separate algae (Mohn 1988).

Molina Grima and Uduman concluded that filtration methods are only suitable for algae cells with diameters over than 10μm. (Molina Grima et al. (2003) and Uduman et al. 2010).

1.13 Centrifugation

A greater force than gravity is applied for driving separation, and almost all microalgae strain can be centrifuged (Mohn 1988).

Disc stack centrifuges are known to apply forces in the range 4000-14000 times gravitational forces (Perry and Chilton 1973), and it consist of shallow cylindrical bowl containing a number (stacks) of closely spaced rotating metal cones (discs). The density of the materials forces them to be separated into thin layers whit the lightest one travelling towards the centre and the heaviest one outwards. Algae with size (3-30 μm) and concentration (0,02-0,05%) of algae cell in growth medium are suited for disc stack centrifuge. This type of centrifuge can separate solid/liquid, liquid/liquid or liquid/liquid/solid on a continuous basis. They demand high energetic input, Uduman exemplified Westfalia HSB400 disc-bowl centrifugal clarifier was limited to 35 m^3h^{-1} yet it has a maximum capacity of 95 m^3h^{-1} (Uduman et al. 2010; Cawdery, D, GEA Westfalia, personal communication 2009). It has been reported an energetic consumption of 1 $kWhm^{-3}$ for concentrating *Scenedesmus* from 0,1 to 1,2% using Westfalia self-cleaning disk stack centrifuge and 1,4 $kWhm^{-3}$ for the disc bowl centrifuge harvesting of algae suspension (Molina Grima et al. 2003; Goh 1984). The energy for centrifugation range about four times the energy available in the available energy in the biodiesel produced (Alfa Laval, personal communication 2009; Milledge and Heaven 2011). The high energy input in the disc stack centrifuges could be reduced by pre-concentration by a combination of separation techniques; Not only use the lipid fraction for production of energy but the entire algae biomass; or elimination by centrifugation of other energy swallowing unit operations in production process of algae biodiesel.

Another known method of algae biomass recovery is the solid bowl centrifuges but is not superior to the disc stack centrifuges, and Decanter centrifuges for separating of microalgae is equivalent in efficiency to the solid bowl (Goh 1984) the average energy consumption at 8 $kWhm^{-3}$ (Molina Grima et al. 2003).

The relative low energetic consumption (0,3 $kWhm^{-3}$) of the Hydro-cyclones, achieves maximum concentration of 0,3%. Their main advantage is the low capital cost, and disadvantages is that the efficiency is highly dependent on solids concentration and only can process few algae strains, and disrupt natural flocs of marine microalgae making difficult even further the harvesting of algae (Origin Oil 2010; Vedhuis et al. 2006).

Evodus manufactured the spiral plate centrifuge, where the suspension, in thin films, flows outwards onto vertical plates, collecting the microalgae at the bottom of the vanes by centrifugal forces. It has been collected 0,025% suspension of *Nannochloropsis* with a DW of 31,5% with an energy usage of 1,9 $kWhm^{-3}$ of dry algae. Which equal 34% of the total combustion energy within the micro algae biomass (Evodus private communication 2011). Milledge concluded that the most effective way to boost the positive energy balance in the production of biodiesel is to exploit the entire harvested microalgae biomass, thus a kg of dry algae biomass containing 20% oil gives approximately 1,9 $kWhm^{-3}$ of biodiesel and it corresponds to 6 kWh of the entire biomass calorific value (Milledge 2010a,b; Sialve et al. 2009; Stephenson et al. 2010) (fig. 11).

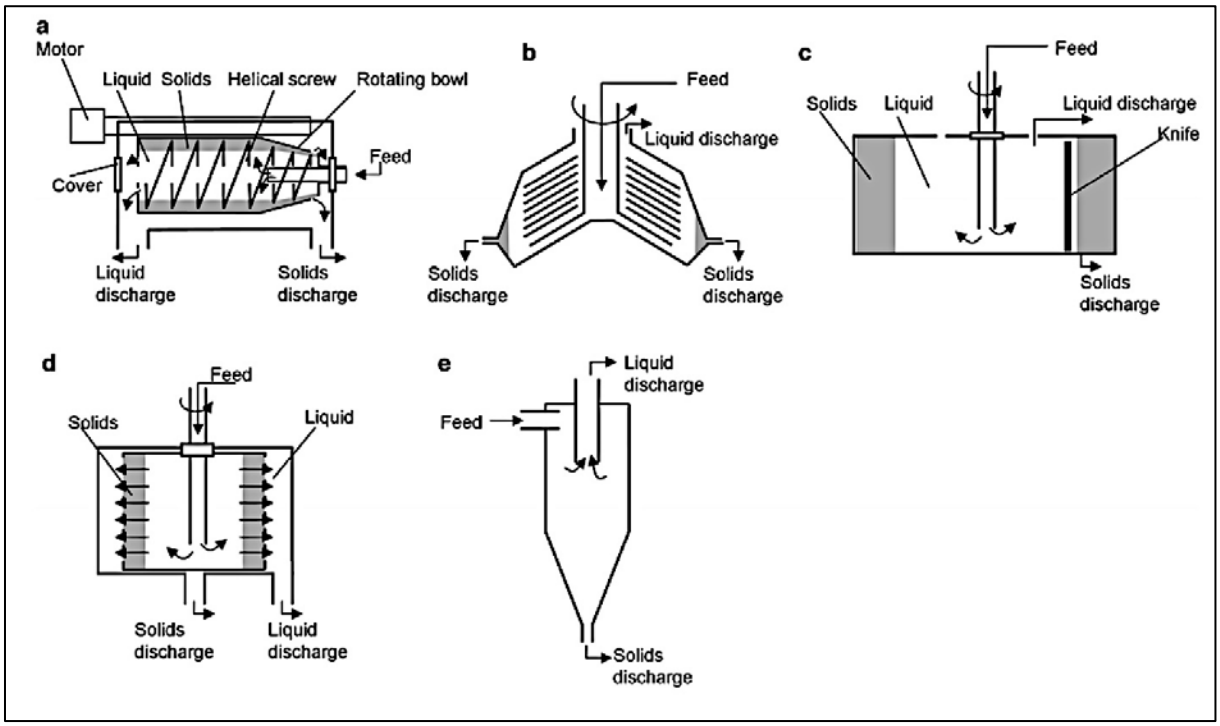

Figure 11- Cross sectional view of a (a) decanter type centrifuge, (b) disc stacked centrifuge, (c) imperforated basket centrifuge, (d) perforated basket centrifuge and (e) hydro-cyclone. Illustration from Borowitzka and Moheimani 2013

1.14 Material handling

Production of microalgae biomass is a synergy of three factors: the harvesting operation must be linked to both growth system and method of exploiting the energy secluded in the organic matter of the algae cell. The considerations of the growth system´s medium and its recycling after harvesting, can increase the cost of energy in the flow of the dilute algae suspension between operation processes. The subsequent treatment and handling of the algae suspension varies in accordance with its concentration (physical properties) and Oswald suggested three categories for algae suspension dilution levels: milk-like (1-2%), suspension cream like (10-12%) and cheese like (15-20%) (Oswald 1988). Each levels of creamlike nature, represents its handling difficulties.

1.15 Drying

After harvesting, comes drying and may be required prior to extraction of energy. Dehydration by evaporation, use to be very intensive. In accordance with the enthalpy for water at 20°C is 84 $kJkg^{-1}$ and for steam at 100°C is 2,676 $kJkg^{-1}$ (Weast 1985;

Mayhew and Rogers 1972). This requires an energy input of approximately 2,6 MJ kg^{-1} or over 700 $kWhm^{-3}$ to heat and evaporate water at atmospheric pressure with 20°C as start temperature. Subsequently to further the processing or energy extraction, several methods have been used to dry the microalgae suspension: from solar drying, roller drying, spray drying and freeze-drying.

Solar drying in spite of zero fossil fuel demands is weather and areal dependent for the drying process (100g dry weight/m^2) (Oswald 1988) and can denaturize the organic constituents of the algae cells (Brennan and Owende 2010).

In the food industry roller, spray and freeze driers has been widely been used with satisfactory results (Molina Grima et al. 2003). High value and fine chemical products from *Dunaliella* has been harvested by means of spray drying, but the production costs are expensive and the powder produced can deteriorate the pigments in the algae cell (Oswald 1988; Molina Grima et al. 2003 and Brennan and Owende 2010).

Freeze drying is more gentle method towards the organic constituents of the algae but increases the costs of production and its rendered to research means (Molina Grima et al. 2003).

Less energy demanding than evaporation is dewatering and is used prior to drying and depending of the final desired product, the selection of the subsequently energy extraction methods.

As illustrated in figure 12, the lowest energy input for microalgae harvesting are sedimentation and flocculation. The harvesting of microalgae is a tailor-made process and strain dependent. The variation in the semi-final product concentration is high: 0,5-27% dry weight, and subsequently processes may be needed to dewater the algae biomass and is highly dependent of the available energy in the algae cell.

The overall conclusion of the different methods of energy extraction from microalgae could be a synergetic growth system with no maximum yield performance, but with better performance in harvesting the biomass algae, which will facilitate through a minimum microalgae biomass concentration, the best extraction process of energy. To reach an efficient harvesting method, further investigation will be need, focusing on the design and operation of both upstream and downstream processes in an integrated overall microalgae production process.

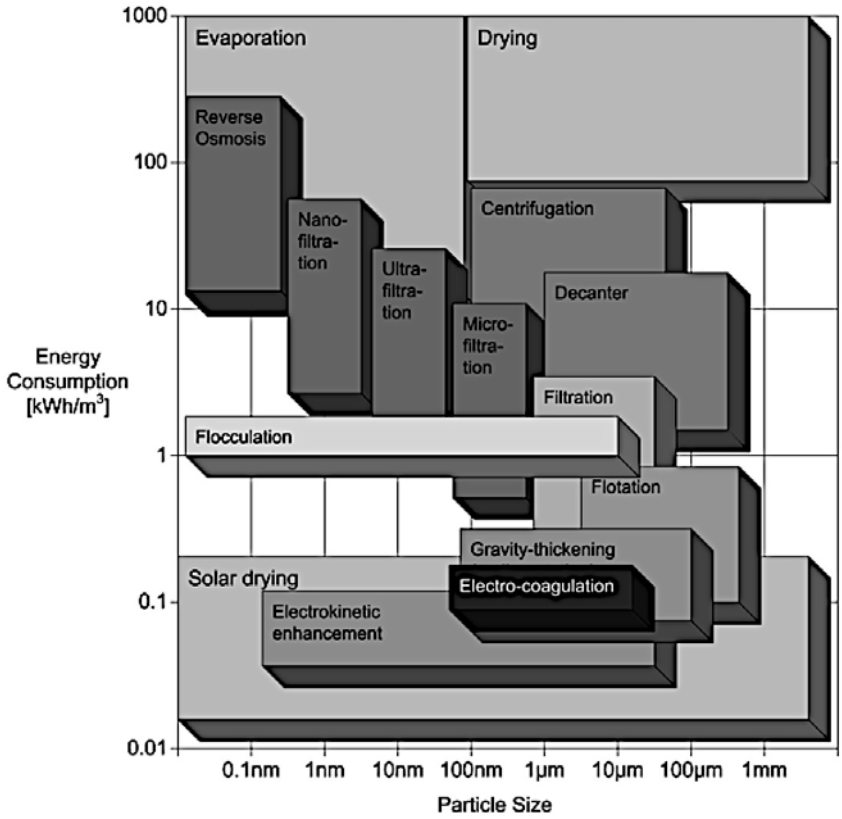

Figure 12 – Energy consumption and particle size for various separation techniques (Illustration from Borowitzka and Moheimani 2013).

2 Experiments

2.1 Introduction

The necessity to develop new strategic devices (TPBR) based on more cleaner, cheaper and more sustainable energy sources, was one of the principal aims of this project. The time was apprehensive and the need of microalgae suspension in high quantities and quality was urgent. The algae biomass concentration was destined to test Salsnes filter in conjugation with flocculation procedures (Sahu et al., 2012). The focus of this master thesis will be on the development of an upscaling method of production, analysis of the registered data and predictions based on this work and work by other authors, of the photosynthetic performance of the TPBRs during a Norwegian summer period (with neither artificial illumination nor heating) and northern geographical location (Ås, SKP, center for climate regulated plant environment, 59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level)

This study pays attention to the control of the temperature of microalgae cultivation in indoors conditions. The possible benefits of a thermic stability (i.e., extra energy input could report in extra biomass energy output) and the drawbacks of a harsh dilution regime were evaluated.

The necessity of make a balance between the produced energy and the supplied energy within the same system is imperative to define the limits of the microalgae productivity and to design the cultivation system of production.

2.2 Material and methods

The low cost polypropylene tray photobioreactor (TPBR) (cover but not sealed by a lid) used in this study contrast to the wide range of engineered system (raceways, closed tubular photobioreactors) and should contribute to lowering the economics: low energetic and monetary costs of production of algae. Raceways are cheaper but prone to high rates of evaporation and contamination. TPBR here could be seen as a hybrid photobioreactor (semi-closed photobioreactor).

In these experiments, the author examines the energetic performance of the TPBRs using natural sunlight and without temperature control (not heating cobs). The TPBR were not coupled in series, each one of them operated as a single unity and remained isolated for two different periods: group 1 operated for 63 days, 200612-230812 (TPBR 1 to TPBR 6, i.e. *Chlorella vulgaris, Dunaliella salinas, Nannochloropsis oculata*) and group 2 operated for 42 days, 110712-230812 (TPBR 7 to TPBR 10, i.e. *Scenedesmus sp., Chlorella wild mix Årungen*). The identification and analysis of energy conversion efficiency of a productive short seasonally average microalgae cultivation. The energy invested in circulating and mixing three of the ten TPBR was analysed.

The biomass productivity from TPBR placed on an east-west-facing orientation reported high values. It´s important to take into account the many obstructive conditions and materials in a greenhouse construction. To mention the first of them, the location of the room which represented shading from the afternoon solar radiation. For the second, the placements of the TPBRs inside the room, represented another challenge, because the TPBR where placed closer to each other (0,50 m apart from each other) on the bench designed for plant production. For the third, the roof structure in iron frames was one of

the most relevant of the obstacles impossible to overcome in solar irradiation exposure, the migrating shade affected directly the measurements of PAR incident on the surface and inside of the TPBRs, reaching the algae suspension. For the fourth, the dilution rate was not an adequate one. The harvesting regime was in occasions too high and the algae culture had problems to overcome it and recover from it. For the sixth, the room in the green house was deployed of both sunscreen and insect mesh. During a rainy day, the roof leaked directly on the TPBRs. The lid partially covered the TPBRs and arrested major damages to the algae culture. The outside surface of the roof area corresponding to the microalgae chamber was painted with a white suspension to contend the high solar radiation and bringing a cooling effect, interpreted as positive in relation with evaporation and overheating of the algae suspension. The contamination by insets was inevitable. The freshwater microalgae TPBRs were compromised by small insects.

The start concentration for each TPBR was calculated by using the first TSS sampled collected, the same day after the inoculation of the TPBR, and divided this value by the total initial volume (V_O =25,75 L), expressed in gL^{-1}.

The start biomass concentration were 0,002135922 gL^{-1} (*Chlorella vulgaris,* TPBR 1), 0,003262136 gL^{-1} (*Chlorella vulgaris,* TPBR 2), 0,001475728 gL^{-1} (*Dunaliella salinas,* TPBR 3), 0,001165049 gL^{-1} (*Dunaliella salinas,* TPBR 4), 0,001087379 gL^{-1} (*Nannochloropsis oculata,* TPBR 5), 0,001009709 gL^{-1} (*Nannochloropsis oculata,* TPBR 6), 0,008970874 gL^{-1} (*Scenedesmus sp.,* TPBR 7), 0,004466019 gL^{-1} (*Scenedesmus sp.,* TPBR 8), 0,005864078 gL^{-1} (*Chlorella wild mix Årungen,* TPBR 9), and 0,004699029 gL^{-1} (*Chlorella wild mix Årungen,* TPBR 10).

Two semi-continuous upscale culture experiments were conducted during 20.06.12-22.08.12 (TPBR 1-6) and 11.07.12-22.08.12 (TPBR 7-10) to estimate the productivity of the TPBR system (Fig. 16).

A promising resource of renewable bio-energy is the use of non-arable land destined for microalgae production. One potential use for microalgal technology is recycling CO_2 emissions liberated to the atmosphere and offset it by production of sustainable, renewable biomass for fuel production (Hulatt et al., 2011). The microalgal physiology in comparison with land plants and crops, reported more benefits because the relatively high efficiency in converting solar energy into chemical energy biomass (William et al. 2010). The same authors calculated the theoretical maximum efficiency of solar energy conversion, i.e., the total solar energy to primary photosynthetic products, for microalgae shown a maximum value of 10%. Hence the metabolic pathways for the synthesis of lipids and proteins production reduced it to 1-3% (William et al., 2010).

2.2.1 Cultivation

2.2.1.1 Microalgae strains used in this experiments

Four of the microalgae strains used in these experiments were provided by IGV, Potsdam, Brandenburg, Germany: *Chlorella vulgaris sp., Dunaliella salinas, Nannochloropsis oculata* and *Scenedesmus sp.,* and the fifth strain was kindly provided by

PhD student Annette Åkerstrøm: *Chlorella wild mix,* from the lake *Årungen*, located in the Ås university campus.

2.2.1.2 Nutrition media + tap freshwater

All five strains were kept separately as growth culture in a liquid medium in sterilized Erlenmeyer glass, from where the inoculum was pipetted out when needed. The medium 3N-BBM+V (Bold-Basal Medium with 3-fold Nitrogen and Vitamins; modified) was used in all stadiums of the experiments: from the stock culture, the batch cultures and the Tray Photo bioreactor (TPBR).

The stock solutions of salts, trace elements and vitamins are shown in table 1 (appendix 2). The stock solutions for the preparation of 3N-BBM+V- medium were prepared in advance and stored at 4 °C until the medium was made. For the recipe of the 3N-BBM+V-medium, see table 2 (appendix 3) (presented in order of addition). The pH of the medium was adjusted to 6.3 before autoclaving the medium. After autoclaving the medium, vitamins were added and cooled at room temperature and stored at 4 °C in dark conditions until the start the experiment.

The stock strain cultures from the suppliers were poured out in the exhaust bench to secure axenic conditions. The stock cultures were incubated in 0.250 l Erlenmeyer flask sealed with aluminium foil that contained 100 ml of the medium. The stock algal culture was kept on a bench in the coolest area of the algae laboratory room, and manually stirred thrice a day, and with a continuous illumination of 150 µmol $m^{-2}s^{-1}$ PDF The temperature was 25 ± 1 °C and the strain was cultivated for 16 days for acclimation before the inoculum was taken and replenished with fresh sterile medium equal to the same removed volume.

2.2.1.3 Inoculum

To start the batch cultures, the protocol for axenic culture of microalgae was followed, to secure an environment free of microorganism. The stock cultures from the Erlenmeyer flask were unsealed only in the exhaust bench. The inoculum of 10 ml were pipetted out with micropipette from the mother culture and poured into a PBR glass with a diameter of 30 mm (max volume 380 ml, figure 12), which contained 320 ml of fresh medium.

The inoculum was taken out of the mother culture using an electronic pipette with 10 mL pipette tips (VWR Serological pipettes). The transferred algae in the PBR glass tube were handled quickly with nitrile gloves, Bunsenbrenner and inside the sterile bench. Each procedure was carefully made.

2.2.1.4 pH measurements

For measuring the pH, a pH-meter (Orion 420A+) was used and calibrated daily. Each medium sample was measured thrice and the average of the three parallels was calculated.

2.2.1.5 Batch cultures

The photobioreactor (PBR) used in this first phase of the upscaling experiment, were available at the algae laboratory room. Each PBR consisted of a glass tube (380 ml). Six PBR were disposed in a hard plastic rack inside a glass aquarium, illuminated from behind the aquarium by 10 cool white fluorescent light tubes (figures 12). The total illumination given had a photon flux density (PFD) of 200µmol $m^{-2}s^{-1}$ and was continuous (24/7). The light intensity was measured with a quantum integral sensor with handheld meter (apogee MQ-100, USA).

The aquarium was filled with 55 L fresh tap water, warmed artificially with heating cobs (Eheim Jäger 3619 Aquarium Heater, 300 W, 220-240 V, Germany). Each PBR were sealed with robber cork. Two perforations in the rubber cork facilitated the gas exchange by means of glass tubes with 3.0 mm inner diameter. One of the tubes served as inlet for the air (mixed with 3% CO_2) and was connected to the gas chamber with a slim rubber hose. The CO_2 enriched gas mixture, was filtered by a SLGS033SB/Millex-GS syringe filter unit, 0,22 µm, mixed cellulose esters, 33 mm ethylene oxide sterilized (Merck Millipore, Merck KGaA, Darmstadt, Germany). The other tube, sealed with a cotton dot, served as outlet, allowed the escape of the O_2 produced by the algae in the PBR and the overflow of CO_2. Six aquariums were connected in series with the heater water chamber by means of a pumping system. The water and temperature environment were kept as uniform as possible. Each aquarium contained six PBRs with their respective microalgal cultures. These PBRs were connected in series to the air CO_2 enriched chamber. The CO_2 levels in the plastic box which served as an air chamber (Samla, transparent plastic box with lid, 78x56x43 cm/130 L, Ikea) were kept at 3% through the whole experiment and measured with a Vaisala CARBOCAP® Carbon Dioxide Transmitter Series GMT220 (Finland). The mixing effect of the bubbling ascending trough the culture in the PBRs was the only turbulence.

The colour of the culture inside the PBR was the parameter taken in account to harvest the broth to continue the next step in the upscaling algae cultivation. The volume of the broth removed was replenished with fresh 3N–BBM+V. After added medium, the PBR restarted the cultivation. If the total volume of the PBR was needed, a new inoculum (10 ml) was pipetted out of the mother culture in the Erlenmeyer glass. All procedures followed the sterile protocol for axenic cultivation of microalgae in the algae laboratory conditions (figs. 13 and 14).

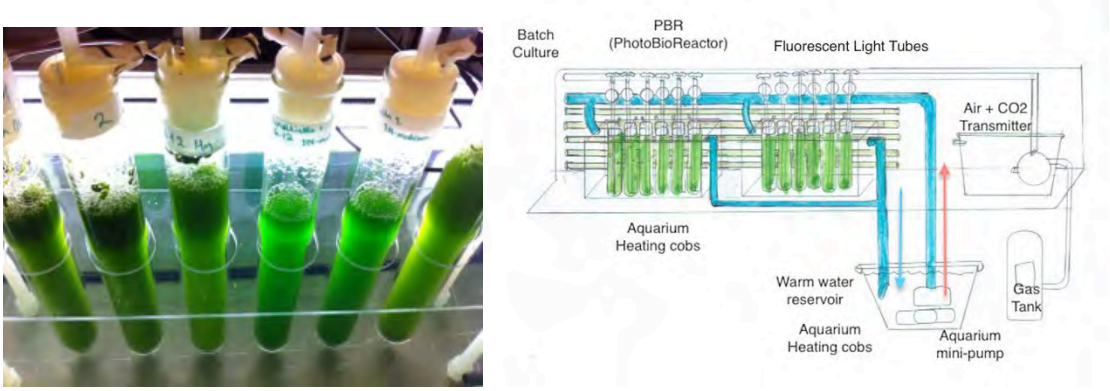

Figure 13 - Batch culture (left: photo) and schematic design of batch culture in the photo bioreactor system (PBR) in the algae room at the plant cell laboratory (right)

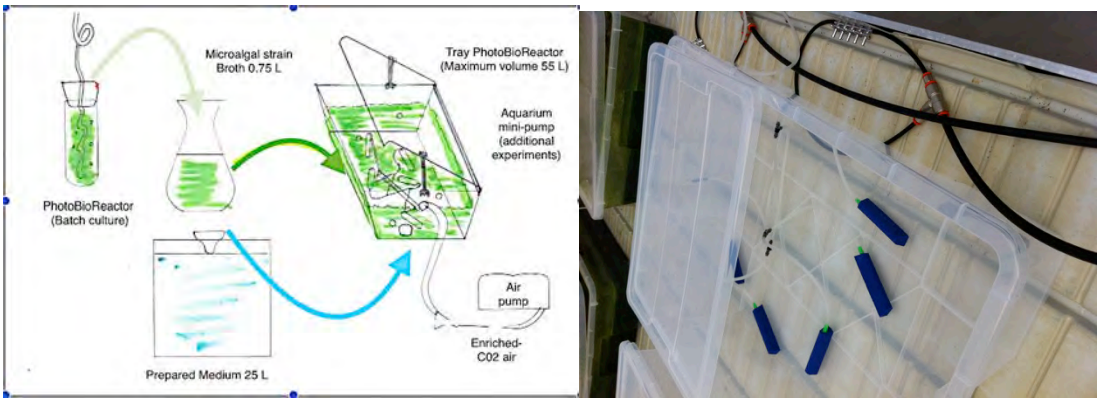

Figure 14 – Illustration of the algae broth collected from the batch PBR and ready for upscaling production in tray photo bioreactor (TPBR) design for greenhouse conditions at NMBU university campus.

2.2.2 Greenhouse room for microalgae upscaling continuous cultivation

The room used for the upscaling of microalgae cultures had no artificial illumination and no shade screens. The roof and the exterior walls were painted with a white solution to filter the solar radiation and to prevent overheating of the room environment. This was essential to maintain the temperature in the aqueous culture inside the PBR under 27 °C (Appendix 9. SKP upscaling room for semicontinuous microalgae cultivation system TPBR)

A second CO_2 air enriched chamber was assembled inside the greenhouse room. The CO_2 levels in the plastic box that served as an air chamber (Samla, transparent plastic box with lid, 78x56x43 cm/130 L, Ikea) were kept between 3 and 5% through the whole phase of this experiment. The mixing effect of the bubbling ascending trough the culture in the PBR was the only turbulence, in all PBR.

The CO_2 enriched chamber was controlled by pressure using a capillary glass tube in a water container. The CO_2 level was measured with an anagas CD 98 plus handheld multigas analyser (Bacharach-Geotechnical Instruments, UK)

The pure CO_2-gas was supplied direct from the main CO_2-tank through tubes and valves into the greenhouse room and connected directly into the air container. The flexible rubber hose was connected to an aquarium air pump (AN 540. 5-10.000 l water, 540 l/h, to outlets, 11W. China) and from this pump the air was injected trough 1 x aquarium fish tank air pump tubing splitter CO_2 five way control valve manifold taps. From each manifold tap, a new rubber hose was connected to a 2-way control valve manifold tap, and subsequently from each manifold tap; a new 5-way control valve manifold taps was connected. Each of the five manifold taps ended in an air stone (Betta aquarium fish tank airstone air bubble wall 6" (150mm). UK)

A capillary tube connected to the CO_2-transport tube, was placed vertically inside a water glass tube. This procedure was necessary to get rid of the CO_2-gass excess. The bubbles blown through the capillary tube would decrease the pressure through the valve, thus lowering the volume of CO_2 added to the air and vice versa.

Figure 15 – Senter for klimaregulert planteforskning (SKP) at the NMBU campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103m over sea level).

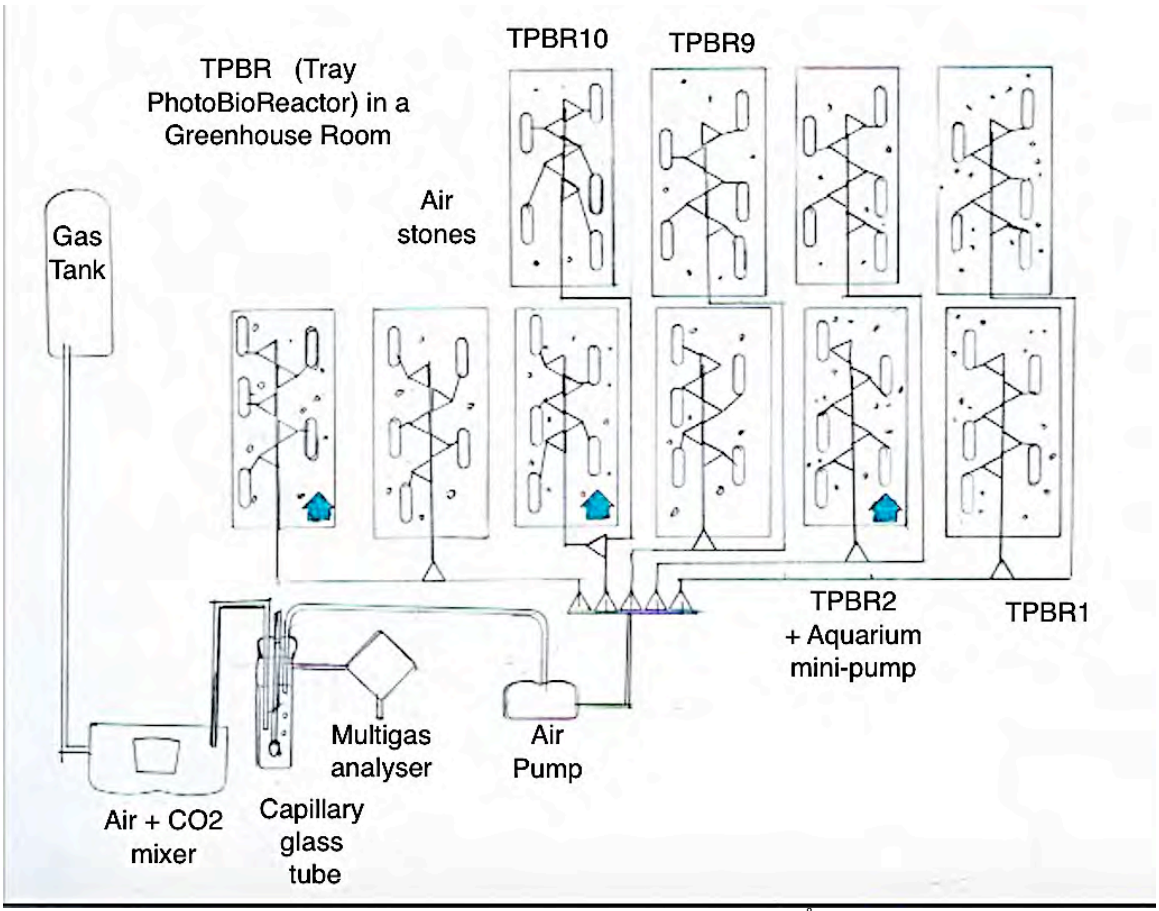

Figure 16 - Schematically design of TPBR system in greenhouse room at NMBU campus, Ås, Norway.

2.2.3 Tray Photobioreactor (TPBR)

The upscaling phase started with the collection of the broth from the PBR in the algae laboratory room. The broth was collected in the sterile bench and brought to the greenhouse room into a new tray photo bioreactor (TPBR). This Polypropylene plastic box suited for the continuous culture intended in this phase of the experiment. (Samla,

transparent plastic box with lid, 78x56x18 cm/55 L, Ikea). The fresh medium, with a volume of 30 L, was temperate to receive the algae. The microalgae was gently dispersed in the medium. The air pump started to bubble the CO_2 enriched air into the TPBR through five air stones in each TPBR (figs 14 and 16).

The lid covering the TPBR was placed on top of two metallic clams opposite to each other, to facilitate high aeration and escaping of the evolved oxygen and avoiding limitations in CO_2 supply, raising the pH values and hence reducing the productivity. There were small variations in pH values registered in the TPBRs algae cultures.

The algae cultures were distributed as follow:
TPBR 1 and TPBR 2 – *Chlorella vulgaris*
TPBR 3 and TPBR 4 – *Dunaliella salinas*
TPBR 5 and TPBR 6 - *Nannochloropsis oculata*
TPBR 7 and TPBR 8 - *Scenedesmus sp.,*
TPBR 9 and TPBR 10 - *Chlorella wild mix Årungen*

2.2.3.1 Truncated rectangular pyramid (algae suspension area)

To calculate the areal system productivity of one TPBR, it was necessary to understand the geometry of the suspension culture inside the TPBR. The water contained in the vessel resembles of a truncated pyramid. The calculation gives a total surface area of 0,913m^2 and a volumetric productivity of 0,031m^3, or 31,314 L. (See appendix 1. Calculations for Eq. 22 and Eq. 23 Figs. 34-37)

In this case the truncated pyramid is inverted. It´s essential to remember that when we calculate the illuminated surface area (ISP), the base or c*d area is omitted. The illuminated area surface was 0,56m^2. The empty space above the algae suspension was (0,913-0,56=0,353 m^2). The ground area occupied by the TPBR, is he c*d area of the truncated pyramid. The parameter evaluated is areal productivity (AP), hence corresponding to 0,913m^2. (See appendix 1. Eq. 24)

2.2.3.2 Turbulence

This additional experiment were performed using aquarium air pump Eheim compact 600, type 1001.220, series 03031, 230V~50Hz, 11W, Hmax m 1,3 I/Std 600 l/h, Germany. Only 3 air pumps were placed in 3 different TPBRs: TPBR 2 (*Chlorella vulgaris*), TPBR 4 (*Dunaliella salinas*), and TPBR 6 (*Nannochloropsis oculata*).

2.2.3.3 Daily measurements

The measurement of dissolved oxygen (DO), was performed with a Multi 3430 SET D, S 940-3, TC 925-3 (Wissenschaftlich-technische Werkstätten (WTW), Germany. The turbidity of the suspended solids in the aliquots was measured with a 2100P Portable Turbidimeter HACH, Germany.

2.2.4 Harvesting algae suspension from TPBR

The suspension was collected from the TPBR by means of a manual vacuum pump for big volumes (over 1 L) or by glass beakers when the volume needed was under 0,5 L.

2.2.4.1 Measurements of TSS (total suspended solids) as growth rate

The dry weight (DW) was determined using GF/F glass microfiber filters (0.7 µm pore size, 50 mm diameter, GE (Whaltman)). Prior to measuring, the filters were washed by vacuum filtration (<35 kPa) with a start volume of 10 ml, diH_2O to remove dust, which could disturb the TSS results. The filters were dried out for 3 hours at 105 °C in an oven (Beschickung, loading model 100-800. Memmert, Germany). The pre-weight of each filter was registered using a 3-decimal weight in mg (Mettler Toledo XP6). The filters were placed separately in sterile petri plastic plates, 55mm diameter, and theirs weight noted on the lid.

TSS was determined with three measurements per sample. Each filter was placed on an all-Glass Filter Holder Assembly with funnel, fritted base, cap, clamp, 47 mm (Cat nr. X1504700, Merck Millipore, USA) and connected to a vacuum pump (Mini Laboport Diaphragm Vacuum Pump (N811 KN.18, KNF N811KN.18, Bintang Instruments. Indonesia). Algae samples were added, maximum 10 ml at a time. The volume of the added algae sample depended on the density of the culture. The vacuum pump was activated and the algae sample was withdraw through the filter. Leaving the pump on, 1x10 ml diH_2O was added to the funnel to rinse off the remains of the algae on the funnel´s walls. The filtered algae sample, was collected with stainless steel forceps and placed on aluminium cups, when all the filtered algae samples were collected on a tray, the filters were dried in the oven for 3 hours at 105 °C. The filters were put in a vacuum desiccator containing silica gel to extract the remaining humidity of the filters. After cooled down to room temperature, the filters were weighed to determine the post-weight and registered. The weight of the algal sample was determined by subtracting the pre-weight of the empty filter from the post-weight of the filter containing the algal biomass

2.2.5 Statistical analysis

The statistical software Minitab 16 (Microsoft Corporation US) was used in the statistical analysis, with the commando One-way Analysis of Variance (ANOVA)(for more details see Appendix 7–ANOVA – One-way. T-T test)

The analysis was conducted with the difference in productivity (g L^{-1} d^{-1}) calculated for the five different algae strains in duplicate, grown under semi-continuous cultivation, for a total of ten TPBRs. The productivity in each TPBR was calculated by dividing the maximum amount of TSS during the experiment by the time (days) required to attain the maximum TSS, during a period between two dilutions. The dilution rate was not equal for all treatments because the algae biomass was harvested when needed for the next phases (flocculation and filtration). These inconsistencies made it difficult to make a good statistical analysis of the experiments.

2.2.6 Validating method of biomass production: TSS

Total suspended solids (TSS = mg/L) where used as an estimative of biomass productivity. The TPBR used for these experiments were low cost polypropylene trays.

Biomass productivity (P, mg/L*day) is the production of algal biomass concentration per unit time in a continuous or semi-continuous culture system. The first factor to limit biomass productivity in a dense cell suspension is algal cell shading each other. This factor is in part caused by the operation conditions of the continuous bioreactor system

(diffusion rate, light intensity); by the selected algal species own maximum growth rate, and possible their genetics and physiological parameters (half-saturation constant for light, biomass absorption coefficient) among others. Stoichiometry of the photosynthetic process of the production of one mole of biomass carbon, one mole of CO_2 is fixed. Therefore can we argue that biomass productivity is directly related to CO_2 bio-fixation?

The objective of this experiment in upscaling research was to produce the highest biomass volume in shortest time possible. The hypothesis is that the maximal biomass productivities are not correlated with the maximum specific growth rate (TPBR VS. Batch PBR). The selection of five different algal strains helped to test the validity of this hypothesis. The measurement of the maximal biomass productivity (at dilution rates in semi-continuous cultures) was the only measurements able to register.

The fractions of the culture suspension removed and replaced with fresh medium were not on daily basis. If the volume removed was more than 1000 ml, this volume was replaced then. The ratio of the rate of the new medium addition (F) to the total culture volume (V) is defined as the dilution rate D (D=F/V). For example: if 2500 mL of suspension are removed from the 7500 mL culture and replaced by 2500 mL of fresh medium that day, the dilution rate is 0,33.

2.2.7 Standardization of parameters

All productivity measurements as well as light energy inputs were normalized by using the calculated values: (*ISA*) 0,56 m^{-2} of overall surface illuminated area and (TSA) total surface area 0,913 m^2 of the upside-down truncated pyramid with a (V_p) predicted volume of 31,314L (See appendix 2, calculations Eq. 21-24), this procedure allowed the computation of photosynthetic efficiency using the solar energy input, and permitted the measurements of productivity to be scalable and interchangeable. Photosynthetic efficiency of the TPBRs, i.e. incident irradiance on the illuminated surface was expressed as (MJ $m^{-2}d^{-1}$).

Based on the work of Hulatt and Thomas (2011), the biomass higher heating value (HHV), was measured by Hulatt and Thomas (2010), by combusting approximately 1g (dry weight) samples of *Scenedesmus oblicuus* in a Parr 1341 oxygen bomb calorimeter, thus 1 g DW $m^{-2}d^{-1}$ generated 22,94 kJ g^{-1}, which was denominated biomass output energy (E_{out}) coefficient and expressed as 0,02294 MJ $m^{-2}d^{-1}$ (Hulatt and Thomas, 2011), i.e. $E_{out,}$ is equal to the illuminated area productivity ($IAP= (P/ISA)* V_o$) and expressed as [g $m^{-2}d^{-1}$] multiplied by 0,02294.

PAR energy measurements were converted from µmol $m^{-2}s^{-1}$ to $Jm^{-2}s^{-1}$ by dividing PAR with the factor 4,57, i.e. 1 $Jm^{-2}s^{-1}$ is equivalent to 4,57 µmol $m^{-2}s^{-1}$ (Thimijan & Heins, 1983; Zhang et al., 2001), and expressed as the equivalent daily energy input MJ $m^{-2}s^{-1}$: 4,57 * 10^{-6} MJ /(1/86,4 * 10^{-6} d) = 0,395 MJ $m^{-2}d^{-1}$, i.e. $E_{in,}$ is equal to the PAR incident irradiation on surface of culture (µmol $m^{-2}s^{-1}$) multiplied by 0,395.

The biomass energy of algae, calculated from the combustion heat was 0,0214MJ g dry $cells^{-1}$. The areal CO_2 fixation rate was calculated by using the equation proposed by Zhang K et al. (2001).

$$0{,}45P * 44 * 12^{-1}\ (g\ CO_2\ m^{-2}d^{-1})$$

Where 0,45 was the carbon content of dried cells (g carbon g biomass^{-1}), *P* was the illuminated area productivity (g biomass m^{2}d^{-1}) and 44 and 12 are the molecular weights of carbon dioxide and carbon, respectively, (Zhang K et al. 2001).

3. Experimental results and discussion on cultivation and upscaling

3.1 Effect of temperature dynamics on biomass productivity (*P*)

At the beginning of the upscaling experiments, the inoculation was performed by dilution of 0,750 L broth algae culture into TPBRs 25 L of fresh prepared 3N-BBM+vitamins media. For the marine algae strains, the broths were diluted in their respective fresh prepared saltwater too. There where little growth in the TPBRs at the beginning of the upscaling experiments, yet the five algae strains in duplicate, behaved independently and differently in each TPBR.

The algae´s intrinsic physiological response shifted and behaved differently among strains, to light and temperature. The target of this study was to produce as highest possible biomass concentration, and as a point of reference, it was assumed it was in the range of 1,5 and 2,5 g L^{-1}. The TSS sampled for TPBR 4, *Dunaliella salinas,* was 1,68 g DW L^{-1} and 1,64 g DW L^{-1} for TPBR 6, *Nannochloropsis oculata*, both marine algae, and in spite of being qualified in the assumed range, the low values reported, it was suspected that the light-limited system (lower limit) impinging the surface of the suspensions, minimized the impact of respiration (upper limit). Not all the cultures remained healthy but they survived the harsh and irregular dilution regimes (Table 3).

It was assumed that the molar carbon: nitrogen (C: N) ratio was stable as a direct result of the replenishment volumes of threefold nitrogen formula, thus it was not observed any symptom (de-coloration) of the algae suspension as result of deficit of nutrients. Maintenance of nutrient replenishment was necessary to get maximum production.

Analysing the data for biomass productivity (*P* = g DW $L^{-1}d^{-1}$) concerning temperature from the table 3, it was possible to conclude following:

Freshwater microalgae *Chlorella vulgaris* TPBR 1, had a higher biomass productivity (*P*) than TPBR 2, with 0,42 contra 0,38 g DW $L^{-1}d^{-1}$, respectively, and registered at 9:00 am, 100812 (day 51-52, age of cultivation, a cloudy day) and at 11:40, 100812 (day 51-52, age of cultivation, a cloudy day), and exposed to 57,67 and 58,86 MJ $m^{-2}d^{-1}$ of PAR incident irradiance input energy (E_{in}) with 19,8 °C and 22,1°C of culture temperature, respectively. The temperature values registered were lower than the average 22,24°C and 23,19°C, respectively (Table 7).

Saltwater strain *Dunaliella salinas* TPBR 4, had a higher biomass productivity (*P*) than TPBR 3, with 1,31 contra 0,81 g DW $L^{-1}d^{-1}$, respectively, and it was registered at 9:15 am, the 100812 (day 51-52, age of cultivation, a cloudy day) and at 16:40, the 120712 (day 22-23, age of cultivation, a cloudy day), exposed to 37,53 and 55,3 MJ $m^{-2}d^{-1}$ of PAR incident irradiance input energy (E_{in}) with 20,5°C and 23,7°C of culture temperature, respectively. The temperature registered was lower and higher to the average 22,35°C and 22,54°C, respectively (Table 7).

Saltwater strain *Nannochloropsis oculata* TPBR 6, had a higher biomass productivity (*P*) than TPBR 5, with 0,77 contra 0,49 g DW $L^{-1}d^{-1}$, respectively, and it was registered at 11:05 am, 090812 (day 50-51, age of cultivation, a sunny day) and at 10:30 am, 030712 (day 13-14, age of cultivation, a cloudy day) exposed to 89,67 and 89,27 MJ $m^{-2}d^{-1}$ of PAR

Table 3 -Comparison of the parameters corresponding to the highest biomass productivity *(P)* registered from each TPBR and it´s respective microalgae strain

TPBR # - Algae Strain	Culture Date	Time Interval (days)	Time of Sampling	Outside weather condition	Algae culture Temp. (°C)	PAR Irradiance input Energy (Ein) (MJ m^{-2} d^{-1})	pH	Dissolved Oxygen (DO) (mg*L^{-1})	Conductivity (mS *cm^{-1})	Salinity) (g/L) ppt	Remaining Culture Volume (Vo) [L]	Dilution rate ($days^{-1}$)	Start Biomass concentration TSS_1/25, 75 L (g DW /L)	Biomass concentration TSS (g DW*L^{-1})	Biomass Productivity (P) (g DW L^{-1} d^{-1})
1 – Chlorella vulgaris	100812	51-52	09:00	Cloudy	19,8	57,67	7,526	12,48	1,625	0,79107	24,8085	0,12092	0,00213	0,79429	0,41650
2 – Chlorella vulgaris [8]	100812	51-52	11:40	Cloudy	22,1	58,855	8,4	15,72	1,486	0,71775	4,746	0,60198	0,00326	0,756	0,37822
3 – Dunaliella salinas	120712	22-23	16:40	Cloudy	23,7	55,3	7,108	10,26	32,8	20,78831	24,685	0,20018	0,00147	0,6425	0,81083
4 – Dunaliella salinas [9]	100812	51-52	09:15	Cloudy	20,5	37,525	8,13	17	35,2	22,44816	18,424	0,75874	0,00116	1,68485	1,30706
5 – Nannochloropsis oculata	030712	13-14	10:30	Cloudy	23,8	89,27	8,128	15,25	34,7	22,10151	16,579	0,28258	0,00108	0,644	0,48781
6 – Nannochloropsis oculata [10]	090812	50-51	11:05	Sunny	22,2	89,665	7,405	10,65	34,5	21,96297	8,1265	0,60482	0,00100	1,644	0,77145
7 – Scenedesmus sp.	140812	34-35	13:20	Sunny	25,3	227,52	7,813	9,29	1,674	0,81705	19,119	0,73061	0,00897	0,336	0,95288
8 – Scenedesmus sp.	160812	36-37	09:35	Sunny	23	137,065	7,584	8,76	1,431	0,68890	6,2915	0,85624	0,00446	0,39459	0,2
9 – Chlorella wild mix Årungen	160812	36-37	10:30	Sunny	22,9	104,28	7,84	13,86	1,516	0,73352	11,243	0,70573	0,00586	1,443	0,2
10- Chlorella wild mix Årungen	90812	29-30	11:40	Sunny	22,8	228,705	7,912	11,5	1,539	0,74564	17,441	0,39145	0,00469	0,37778	0,50330

Computations based on Appendix 3, Tabs. 15-45, Appendix 5, Tabs. 46-59, Appendix 6, Tabs. 60-79.

[8] TPBR 2 equipped with aquarium pump to perform turbulence and aeration experiment towards TPBR 1. Both TPBRs contained freshwater microalgae strain *Chlorella vulgaris,* cultivated indoors for 62 days in semi-continuous production system (20.06.12-22.08.12), Norwegian green house at northern geographical location (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level).

[9] TPBR 4 equipped with aquarium pump to perform turbulence and aeration experiment towards TPBR 3. Both TPBRs contained marine microalgae strain *Dunaliella salinas,* cultivated indoors for 62 days in semi-continuous production system (20.06.12-22.08.12), in Norwegian green house at northern geographical location (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level).

[10] TPBR 6 equipped with aquarium pump to perform turbulence and aeration experiment towards TPBR 5. Both TPBRs contained marine microalgae strain Nannochloropsis oculata, cultivated indoors for 62 days in semi-continuous production system (20.06.12-22.08.12), in Norwegian green house at northern geographical location (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level).

Incident irradiance input energy (E_{in}) with 22,2°C and 23,8°C of culture temperature, respectively. The temperature registered was lower and higher to the average 22,50°C and 23,64°C, respectively (Table 7).

Freshwater strain *Scenedesmus sp.* from TPBR 7, had a higher biomass productivity (*P*) than TPBR 8, with 0,95 contra 0,2 g DW $L^{-1}d^{-1}$, respectively, and it was registered at 13:20, 140812 (day 34-35, age of cultivation, a sunny day) and at 9:35, 160812 (day 36-37, age of cultivation, a sunny day) exposed to 227,52 and 137,10 MJ $m^{-2}d^{-1}$ of PAR incident irradiance input energy (E_{in}) with 25,3°C and 23°C of culture temperature, respectively. The temperature registered was lower than the average 22,68°C and 23,90°C, respectively (Table 7).

For freshwater algae *Chlorella wild mix Årungen,* from TPBR 10 had a higher biomass productivity (*P*) than TPBR 9, with 0,50 contra 0,2 g DW $L^{-1}d^{-1}$, respectively, and it was registered at 11:40, 090812 (day 29-30, age of cultivation, a sunny day) and at 10:30, 160812 (day 36-37, age of cultivation, a sunny day), and exposed to 228,71 and 104,28 MJ $m^{-2}d^{-1}$ of PAR incident irradiance input energy (E_{in}) with 22,8°C and 22,9°C of culture temperature, respectively. The temperature registered was higher than the average 22,60°C and 22,67°C, respectively (Table 7).

Among the freshwater strains, *Scenedesmus sp.* TPBR 7 had the highest biomass productivity (*P*) with 0,95 g DW $L^{-1}d^{-1}$ with the highest temperature of 25,3°C. among the marine microalgae species, *Dunaliella salinas* TPBR 4 topped the overall highest biomass productivity (*P*) with 1,31 g DW $L^{-1}d^{-1}$ and the lowest temperature of 20,5°C.
It seems that the temperature play a direct role in the productivity when the coolest temperature in aquatic systems reported the highest biomass productivity of all strains in this study. 23,4% (25,3*100/20,5) of lower culture temperature increased the biomass productivity in 37,9% (1,31*100/0,95).

3.2 Effects of irradiance and temperature on specific growth rate (μ) and daily growth

Based on the data for the computation of the specific growth rate (Appendix 3, Tables 15-45 and Figures 38-67)(Appendix 5: Figures 78-91 and Tables 46-59). The highest consecutives reported TSS biomass concentrations were extracted from the parameters daily recording and used to calculate the specific growth rate.

Analysing the data for specific growth rate (μ = h^{-1}) concerning irradiance and temperature from the table 4, it was possible to conclude following:

Freshwater microalgae *Chlorella vulgaris* from TPBR 1 had a higher specific growth rate than TPBR 2, with 0,011 and 0,008h^{-1}, respectively, and it was registered 15-16/08 (day 55/56, age of culture) and 16-17/08 (day 58/59, age of culture) with 153 and 159 µmol m^2s^{-1} of irradiance (I_o), 20,55°C and 25°C of culture temperature, from a remaining culture volume of 24,75 and 29,71L and reporting a productivity (*P*) of 0,19 and 0,24 g DW $L^{-1}d^{-1}$, with 8,4 and 12,90 g $m^{-2}d^{-1}$ of illuminated areal output, 0,24 and 0,27 g $L^{-1}d^{-1}$ of volumetric output and 5,15 and 7,91 g $m^{-2}d^{-1}$ of areal productivity. 39,7% (153*100/109,5) higher irradiance and 21,7% (25*100/20,55) lower culture temperature gave 37,5% (0,011*100/0,008) higher specific growth rate.

For the marine algae, *Dunaliella salinas* from TPBR 3 had a higher specific growth rate than TPBR 4, with 0,024 and 0,014h^{-1}, respectively, and it was registered 16-17/08 (day 58/59, age of culture) and 12-13/07 (day 21/22, age of culture) with 220 and 107 µmol m^2s^{-1} of irradiance (I_o), with 23,95°C and 22,95 of culture temperature, from a remaining culture volume of 14,38 and 24,70L and reporting a productivity (P) of 0,44 and 0,21 g DW $L^{-1}d^{-1}$, with 11,25 and 9,15 g $m^{-2}d^{-1}$ of illuminated areal output, 0,96 and 0,26 g $L^{-1}d^{-1}$ of volumetric output, with 2,90 and 5,61 g $m^{-2}d^{-1}$ of areal productivity. Over 200% (220*100/107) higher irradiance and 7,65% (23,95*100/22,25) higher culture temperature gave 71,4% (0,024*100/0,014) higher specific growth rate.

For marine algae *Nannochloropsis oculata* from TPBR 5 had a higher specific growth rate than TPBR 6, with 0,014 and 0,008h^{-1}, respectively, and it was registered 16-17/08 (day 58/59, age of culture) and 16-17/08 (day 58/59 age of culture), with 210,5 and 106 µmol m^2s^{-1} of irradiance (I_o), with 24,50°C and 23,85°C of culture temperature, from a remaining culture volume of 21,34 and 36,93 L and reporting a productivity (P) of 0,11 and 0,18 g DW $L^{-1}d^{-1}$, with 3,98 and 11,83 g $m^{-2}d^{-1}$ of illuminated areal output, 0,16 and 0,15 g $L^{-1}d^{-1}$ of volumetric output and 2,44 and 7,26 g $m^{-2}d^{-1}$ of areal productivity. 98,5% (210,5*100/106) higher irradiance and 2,73% (24,5*100/23,85) higher culture temperature gave 175% (0,014*100/0,008) higher specific growth rate.

For freshwater algae, *Scenedesmus sp.*, from TPBR 7 had a higher specific growth rate than TPBR 8, with 0,05 and 0,01h^{-1}, respectively, and it was registered 14-15/08 (day 34/35, age of culture) and 21-22/08 (day 40/41, age of culture) with 398,5 and 197,0 µmol m^2s^{-1} of irradiance (I_o), with 22,8°C 23°C of culture temperature, from a remaining culture volume of 19,13 and 21,25L and reporting a productivity (P) of 0,52 and 0,08 g DW $L^{-1}d^{-1}$, with 17,61 and 1,96 g $m^{-2}d^{-1}$ of illuminated areal output, 0,85 and 0,12 g $L^{-1}d^{-1}$ of volumetric output and 10,80 and 1,82 g $m^{-2}d^{-1}$ of areal productivity. Over 200% (398,5*100/197) higher irradiance and 0,88% (23*100/22,80) higher culture temperature gave 500% (0,05*100/0,01) higher specific growth rate.

For freshwater algae *Chlorella wild mix Årungen,* from TPBR 9 had a higher specific growth rate than TPBR 10, with 0,03 and 0,01h^{-1}, respectively, and it was registered 14-15/08 (day 34/35, age of culture) and 15-16/08 (day 35/36, age of culture) with 303 and 261 µmol m^2s^{-1} of irradiance (I_o), 22,70°C and 21,5°C of culture temperature, from a remaining culture volume of 15,28 and 14,39L and reporting the lowest productivity (P) of 0,003 contra 0,343 g DW $L^{-1}d^{-1}$, with 1,27 contra 0,34 g $m^{-2}d^{-1}$ of illuminated areal output, -0,13 contra 1,07 g $L^{-1}d^{-1}$ of volumetric output and 0,78 contra 6,1 g $m^{-2}d^{-1}$ of areal productivity. 16,1% (303*100/261) higher irradiance and 5,6% (22,7*100/21,50) higher culture temperature gave 300% (0,03*100/0,01) higher specific growth rate.
(The cultures in both TPBR had to be refilled with new algae broth and these values are just for orientation and not taken into analysis account)(For more details see appendix 6, tables 60-79).

All the strains specific growth were affected positively by higher irradiation energy input in combination with a moderate culture temperature around 23°C, however, among the freshwater microalgae strains, *Scenedesmus sp.* TPBR 7 had the highest specific growth rate with 0,05 h^{-1} and showed a fivefold increase when doubling the irradiation energy input and temperature kept at 23°C. Among the marine microalgae species, *Dunaliella salinas* TPBR 3 had the highest specific growth rate with 0,024h^{-1} and

Table 4 – Computation for all TPBRs, of population specific growth rate (μ), R^2 value, temperature (C) and incident irradiation directly on algae culture (I_o) for the selected biomass concentration sampled. The data was extracted from TPBRs register, for group 1 (20.06.12-23.08.12) and group 2 (11.07.12-23.08.12) for a total of 63 and 42 days of culture (age of culture) respectively, grown in semi-continuous system production. Those TPBR that appear with two samples on the table below, indicate that both samples of the algae biomass concentration was increasing and both were taken into account. Otherwise, just one data was selected.

Date (2012)	Age of culture (days)	Gr. #	Algae strain	TPBR #	Irradiation on culture (I_o) (μmol $m^{-2}s^{-1}$)*	Temp. Algae Culture (°C) *	Remaining Culture Volume (L)*	Productivity P (g L^{-1} d^{-1}) *	Illuminated Areal output (g $m^{-2}d^{-1}$) *	Volumetric Output (g $L^{-1}d^{-1}$) *	Areal Productivity (g $m^{-2}d^{-1}$) *	Specific growth rate (μ) (h^{-1})	R^2 –value *
10-11/08	19/20	1	*Chlorella vulgaris*	1	605,0	22,85	20,05	0,038	1,186	0,072	0,7273	0,00322	0,99997
15-16/08	55/56	1	*Chlorella vulgaris*	1	153,0	20,55	24,75	0,190	8,400	0,241	5,1526	0,01074	1,00006
16-17/08	58/59	1	*Chlorella vulgaris*	2	109,5	25,00	29,71	0,244	12,889	0,265	7,9052	0,00827	0,99997
16-17/08	58/59	1	*Dunaliella salinas*	3	220,0	23,95	14,38	0,439	11,252	0,956	2,9010	0,02352	0,99998
12-13/07	21/22	1	*Dunaliella salinas*	4	107,0	22,25	24,70	0,208	9,146	0,263	5,6100	0,01411	1,00013
16-17/08	58/59	1	*Dunaliella salinas*	4	192,5	25,05	23,33	0,188	4,936	0,159	3,0275	0,00614	1,00007
27-28/06	06/07	1	*Nannochloropsis oculata*	5	188,5	21,09	3,94	0,262	1,841	2,084	1,1293	0,00416	0,99997
16-17/08	58/59	1	*Nannochloropsis oculata*	5	210,5	24,50	21,34	0,106	3,981	0,161	2,4415	0,01423	0,99997
11-12/07	21/22	1	*Nannochloropsis oculata*	6	43,5	25,55	27,41	0,139	6,810	0,159	4,1765	0,00726	1,00008
16-17/08	57/58	1	*Nannochloropsis oculata*	6	106,0	23,85	36,93	0,179	11,831	0,152	7,2566	0,00752	0,99997
14-15/08	34/35	2	*Scenedesmus sp*	7	398,5	22,80	19,13	0,516	17,610	0,845	10,8016	0,04765	0,99981
21-22/08	40/41	2	*Scenedesmus sp*	8	197,0	23,00	21,25	0,078	1,961	0,115	1,8160	0,01209	0,99988
14-15/08	34/35	2	*Chlorella wild mix Årungen*	9	303,0	22,70	15,28	0,0025	1,272	-0,131	0,7803	0,02937	1,00026
15-16/08	35/36	2	*Chlorella wild mix Årungen*	10	261,0	21,50	14,39	0,343	9,938	1,069	6,0955	0,01187	0,99995

Computations based on Appendix 2: Eq. 21-34, and 25)(Appendix 3: Figs. 38-67, Tabs. 15-45)(Appendix 5: Figs. 78-91 and Tabs. 46-59

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* Average value of two registered data

Showed a 71,4% increase when doubling the irradiation energy input and temperature kept at 23°C.

3.3 Effect of weather conditions in conjugation with irradiation and temperature on oxygen evolution

The evolution of (dissolved) oxygen, pH, conductivity and salinity levels were analysed in table 3.

Among freshwater strains, cloudy days with the fourth lowest register of 58,86 MJ m^{-2} d^{-1} PAR incident irradiance in combination with second lowest algae culture temperature of 22,1°C, gave highest values in pH (8,4), evolved oxygen (15,72 mg L), salinity (0,72 ppt) and conductivity (1,49 mS cm^{-1}), which were registered for *Chlorella vulgaris* (TPBR 2). In contrast sunny days with the next highest register of 227,52 MJ m^{-2} d^{-1} PAR incidence irradiance input energy (E_{in}) in combination with highest temperature of 25,3°C, with 7,81 (pH) evolved 9,29 mg L^{-1} of oxygen, at 1,67 mS cm^{-1} of conductivity and 0,82 ppt of salinity, which were registered for *Scenedesmus sp.,* TPBR 7.

Among saltwater strains, cloudy days with the low register of 37,52 MJ m^{-2} d^{-1} PAR incident irradiance in combination with low algae culture temperature of 20,5°C, gave highest values in pH (8,13), evolved oxygen (17 mg L), salinity (22,45 ppt) and conductivity (35,2 mS cm^{-1}), which were registered for marine strain *Dunaliella salinas* (TPBR 4). In contrast sunny days, with 89,67 MJ m^{-2} d^{-1} registered as lowest PAR irradiance input energy (E_{in}) in combination with lowest temperature of 22,2°C, gave next lowest pH (7,41) and evolved 10,65 mg L^{-1} of oxygen, 34,5 mS cm^{-1} of conductivity and 21,96 ppt of salinity) which were recorded to marine strain *Nannochloropsis oculata,* TPBR 6.

The sum of the five strains data (Table 3) for cloudy days gave a total of 70,71 mgL^{-1} of dissolved oxygen (DO) generated by a PAR irradiation sum of 298,62 MJ $m^{-2}d^{-1}$ mean while the sum for the sunny days gave 54,06 mgL^{-1} of dissolved oxygen (DO) mgL^{-1} of dissolved oxygen (DO) generated by a PAR irradiation sum of 787,24 MJ $m^{-2}d^{-1}$. By lowering the PAR irradiation 2,64 times it was generated 31% higher dissolved oxygen.

The cloudy weather conditions in combination with lower PAR irradiance and lower culture temperature affected directly the algae cell´s O_2 evolution, which was emitted at higher volumes than when the sunny weather combined with higher irradiation and higher temperature.

Fernandez et al., (2003) shown that photoinhibition and temperature stresses have significant impacts on the photosynthetic efficiency of outdoor mass algae cultivation. The stability observed and analysed in these experiments was possible, perhaps to the light: dark ratio of 35% (8,4 h, photo-stage structure of the volume reached by light-hours), and the bulk volume of culture, which minimized light over-exposure and temperature fluctuations due to heating capacity of water, and biomass concentration.

3.4 Effects of dilution regime on biomass output per TPBR

The daily productivity was computed based on the recorded values for biomass concentration of two following days (Appendix 3, Tabs. 15-45, Figs. 38-67)(Appendix 5, Tables 78-91 figures 46-59)(Appendix 6, Tables 89-108) and summarized in Table 5. The semi-continuous production system of the algae cultures in the TPBRs´ was disturbed by the irregular and harsh dilution rate and the light-limited stage was not easy to determine. The yield or biomass output (g DW L^{-1}) per TPBR was analyzed and presented in Table 5 (Appendix 6, Figs. 60-79).

Analysing the data for the effects of dilution (d^{-1}) regime on the total biomass output (g DW) per TPBR, presented in table 5, it was possible to conclude following:

For freshwater microalgae *Chlorella vulgaris* from TPBR 1 was registered a higher biomass output than TPBR 2, with 23,65 contra 21,32 g DW, which were extracted by vacuum filtration (TSS sampling) from 1,087 and 1,12 L, respectively, with a specific growth rate (μ) of 0,011 and 0,008 h^{-1}, which yielded per TPBR a total productivity (P) of 0,35 and 0,31 g $L^{-1}d^{-1}$ which corresponded to a yielded biomass concentration of 21,76 and 19,03 gL^{-1} and the growth rate (μ)/TPBR/d, was 0,014 and 0,013 g $L^{-1}h^{-1}$, with a volumetric output of 0,012 and 0,011 g $L^{-1}d^{-1}$, respectively. For the TPBR1 and TPBR 2 the number of dilutions was 6 and 9, which reported an average dilution rate (d^{-1}) of 0,353 and 0430 d^{-1}, respectively. The lowest average dilution rate gave higher biomass output. Calculating a prediction of volume of nutrition medium required for growing 1Kg of product at the same rates and TPBR conditions, TPBR 1 and TPBR 2 will require 228260 and 437092 L d^{-1}, respectively. Less dilution rate will need 91,2% less volume to achieve the same biomass output. The effect of dilution on biomass output is 22% (0,430/0,353) less harsh dilution gives 11% (23,65/21,32) higher algae output.

For saltwater microalgae *Dunaliella salinas* from TPBR 3 was registered a higher biomass output than TPBR 4, with 27,87 contra 21,31 g DW, which were extracted by vacuum filtration (TSS sampling) from 1,46 and 1,57 L, respectively, with a specific growth rate (μ) of 0,024 and 0,014 h^{-1}, which yielded per TPBR a total productivity (P) of 0,30 and 0,22 g $L^{-1}d^{-1}$ which corresponded to a yielded biomass concentration of 19,15 and 13,56 gL^{-1} and the growth rate (μ)/TPBR/d, was 0,013 and 0,009 g $L^{-1}h^{-1}$, with a volumetric output of 0,014 and 0,011 g $L^{-1}d^{-1}$, respectively. For the TPBR3 and TPBR 4 the number of dilutions was 4 and 9, which reported an average dilution rate (d^{-1}) of 0,55 and 056d^{-1}, respectively. The lowest average dilution rate gave higher biomass output. Calculating a prediction of volume of nutrition medium required for growing 1Kg of product at the same rates and TPBR conditions, TPBR 3 and TPBR 4 will require 219572 and 673256 L d^{-1}, respectively Less dilution rate will need 3,1 times less volume to achieve the same biomass output. The effect of dilution on biomass output is 3% (0,564/0548) less harsh dilution gives 31% (27,87/21,31) higher algae output.

For saltwater microalgae *Nannochloropsis oculata* from TPBR 5 was registered a higher biomass output than TPBR 6, with 33,67 contra 29,11 g DW, which were extracted by vacuum filtration (TSS sampling) from 1,89 and 1,90 L, respectively, with a specific growth rate (μ) of 0,014 and 0,008 h^{-1}, which yielded per TPBR a total productivity (P) of 0,28 and 0,24 g $L^{-1}d^{-1}$ which corresponded to a yielded biomass concentration of 17,80 and 15,31 gL^{-1} and the growth rate (μ)/TPBR/d, was 0,012 and 0,010 g $L^{-1}h^{-1}$, with a volumetric output of 0,017 and 0,015 g $L^{-1}d^{-1}$, respectively. For the TPBR 5 and TPBR 6

the number of dilutions was 8 and 13, which reported an average dilution rate (d^{-1}) of 0,44 and 0,62 d^{-1}, respectively. The lowest average dilution rate gave higher biomass output. Calculating a prediction of volume of nutrition medium required for growing 1Kg of product at the same rates and TPBR conditions, TPBR 5 and TPBR 6 will require 393085 and 614610 L d^{-1}, respectively Less dilution rate will need 44% less volume to achieve the same biomass output. The effect of dilution on biomass output is 41% (0,62/0,44) less harsh dilution gave 16% (33,67/29,11) higher algae output.

For freshwater microalgae *Scenedesmus sp.,* from TPBR 7 was registered a higher biomass output than TPBR 8, with 15,95 contra 7,97 g DW, which were extracted by vacuum filtration (TSS sampling) from 0,70 and 0,52 L, respectively, with a specific growth rate (μ) of 0,048 and 0,012 h^{-1}, which yielded per TPBR a total productivity (P) of 0,54 and 0,36 g $L^{-1}d^{-1}$ which corresponded to a yielded biomass concentration of 22,82 and 15,30 gL^{-1} and the growth rate (μ)/TPBR/d, was 0,023 and 0,015 g $L^{-1}h^{-1}$, with a volumetric output of 0,012 and 0,006 g $L^{-1}d^{-1}$, respectively. For the TPBR 7 and TPBR 8 the number of dilutions was 7 and 7, which reported an average dilution rate (d^{-1}) of 0,53 and 073d^{-1}, respectively. The lowest average dilution rate gave higher biomass output. Calculating a prediction of volume of nutrition medium required for growing 1Kg of product at the same rates and TPBR conditions, TPBR 7 and TPBR 8 will require 201197 and 400412 L d^{-1}, respectively. Less dilution rate will need 99% less volume to achieve the same biomass output. The effect of dilution on biomass output is 39% (0,733/0,526) less harsh dilution gives 200% (15,95/7,97) higher algae output.

For freshwater microalgae *Chlorella wild mix Årungen,* from TPBR 9 was registered a higher biomass output than TPBR 10, with 15,53 contra 14,19 g DW, which were extracted by vacuum filtration (TSS sampling) from 0,555 and 0,450 L, respectively, with a specific growth rate (μ) of 0,029 and 0,012 h^{-1}, which yielded per TPBR a total productivity (P) of 0,67 and 0,75 g $L^{-1}d^{-1}$ which corresponded to a yielded biomass concentration of 27,98 and 31,54 gL^{-1} and the growth rate (μ)/TPBR/d, was 0,028 and 0,031 g $L^{-1}h^{-1}$, with a volumetric output of 0,012 and 0,011 g $L^{-1}d^{-1}$, respectively. For the TPBR 9 and TPBR 10 the number of dilutions was 9 and 7, which reported an average dilution rate (d^{-1}) of 0,61 and 67d^{-1}, respectively. The lowest average dilution rate gave higher biomass output. Calculating a prediction of volume of nutrition medium required for growing 1Kg of product at the same rates and TPBR conditions, TPBR 9 and TPBR 10 will require 246622 and 164114 L d^{-1}, respectively. In contrast to the other TPBRs predictions, less dilution rate will need 50% more volume to achieve the same biomass output. The effect of dilution on biomass output is 12% (0,75/0,67) more harsh dilution gives 10% (15,53/14,19) lower algae output. The cultures in TPBR 9 and TPBR 10 underwent collapsing due to repetitive irregular high dilution rates over 0,85 d^{-1} (for more details, see appendix 6, table 105 and 107).

The rate of flow of medium into a continuous culture system was defined as the dilution rate (Barsanti and Gualtieri, 2006) and is equal the rate of cell division in the culture, as the cells being removed by the outflow of medium are being replaced by an equal number through the cell division in the culture. As long as the dilution rate is lower than the maximum growth rate attainable by the algae species, the cell density will increase to a point at which the cell division rate ("birth rate") exactly balances the cell washout rate ("death rate"), and this steady-state cell density characterizes the constancy of all metabolic and growth parameters. When the golden rule of approximately 20% of culture volume per day exceeded the maximum cell division rate, the cells have been

Table 5 - Computation for all TPBRs, summarized values for all algae populations data, in respect to specific growth rate ($\mu=h^{-1}$), for the selected biomass concentration sampled for the whole cultivation period. The productivy and growth rate per TPBR, prediction for 1kg biomass production, volumetric output and average dilution rate based on number of dilutions per TPBR. The data was extracted from TPBRs register, for group 1 (20.06.12-23.08.12) and group 2 (11.07.12-23.08.12) for a total of 63 and 42 days of culture (age of culture) respectilvely, grown in semi-continuous system production. (Appendix 1: Equations 21, 22, 23, 24). Optical path 9 cm. Total illuminated reactor surfaces required to produce 1 kg product d^{-1} (0,56 m^{-2}). Volume 31,314 L.

Gr. #	Algae strain	TPBR #	Total Harvested volume (L) for SF	Total Used Medium 3N-BBM+V (L)	Specific Growth Rate (μ) (h^{-1})[12]	Algae suspension volume (L) for TSS	Total Biomass Output per TPBR (g DW)	Total Productivity P per TPBR (g L^{-1} d^{-1})[13]	Yielded algae Biomass concentration (g L^{-1})[14]	Growth rate (μ)/ TPBR /d (g L^{-1} h^{-1})[15]	Volume (L) required to1 Kg product d^{-1}[16]	Volumetric Output (g L^{-1} d^{1})[17]	Average Dilution rate (d^{-1})[18]	Number of dilutions
1	*Chlorella vulgaris*	1	76,9	78,75	0,01074	1,087	23,6545	0,345	21,76	0,014	228260	0,012	0,353	6
1	*Chlorella vulgaris*	2	123,0	133,75	0,00827	1,120	21,3175	0,307	19,03	0,013	437092	0,011	0,430	9
1	*Dunaliella salinas*	3	51,0	66,75	0,02352	1,455	27,8688	0,304	19,15	0,013	219572	0,014	0,548	4
1	*Dunaliella salinas*	4	121,0	144,75	0,01411	1,571	21,3100	0,215	13,56	0,009	673256	0,011	0,564	9
1	*Nannochloropsis oculata*	5	105,1	110,85	0,01423	1,892	33,6681	0,282	17,80	0,012	393085	0,017	0,436	8
1	*Nannochloropsis oculata*	6	130,6	149,35	0,00752	1,902	29,1108	0,243	15,31	0,010	614610	0,015	0.615	13
2	*Scenedesmus sp*	7	84,5	109,25	0,04765	0,699	15,9543	0,543	22,82	0,023	201197	0,012	0,526	7
2	*Scenedesmus sp*	8	124,0	145,75	0,01209	0,521	7,9731	0,364	15,30	0,015	400412	0,006	0.733	7
2	*Chlorella wild mix Årungen*	9	140,0	164,25	0,02937	0,555	15,5310	0,666	27,98	0,028	246622	0,012	0,612	9
2	*Chlorella wild mix Årungen*	10	100,0	123,25	0,01187	0,450	14,1915	0,751	31,54	0,031	164114	0,011	0,669	7

Computations based on Appendix 2, Eq. 21-34, Figs. 34-37, Appendix 6,Tables 60-79. Demarked zone corresponds to marine microalgae strains in their respective TPBRs in duplicate.

[12] Specific growtn rate ($\mu = h^{-1}$)from table 4

[13] Productivity (*P*) per TPBR/d (g $L^{-1}d^{1}$): total biomass output per TPBR (g DW) divided by algae suspension volume (L) for TSS (L) and by the total number of days of cultivation, 63 and 42 days, for group 1 (TPBR 1 to 6) and group 2 (TPBR 7 to 10), respectively.

[14] Yielded algae biomass concentration (gL^{-1}): total biomass output per TPBR (g DW) divided by algae suspension volume (L) for TSS

[15] Growth rate (μ) per TPBR/d (g $L^{-1}h^{1}$): total productivity per TPBR (g $L^{-1}d^{1}$) divided by 24 hours.

[16] Volume (L) required to 1Kg of product d^{-1}: total used medium 3N+BBM+V (L) divided by total productivity (P) per TPBR (g $L^{-1}d^{-1}$) and multiplied by 1000 g, it gives the amount of L d^{-1} needed.

[17] Volumetric output (g $L^{-1}d^{1}$): total biomass output per TPBR (g DW) divided by V_p which is the predicted volume for the illuminated area of 0,56 m^2, and equal to 31,314 L, and by the total number of days of cultivation, 63 and 42 days, for group 1 (TPBR 1 to 6) and group 2 (TPBR 7 to 10), respectively.

[18] The dilution regime should be not higher than 0,25 as recommended in the literature. Here the lowest was 0,353 (TPBR 21, average value of 6 dilutions), and the highest was 0,733 (TPBR 8, average value of 8 dilutions.

Removed faster than they are produced and the total washout of the entire cell population occurred (Barsanti and Gualtieri, 2006). The fresh medium, replenishing the removed volume, is delivered to the culture all at once, as happened in these experiments, is known as semi-continuous culture system operation.

Summarizing the effects of irregular repetitive harsh dilution regime, none of the data reported from the TPBRs with their respective algae cultures, obey the 0,20 dilution rate regime. There were reported values of 0,353 and 0,733 d^{-1} for lowest and highest dilution rates, respectively. Among the freshwater microalgae strains, *Scenedesmus sp.* TPBR 7 had the highest biomass yield doubling its g DW when the dilution rate was 39% less harsh than the dilution regime for the same algae strain in the respective TPBR 8. The benefits were of magnitude 5 (200/39). Among the marine microalgae species, *Dunaliella salinas* TPBR 3 had the highest biomass yield increased by 31% of it´s g DW when the dilution rate was 41% less harsh than the dilution regime for same algae strain in the respective TPBR 4. The benefits were of magnitude 10,3 (31/3).
The cultures of *Chlorella wild mix Årungen*, freshwater strain, were washout due to the over dilution the TPBR volumes were removed and replenished. The cultures were thrice inoculated with fresh broth from the backup batch cultures in the algae laboratory room.

3.5 Effects of irradiance on photosynthetic efficiency

The photosynthetic efficiency (PE) for each sampled point was calculated based on the values for the highest biomass productivity (*P*) from each TPBR, and summarised in the table 6, and by using the equation proposed by Thimijan & Heins (1983) and Zhang et al., (2001), the values for PE = $100(E_{out}/E_{in})$, where E_{out} is the biomass output energy (MJ $m^{-2}d^{-1}$) and E_{in} is the PAR incident irradiance input energy (MJ $m^{-2}d^{-1}$)(all parameters were measured at the sampling time denoted in table 3) (Hulatt et al. 2011). The average PE was 0,61% (of ten values) and there was no significant effect on mean daily irradiance on PE (R^2=0,00231)(fig. 17)(Appendix, Eq. 29-34, Figs. 34-37 and Tabs. 60-79).

Analysing the data for the effects of PAR irradiance input energy ($E_{in,}$ MJ $m^{-2}d^{-1}$) (incident on the algae culture surface) on the photosynthetic efficiency ($PE, 100*E_{out}/E_{in}$) for the highest biomass productivity rate ($P, g\ DW\ L^{-1}d^{-1}$) registered for the five microalgae strains in duplicate for a total of ten TPBR, and presented in table 6, it was possible to conclude following:

For freshwater microalgae *Chlorella vulgaris* from TPBR 1 was registered a higher photosynthetic efficiency than TPBR 2, with 0,73 contra 0,49, which were both collected at 9:40 and 11:40, 100812 (day 51-52 age of culture, cloudy day), with a growth rate of 0,73 and 0,69 (r), 1,16 and 1,08 doublings per day (k) and 0,86 and 0,92 doubling time (T_2), respectively. 0,42 and 0,38 g DW $L^{-1}d^{-1}$ of biomass productivity, 18,45 and 3,24 g $m^{-2}d^{-1}$ of illuminated area productivity, 0,53 and 2,57 g $L^{-1}d^{-1}$ of volumetric productivity, 11,32 and 1,99 g $m^{-2}d^{-1}$ of areal productivity, reporting 0,42 and 0,07 MJ $m^{-2}d^{-1}$ of biomass input energy (E_{out}), 57,67 and 68,73 MJ $m^{-2}d^{-1}$ of PAR irradiance input energy (E_{in}) (on the algae culture surface), reporting 30,45 and 24,46 g CO_2 $m^{-2}d^{-1}$ of areal CO_2 fixation rate, respectively. Lower PAR irradiance input energy (E_{in}) gave higher photosynthetic efficiency, higher biomass productivity (P), higher biomass output

energy (E_{out}), higher areal productivity (AP), higher areal CO_2 fixation rate (ACO_2FR) and lower volumetric productivity (VP).

For saltwater microalgae *Dunaliella salinas* from TPBR 3 was registered a higher photosynthetic efficiency than TPBR 4, with 1,48 contra 1,24, which were collected 120712 (at 16:40, day 22-23 age of culture, cloudy day) and 100812 (at 9:15, day 51-52 age of culture, cloudy day), respectively, and with 0,82 and 1,50 of growth rate (r), 1,28 and 2,34 doublings per day (k) and 0,78 and 0,43 doubling time (T_2), respectively. 0,81 and 1,31 g DW $L^{-1}d^{-1}$ of biomass productivity, 35,74 and 43,00 g $m^{-2}d^{-1}$ of illuminated area productivity, 1,03 and 2,22 g $L^{-1}d^{-1}$ of volumetric productivity, 21,92 and 26,38 g $m^{-2}d^{-1}$ of areal productivity, reporting 0,82 and 0,99 MJ $m^{-2}d^{-1}$ of biomass input energy (E_{out}), 55,3 and 79,40 MJ $m^{-2}d^{-1}$ of PAR irradiance input energy (E_{in}) (on the algae culture surface), reporting 59,00 and 71,00 g CO_2 $m^{-2}d^{-1}$ of areal CO_2 fixation rate, respectively. Lower PAR irradiance input energy (E_{in}) gave higher photosynthetic efficiency, lower biomass productivity (P), lower biomass output energy (E_{out}), lower areal productivity (AP), lower areal CO_2 fixation rate (ACO_2FR) and lower volumetric productivity (VP).

For saltwater microalgae *Nannochloropsis oculata* from TPBR 5 was registered a lower photosynthetic efficiency than TPBR 6, with 0,37 contra 1,17, which were collected 030712 (at 10:30, day 13-14 age of culture, cloudy day) and 090812 (at 11:05, day 50-51 age of culture, sunny day), respectively, and with 0,56 and 0,63 of growth rate (r), 0,88 and 0,99 doublings per day (k) and 1,13 and 1,01 doubling time (T_2), respectively. 0,49 and 0,77 g DW $L^{-1}d^{-1}$ of biomass productivity, 14,44 and 45,56 g $m^{-2}d^{-1}$ of illuminated area productivity, 0,92 and 0,73 g $L^{-1}d^{-1}$ of volumetric productivity, 8,86 and 27,95 g $m^{-2}d^{-1}$ of areal productivity, reporting 0,33 and 1,05 MJ $m^{-2}d^{-1}$ of biomass input energy (E_{out}), 89,27 and 89,67 MJ $m^{-2}d^{-1}$ of PAR irradiance input energy (E_{in}) (on the algae culture surface), reporting 23,83 and 75,18 g CO_2 $m^{-2}d^{-1}$ of areal CO_2 fixation rate, respectively. Lower PAR irradiance input energy (E_{in}) gave lower photosynthetic efficiency, lower biomass productivity (P), lower biomass output energy (E_{out}), lower areal productivity (AP), lower areal CO_2 fixation rate (ACO_2FR) and higher volumetric productivity (VP).

For freshwater microalgae *Scenedesmus sp.,* from TPBR 7 was registered a higher photosynthetic efficiency than TPBR 8, with 0,33 contra 0,06, which were collected 140812 (at 13:20, day 34-35 age of culture, sunny day) and 160812 (at 9:35, day 36-37 age of culture, sunny day), with a growth rate of 1,34 and 0,41 (r), 2,10 and 0,64 doublings per day (k) and 0,48 and 1,56 doubling time (T_2), respectively. 0,95 and 0,20 g DW $L^{-1}d^{-1}$ of biomass productivity, 32,53 and 2,25 g $m^{-2}d^{-1}$ of illuminated area productivity, 1,56 and 1,00 g $L^{-1}d^{-1}$ of volumetric productivity, 19,95 and 1,38 g $m^{-2}d^{-1}$ of areal productivity, reporting 0,75 and 0,05 MJ $m^{-2}d^{-1}$ of biomass input energy (E_{out}), 227,52 and 79,79 MJ $m^{-2}d^{-1}$ of PAR irradiance input energy (E_{in}) (on the algae culture surface), reporting 55,68 and 3,71 g CO_2 $m^{-2}d^{-1}$ of areal CO_2 fixation rate, respectively. Higher PAR irradiance input energy (E_{in}) gave higher photosynthetic efficiency (PE), higher biomass productivity (P), higher biomass output energy (E_{out}), higher areal productivity (AP), higher areal CO_2 fixation rate (ACO_2FR) and higher volumetric productivity (VP).

For freshwater microalgae *Chlorella wild mix Årungen,* from TPBR 9 was registered a lower photosynthetic efficiency than TPBR 10, with 0,07 contra 0,16, which were collected 160812 (at 10:30, day 36-37 age of culture, sunny day) and 090812 (at 11:40,

day 29-30 age of culture, sunny day), with a growth rate of 0,05 and 2,32 (r), 0,08 and 0,85 doublings per day (k) and 12,95 and 1,22 doubling time (T_2), respectively. 0,20 and 0,50 g DW $L^{-1}d^{-1}$ of biomass productivity, 4,02 and 15,68 g $m^{-2}d^{-1}$ of illuminated area productivity, 0,56 and 0.93 g $L^{-1}d^{-1}$ of volumetric productivity, 2,46 and 9,61 g $m^{-2}d^{-1}$ of areal productivity, reporting 0,09 and 0,35 MJ $m^{-2}d^{-1}$ of biomass input energy (E_{out}), 130,75 and 228,71 MJ $m^{-2}d^{-1}$ of PAR irradiance input energy (E_{in}) (on the algae culture surface), reporting 6,63 and 25,86 g CO_2 $m^{-2}d^{-1}$ of areal CO_2 fixation rate, respectively. Lower PAR irradiance input energy (E_{in}) gave lower photosynthetic efficiency (PE), lower biomass productivity (P), lower biomass output energy (E_{out}), lower areal productivity (AP), lower areal CO_2 fixation rate (ACO_2FR) and lower volumetric productivity (VP).

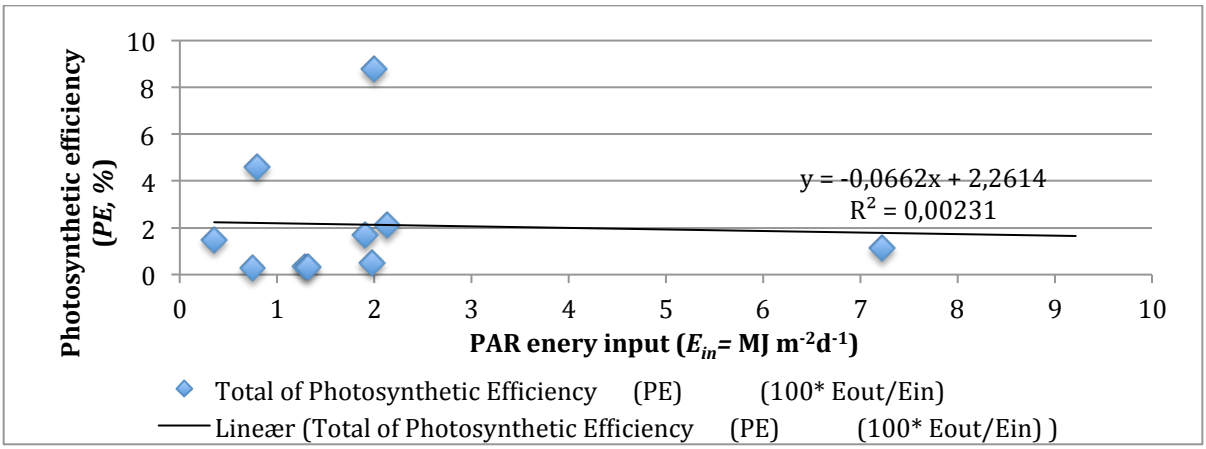

Figure 17 – Relationship between PAR energy input (MJ $m^{-2}d^{-1}$) and total PAR photosynthetic efficiency (*PE, %*) during the cultivation period (data plotted from Table 7 in this study).

Among freshwater strains, *Chlorella wild mix Årungen,* TPBR 10, registered the highest PAR incident irradiance input energy (E_{in}), gave the lowest photosynthetic efficiency (PE), with 228,71 MJ m^{-2} d^{-1} and 0,16%, respectively, on the contrary, *Chlorella vulgaris*, TPBR 1, registered the lowest irradiance gave the highest photosynthetic efficiency, with 57,67 MJ m^{-2} d^{-1} and 0,73%, respectively. Reporting as well the highest

Among saltwater strains, *Nannochloropsis oculata, TPBR 5*, registered the next highest PAR incident irradiance input energy (E_{in}), gave the lowest photosynthetic efficiency (PE), with 89,27 MJ m^{-2} d^{-1} and 0,37%, respectively, on the contrary, *Dunaliella salinas,* TPBR 3, the lowest irradiance gave the highest photosynthetic efficiency, with 55,3 MJ m^{-2} d^{-1} and 1,48%, respectively.

It seems that the level of irradiance had an inverted effect on the photosynthetic efficiency, i.e. lower irradiance absorbed by the high dilute algae culture gave higher photosynthetic efficiency.

Table 6 - Comparison of productivity between the highest biomass productivity values *(P)* registered on 5 microalgae strains in duplicate TPBR experiments

TPBR # Algae Strain	Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1-LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931/ r	Biomass Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1})	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1})	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1})	Biomass Output Energy (E_{out}) (MJ $m^{-2}d^{-1}$)	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$)	Photosynthetic Efficiency (PE) (100*E_{out}/E_{in})	Areal CO_2 Fixation rate (0,45P*(44/12) (g CO_2 m^{-2} d^{-1})
1 – Chlorella vulgaris	100812	51-52	Cloudy	0,74313	1,16277	0,86000	0,41650	18,45157	0,52572	11,31750	0,42327	57,67	0,73396	30,44509
2 – Chlorella vulgaris [19]	100812	51-52	Cloudy	0,69372	1,08547	0,92125	0,37822	3,24121	2,46792	1,98803	0,07435	68,73	0,49471	24,45643
3 – Dunaliella salinas	120712	22-23	Cloudy	0,81624	1,27718	0,78297	0,81083	35,74182	1,02857	21,92269	0,81991	55,3	1,48267	58,97400
4 – Dunaliella salinas [20]	100812	51-52	Cloudy	1,49511	2,33941	0,42745	1,30706	43,00255	2,22153	26,37615	0,98647	79,395	1,24249	70,95421
5 – Nannochloropsis oculata	030712	13-14	Cloudy	0,56388	0,88230	1,13339	0,48781	14,44203	0,92137	8,85820	0,33130	89,27	0,37112	23,82935
6 – Nannochloropsis oculata [21]	090812	50-51	Sunny	0,63346	0,99118	1,00888	0,77145	45,56451	0,73036	27,94756	1,04524	89,665	1,16572	75,18144
7 – Scenedesmus sp.	140812	34-35	Sunny	1,34442	2,10362	0,47537	0,95288	32,53264	1,56068	19,95430	0,74629	227,52	0,32801	53,67886
8 – Scenedesmus sp.	160812	36-37	Sunny	0,41002	0,64155	1,55870	0,2	2,24696	0,99543	1,37820	0,05154	79,79	0,06460	3,70749
9 – Chlorella wild mix Årungen	160812	36-37	Sunny	0,04935	0,07721	12,95009	0,2	4,01535	0,55703	2,46286	0,09211	130,745	0,07045	6,62533
10- Chlorella wild mix Årungen	90812	29-30	Sunny	2,33225	0,84683	1,22172	0,50330	15,67513	0,93036	9,61453	0,34958	228,705	0,15722	25,86396

Computations based on Tabs. 3 and 4

[19] TPBR 2 equipped with aquarium pump to perform turbulence and aeration experiment towards TPBR 1. Both TPBRs contained freshwater microalgae strain *Chlorella vulgaris*, cultivated indoors for 62 days in semi-continuous production system (20.06.12-22.08.12), Norwegian green house at northern geographical location (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level).

[20] TPBR 4 equipped with aquarium pump to perform turbulence and aeration experiment towards TPBR 3. Both TPBRs contained marine microalgae strain *Dunaliella salinas*, cultivated indoors for 62 days in semi-continuous production system (20.06.12-22.08.12), in Norwegian green house at northern geographical location (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level).

[21] TPBR 6 equipped with aquarium pump to perform turbulence and aeration experiment towards TPBR 5. Both TPBRs contained marine microalgae strain Nannochloropsis oculata, cultivated indoors for 62 days in semi-continuous production system (20.06.12-22.08.12), in Norwegian green house at northern geographical location (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level).

3.6 Effects of irradiance on areal CO_2 fixation rate (g CO_2 $m^{-2}d^{-1}$)

Nagajara (1984) had shown than the total solar radiation for this period June-July-October is in average 21,68 MJ m^{-2}. Thus the energetic relationship between PAR and total solar radiation in this study accounts for 22,73% (4,93*100/21,69 MJ m^{-2} d^{-1}) of total irradiance, which is the half (45,7%), reported by Nagajara (1984). The energy conversion efficiency of the TPBRs was 0,5% of the full solar spectrum which is approximately the 5% of the absolute theoretic maximum for microalgal photosynthesis presented by Williams and Laurens (2010) with their glass and transparent PBR. The lower value for energy conversion efficiency in this study is understandable because the whitish non-transparent polypropylene walls of the TPBRs with an optical path of 9 cm, and could be much higher if the TPBRs material made of glass too.

The biomass density is a key parameter to be maintained stable, and when the dilution rate is higher than 20% of the total suspension volume, the light dilution will waste the solar energy impinging the suspension by allowing some irradiation to pass through the media without being absorbed by the photosynthetic pigments. On the other hand dense suspensions reduce productivity by arisen respiration by heating up the algae culture. The cell density produced did not affect significantly the performance of the system, and its possible to assume that the TPBR was operated within an appropriate cell concentration range allowing the best use possible for the energetic convention. Mean temperature and mean light values on the sampled days had slightly correlation.

Greenhouses exert passive control of temperature with their insulating polythene structures and plus the properties of the polypropylene, resulted in interference in energy efficiency reported in too diluted transmission light levels into the algae suspension.

After Campbell et al., (2011) the most productivity values reported for microalgae growth are 15-30 g DW $m^{-2}d^{-1}$. These experiments reported a maximum productivity of 27,95 g DW $m^{-2}d^{-1}$ of areal productivity, which corresponds to a sunny day (090812, TPBR 6, *Nannochloropsis oculata*) (Table 6). This growth value was not sustained for the cultivation period. The highest total areal productivity was recorded again for TPBR 6 average, 46,79 g DW $m^{-2}d^{-1}$, which corresponds to 1,79 g DW $m^{-2}d^{-1}$ (46,79/26) of daily production. (Table 7), which is almost 8,4 times lower that the values reported by Campbell.

The lowest and highest areal CO_2 fixation rate (g CO_2 $m^{-2}d^{-1}$) among the freshwater strains were 3,70 and 53,68 g CO_2 $m^{-2}d^{-1}$ as well as the values for biomass productivity 0,2 and 0,95 g DW $L^{-1}d^{-}$ for *Scenedesmus sp.,* TPBR 8 and TPBR 7, respectively, and among the saltwater strains were 23,83 and 75,18 g CO_2 $m^{-2}d^{-1}$ as well as the values for biomass productivity 0,49 and 0,77 g DW $L^{-1}d^{-}$ for *Nannochloropsis oculata,* TPBR 5 and TPBR 6, respectively.

Using in these experiments, the mean higher heating value (22,49 KJ g^{-1}) recorded by Hulatt et al., (2011), and comparing the results in this study, the mean $C0_2$ fixation rate was 55,44 (range 3,71-75,18) g CO_2 $m^{-2}d^{-1}$ (Table 6), which is 2,6 times higher values than found by Hulatt (2011).

3.7 Effects of mixing (turbulence) on biomass productivity (P)

Early studies of mechanism of photosynthesis, has been demonstrated that the yield of oxygen per photon was greater in the intermittent light (Emerson and Arnold, 1932) because a single 400 chlorophyll photon collector can process around 2000 excitations per second mean while the enzymatic processes for carbon assimilation, at best have 100-200 electrons per second (Radmer and Kok, 1977) and during the dark period, between the flashes, the different speeds of photochemical reactions is reached by the enzymatic reactions, being calculated 50ms as the optimum dark period and it´s temperature dependent. Williams and Laurens (2010) assumed that to have a significant effect, the dark period should be 10 times greater than the light, and that the gains efficiency with flash periods (Kok 1953) shorter than 10ms get lost, and in dense cultures more than 90% of algae cells are in effective darkness. Grobelaar et al., (1996) found that in dense algae cultures (3-6 kg m^{-3}) the flashing light effect was neutralized at 10 Hz, but Molina Grima et al. (2000) set this limit to 1 Hz. By increasing the turbulence in TPBR, the production rates will have the potential for improvement when mixing frequency of 0,5 to 1 Hz will increase the areal production, exposing more cell surfaces to irradiation hence to flash periods (Laws et al., 1953). Laws et al. (1986) reported sustained production rates of 40 g m^{-2} d^{-1}, with short terms and 60-70 g m^{-2} d^{-1} at maximum rates, achieving these values by means of raceways.

The effects of culture parameters of the indoor experiments on the biomass productivity were studied during the experimental period. The highest daily volumetric productivity of 2,47 g $L^{-1}d^{-1}$ which was calculated from the change in cell concentration during one day shown in table 6 was obtained on 10.08.12, a cloudy day (TPBR 2 – *Chlorella vulgaris* – 51-52 day of culture)

According to Hu Q, et al., (1996) mixing is the factor, which enhances growth of algal culture and therefore its biomass productivity. In this study, was investigated the relationship between biomass productivity and the turbulence created by mixing the algae suspension by means of aquarium pump placed inside the TPBR 2 (*Chlorella vulgaris*), TPBR 4 (*Dunaliella salinas*), and TPBR 6 (*Nannochloropsis oculata*).

Analysing the data for the effects of turbulence (mixing), provided by an aquarium pump, on biomass productivity rate (P) (*P, g DW $L^{-1}d^{-1}$*) registered for the three microalgae strains in duplicate for a total of six TPBR, and presented in table 6, it was possible to conclude following:

For freshwater strain, *Chlorella vulgaris,* TPBR 2, had lower biomass productivity that TPBR 1, with 0,38 and 0,42 g DW $L^{-1}d^{-1}$, respectively, with 8,4 and 7,53 of pH, with 15,72 and 12,48 mgL^{-1} of dissolved oxygen, with 4,75 and 24,81L of remaining culture volume, and with 0,60 and 0,13 d^{-1} of dilution rate. The turbulence effect was no positive, due to the harsh dilution rate combined with low culture volume, and it reported a decrease of -11% (0,38*100/0,42) of biomass productivity.

For saltwater strain, *Dunaliella salinas,* TPBR 4, had higher biomass productivity that TPBR 3, with 1,31 and 0,81 g DW $L^{-1}d^{-1}$, respectively, with 8,13 and 7,11 of pH, with 17 and 10,26 mg L^{-1} of dissolved oxygen, with 18,42 and 24,69 L of remaining culture volume and with 0,76 and 0,20 d^{-1} of dilution rate. The turbulence effect was positive,

though the harsh dilution rate the remaining volume higher, and it reported an increase of 61,72% (1,31*100/0,81) of biomass productivity.

For saltwater strain, *Nannochloropsis oculata,* TPBR 6, had higher biomass productivity that TPBR 5, with 0,77 and 0,49 g DW $L^{-1}d^{-1}$, respectively, with 7,4 and 8,13 of pH, with 10,65 and 15,25 mg L^{-1} of dissolved oxygen, with 8,13 and 16,58 L of remaining culture volume and with 0,60 and 0,28 d^{-1} of dilution rate. The turbulence effect was positive, though the harsh dilution rate combined with a higher remaining culture volume, and it reported an increase of 57,14% (0,77*100/0,49) of biomass productivity.

The benefits of a turbulence regime should have been higher if the dilution regime had been lower (approximately 0,20 d^{-1}) than the operated in this study, as well as the remained culture volume, which affected severely the biomass output. Keeping a semicontinuous cultures system in adequate volumes and at stable low dilution rate, will influence the biomass productivity yield increasing it manifold times.

3.8 Effects of outside weather conditions on TPBRs´ energetic efficiency

The cell concentration, biomass productivity, CO_2 fixation rate and daily irradiation registered during two culture periods of five microalgae strains are shown in table 6. Irradiation and cell concentration were the basic parameters to calculate the overall productivity. The initial concentration of biomass in the algae broth (0,75 L) added to the 25 L of start fresh media (3N-BBM+vitamins) and after performed the first TSS, the reported dilution start rate (D_O) in the range 0,001 to 0,009 d^{-1} (table 3)

The supply of carbon to the culture though the harsh dilution rates combined by the not-sealed TPBRs cultivation strategy maintained the demand for cell growth, even at low irradiation reaching the culture, at the beginning of the culture periods, the low cell concentration affected the biomass productivity. In spite of the relative low amount of grow experiments performed on five microalgae strain, it was reported one example from each of the ten different TPBRs. (see appendix 6. Tables 60 to 79).

The parameters shown in table 7 concern the evaluation of all reactors efficiency (TPBR 1 – TPBR 10), based on productivity registered on five microalgae strains in duplicate, where the effect of irradiation (µmol $m^{-2}s^{-1}$) registered inside the TPBR but above the algae culture, combined with temperature inside the algae suspension (°C) had a direct influence in the productivity (P = g/L*d) increasing or decreasing the biomass concentration harvested for those days.

Chlorella vulgaris cultivated for 63 days, in TPBR 1 and TPBR 2, and calculations based on 26 observed samples reported 15,37 and 20,09 g m^{-2} d^{-1} of total areal productivity, with 25,06 and 32,77 g m^{-2} d^{-1} of total illuminated area productivity, which received 908,9 and 1011 MJ m^{-2} d^{-1} of total PAR irradiance input energy, respectively. Hence, the average PAR irradiance input for TPBR 1 and TPBR 2 was 34,96 and 37,44 MJ m^{-2} d^{-1}, respectively, and a daily PAR irradiance of 2,33 and 2,70 MJ m^{-2} d^{-1}, respectively. The totals for photosynthetic efficiency (PE) and areal CO_2 fixation rate were 1,683% and 41,349 g CO_2 m^{-2} d^{-1} reporting en total biomass output energy (E_{out}) of 0,575 MJ m^{-2} d^{-1} for TPBR1 and 8,781% and 110,703 g CO_2 m^{-2} d^{-1} reporting a total biomass output energy of 0,752 MJ m^{-2} d^{-1} for TPBR2, respectively. The Irradiation utilization efficiency for TPBR 1 and TPBR 2 were 0,72 and 0,81 g MJ^{-1}, respectively.

Table 7 - Comparison of parameters concerning evaluation of TPBRs´ efficiency based on productivity registered for 5 microalgae strains

TPBR #	Algae strain	Mean Algae culture Temperature (°C)	Mean pH	Mean Conductivity (mS cm^{-1})	Mean Salinity (g L^{-1})	Total Dissolved Oxygen (DO) (mg L^{-1})	Total Areal productivity AP=(P/TSA)*Vo (g * m^{-2} * d^{-1}) [22]	Total Illuminated area productivity IAP=(P/ISA)*Vo (g * m^{-2} * d^{-1}) [23]	Total PAR Irradiance input Energy (TPEin) MJ m^{-2} d^{-1})	Average PAR Irradiance input Energy (APIEin) MJ m^{-2} d^{-1})	Mean of 15h daily PAR Irradiance input energy (MJ m^{-2} d^{-1})	Observations of samples collected (n)	IUE or Productivity per unit irradiance (PPI) = (IAP/APIEin) (g*MJ^{1}) [24]	Total Biomass Output Energy (Eout) MJ m^{-2} d^{-1})	Total of Photosynthetic Efficiency (PE) (100* Eout/Ein)	Total of Areal CO2 fixation rate 0,45P*(44/12) (g CO_2 m^{-2} d^{-1})
1	*Chlorella vulgaris*	22,24	7,58	1,47	0,71	391,88	15,371	25,060	908,900	34,957	2,33	26	0,716	0,575	1,683	41,349
2	*Chlorella vulgaris*	23,19	7,63	1,49	0,72	416,46	20,099	32,769	1011,000	40,44	2,70	25	0,810	0,752	8,781	110,703
3	*Dunaliella salinas*	22,35	7,37	34,30	21,82	285,91	34,893	56,887	1601,725	61,604	4,11	26	0,923	1,305	4,623	93,864
4	*Dunaliella salinas*	22,54	7,37	33,28	21,13	397,22	15,217	24,809	1412,520	54,327	3,62	26	0,456	0,569	0,336	40,936
5	*Nannochloropsis oculata* [25]	22,50	7,47	35,11	22,40	436,21	18,873	30,769	1562,225	60,085	4,01	26	0,512	0,706	0,326	50,769
6	*Nannochloropsis oculata*	23,64	7,55	33,73	21,44	109,27	46,797	76,297	1458,233	56,085	3,74	26	1,360	1.818	2,125	125,889
7	*Scenedesmus sp*	22,68	7,69	1,64	0,80	224,78	44,038	71,798	1377,760	98,411	6,56	14	0,730	1,647	1,491	118,466
8	*Scenedesmus sp*	23,90	7,55	1,43	0,69	140,55	3,448	5,622	1620,685	115,763	7,72	14	0,049	0,129	0,513	9,276
9	*Chlorella wild mix Årungen*	22,60	7,70	1,53	0,74	221,47	12,193	19,879	1043,195	74,513	4,97	14	0,267	0,456	1,139	32,801
10	*Chlorella wild mix Årungen*	22,67	7,66	1,53	0,74	239,42	11,884	19,376	1306,265	93,304	6,22	14	0,208	0,444	0,290	31,970

Computations based on Appendix 6, Tabs. 60-79

[22] Total areal productivity (*TAP*) in Nos. 1-10 was calculated according to Σ *AP / TSA*, where *AP* is daily productivity of each TPBR (g day^{-1}) and *TSA* is the total area of the TPBR included the empty space (area) above of the algae suspension (0,913 m^{-2}). *TAP* is expressed in (g m^{-2}d^{-1}).

[23] Total illuminated area productivity (*TIAP)* in Nos. 1-10 was calculated according to Σ *IAP / ISA*, where *IAP* is daily productivity of each TPBR (g day^{-1}) and *ISA* is the illuminated surface area of each TPBR exposed to the incident irradiation (0,56 m^{-2}). *TIAP* is expressed in (g m^{-2}d^{-1}).

[24] Productivity per unit irradiance *(PPI)* in Nos. 1-10 was calculated according to *IAP / APIE$_{in}$*, where *IAP* is daily productivity of each TPBR (g day^{-1}) and (*APIE$_{in}$*) is the average PAR irradiation input energy (MJ m^{-2} d^{-1}). PPI is expressed in (g MJ^{-1}). Also known as Irradiation utilized efficiency *(IUE)*

[25] *Nannochloropsis oculata* was cultivated in TPBR 6, and reported a negative growth rate of -0,89 d^{-1}, on day 28-29 of cultivation. This was a big draw back to the culture, yet the culture survived and continued to grow. For better clearance of the results, the negative sign was omitted, hence the low values reported and calculated for the TPBR 5

Chlorella vulgaris, TPBR 2 performed 12,5 % more efficiently than the same strain in TPBR 1.

Dunaliella salinas cultivated in TPBR 3 and TPBR 4, cultivated for 63 days and calculations based on 26 observed samples reported 34,89 and 15,22 g m^{-2} $d^{-1}$of total areal productivity, with 56,89 and 24,81 g m^{-2} $d^{-1}$of total illuminated area productivity, which received 1601,72 and 1412,52 MJ m^{-2} $d^{-1}$of total PAR irradiance input energy, respectively. Hence, the average PAR irradiance input for TPBR 3 and TPBR 4 was 155,96 and 137,54 MJ m^{-2} d^{-1}, respectively, and a daily PAR irradiance of 4,11 and 3,62 MJ m^{-2} d^{-1}, respectively. The totals for photosynthetic efficiency (PE) and areal CO_2 fixation rate were 4,623% and 93,864 g CO_2 m^{-2} d^{-1} reporting a total biomass output energy (E_{out}) 1,305 MJ m^{-2} d^{-1} for TPBR3 and 0,336% and 40,936 g CO_2 m^{-2} d^{-1} reporting a total biomass energy output (E_{out}) of 0,569 MJ m^{-2} d^{-1} for TPBR4, respectively. The Irradiation utilization efficiency for TPBR 3 and TPBR 4 was 0,923 and 0,456 g M J^{-1}, respectively. *Dunaliella salinas,* TPBR 3 performed twice as efficient as the same strain in TPBR 4.

Nannochloropsis oculata cultivated in TPBR 5 and TPBR 6, cultivated for 63 days and calculations based on 26 observed samples reported 18,87 and 46,79 g m^{-2} d^{-1} of total areal productivity, with 30,77 and 76,29 g m^{-2} $d^{-1}$of total illuminated area productivity, which received 1562,22 and 1458,23 MJ m^{-2} $d^{-1}$of total PAR irradiance input energy, respectively. Hence, the average PAR irradiance input for TPBR 5 and TPBR 6 was 152,11 and 54,01 MJ m^{-2} d^{-1}, respectively, and a daily PAR irradiance of 4,01 and 3,74 MJ m^{-2} d^{-1}, respectively. The totals for photosynthetic efficiency (PE) and areal CO_2 fixation rate were 0,326% and 50,769 g CO_2 m^{-2} d^{-1} reporting a total biomass output energy (E_{out}) 0,706 MJ m^{-2} d^{-1} for TPBR5 and 2,125% and 125,889 g CO_2 m^{-2} d^{-1} reporting a total biomass energy output (E_{out}) of 1,818 MJ m^{-2} d^{-1} for TPBR6, respectively. The Irradiation utilization efficiency for TPBR 5 and TPBR 6 was 0,512 and 1,26 g M J^{-1}, respectively. *Nannochloropsis oculata,* TPBR 6 utilized the irradiation almost three times more efficiently than the same strain in TPBR 5.

Scenedesmus sp., cultivated in TPBR 7 and TPBR 8, cultivated for 42 days and calculations based on 14 observed samples reported 44,04 and 3,45 g m^{-2} $d^{-1}$of total areal productivity, with 71,79 and 5,62 g m^{-2} $d^{-1}$of total illuminated area productivity, which received 1377,76 and 1620,68 MJ m^{-2} $d^{-1}$of total PAR irradiance input energy, respectively. Hence, the average PAR irradiance input for TPBR 7 and TPBR 8 was 98,41 and 115,76 MJ m^{-2} d^{-1}, respectively, and a daily PAR irradiance of 6,56 and 7,72 MJ m^{-2} d^{-1}, respectively. The totals for photosynthetic efficiency (PE) and areal CO_2 fixation rate were 1,491% and 118,466 g CO_2 m^{-2} d^{-1} reporting a total biomass output energy (E_{out}) 1,647 MJ m^{-2} d^{-1} for TPBR7 and 0,513% and 9,276 g CO_2 m^{-2} d^{-1} reporting a total biomass energy output (E_{out}) of 0,129 MJ m^{-2} d^{-1} for TPBR8, respectively. The Irradiation utilization efficiency for TPBR 7 and TPBR 8 was 0,73 and 0,05 g M J^{-1}, respectively. *Scenedesmus sp.,* TPBR 7 utilized the irradiation almost fifteen times more efficiently than the same strain in TPBR 8.

Chlorella wild mix Årungen, cultivated in TPBR 9 and TPBR 10, cultivated for 42 days and calculations based on 14 observed samples reported 12,19 and 11,88 g m^{-2} $d^{-1}$of total areal productivity, with 19,88 and 19,38 g m^{-2} $d^{-1}$of total illuminated area productivity, which received 1043,19 and 1306,26 MJ m^{-2} $d^{-1}$of total PAR irradiance input energy, respectively. Hence, the average PAR irradiance input for TPBR 9 and TPBR 10 was

74,51 and 93,30 m^{-2} d^{-1}, respectively, and a daily PAR irradiance of 4,97 and 6,22 MJ m^{-2} d^{-1}, respectively. The totals for photosynthetic efficiency (PE) and areal CO_2 fixation rate were 1,139% and 32,801 g CO_2 m^{-2} d^{-1} reporting a total biomass output energy (E_{out}) 0,456 MJ m^{-2} d^{-1} for TPBR9 and 0,29% and 31,97 g CO_2 m^{-2} d^{-1} reporting a total biomass energy output (E_{out}) of 0,444 MJ m^{-2} d^{-1} for TPBR10, respectively. The Irradiation utilization for TPBR 9 and TPBR 10 was 0,27 and 0,21 g M J^{-1}, respectively. *Chlorella wild mix Årungen,* TPBR 9 utilized the irradiation almost 29 % more efficiently than the same strain in TPBR 10.

The reason for the differences in TPBR performances could be the uneven and harsh dilution rate (see sections 3.4 and 3.7 in this study) combined with the opacity of the TPBRs polypropylene material, which reacted differently in each TPBR, and the cultivated algae strain behaved physiological differently. If there were any production of auto-inhibitors due to physiological stress, it was not possible to corroborate, and further biochemical investigations of the algae constituents as well as testing of the polypropylene material subjected to scruff to remove the algae from the lid, are needed to find out the real causes of the differences in performances.

The overall irradiation utilization efficiency (*IUE*) provided by the TPBR on these experiments shows that the saltwater strain, *Nannochloropsis oculata,* TPBR 6, performed the highest values of all TPBRs, with 1,37 g M J^{-1} utilization efficiency of the irradiation that penetrated the walls and lid of the TPBR 6. This value is too low compared to 5.1 and 6.4 g M J^{-1}, values recorded by Zhang (1999, 2000). TPBR3, saltwater strain *Dunaliella salinas,* reported the next highest efficiency with 0,923 g M $J^{-1}$was even lower. Comparing the results for performance by TPBR6 and TPBR 3, 1,36 and 0,923 g M J^{-1} with the average value of 5,75 g M J^{-1} (Zhang 1999, 2000), showed that Zhang´s flat PBR excelled in performance approximately five times better that the TPBR of this experiments. The differences could be explained in the optical pathway for the TPBR in this study and Zhang´s flat PBR, 0,09 and 0,01 cm, respectively.

High productivity was obtained when the culture reached the highest biomass concentration, and when the cell concentration was kept between 1-2 g L^{-1}. The maximum areal biomass productivity of 45,56 g $m^{-2}d^{-1}$from the TPBR 4 (*Dunaliella salinas* marine microalgae*)* was recorded on 100812, a cloudy day (Table 6). The average productivity for the TPBR 1 to TPBR 5 during the cloudy days was 122,11 MJ $m^{-2}d^{-1}$ and for the TPBR 6 to TPBR 10 under sunny days was 175,30 MJ $m^{-2}d^{-1}$. The average and maximum $C0_2$ fixation rates calculated after Equation (15) for the TPBRs 55,45 and 125,89 g $C0_2$ $m^{-2}d^{-1}$, respectively. The corresponding value for average areal productivity was 36,33 g biomass $m^{-2}d^{-1}$. These values were quite similar to the values shown by Zhang, *et al.* (1999 and 2000). 31,5 g biomass $m^{-2}d^{-1}$, which corresponded to a CO_2 fixation rate of 51 g CO_2 $m^{-2}d^{-1}$, sustainable in the northern region of Japan during the winter time (January and February). The Norwegian values for the average productivity and the CO_2 fixation rate were slightly higher than the Japanese ones, 15% and 8,73%, respectively.

The irradiation utilization *(IUE)* was defined as the efficiency of solar irradiation conversion to biomass energy (Zhang et al., 2000), was calculated to describe the impact of climatic conditions on the biomass productivity (Fig. 18).

When the cell concentration started to rise, IUE dropped because some of the incident irradiation was not being transmitted through the culture (0,09 m of light pathway, I.e. depth of culture) but was being absorbed by the algal cells, until the next removal of volume (harvest) and following replenishment of fresh media was added to the suspension (dilution rate). With increasing of cell concentration (0,46 g L^{-1}, TPBR 6), the IUE was inconsistently low (1,37 g MJ^{-1}), because just 41,3% of all the light impinging on the suspension surface was absorbed for photosynthesis. There were more variations during the cloudy than the sunny weather conditions. IUE was higher during the cloudy days and hence the biomass concentration produced higher. During the cloudy days (*Chlorella vulgaris,* TPBR 1 and TPBR 2, *Dunaliella salinas*, TPBR 3 and TPBR 4 and *Nannochloropsis oculata,* TPBR 5) contra the sunny days (*Nannochloropsis oculata,* TPBR 6, *Scenedesmus sp.,* TPBR 7 and TPBR 8, and *Chlorella wild mix Årungen,* TPBR 9 and TPBR 10), produced par TPBR/weather group, cloudy contra sunny 4,48 g L^{-1} and 4,18 g L^{-1}, respectively. The average diary biomass concentration, for the same periods was 20,14 g L^{-1} contra 17,61 gL^{-1}, respectively (table 7).

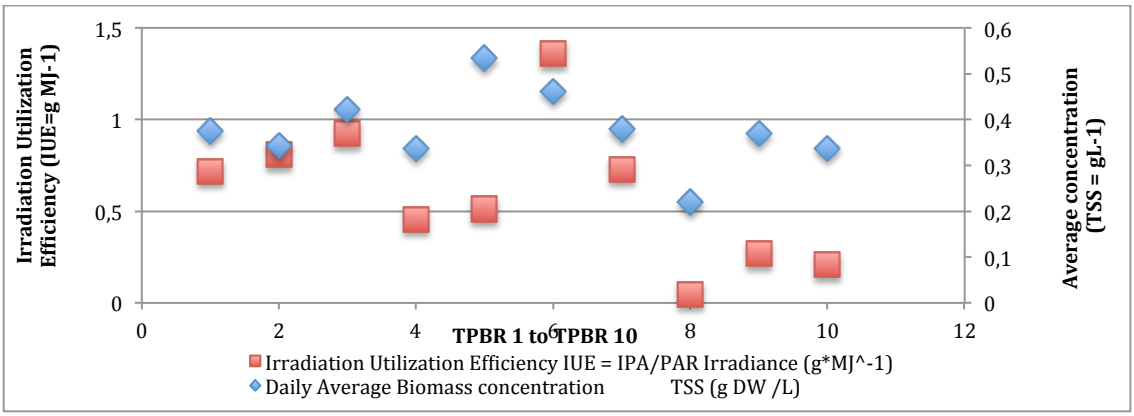

Figure 18 –Irradiation Utilization Efficiency (*IUE*) for various weather conditions and average cell concentration. *IUE*: biomass productivity (g biomass $m^{-2}d^{-1}$)/Average PAR irradiance input energy (E_{in}) (MJ $m^{-2}d^{-1}$). TPBR 1 to TPBR 5 reported an average irradiation of 122,11 MJ $m^{-2}d^{-1}$ (cloudy weather). TPBR 6 to TPBR 10 reported an average irradiation of 175,30 MJ $m^{-2}d^{-1}$ (sunny weather).

In contrast, the sunny days showed higher productivity than in cloudy days (table 6). The highest illuminated areal productivity (IAP) was recorded on TPBR 6 (*Nannochloropsis oculata*) 090812, a sunny day. During the acclimation of the algae cell to average light, Grobelaar et al. (1995) showed that this process from the moment of the inoculation from high light (HL) to low light (LL) following the densification on the culture suspension at it grows. During LL- acclimation, the cell utilized the light more efficiently, had a low *(P_{max})* light-saturated photosynthetic rate hence the size of the antenna was bigger. The relative low level of irradiation less light, was utilized more efficiently and less radiant energy were wasted due to the low P_{max} rate.

Compared to the productivity per unit irradiation from other works 5,1 g M J^{-1} (Zhang *et al*. 1999) and 6,4 g M J^{-1} (Zhang *et al.* 2000), this study reported poor values, with the highest being 1,37 g M J^{-1}. These comparisons show 3,6 and 5,6 times lower to the data reported by Zhang (2010).

4 Conclusions for cultivation and upscaling

The summary of desirable properties of microalgae for large-scale culture destined for energy production, are: rapid growth, high lipid content (for biodiesel production), temperature optimum, maximum temperature tolerated, high photosynthetic efficiency, ability to tolerate high irradiances, salinity, shear tolerance, non-"sticky" cells, grows in a "selective" environment, reduced sensitivity of photosynthesis (Rubisco) to high 0_2 concentration, lower intrinsic respiratory rate, large cell size or colonial or filamentous morphology, high specific gravity, weak cell covering or no cell wall, no production of auto-inhibitors, lipid composition (for biodiesel).
TPBR are not closed to the atmosphere and do not protect the cultivated algae to some extent to avoid contamination. Growth parameters i.e. temperature can be better controlled, higher surface-to-volume (S/V) ratio, hence higher volumetric productivities and cell concentrations, elimination or strongly reduction of evaporation in TPBR is a disadvantage, as well as the flexibility of the TPBR for improvement in performance by redesigning it, is advantageous.
Adequate mixing improve algae cells light-dark cycle and avoid bio-fouling, efficiently CO_2 supply preventing O_2 build-up and providing high mass transfer capacity, high S/V ration increases the cell concentration an volumetric productivity, optimization of growth conditions by regular thermic, pH, CO_2 and nutrient concentrations controls, and adequate harvesting regime to maintain the optimal population density.

Increase in productivity per unit irradiation utilized energy (IUE) is dependent of the light-path or culture depth, not direct proportional to the increase of the illuminated area. Raising the volume of liquid in a TPBR of the characteristics used in this study, will increase the light-path and will lead to decreased illumination for the inner layers of the water column. Reducing the mixing will lead to decreased rate of growth due to lower light flashing effect exerted on cell algae.

The relationship between energy inputs and outputs are of complex character, and the role played by the light transmission capacity of the material TPBR is build of, is essential. The weather conditions for the cultivation period needed not artificial heating to maintain a high cell concentration and areal middle productivity. Further studies need to operate long-term experiments to achieve better understanding of bio-energetic productivities for the same type of TPBR or of a newer design and materials. The low irradiances registered inside the TPBR were of positive impact in the growth rates of the different algae strains. Is possible to produce large volumes of microalgae suspension at temperate northern summer weather conditions with satisfactory results. With better equipment and improved procedures, the microalgae biomass yield could be boosted from the results of these experiments. Low cost production without extra illumination not heating is already proved in this master thesis.

5 Material and methods on harvesting (flocculation and SF filtration)

5.1 Introduction

The production of almost all microalgae products implies the recovery and processing of microalgae biomass from culture media is an essential component. Harvesting, thickening and dewatering of microalgae biomass represents a high cost (Borowitzka 1999, Molina Grima et al. 2003; Uduman et al. 2010; Fon sing et al. 2011). The factors determinants of harversting and consequently the high biomass recovery costs imply difficulties:

(i) The nature of microalgae cells (i.e.) size, specific gravity, charge and morphology

(ii) The low concentration of biomass typical in large-scale culture systems, and

(iii) High capital equipment costs, especially, for the cultivation systems for saltwater microalgae.

The technologies and assess the technical and economic considerations for the wide range of solid-liquid separation techniques have been presented in this study (see 1.8 to 1.16). Selection of the appropriate technology for the end product from microalgae is the main challenge because the traditional microalgae concentration processes used energy-intensive and expensive unit operations, whilst no revolutionary advances have been reported, reviews reported of process optimization have been proved to reduce operating cost significantly (Golueke and Oswald, 1965; Henderson et al. 2008; Mohn 1980, 1988; Shelef et al. 1984; Mohn and Contreras, 1990; Molina Grima et al. 2004; Uduman et al., 2010).

The main obstacle in the microalgae cultivation system is the low concentration of the biomass produced, in the range of 0,1 to 4,0 g L^{-1} ash free dry weight (AFDW). The main goal is to produce concentrated slurry, a paste or a dried solid, which will satisfy the requirements and/or the economic packaging and transportation of microalgae products (i.e. logistics) of downstream processing operations.

The effectiveness of a solid-liquid separation process can be described in terms of the recovery efficiency *(RE)* and the concentration factor *(CF)*(Lee et al. 2009). RE is defined as the ratio of the mass of cells recovered in the final product to the total mass of cells in the initial culture (Eq. 18). CF is the ration of the concentration of microalgae biomass in the final product to the initial concentration in culture (Eq. 19).

$$Recovery\ efficiency\ (RE) = \frac{mass\ of\ cells\ recovered}{mass\ of\ cells\ initial\ culture} \qquad \text{Eq. 18}$$

$$Concentration\ factor\ (CF) = \frac{concentration\ of\ algae\ in\ final\ product}{initial\ concentration\ of\ algae\ in\ culture} \qquad \text{Eq. 19}$$

The industrial solid-liquid processes include, filtration, sedimentation and flotation shown in fig.18, and described in 1.8 to 1.16 on this study, implies that the harvesting and concentration of microalgae (i.e. solids) from the culture fluid (i.e. liquid) in any microalgal biomass recovery system must:

(i) Be able to handle extremely large volumetric throughputs
(ii) Be highly reliable
(iii) Have low capital and operating costs
(iv) Be easily managed
(v) Be constructed with materials that are compatible with the culture media
(vi) Allow growth media recycling, and
(vii) Be sufficiently flexible to handle any upstream physical, biological and environmental changes.

The potential output changes include volumetric throughput, biomass/solids concentration, particle properties (size, specific gravity, zeta-potential) and the fluid properties (i.e. nutrients, ionic strength, salinity, pH, dissolved gases, specific gravity,

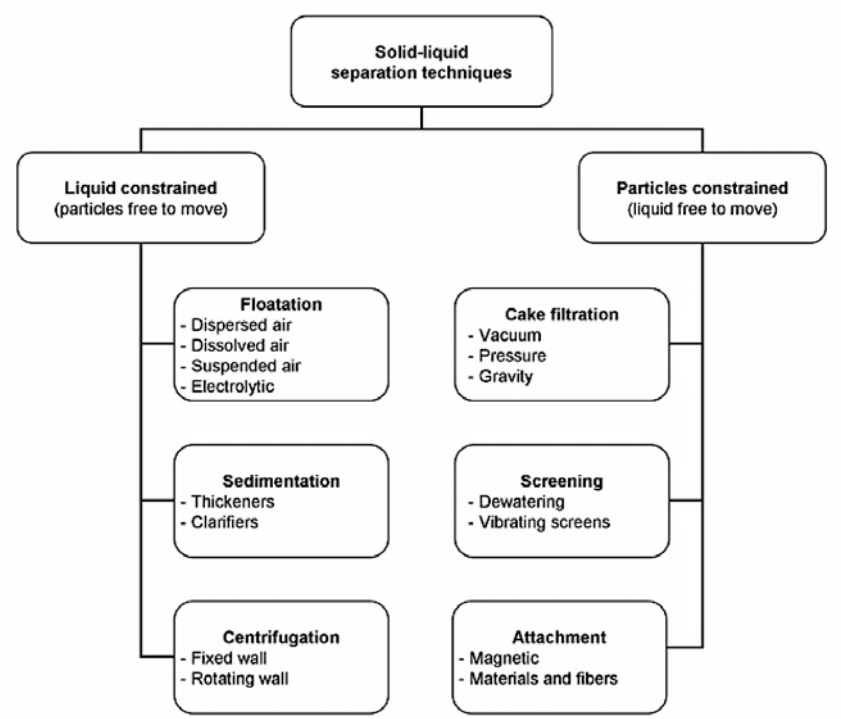

Figure 19 – Common industrial solid-liquid separation techniques (Illustration from Borowitzka and Moheimani 2013).

Temperature and composition) the main attention has to be also focused on the quality of the end product aiming to avoid its deterioration. (Borowitzka and Moheimai, 2007)

The recovery techniques of microalgae biomass can be used individually (single-stage) or in combination (multi-stage), depending the choice of the microalgae strain in question, final product´s quality and concentration.
The multi-stage or sequential processing steps have to be designed to increase the concentration of the algae tailor-made in aspect such suitability, economical and sustainability for each method in each stage, aiming to reduce the impact on the subsequent stage (fig. 19-20).

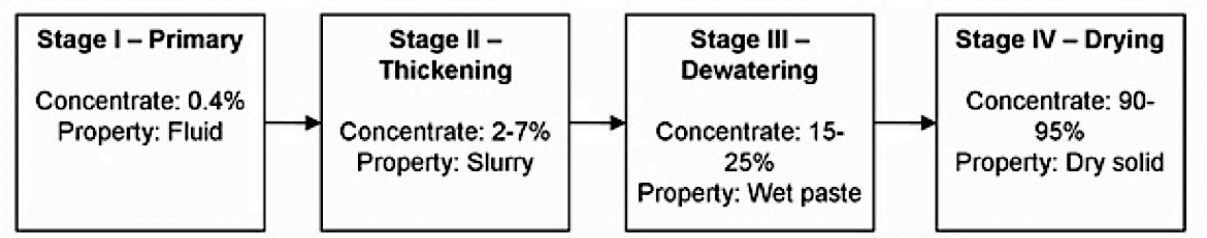

Figure 20 – Block flow diagram for the recovery of microalgae biomass, showing typical biomass concentration achieved and the physical state of the output stream (Borowitzka and Moheimani, 2013).

(i) Stage I (Harvesting/primary concentration): the material retains its fluid like consistency, in spite the concentration by a factor 0f 10-20 has been increased

(ii) Stage II (Thickening): the thickened slurry-like consistency material is being generated from the primary concentrate by an additional concentration factor of 10

(iii) Stage III (Dewatering): The wet paste has been generated, by dewatering the thickened biomass to 15-25% solids

(iv) Stage IV (Drying): The dry solids, has been generated by removing unbound and possibly bound water, thus increasing the stability by minimizing spoilage

The morphological properties of microalgae influences theirs recovery and they are:

(i) Particle shape (filamentous, rods, spheres, or chains)

(ii) Particle size (in the range 2 to 20μm)

(iii) Specific weight (in the range 1,05 to 1,1)

(iv) Charge (usually negative)

(v) Those properties depend on strain, growth conditions and age of culture.

Most of the solid-liquid separation techniques do not function effectively without microalgae pre-treatment or conditioning, due to the colloidal nature, microscopic size of microalgae and the small density difference between live microalgae and the culture fluid. Destabilization and aggregation of the microalgae cells by coagulation and flocculation is a pre-treatment that can improve the recovery efficiency (RE) and concentration facto (CF) (Borowitzka and Moheimani, 2013).

Microalgae suspensions are colloidal and consist of discontinuous phase (highly disperse particles) and continuous phase (distributed uniformly throughout a dispersion medium). An electrical surface charge at the cellular level result when at the surface of the microalgae cell, the interfacial effects interact with the dissolved ions in the continuous phase, and its dependent of the combination of these parameters:

(i) Preferential adsorption of the dissolved ions in solution

(ii) Adsorption-desorption of lattice ions

(iii) Direct dissociation or ionization of active surface groups and

(iv)Have charge-defective lattice (isomorphs substitution) (Borowitzka and Moheimani, 2013).

The slightly negative surface charge exhibit by the microalgae cell repels negative ions (co-ions) in the solution and attracts positive ions (counter-ions) from the solution, hence creating a tightly bound layer of ions called the *Stern layer.* The charges achieve a dynamic equilibrium (co-ions and counter-ions) motion-free around the Stern layer to create *diffuse layer*, which extends from the edge of the Stern layer into the surrounding media until the concentration of counter-ions and co-ions equalizes producing a zero net charge. This net result forms around the microalgae cell, an electrical double layer (EDL) and any difference in ion concentration create an electrical potential and it´s magnitude can be visualized as a function of distance (fig 21) (Shelef et al., 1984; Molina Grima et al., 2003).

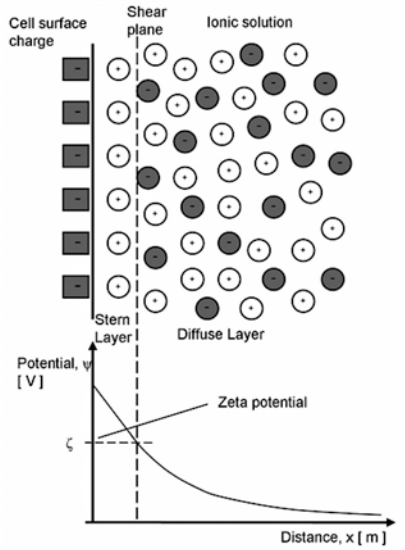

Figure 21 – Visualization of the Electric Double Layer (EDL) (Illustration from Borowitzka and Moheimani, 2013).

Colloids have a special parameter, the *zeta potential,* which is the potential at the interface (share plane) of the Stern layer and diffuse layer, and it is negative in the range -10 to -35 mV (Henderson et al., 2008), and it´s dependent of pH and ionic strength of the culture media (Conductivity), and it´s reduced by salinity (fig. 22)

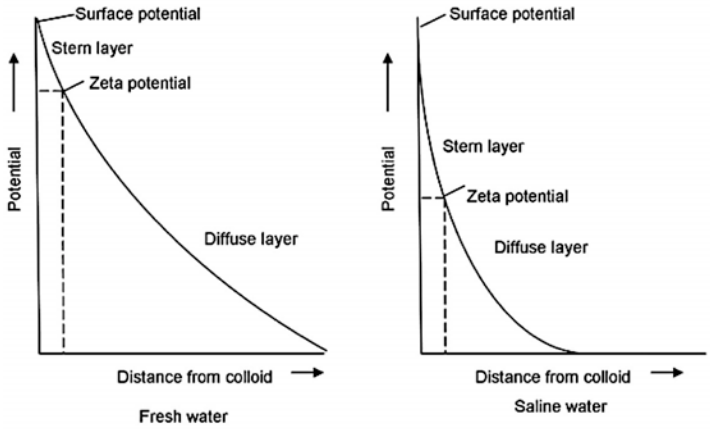

Figure 22 – Effects of salinity on zeta potential (Illustration from Borowitzka and Moheimani, 2013).

The liquid constrained system around a microalgae cell includes centrifugation, sedimentation and flotation and requires a density different between the cell and the surrounding media. The gravitational forces: external force (F_E), buoyance force (F_B) and drag force (F_D) exerted on a single cell is shown in fig 23. The drag force appears wherever there is a relative motion between the algae cell and fluid, the external force acts parallel to buoyance force but in opposite direction.

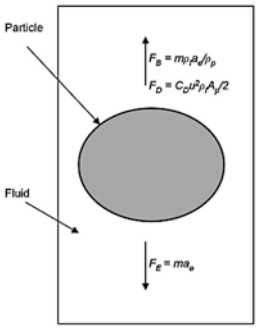

Figure 23 – Forces exerted on a particle (Illustration from Borowitzka and Moheimani 2007)

Stokes Law dictates that in a Newtonian fluid, a small inert spherical isolated particle, as some specimens of microalgae are, the sedimentation rate is governed by, being the algae´s terminal velocity:

$$V = \frac{g(\rho_s - \rho_f)d^2}{18\mu} \qquad \text{Eq. 20}$$

Where V is the terminal velocity (m s^{-1}), g is the acceleration due to gravity (m s^{-2}), ρ_s is the density of the particle (kg m^{-3}), ρ_f is the density of the fluid (kg m^{-3}), d is the diameter of the spherical particle (m) and μ is the fluid viscosity (kb m^{-1} s^{-1}). Other parameters to take into account are: cell motility, water turbulence and water upwelling (Eq. 20) (Borowitzka et Moheimani, 2013).

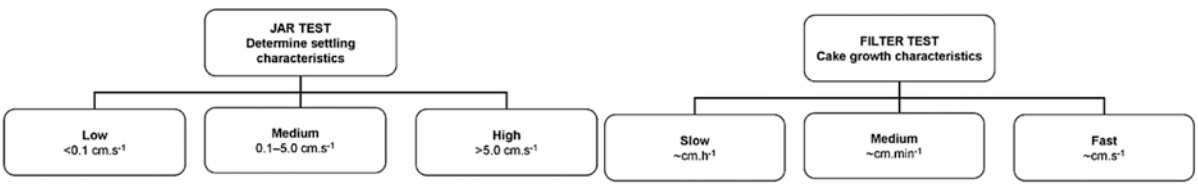

Figure 24 – The settling characteristics can be determine by a jar test (left) and the cake growth and filtration rate can be determined from a leaf vacuum test (right) (Illustration from Borowitzka and Moheimani 2007)

Sedimentation and filtration rates (fig. 24) can be used to design sedimentation and filtration systems. To develop an understanding of the flocculation, flotation, filtration, or sedimentation ability of the microalgae in question under various process conditions, flocculation jar test apparatus, settlings rubes (Imhoff cones) and leaf filters can be used hence developing the requirement in terms of scale (throughput), operation and performance thus the specification for the harvesting equipment (Fig. 25) (Borowitzka et Moheimani, 2013).

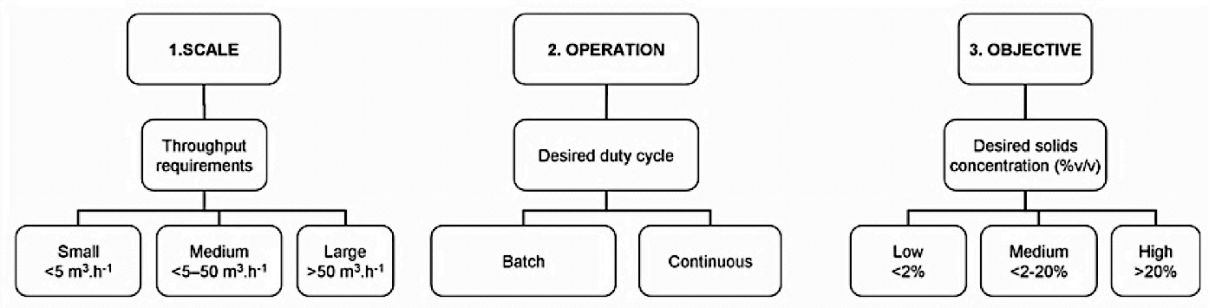

Figure 25 – Duty specification for harvesting system (Illustration from Borowitzka and Moheimani, 2013)

The information and results registered from laboratory jar test apparatus assist in the duty specification aiming to suggest the suitable equipment for selection, taking into account the predicted energy consumption using as guide the figure 11, and the capital cost, expected downtime and equipment requirements for maintenance and the health hazards created by equipment operation and processes (i.e. moving and rotating items, chemicals toxicity and noise) and further more the creation of the respective protocol referring to the applied standards in the specific design of the equipment (Borowitzka et Moheimani, 2013).

The primary treatment of municipal wastewater and from industrial wastewater, the removal of suspended solids (SS) both use the commercial Salsnes Filter (SF) micron [μm] mesh sieve technology (Rusten and Ødegaard, 2006). Adding flocculants and/or coagulants to the wastewaters enhances the performance of particles removal efficiency. The ecological impact and the flexibility of the SF technology are recorded in many reports on less consuming energy hence footprint reducing systems and the possibility to adds units to the enlarge the already establish treatment plant (Salsnes Filter, 2004) (Sahu, AK, et al., 2015).

Based on the experiences of SF technology in wastewaters processes, with removal efficiencies of ≥ 90%, the overall objective of these experiments was of find improved methods of microalgae harvesting processes. The achievements for this purposes includes the use of available commercial flocculants and the SF technology and their synergetic effects when combined, resulting in a high removal efficiency rates of algae biomass from the water phase. The Salsnes Water to Algae Treatment technology, SWAT, is a combined technology demands specific objectives of determination, calibration and optimisation for minimal amount of flocculant dosage for the specific SF. Both bench and pilot scale flocculation technology for five microalgae strains were investigated in these experiments. Achievement for SWAT technology´s redesign and upscaling will need further investigation and research to accomplish the overall objective (Sahu, AK. et al., 2015).

This study investigated five different microalgae strains. Aliquots of 10 ml for four commercial microalgae were provided from IGV GmBH (Potsdam, Germany) and the fifth one, (Chlorella wild mix Årungen lake at the NMBU campus, Ås, Norway) was kindly provided by NMBU PhD student Annette Åkerstrøm. The aliquots were first cultivated in sterile batch conditions at the algae laboratory at the plant laboratory at the institute for controlled climate environments (SKP: Senter for klimaregulert plantevitenskap). The algae were cultivated separately in 380 ml glass tubes, and kept in aquariums with a total of 55 L of heated tap freshwater to keep a constant surrounding aquatic

temperature of 23°C. The inoculum were cultivated in sterile prepared in situ nutrition media (3N-BBM+Vitamins) and supplied with a continuous mixture of air and carbon dioxide (3%) and continuous fluorescent illuminated. The algae broth was collected and inoculated the 65 L polypropylene, open tray photoreactor (TPBR) for the following upscaling step and hence cultivated at not sterile conditions prepared in situ from stock solutions of 3N-BBM+Vit nutrition media dissolved in tap freshwater, under natural light conditions at greenhouse indoors environment provided by the institute of plant sciences (IPV: institutt for plantevitenskap), and grown with enriched CO2 (3-5%) air mixture. The growth of the five microalgae strains was monitored dialy, registering the following parameters: time and volume of collected sample for TSS, pH, dissolved oxygen (DO), salinity, conductivity, outside temperature and weather conditions, culture temperature, light intensity, total suspended solids concentration (TSS). The data for the parameters and growth rates of the microalgae are documented in this study (Sahu, AK. et al., 2015).

5.2 Particle size analysis

A portable fluid imaging device aka, FlowCam (Yarmouth, Maine, USA) was use to size analysis and morphology determination for the microalgae cells before and after flocculation. Area Based Diameter (ABD) was used for all data analysis. Particle size distribution was based on ABD and all results were tested for one minute run time with no restricted particles number (Sahu, AK. et al., 2015).

By using a 20x objective and 50µm flowcell opening, the microalgae cells were measured before flocculation. The objective made use of a collimeter and a 500µL syringe. Dilution 1:100, was needed to avoid clogging of the flowcell, as well as continually flushing with about 1mL of the sample through the flowcell for focus clearance before sample measurement. The dilution factor was taken into results account. The selection of images was based on optically resemblance with samples for consistency. Not matching images with the pure culture images were deleted (Sahu, AK. et al., 2015).

By using a 4x objective or a 2x objective and 1000µm flowcell opening, the flocculated microalgae was measured. A peristaltic pump attached to the outlet of the flowcell was used to drawn the samples into the flowcell, avoiding the destruction of the flocs prior to the measurement. Pump flowrate was set to the range 10,8-12,9 $mLmin^{-1}$ using the C57 tubing of the FlowCam. Image distilled for flocs and individual particle and statistically similar particles were chosen for analysis (Sahu et. al., 2015)

5.3 flocculant and G-value estimation

Bench scale jar test flocculator and pilot scale 20L flocculator were investigated in this study, and they were operated in batch mode. 17 commercial and industrial Polycationic polymers (BASF, Germany; Kemira, Finland; Xiato, China) and two chemicals, PAX (Kemira, Fredrikstad, Norway), chitosan (Norwegian Chitosan, Kløfta, Norway) were investigated for each microalgae specie to determine harvesting efficiency in each case (Sahu, AK, et al., 2015)(Appendix 1, Table 14).

The factor taking in account for choosing of the polymers and chemicals were: commercial availability in water treatment (potable and/or wastewater), availability easiness, consistency of examined in previous reference of their utilization in microalgae harvesting (Bulena et al., 1990; Harith et al., 2009; Knuckey et al., 2006; Lee et al., 2009), low level of toxicity and approved by the EU and the BAFTA regulations in the food industry. In spite of the price was

not taken into account for the selection criteria, the overall goal was the use of lowest dosage as possible for maximal removal performance of algae biomass. The dosages were reported here as mg polymer/g SS of algae in the water phase (Sahu, AK, et al., 2015).

5.3.1 Jar test flocculator

By using a bench scale jar test flocculator device by Kemira Kemwater (Helsinki, Finland), consisting this semi-automatic device of six parallel agitators controlled by a microprocessor, that allows rapid mixing regimes and slow stirring speeds and times to be predefined. The volume of well-mixed fresh microalgae suspension collected from the TPBR was one litre transfer into a one-litre jar test flocculator beaker for all tests (Sahu, AK, et al., 2015)(fig 26).

Figure 26 - Jar Test apparatus, by Kemira, Finland, with six beakers of 1L in process of mixing algae suspension with pipetted flocculant dosage (left). Flocs still in movement after stirring with stirring paddle (center). Settled flocs and the bottom of the beaker of 1L treated microalgae, and the water phase (right).

5.3.2 20 L flocculator

A polyethylene container with a capacity of 25 L was used, and 20 L were kept as working volume, and by using a commercial paint stirrer motored by a machine drill (Biltema, Oslo), and with a 150 mm as stirrer base, the flocculation process of the microalgae was accomplished. The optimised polymer dosage was calculated based on the TSS concentration measured, in advance, from the small volume collected from the TPBR (Sahu, AK. et al., 2015)(fig 27).

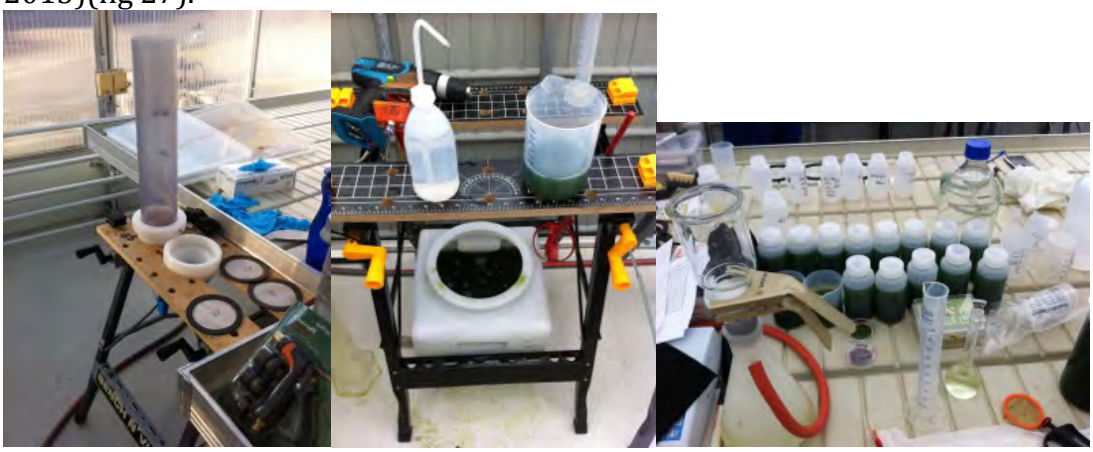

Figure 27 – Scale bench Salsnes Filter device with filter mesh sieves displayed on the bench before use (left). A 25L plastic container placed under the bench, containing the flocculated microalgae after being stirred (center). Samples ready for subsequently turbidity, pH analysis and TSS measurement.

5.3.3 G-value estimation

The principle parameters governing the effective degree of flocculation are the retention time and velocity gradients (G-value) are applied for a given destabilized water and flocculation device. The type of raw water and the mode of destabilization on the type of coagulant and flocculant used, determines the kinetics of the flocculation process (Bratby, 2008). Kemira Kemwater kindly provided the G-values for the jar test

flocculator in relation to stirrer speed (for details about G-values, see glossary for definition)(Sahu, AK, et al., 2015).

In the estimation of the G-values to determine the relationship of speed of the stirrers in relation to power consumed by the stirrer of the 20L flocculator, were utilized the equation for G-value proposed by Camp and Stein (1943) together with the equation for power consumption of the mixer (Leentvaar and Ywema, 1980) (Sahu, AK. et al., 2015).
The power supply to the drill for mixing was generated by a DC power source coupled to the electrical drill with crocodile clips (fig. 25). By using Ohms Law power inputs were calculated based on voltage and resistance measures recorded with a multimeter (Fluke, USA) both in air and in algae suspension at many different stirrer speeds. The power actually transferred from the stirrer to the liquid gave the difference in power input. Mimicking the same G-values as for the jar test device, the voltage and current were adjusted to get the approximate power input to the drill, during pilot scale testing (Sahu, AK. et al., 2015).

5.4 Bench scale SF

Salsnes filter AS (Namsos, Norway) supplied the bench scale test filter kit with different mesh openings (Rusten and Lundar, 2006) (fig. 28). The size in microns for the mesh used was: 11, 15, 18, 33, 55, 74, and 90, 210, 250, 300, 350. The "mat formation" was not observed in any of the conducted experiments (Rusten and Lundar, 2006). The full scale SF dislodges the particles from the rotating sieve cloth by using of a patented air blade (Salsnes Filter, 2014). The prototype of bench scale SF using fine mesh sieves, used in this study, dislodged the particles from the sieve by using either compressed air (2 bars)(portable compressor, Biltema, Norway) and/or by using small amount of water jet dislodging and cleaning the entire mesh. This stadium of the experiment is relevant for microalgae harvesting (Sahu, AK. et al., 2015).

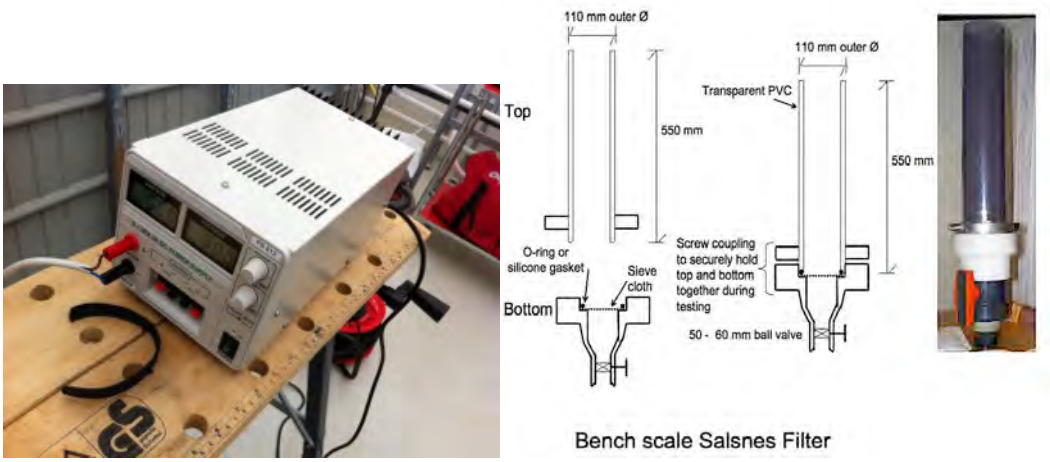

Figure 28 – Energy supplier Device used to supply power to the stirrer for the 20L mixing of the scale bench flocculator (left) and the bench scale Salsnes Filter (SF)(right).

5.5 Flocculation + Filtration tests

The comparing simulations with empirical data (benchmark) for microalgae biomass removal efficiency from water phase were set to ≥ 90%. By using the bench scale SF, particle size analysis was performed on the cultures followed by gravity filtration. Registered low percentage recoveries, implied addition of polymer of chemicals in conjunction with jar test flocculation. The stock solution was prepared in situ by dissolving $1gL^{-1}$ of powder polymer, into distilled water and used within two days otherwise the loss of activity increases as time passes (Sahu, AK, et al., 2015).

A rapid mixing of 300 rpm for 20 sec followed by slow mixing of 50 rpm for 10 min and settling for 10 min was performed and adopted for initial screening of chemicals and/or polymers (dosages 1 mgL^{-1} and 5 mgL^{-1}). To make a decision on selection of polymer, percentage turbidity removal and visual representation of the bulk water phase after settlings was used. One or two polymer/chemicals for each microalgae strain were chosen based on the two best performances in algae removal efficiencies (Sahu, AK. et al., 2015).

The highest removal rates were found, by testing the different dosages of selected polymers or chemicals, in a range of 0,25 mgL^{-1} to 10 mgL^{-1}, hence plotting the recorded results. The optimise dose was determined as the best dosage achievement the maximum removal with minimum dosage, and these results gave base when the best speeds in jar tests settings were optimised for floc production to determine an specifically optimum G-value microalgae strain in respect to the chosen polymer/chemical dosage. Each microalgae strain were exposed to jar testing treatment using two different rapid mixing speeds (300 rpm and 400 rpm) with varying times (10sec and 20sec) and two different slow mixing speeds (30 rpm and 50 rpm) with varying times (5min, 10min and 15min) using an optimised polymer dosage individually per algae strain. A bulk turbidity of the solution was measured before polymer/chemical addition and after settling, and a10 minutes settling time was adopted and kept for all the experiments (Sahu, AK, et al., 2015).

The optimised setting was determined by the best turbidity results (different between starting and after settling) and it was taken into account the pictorial view for the formation of flocs and minimum settings conditions in terms of time and speed. Based on the turbidity results and taking into account, the energetic assumptions that higher speed and longer mixing requires higher energetic demands and larger foot-print, an attempt was made to select the lowest mixing speeds and shortest mixing times wherever possible (Sahu, AK. et al., 2015).
The FlowCam instrument made possible to conduct floc size analysis on the optimised dosage and speed settings recorded by means of the jar test device. Means while the solution was drawn through the flowcell, the flocs, after completion of optimised flocculation time, were kept in suspension by continuous slow mixing making possible to obtain representative samples of the flocs formed at optimised setting (Sahu, AK, et al., 2015).

20L of fresh microalgae suspension was removed from the respective TPBR and poured into
A 25L plastic containers for flocculation at optimized settings (polymer dose, G-values and mixing times), and the flocculant was calculated based on the initial TSS of fresh microalgae.
Achieved the flocculation process, the stirrer was stopped, the flocculated microalgae was gently mixed, to obtain a representative sample, before 1L sample was collected for gravity filtration, and further investigations, testing each SF mesh sieve (Sahu, AK, et al., 2015).

To express the effectiveness of solid-liquid separation process, Path et al., (2013) used recovery efficiency (RE) and concentration factor (CF) (see section 5.1 and eq. 15, eq. 16 of this study). To express RE, in this study, suspended solids (SS) was used to measure the concentrations and percentage SS removal (ratio of difference of SS in final and initial concentration to initial algae concentration). SS was measured using Standard Methods (Standard Methods, 2005). To determine the specific flowrate, the recorded time and volume of the collected filtrate was used. In many processes, the flocculated algae suspension clogged the filter and the time until the clogged moment happened was also recorded. No pH adjustments were done for any experiments, and autoflocculation blanks were used as control. 1L of fresh microalgae suspension without flocculants were treated for gravity filtration (Sahu, AK, et al., 2015).

6 Results and discussions

6.1 Particle size analysis and gravity filtration

Table 1 in this study show the results for microalgae strains´ particle size analysis and morphology observed with FlowCam instrument (tab 8). The results for gravity filtration registered with bench scale SF use for five microalgae strains yielded no significance. The removal rates for *Scenedesmus sp.,* was 40% and 20% for the other microalgae strains. Further information about size and morphology of the microalgae strains of this study, reported by FlowCam device, (fig. 29) (Sahu, AK. et al., 2015), and images registered by SEM technology (fig. 33)(Appendix 8 SEM).

Table 8 – Microalgae characteristics and optimized dosages in mg polymer/g SS algae in water phase (Sahu et al. 2012)

Species	**Shape**[3]	**Media**	**Average particle size**[1]	**# Of particle analysed**	**Area Based Diameter range**	**Optimised dosage**	**Area Based Diameter for flocs range**
			mm		mm	mg/g SS	mm
Chlorella vulgaris	Spherical	FW	5.07	3838	3.99±1.26	18	72.5±14.2
Dunaliella salinas	Irregular	M	7.26	646	4.84±3.7	5.4+52[2]	41.9±9.9
Nannochloropsis oculata	Spherical	M	4.80	3805	3.76±0.94	3.5	35.3±8.7
Scenedesmus sp	Rod	FW	20.01	440	14.7±6.61	2.3	40.7±12.4
*Chlorella wild mix Årungen**	Oval	WW	50.43	54	14.8±7.83	32	52.3±14.5

1- Based on length; FW-fresh water; M-Marine; WW-Wastewater; 2- mg Al/L from PAX; Typical starting concentration of microalgae ranged from 1000-2500 mg/L., *-media used for growth is same as for *Chlorella vulgaris*

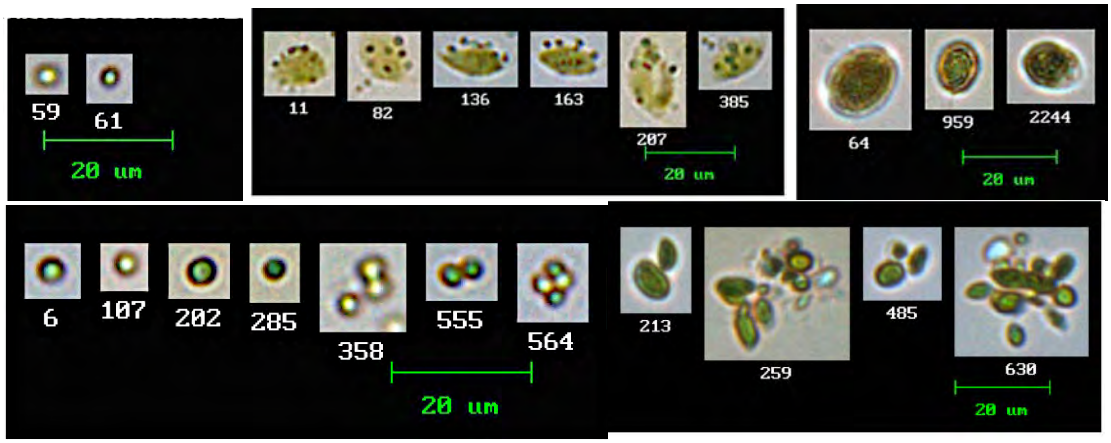

Figure 29 – FlowCam reported images and sizes (clockwise and from left to right) for *Chlorella vulgaris, Dunaliella salinas, Chlorella wild mix Årungen, Nannochloropsis oculata* and *Scenedesmus sp.,* by using a portable fluid imaging device aka, FlowCam (Yarmouth, Maine, USA (Illustration from Sahu et al. 2015)

6.2 Flocculation screening

Auto-flocculation in the absence of flocculants were performed by collected 1L microalgae suspension and mixing it in jar test apparatus at 300 rpm rapid mixing for 20s followed by 50 rpm slow mixing for 10 min and 10 min settling. By turbidity measurement, the registered removal percentage was <15% (very poor) hence the addition of chemical or polymer as flocculant to the water phase, was imperative. Based on turbidity, all flocculation tests did get the same procedure of removal percentage (Sahu, AK, et al., 2015) (for more details about flocculants, see appendix 1, Tab, 14).

Aiming to investing the flocculant removal achievement, starting doses of 1,9 and 5,7 mg AlL^{-1}, and 1 and 5mgL^{-1} were examined for PAX and chitosan, respectively. The highest removal achieved with PAX or chitosan as flocculants with the tested dosages (~50%) were registered for freshwater algae *Chlorella vulgaris, Scenedesmus sp* and *Chlorella wild mix Årungen,* and further different dosages of PAX underwent examination to achieve the optimum dosage for the same algae strains (Sahu, AK, et al., 2015).

For *Chlorella vulgaris*, was determined the optimum dose of 34 mg of Alg^{-1} SS of algae (48 mg AlL^{-1}) achieving 34% removal efficiency from the water phase, and due the high dosage and low removal rates reported by PAX, this flocculant is not suitable for pilot scale testing of the same algae strain (Sahu, AK, et al., 2015).

For *Scenedesmus sp,* was determined the range of 15 to 30 mg Alg^{-1} SS of algae in water phase was the indicated to achieve the highest removal efficiency of 96%, due to the relatively flat percentage removal achieved for a dose of 32 mg Alg^{-1} SS of algae, thus the disadvantageous high dosage used for this algae strain, PAX is partially recommended (Sahu, AK, et al., 2015).

For *Chlorella wild mix Årungen,* was determined an optimum dose of 16 mg Alg^{-1} SS of algae
With 33% of removal efficiency from the water phase, therefore, for this algae strain, PAX was not recommended for pilot scale testing (Sahu, AK, et al., 2015).

For saltwater algae strain *Nannochloropsis oculata,* was reported the highest (> 90%) of removal efficiency from water phase and a value of 98% was achieved which determined the dose of 5,7 mg Alg^{-1} SS of algae as the optimum dosage (Sahu, AK, et al., 2015).

Table 9 – Estimation of G values for optimized speed settings for jar test flocculation (Sahu et al. 2012)

Microalgae species	**Best polymer/ Chemical**	**Optimised dosage**	**Starting SS**	**% Turbidity removal in clear phase**	**Rapid mixing**			**Slow mixing**		
		mg polymer per g of SS algae in water phase	mg/L		rpm	s	G s^{-1}	rpm	min	G s^{-1}
Chlorella vulgaris	Cationic – Kemira	7.1	704	99.0	300	10	334	50	5	31
Dunaliella salinas	Anionic – BASF + PAX	5.4[1]	920	98.8	300	10	334	30	5	17
Nannochloropsis oculata	Cationic-Kemira	1.4	993	86.0	300	20	334	50	10	31
Scenedesmus sp.	Cationic-BASF	15.7	487	98.9	300	20	334	50	5	31
Chlorella wild mix Årungen	Cationic-BASF	6.9	720	96.0	300	10	334	50	10	31

For *Dunaliella salinas,* the dosage of PAX or chitosan as flocculants reported the poorest removal efficiency of ~15%. Molina et al. (2003) pointed out that high water salinity reduces severely chitosan´s flocculation power. In this study was experiment that by adding first an anionic polymer and secondly PAX, to the algae suspension, during rapid mix phase reported the highest removal efficiency of all experiments. It was determined 5,4 mg polymer + 52 mg Alg^{-1} SS of algae in water phase, with 99% of removal efficiency from the water phase, as the optimum dosage (Sahu, AK, et al., 2015).

Based on the studies of Buelna et al., (1990), who reported the negatively charged surface of the microalgae cell cationic polymers were used in this investigation. Polycationic polymer Zetag 63, 5& mgL^{-1} achieved >99% removal efficiency. BASF and Kemira Polycationic polymer used in this study, reported similar removal rates for *Chlorella vulgaris* (Table 9).

It´s necessary to observe the floc size formation during the jar tests, prior determination of the jar test optimisation on speed of the stirrer for rapid and slow mixing. Smaller flocs are preferred contra larger flocs, because they will not break due to pressure on the sieve cloth nor in turbulent transfer neither be forced through the openings in the sieve cloth. In theory, Large and weak flocs are built with low G-values and long flocculation times, meanwhile small and strong floc can be built at higher G-values. By using the optimum polymer dosage specific for each algae strain, the floc sizes were obtained, as shown in Table 7.1, and further investigated by using the SF sieve cloths, the floc strength were put to test for filtration (Sahu, AK, et al., 2015).

6.3 Microalgae harvesting using SWAT technology

The investigated flocculators (bench and pilot scale) were put to test with optimised polymer and chemical dosages, and close G-values for SF. The extent and the rate of the flocculation process are directly influenced by the critical parameter: concentration of the flocculant (Buelna et al., 1990). To determine the flocculant dosage prior to flocculation for these experiments, SS of raw algae was initially measured, and based on the overall floc sizes (Table 7.1) seven different sieve sizes were selected for these experiments (18, 33, 74, 90, 158, 250, 350 µm). The volumes collected were 1L for the jar test flocculator for each sieve and 1L grab sample extracted from the 20L container and used for each sieve (Sahu, AK, et al., 2015).

6.4 Jar test flocculator + SF

For freshwater microalgae strain *Chlorella vulgaris*, 7,1 mg cationic polymer g^{-1} SS were pipetted in 1L of algae suspension, with 1185 mg L^{-1} initial SS concentration, 7,97 pH value, 24,6˚C of culture temperature, achieving 42% as the highest SS removal with a 33µm mesh size (fig. 26). For filters size ≤33µm the sieve clogged, and for filter size <74µm the compressed air alone was enough to clean the filters and both and water were needed for cleaning. A second experiment was performed with increased polymer dosage from 7,1 mg g^{-1} SS to 18 mg g^{-1} SS (fig. 26) and the dosage increasing was tested stepwise (step data not shown). With 1221 mgL^{-1} as starting SS concentration, and gradually increasing algae SS removal up to 92% was observed at 33µm (fig. 26) and 2,7 $m^3\ m^{-2}\ h^{-1}$ value for specific flowrate was measured (fig. 27). Experiments with filters from 350µm went clogged hence stopped at 33µm. When increasing the flocculant dosage by 2,5 times, there were significant clogging of filter size 33µm, otherwise any increase of dosage under that limit reported un improvement in the removal rates from 42% to 92%. At higher flocculant dosage, the 33µm mesh cleaning was difficult, but easier with 74µm mesh, both mesh reporting similar % SS removal, and reduction in specific flowrate has similar effect (fig. 27) (Sahu, AK, et al., 2015).

For marine microalgae strain *Dunaliella salinas,* 1920 mg L^{-1} as starting SS concentration, a 7,96 pH value, 26,7˚C of culture temperature, achieving 82% SS with a 55µm mesh size (fig. 28), and 1,1 $m^3\ m^{-2}\ h^{-1}$ value for specific flowrate was measured (fig. 27). For mesh size ≤250µm the sieve clogged simultaneously decreasing in specific

flowrate (fig. 27). The fluffy and soft consistency of the flocs produced by the presence of PAX, were advantageous in respect to mesh cleaning. Ben-Amotz and Avorn (1987) reported fruitless results when *Dunaliella salinas* were subjected to filtration through sand filters, cellulose fibers and other material, due to the bacterial size of the algae (Sahu, AK, et al., 2015).

For marine microalgae strain *Nannochloropsis oculata,* 1.4 mg cationic polymer g^{-1} SS was pipetted in 1L of algae suspension with 2000 mg L^{-1} of starting SS concentration, 8,05 pH value, 22.5˚C of culture temperature, achieving 75% as the highest SS removal with a 18µm mesh size (fig. 28). 74µm mesh size reported 7,0 m^3 m^{-2} h^{-1} value for specific flowrate was measured (fig. 27), and the cleaning was easy without air alone no water spray. An increase of flocculant from 1,4 mg g^{-1} to 3,5 mg g^{-1} was needed to improve the removal rate performance >80%, resulting in dosage increase tested stepwise (the data is not shown here). With a starting concentration of 1403 mg L^{-1} for 3,5 mg polymer g^{-1} SS, the 74µm sieve size, reported a significant increase in removal of SS from 28% to 78%, compared to 1.4 mg g^{-1} SS (Sahu, AK, et al., 2015).

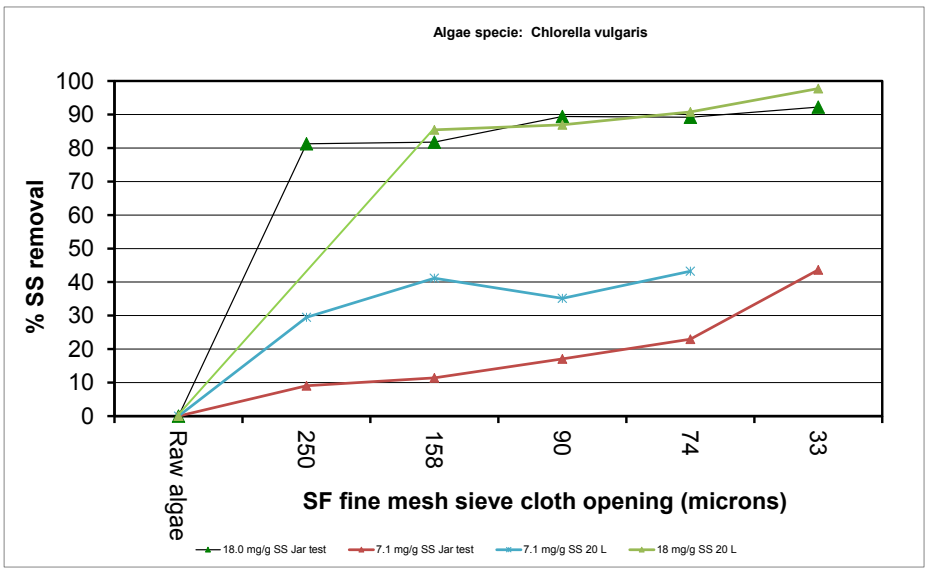

Figure 30 – Effect of increased polymer dose on harvesting of *Chlorella vulgaris* using two different flocculators and SF fine mesh sieves (Sahu et al. 2012)

It was demonstrated in this study that selecting a larger mesh opening combined with a higher flocculant dosage, removed the algae as well as this facilitated a better cleansing of the mesh sieve, reporting the same low 78-80% as highest algae removal. Remember the specificity of the flowrate strong related to the mesh size, and the flocculant dosage, when upscaling the microalgae harvesting operation system, thus larger mesh size implies larger flowrate, when higher flocculant dosage is set off by the significantly smaller sieve size (Sahu, AK, et al., 2015).
For freshwater microalgae strain *Scenedesmus sp.,* it was determined 15,7 mg g^{-1} SS as optimum dosage, and 20,3 mg g^{-1} SS of flocculant was also tested. With 1289 mg L^{-1} as starting SS concentration, 7,98 pH value and 23,2˚C of culture temperature, achieving 98% SS as highest removal with 55µm mesh size (fig. 28), reporting 7,5 m^3 m^{-2} h^{-1} as specific flowrate when measured a 55µm mesh sieve (fig. 27). 30m^3 m^{-2} h^{-1} was the flowrate reported for 250µm mesh size with 95% SS algae removal. Scrubbing was needed for 33µm filter mesh, and for 90µm mesh filter, the jet air stream was use with

easiness in absence of water. The paddle of the jar test flocculator was "glued" by the flocculated algae (Sahu, AK, et al., 2015).

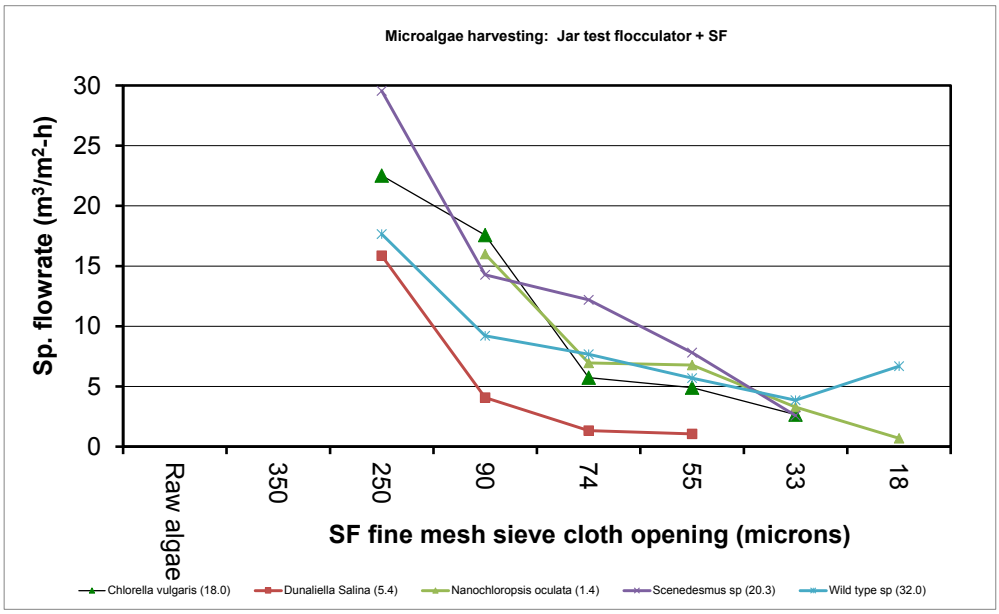

Figure 31 – Specific flow rates obtained on microalgae harvesting using jar test flocculator and bench scale SF. Concentration of polymer in mg/g SS are given in parentheses for each specie (Sahu et al. 2012)

For the freshwater microalgae species *Chlorella wild mix Årungen*, 1L of algae with 1415 mg L^{-1} as starting SS concentration was pipetted with 6,9 mg g^{-1} SS of flocculant dosage, 8.13 of pH value and 21.8˚C of culture temperature, achieving 40% SS as highest removal with 90µm mesh size (data not shown). For mesh sizes ≤250µm the filters got clogged. The 33µm mesh sieve was possible to dislodge the clogged algae by spray water stream after being scrubbed. A stepwise increase of 5 mg L^{-1}, in polymer dosage from 6,9 mg g^{-1} SS to 32 mg g^{-1} SS was performed to improve the removal rate to > 40% (data not shown here). With 1600 mg L^{-1} as starting SS concentration, for 6,9 mg g^{-1} SS, the 250µm sieve size, reported a significant increase in removal of SS from 40% to 80%, compared to the higher dosage of 32 mg g^{-1} SS (fig. 28), reporting 17,7 m^3 m^{-2} h^{-1} as specific flowrate when measured with a 250µm mesh sieve (fig. 27). A 10 mL inoculum of *Chlorella wild mix Årungen*, grown originally in sludge liquor, was transferred to batch culture (5 tube glass à 380 mL) and grown in sterile nutrition media, 3N-BBM+Vitamins. FlowCam registered the ovoid morphology of this species (fig. 26) (Sahu, AK, et al., 2015).

6.5 20 L flocculator + SF

The cultivated microalgae species were harvested from its respective TPBR and flocculated in 20L flocculator followed by SF. The reported G-values from the jar test flocculator were adopted as standard flocculation to obtain good floc size. The results are summarised in Table 9. The grab samples collected from the TPBR, for the 20L batch mode performance, were hot homogenous like the treated ones in the jar test flocculator, reporting uniform stirring and speed per sample when each sample was treated individually for each sieve. Mimicking industrial scenario, the 20L batch flocculation process presented a floc size distribution with more uniformity compared to the grab sample. SF will be used continuously in industrial microalgae harvesting.

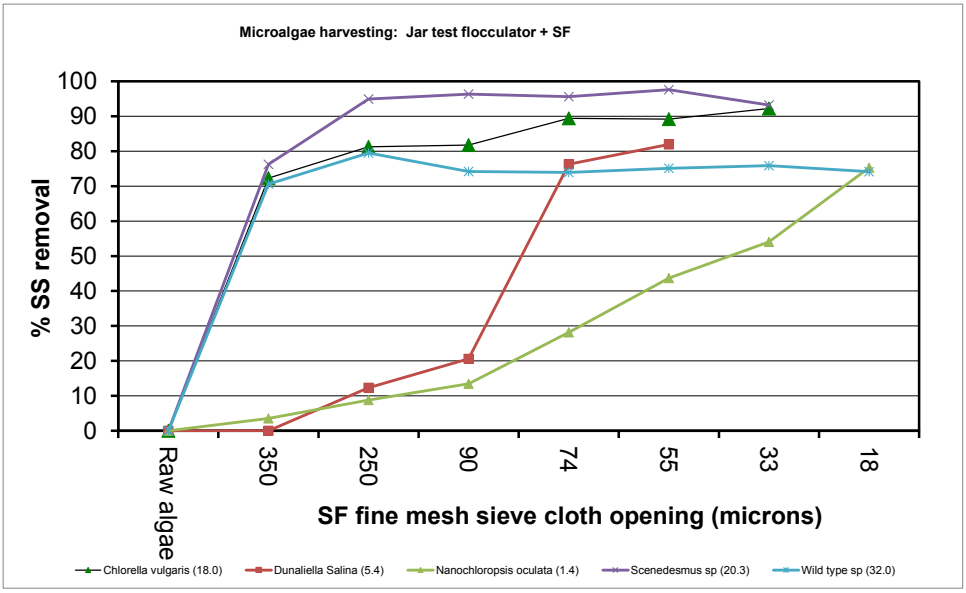

Figure 32 – Microalgae harvesting using jar test flocculator and bench scale SF. Concentration of polymer in mg/g SS are given in parentheses for each algae strain (Sahu et al. 2012)

Table 10 – Summary of 20 L pilot scale testing of flocculation + SF for different microalgae (Sahu et al. 2012)

Microalgae species	**Polymer Dosages**	**Raw algae SS**	**SS removal**	**Sieve size**	**Sp. flow rate**	**Mixing conditions in 20 L microalgae**					
						Rapid mixing			**Slow mixing**		
						Power Input	Sp.	G	Power input	Sp.	G
	mg/ g SS	mg/L	%	mm	m^3/m^2-h	W	rpm	s^{-1}	W	rpm	s^{-1}
Chlorella vulgaris	18	1224	98	33	6.2	15.3	249	414	2.89	94	97
Dunaliella salinas	5.4 + 52 Al	1056	30	15	-	13.9	236	382	2.39	84	81
Nannochloropsis oculata	1.4	1325	92	15	15.2	14	237	385	2.34	83	79
Scenedesmus sp	31	357	95	158	-	13.5	232	373	1.95	74	67
Chlorella wild mix Årungen	6.9	338	84	90	0.3	14.7	249	400	1.49	62	51

For the 20L flocculation, the G-values reported for the jar tested were adapted, by measuring the amount of power supplied to the stirrer (Table 9) (Sahu, AK, et al., 2015). For *Chlorella vulgaris*, the same polymer dosage, especially at the highest 18 mg g^{-1} SS was achieved similar removal performances (fig. 32), and it was observed up to 98% removal for many algae species. This study corroborated that the SWAT filter technology is apt for harvesting many of the algae strains with some exceptions, like the halophile *Dunaliella salinas* when the addition of PAX as flocculant aggregated the particles into flocs with not optimal consistency with did not supported compress on filtration nor were able to be retained on the mesh sizes, and for some sieves, the removal reported negative values (complete data set not shown here) due to higher production of particles that the raw sample, especially at this 20L scale (zero flocculation effect observed at 1L jar test). When only anionic polymer was used as flocculant, 16% of removal rate was achieved with 18µm mesh size (Sahu, AK. et al., 2015).

For this marine microalgae strain biomass recovery from water, it was imperative to create the synergy effect produced by the combination of PAX and Polycationic polymer (Sahu, AK, et al., 2015).

7 Conclusions for harvesting (flocculation and SF filtration)

By using the SWAT Salsnes Filter technology, four commercial and one free available microalgae strains were harvested, by means of induced flocculation processes in combination with filtration process. The achievement of ≥ 90 % of biomass microalgae removal efficiencies from the water phase, by using existing commercial sieve technology was the overall aim of this study (Sahu, AK, et al., 2015).

The process of harvesting demanded high volumes of microalgae, from the batch stadium to the TPBR, up-scaled semi-continuous cultivation system operated indoors at greenhouse conditions. The collected grab volume samples where subjected to the effects of polymer/chemical as flocculator, therefore the induced algae flocculation was harvested using SF fine mesh sieves. The investigation demanded two flocculators, bench scale jar tester and a pilot scale 20L flocculator. To identify the microalgae morphology and size characteristics a particle size analysis was used. Subsequently, the poor removal efficiencies reported from gravity filtration using SF, corroborated the requirements for commercial Polycationic polymers and chemicals (PAX and chitosan) as flocculants. For some of the strains, PAX and chitosan reported high removal efficiencies, but the disadvantageous fluffy consistency of the flocs created and the poor filterability characteristics, obliterated the following use of these chemicals compounds from the experiments of this study. The optimization for dosages and stirrer speed were tested for the predicted removal efficiencies of the cationic commercial polymers in question for most of the species. For the 20L pilot flocculator, G-values were estimated, using the power inputs and empirical equations, and these values were used for the up-scaling processes for rapid and slow mixing. Using various flocculators devices, polymer dosages and fines mesh sizes, ≥ 95% of high removal efficiencies from the water phase, were achieved with the freshwater algae strains C*hlorella vulgaris, Nannochloropsis oculata, Scenedesmus sp.*

Harvesting biomass microalgae cells demands essential requirements: the specific G-values, polymer/chemical (flocculant) dosage and fine mesh sieve size used have to be determined as well as their synergetic relationship. Microalgae harversting technology needs further investigation, and this study can be a good guide for future testing of upscaling commercial continuous flow SF for harvesting of large microalgae volumes (Sahu, AK, et al., 2015)

8 Overall conclusions

The overall conclusion of the different processes and methods of harvesting high microalgae biomass concentration for subsequently energy extraction could be a synergetic growth system with no maximum yield performance, but with better performance in harvesting the biomass algae, which will facilitate through a minimum microalgae biomass concentration, the best extraction energy processes.

To achieve an efficient automatized upscaling cultivation system with transparent build TPBR to secure a higher algae biomass output with adequate consistence aimed to facilitate a low cost harvesting method without the addition of chemical flocculants, thus in spite of the high removal rate presented in this study, the risk of toxicity levels, not analysed in this study, make the algae yield unsuitable for human or animal consumption. Further investigation will be need, focusing on the design and operation of both upstream and downstream processes in an integrated overall microalgae production process. The other fields where the flocculated (contaminated) biomass output can be destined for is for energy production but the requirement for desirable properties has to be satisfied before starting the large-scale culture intended for energy production: rapid growth, high lipid content (for biodiesel production), temperature optimum, maximum temperature tolerated, high photosynthetic efficiency, ability to tolerate high irradiances, salinity, shear tolerance, non-"sticky" cells, grows in a "selective" environment, reduced sensitivity of photosynthesis (Rubisco) to high 0_2 concentration, lower intrinsic respiratory rate, large cell size or colonial or filamentous morphology, high specific gravity, weak cell covering or no cell wall, no production of auto-inhibitors, lipid composition (for biodiesel).

Redrawn and redesigned TPBR to avoid contamination, combined with an adequate mixing improve algae cells light-dark cycle and avoid bio-fouling, efficiently CO_2 supply preventing O_2 build-up and providing high mass transfer capacity, high S/V ration increases the cell concentration and volumetric productivity. The optimization of growth conditions and parameters reading and control by regular or automatized thermic, pH, CO_2 and nutrient concentrations, together with an adequate harvesting and dilution regime (0,20) to maintain the optimal population density.

It was found in this study that the comparison of two TPBR 7 and TPBR 8 containing the same strain, 39% lower dilution rate in freshwater strain *Scenedesmus sp.,* boosted the productivity twofold, a benefit ratio of 5,1 (200/39). On the other hand with 1,8% lower dilution rate in saltwater strain *Dunaliella salinas*, gave a 31% higher biomass output, a benefit ratio of 17,2 (31/1,8)

TPBR build with transparent materials will increase the unit irradiation utilized energy (IUE), which is dependent of the light-path (0,09 cm in this study), not direct proportional to the increase of the illuminated area. Raising the volume of liquid in a TPBR of the characteristics used in this study, will increase the light-path and will lead to decreased illumination for the inner layers of the water column. Reducing the mixing will lead to decreased rate of growth due to lower light flashing effect exerted on cell algae.

Among the freshwater strains, *Scenedesmus sp.* TPBR 7 had the highest biomass productivity (*P*) with 0,95 g DW $L^{-1}d^{-1}$ with the highest temperature of 25,3°C. among the

marine microalgae species, *Dunaliella salinas* TPBR 4 topped the overall highest biomass productivity (*P*) with 1,31 g DW $L^{-1}d^{-1}$ and the lowest temperature of 20,5°C.
It seems that the temperature play a direct role in the productivity when the coolest temperature in aquatic systems reported the highest biomass productivity of all strains in this study. 23,4% (25,3*100/20,5) of lower culture temperature increased the biomass productivity in 37,9% (1,31*100/0,95), and it was registered for saltwater strain, *Dunaliella salinas* TPBR 4, which topped the overall highest biomass productivity (*P*) with 1,31 g DW $L^{-1}d^{-1}$ with the highest temperature of 25,3°C and freshwater strain, *Scenedesmus sp.* TPBR 7 with the second highest biomass productivity (*P*) with 0,95 g DW $L^{-1}d^{-1}$ and the lowest temperature of 20,5°C.

The sum of the five strains data (Table 3) for cloudy days gave a total of 70,71 mgL^{-1} of dissolved oxygen (DO) generated by a PAR irradiation sum of 298,62 MJ $m^{-2}d^{-1}$ mean while the sum for the sunny days gave 54,06 mgL^{-1} of dissolved oxygen (DO) mgL^{-1} of dissolved oxygen (DO) generated by a PAR irradiation sum of 787,24 MJ $m^{-2}d^{-1}$. By lowering the PAR irradiation 2,64 times it was generated 31% higher dissolved oxygen.
The cloudy weather conditions in combination with lower PAR irradiance and lower culture temperature affected directly the algae cell´s O_2 evolution, which was emitted at higher volumes than when the sunny weather combined with higher irradiation and higher temperature.

All the strains specific growth were affected positively by higher irradiation energy input in combination with a moderate culture temperature around 23°C, however, among the freshwater microalgae strains, *Scenedesmus sp.* TPBR 7 had the highest specific growth rate with 0,05 h^{-1} and showed a fivefold increase when doubling the irradiation energy input and temperature kept at 23°C. Among the marine microalgae species, *Dunaliella salinas* TPBR 3 had the highest specific growth rate with 0,024h^{-1} and showed a 71,4% increase when doubling the irradiation energy input and temperature kept at 23°C.

It seems that the level of irradiance had an inverted effect on the photosynthetic efficiency (PE), i.e. lower irradiance absorbed by the high dilute algae culture gave higher photosynthetic efficiency. Saltwater strains *Nannochloropsis oculata, TPBR 5*, registered the next highest PAR incident irradiance input energy (E_{in}), gave the lowest photosynthetic efficiency (PE), with 89,27 MJ m^{-2} d^{-1} and 0,37%, respectively. *Dunaliella salinas,* TPBR 3, with the lowest irradiance gave the highest photosynthetic efficiency, with 55,3 MJ m^{-2} d^{-1} and 1,48%, respectively.

More promising results were achieved in respect of carbon sequestration levels, and by using in our experiments, the mean higher heating value (22,49 KJ g^{-1}) recorded by Hulatt et al., (2011), and comparing the results with this study, the mean $C0_2$ fixation rate was 55,44 (range 3,71-75,18) g CO_2 $m^{-2}d^{-1}$ (Table 6), which is 2,6 times higher values than found by Hulatt (2011).

The turbulence effect was negative, due to the harsh dilution rate combined with low culture volume, and it reported a decrease of -11% (0,38*100/0,42) of biomass productivity, for freshwater strain, *Chlorella vulgaris,* TPBR 2 (mixed with pump), had lower biomass productivity that TPBR 1 (no pump), with 0,38 and 0,42 g DW $L^{-1}d^{-1}$, respectively, with 4,75 and 24,81L of remaining culture volume, and with 0,60 and 0,13 d^{-1} of dilution rate, respectively.

The turbulence effect was positive, though the harsh dilution rate the remaining volume higher, and it reported an increase of 61,72% (1,31*100/0,81) of biomass productivity, for saltwater strain, *Dunaliella salinas,* TPBR 4 (mixed with pump), had higher biomass productivity that TPBR 3 (no pump), with 1,31 and 0,81 g DW $L^{-1}d^{-1}$, respectively, with 18,42 and 24,69 L of remaining culture volume and with 0,76 and 0,20 d^{-1} of dilution rate, respectively.

The turbulence effect was positive, though the harsh dilution rate combined with a higher remaining culture volume, and it reported an increase of 57,14% (0,77*100/0,49) of biomass productivity for saltwater strain, *Nannochloropsis oculata,* TPBR 6 (mixed with pump), had higher biomass productivity that TPBR 5 (no pump), with 0,77 and 0,49 g DW $L^{-1}d^{-1}$, respectively, with 8,13 and 16,58 L of remaining culture volume and with 0,60 and 0,28 d^{-1} of dilution rate, respectively.

The most striking fact in this study is that when investigating the TPBR performance efficiency, even the low irradiation registered inside the TPBR and above the algae culture surface, but not registered on the TPBR propylene walls, was absorbed efficiently and when comparing with this productivity per unit irradiation, i.e. irradiation utilization efficiency (IUE): 5,1 g M J^{-1} (Zhang *et al*. 1999) and 6,4 g M J^{-1} (Zhang *et al.* 2000), this study reported poor values, with the highest being 1,40 g M J^{-1}. These comparisons show 3,6 and 5,6 times lower to the data reported by Zhang (2010) yet the optical path was 1/9 of the used in this study. Our TPBR are of significance performance efficiency!!! Refraction index for Polypropylene (0,900), water at 20°C (1,332) and Plexiglas (1,488)(Barsanti and Gualtieri, 2006) have to be taken into account for further investigation of the suitability of the TPBR used in this study.

The news in this study is the corroboration that it is possible to grow microalgae at our northern geographical location (Ås, SKP, center for climate regulated plant environment, 59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level), under Norwegian summer period, with neither artificial illumination nor heating, and using low cost materials as polypropylene (TPBR) and manually operated by one person (upscaling) and three operators (harversting).

By using the SWAT Salsnes Filter technology, four commercial and one free available microalgae strains were harvested, by means of induced flocculation processes in combination with filtration process. The achievement of $\geq$ 90 % of biomass microalgae removal efficiencies from the water phase, by using existing commercial sieve technology was the overall aim of this study. The optimization for dosages and stirrer speed were tested for the predicted removal efficiencies of the cationic commercial polymers in question for most of the species. For the 20L pilot flocculator, G-values were estimated, using the power inputs and empirical equations, and these values were used for the up-scaling processes for rapid and slow mixing. Using various flocculators devices, polymer dosages and fines mesh sizes, $\geq$ 95% of high removal efficiencies from the water phase, were achieved with the freshwater algae strains C*hlorella vulgaris, Nannochloropsis oculata, Scenedesmus sp.* For *Dunaliella salinas,* the poor removal efficiencies reported from gravity filtration using SF, corroborated the requirements for commercial Polycationic polymers and chemicals (PAX and chitosan) as flocculants, to achieve high removal efficiencies, but the disadvantageous fluffy consistency of the flocs created and the poor

filterability characteristics, obliterated the following use of these chemicals compounds from the experiments of this study.

Alternative solutions to the use of Salsnes Filter technology, commonly applied to wastewater treatment, were investigated and proved with satisfactory results but with the use of flocculants was essential to achieve it. Further investigation is needed, to avoid the use of flocculants (chemicals organic or inorganic (polymers)) to in the harvesting processes. The back draws of energy input in the production of flocculants and the de-contamination of the yielded microalgae biomass were not taken into account in this study. The need of figure out alternative uses based on more environmental friendly and cheaper energetic consumption processes need further research and development.

This study is the core of a master thesis, which is intended to bring knowledge, guidance and joy about the one of the oldest living autotrophic organisms on our living planet.

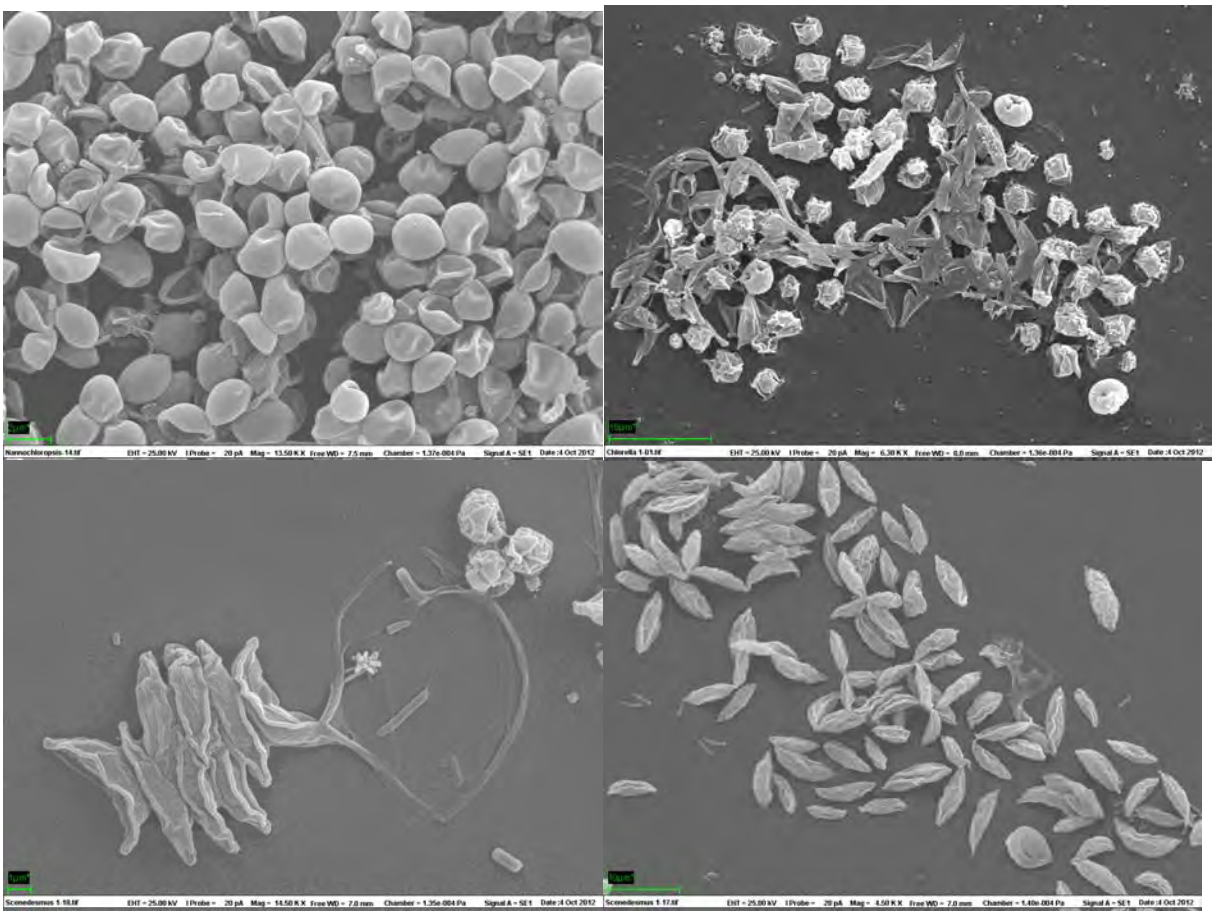

Figure 33 – SEM micrograph of Dunaliella salinas and Chlorella wild mix Årungen (Upper) and Scenedesmus sp., (Down)

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10 Appendix 1. Nutrition media protocol and flocculants (Tabs. 11-14)

10.1 Protocol for 3N-BBM + Vitamins media preparation

The marine medium contained growth medium details for the respective microalgae For the batch culture (0.380 ml glass tube incubator), the recipe for 3N-BBM + V (3N-BBM+V (Bold-Basal Medium with 3-fold Nitrogen and Vitamins; modified)) was strictly followed.

http://www.ccap.ac.uk/media/recipes/3N_BBM_V.htm

Table 11 – Stock solutions for 3N-BBM+Vit (from http://www.ccap.ac.uk/)

Name: 3N-BBM+V (Bold-Basal Medium with 3-fold Nitrogen and Vitamins; modified)		
Description:	Freshwater	algae
Stocks	per	litre
(1)	$NaNO_3$	(25.0gL^{-1})
(2)	$MgSO_4.7H_2O$	(7.5gL^{-1})
(3)	NaCl	(0.5gL^{-1})
(4)	$K_2HPO_4{\cdot}3H_2O$	(7.5gL^{-1})
(5)	KH_2PO_4	(17.5gL^{-1})
(6) $CaCl_2.2H_2O$ (2.5 g L^{-1})		
(7) Trace elements solution		
Added to 1 litre of distilled water 0.75 g Na_2EDTA and the minerals below in exactly the following sequence:		
$FeCl_3{\cdot}6H_2O$ (97.0 mg L^{-1})		
$MnCl_2{\cdot}4H_2O$ (41.0 mg L^{-1})		
$ZnCl_2{\cdot}6H_2O$ (5.0 mg L^{-1})		
$CoCl_2{\cdot}6H_2O$ (2.0 mg L^{-1})		
$Na_2Mo_4{\cdot}2H_2O$ (4.0 mg L^{-1})		
(8) Vitamin B_1 - 0.12 g Thiaminhydrochloride in 100 ml distilled water. Filter sterile.		
(9) Vitamin B_{12} - 0.1 g Cyanocobalamin in 100 mg distilled water, take 1 ml of this solution and add 99 ml distilled water. Filter sterile.		
Medium		
Stock solution 1 (30.0 ml)		
Stock solutions 2 to 6 (10.0 ml each)		
Stock solution 7 (6.0 ml)		
Stock solutions 8 to 9 (1.0 ml each)		
Method		
Make up to 1 litre with deionized water. Autoclave at 15 psi for 15 minutes.		

For the tray bioreactor cultures, the autoclave procedures and the sterile filtration of the vitamins were omitted. 5 different microalgae species cultured in 2 trays each.

Table 12 – Medium details for freshwater preparation of 25L final medium, taken from stock solutions for 3N-BBM+Vit (from http://www.ccap.ac.uk/).

Medium details for 25 Lt final medium					
3N-BBM+V for fresh water micro algal species: *Chlorella vulgaris*				3N-BBM+V Total volume: 2.200 ml	
$NaNO_3$	750 ml	$K_2HPO_4{*}3H_20$	250 ml	Trace elements	150 ml
$CaCl_2{*}2H_20$	250 ml	KH_2PO_4	250 ml	Vitamin B_1	25 ml
$MgSO_4{*}7H_20$	250 ml	NaCl	250 ml	Vitamin B_{12}	25 ml
Growth conditions for: *Chlorella vulgaris,* Tray nr. 1 and 2 - Inoculum (750 ml suspension) diluted in 25 Lt growing media					
Scenedesmus sp, Tray nr. 7 and 8 - Inoculum (750 ml suspension) diluted in 25 Lt growing media					
Chlorella wild mix Årungen Tray nr. 9 and 10 - Inoculum (750 ml suspension) diluted in 25 Lt growing media					
Trace elements for 1 Lt Stock solution					
$Na_2EDTA{*}2H_20$	0.750g/Lt	$MnCl_2{*}4H_20$	0.041g/Lt	$CoCl_2{*}6H_20$	0.020g/Lt
$FeCl_3{*}6H_20$	0.097g/Lt	$ZnCl_2{*}6H_20$	0.050g/Lt	$Na_2MoO_4{*}2H_20$	0.004g/Lt

Tap water volume: 22.800 ml. total volume of the final media: 25 Lt.

For the marine species, the artificial salt water was calculated to achieve pH 6.30
With the dissolution of 27 g of salt / 1 Lt tap water

Calculations:
25L of tap water X 27 g salt give 675 g/25L.
An optimal artificial marine media is composed by 60 % of salt water and 40 % of fresh water. There were mixed just 405 g of salt (60 % of the total salt to be dissolved) in 22,800 Lt of tap water. The final addition of the 3N-BBM+V (2,200 ml) gives a total volume of 25,000 ml.

Table 13 - Medium details for saltwater preparation of 25L final medium, taken from stock solutions for 3N-BBM+Vit (from http://www.ccap.ac.uk/).

Medium details for 25 Lt final medium					
3N-BBM+V for marine (salt water) micro algal species: *Dunaliella salina, Nannochloropsis oculata*				3N-BBM+V Total volume: 2.200 ml	
$NaNO_3$	750 ml	$K_2HPO_4{*}3H_20$	250 ml	Trace elements	150 ml
$CaCl_2{*}2H_20$	250 ml	KH_2PO_4	250 ml	Vitamin B_1	25 ml
$MgSO_4{*}7H_20$	250 ml	NaCl	250 ml	Vitamin B_{12}	25 ml
Growth conditions for marine species *Dunaliella salina*					
Tray nr 3 – inoculum (750 ml suspension) diluted in 25 L growing media					
Tray nr 4 – inoculum (750 ml suspension) diluted in 25 L growing media					
Nannochloropsis oculata					
Tray nr 5 – inoculum (750 ml suspension) diluted in 25 L growing media					
Tray nr 6 – inoculum (750 ml suspension) diluted in 25 L growing media					
Trace elements for 1 L Stock solution					
$Na_2EDTA{*}2H_20$	$0.750gL^{-1}$	$MnCl_2{*}4H_20$	$0.041gL^{-1}$	$CoCl_2{*}6H_20$	$0.020gL^{-1}$
$FeCl_3{*}6H_20$	$0.097gL^{-1}$	$ZnCl_2{*}6H_20$	$0.050gL^{-1}$	$Na_2MoO_4{*}2H_20$	$0.004gL^{-1}$

10.2 Chemical flocculants (organic and inorganic)

Polymers 20 types were prepared 1g/L for the powder form. For the flocculants in emulsion condition, and for chitosan, the dilution procedure was some different

Table 14 – List of 20 different organic and/or inorganic flocculants were prepared 1g dry matter/L distilled water (diO) (Notation: * CPAM - Cationic polyacrylamide).

Nr	Code	Made by	Chemical Family	Material Safety Data Sheet (MSDS)
1	C5005	Xitao	CPAM *	http://xitao.en.alibaba.com/product/341116597-200858430/Weak_cationic_charge_polyacrylamide_C5005_in_papermaking.html (Read 20.06.12)
2	C5035	Xitao	CPAM	http://xitao.en.alibaba.com/product/341117313-200858430/Cationic_Polyacrylamide_Powder_C5035_for_belt_press.html (read 20.06.12)
3	C5630	Xitao	CPAM	http://xitao.en.alibaba.com/product/238777144-200858430/Sludge_Dewatering_Cationic_flocculant_C5630_for_centrifuge.html (read 20.06.12)
4	C491	Kemira	CPAM	http://dsichemicals.com/msds11/Superfloc%20C-496.pdf (read 20.06.12)
5	C492	Kemira	CPAM	http://www.strykerchem.com/docs/SuperflocC-496TDS.pdf (read 20.06.12)
6	C496	Kemira	CPAM	http://dsichemicals.com/msds11/Superfloc%20C-496.pdf (read 20.06.12)
7	Magnafloc LT22S	BASF	Copolymer of a quaternary acrylate salt and acrylamide	http://www.hillbrothers.com/msds/pdf/n/magnafloc-lt22s.pdf (read 20.06.12)
8	Magnafloc LT32	BASF	Polyamine	http://www.fhi.no/dokumenter/f2951c184e.pdf (read 20.06.12)
9	Magnafloc LT37	CIBA		(Read 20.06.12)
10	Kitoflocc			(Read 20.06.12)
11	Zetag7550	Ciba		(Read 20.06.12)
12	Zetag7587	Ciba	Copolymer of a quaternary acrylate salt and acrylamide	http://www.marquisalliance.com/sites/default/files/data-sheet/Zetag%207587.pdf (read 20.06.12)
13	Zetag8125			http://worldaccount.basf.com/wa/NAFTA~en_US/Catalog/WaterSolutions/pi/BASF/Type/cationic_powder (read 20.06.12)
14	Zetag8160			Same as above
15	Zetag8165			Same as above
16	Zetag8180			Same as above
17	Zetag8185			Same as above
18	Zetag9014	BASF	Cationic emulsion	http://www.basf.com/group/corporate/en/overview-page:/Brand+Zetag+Cationic+Emulsion (read 20.06.12)
19	Zetag9048FS	BASF	Same as above	Same as above
20	PAX XL 60			(Read 20.06.12)

11 Appendix 2. Calculations and equations (Eqs. 21-34 and Figs. 34-37)

11.1 Equation 21

Specific growth rate μ = (g/L*d)

$$\mu = \frac{\ln (TSS1 - TSS\circ)}{T1 - T1\circ}$$

11.2 Equation 22

During exponential growth, the rate of increase in population (biomass here) per unit time is proportional to the number of population present in the culture at the beginning of any unit of time. Population growth follows this equation (Guillard used *r* for the exponential growth rate of the population, equal to "K_e" (Guillard, 1973):

$$\frac{dx}{dt} = rX$$

The solution of this is

11.3 Equation 23

$$X_1 = X_0 e^{rt}$$

Where X_0 is the population size (TSS) at the beginning of a time interval, X_1 is the population size at the end of the time interval and *r* is the proportional rate of change, also called *the intrinsic rate of increase*, the *Malthusian parameter,* or the *instantaneous rate of increase* (Gotelli 1995). Units of *r* are always expressed per unit time (t^{-1})

11.4 Equation 24

$$r = \frac{\ln(X1 - Xo)}{\Delta t} = \frac{\ln X1 - \ln Xo}{\Delta t}$$

11.5 Equation 25

$$r = \mu - m$$

r is equal to μ, the specific growth rate, when mortality (m) is zero. When "t" is expressed in days, then the growth *r* can be converted to doublings per day (k) by dividing *r* by the natural log of 2.0, according to the equation

11.6 Equation 26

$$k = r / 0.6931$$

Doublings per day (k) can be calculated directly from estimates of X with the use of this equation

11.7 Equation 27

$$\mathrm{k} = \frac{\log_2\left(\frac{X_1}{X_0}\right)}{\Delta t} = \frac{log\,\frac{\left(\frac{X_1}{X_0}\right)}{\log_2}}{\Delta t}$$

This last part of the equation is also know as the *exponential growth or decay, accelerating growth in terms of doubling time.*

11.8 Equation 28

Doubling time T_2, for the culture, expressed in the same units of time as *r,* can be calculated from an estimate of r with use of this equation

$$T_2 = \frac{0{,}6931}{r}$$

11.9 Equation 29

At steady state, the specific growth rate (μ) of the population within either type of continuous culture is determined by the dilution rate:

$$\mu = \frac{\mathrm{F}}{V} = D$$

Where F is the fresh medium added to the culture vessel, for replenishing the removed suspension from the vessel, V is the volume of the culture remaining in the vessel (L). D is the dilution rate.

11.10 Equation 30

Graph to inverse functions

$f(x) = 2$ (Eq. 30.1)

$\Leftrightarrow y = 2^x$

$\Leftrightarrow \lg y = \lg 2^x$

$\Leftrightarrow \lg y = x*\lg 2$ because $\lg 2 = 1$

$\Leftrightarrow x = \lg y$ (Eq. 30.2)

The inverted function to *f* is then

$g(y) = \lg y$

$D_{lg}\ V = \langle 0, \rightarrow \rangle\ V_{lg} = D_f = R$ (see tables 16 and 17)

When the graphs of two inverted functions lay symmetrically to each other on a line, it tells about theirs continuity too. If one graph is continuous, the other is it, too.

11.11 Equation 31

Productivity (P) or Yield (Y) = (g/L*d) the *output rate* in continuous culture

$$P = \frac{\mathrm{TSS1} - \mathrm{TSS}\circ}{\mathrm{t1} - \mathrm{t}\circ} = \frac{\mathrm{TSS1} - \mathrm{TSS}\circ}{\Delta t}$$

Where TSS = total suspended solids, Δt is the length of the time interval (t_1 - t_0).

11.12 Truncated rectangular pyramid

To calculate the areal system productivity of one TPBR is necessary to understand the geometry of the suspension culture inside the TPBR. The water contained in the vessel resembles of a truncated pyramid. The calculation gives a total surface area of 0,913 m^2 and a volumetric productivity of 0,031m^3 or 31,314L (Figures. 31-34)

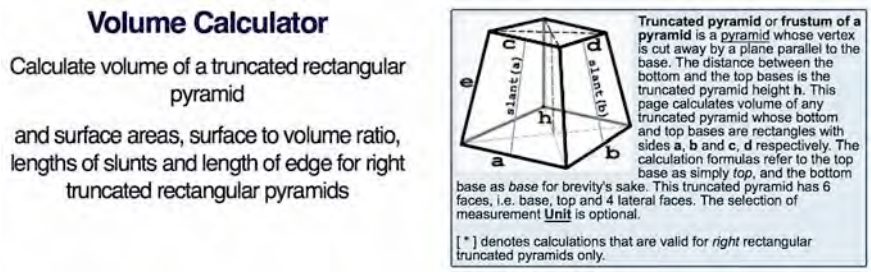
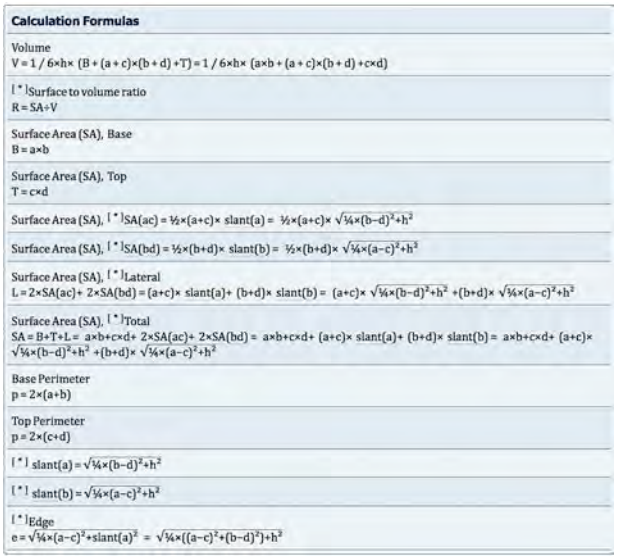


Figure 34 – Volume calculator for the area contained the microalgae suspension for a volume of 30,113L (http://www.aqua-calc.com/calculate/volume-truncated-pyramid, accessed January 2015)

In this case the truncated pyramid is inverted. It´s essential to remember that when we calculate the illuminated surface area (ISP), the base or c*d area is omitted. Otherwise we include it and now we are calculating the grown area occupied by the TPBR, and the parameter evaluated is areal productivity (AP).



Calculation Formulas

Volume
$V = 1 / 6 \times h \times (B + (a + c) \times (b + d) + T) = 1 / 6 \times h \times (a \times b + (a + c) \times (b + d) + c \times d)$

[*] Surface to volume ratio
$R = SA \div V$

Surface Area (SA), Base
$B = a \times b$

Surface Area (SA), Top
$T = c \times d$

Surface Area (SA), [*] $SA(ac) = \frac{1}{2} \times (a+c) \times \text{slant}(a) = \frac{1}{2} \times (a+c) \times \sqrt{\frac{1}{4} \times (b-d)^2 + h^2}$

Surface Area (SA), [*] $SA(bd) = \frac{1}{2} \times (b+d) \times \text{slant}(b) = \frac{1}{2} \times (b+d) \times \sqrt{\frac{1}{4} \times (a-c)^2 + h^2}$

Surface Area (SA), [*] Lateral
$L = 2 \times SA(ac) + 2 \times SA(bd) = (a+c) \times \text{slant}(a) + (b+d) \times \text{slant}(b) = (a+c) \times \sqrt{\frac{1}{4} \times (b-d)^2 + h^2} + (b+d) \times \sqrt{\frac{1}{4} \times (a-c)^2 + h^2}$

Surface Area (SA), [*] Total
$SA = B + T + L = a \times b + c \times d + 2 \times SA(ac) + 2 \times SA(bd) = a \times b + c \times d + (a+c) \times \text{slant}(a) + (b+d) \times \text{slant}(b) = a \times b + c \times d + (a+c) \times \sqrt{\frac{1}{4} \times (b-d)^2 + h^2} + (b+d) \times \sqrt{\frac{1}{4} \times (a-c)^2 + h^2}$

Base Perimeter
$p = 2 \times (a+b)$

Top Perimeter
$p = 2 \times (c+d)$

[*] $\text{slant}(a) = \sqrt{\frac{1}{4} \times (b-d)^2 + h^2}$

[*] $\text{slant}(b) = \sqrt{\frac{1}{4} \times (a-c)^2 + h^2}$

[*] Edge
$e = \sqrt{\frac{1}{4} \times (a-c)^2 + \text{slant}(a)^2} = \sqrt{\frac{1}{4} \times ((a-c)^2 + (b-d)^2) + h^2}$

Figure 35 - – List of the formula used for volume calculation of the area contained the microalgae suspension for a volume of 30,113L (http://www.aqua-calc.com/calculate/volume-truncated-pyramid, accessed January 2015)

a: 0.5 b: 0.72 c: 0.48 d: 0.7 h: 0.09 meter

precision: 3 Calculate × Formulas | Surface to volume | Reference

Convert: volume to weight | volume to weight (food calculator)

show all units

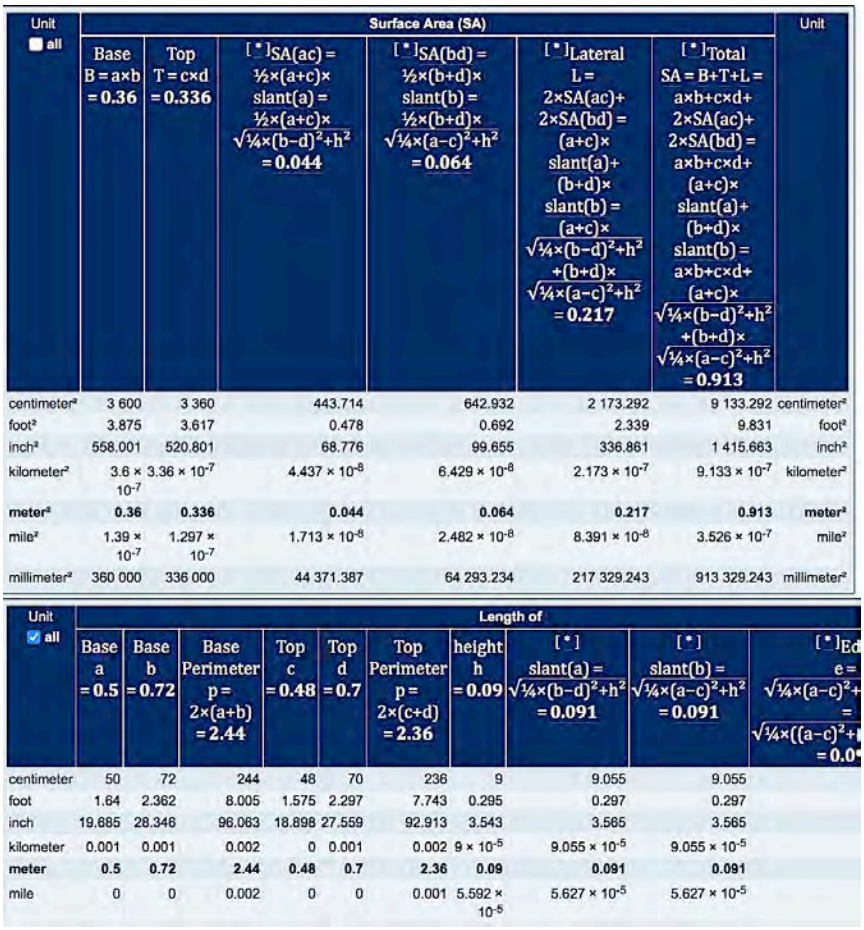
Volume
$V = 1 / 6 \times h \times (B + (a + c) \times (b + d) + T) = 1 / 6 \times h \times (a \times b + (a + c) \times (b + d) + c \times d) = 0.031$

angstrom³	$3.131 \times 10^{+28}$	microinch³	$1.911 \times 10^{+21}$
astronomical unit³	9.353×10^{-36}	micrometer³	$3.131 \times 10^{+16}$
centimeter³	31 314	micron³	$3.131 \times 10^{+16}$
chain³	3.846×10^{-6}	mil³	$1.911 \times 10^{+12}$
decimeter³	31.314	mile³	7.513×10^{-12}
dekaliter	3.131	milliliter	31 314
dekameter³	3.131×10^{-5}	millimeter³	31 314 000
fathom³	0.005	nanometer³	$3.131 \times 10^{+25}$
foot³	1.106	nautical mile³	4.93×10^{-12}
furlong³	3.846×10^{-9}	oil barrel	0.197
gigaliter	3.131×10^{-8}	parsec³	1.066×10^{-51}
hectometer³	3.131×10^{-8}	picometer³	$3.131 \times 10^{+34}$
Imperial gallon	6.888	teraliter	3.131×10^{-11}
inch³	1 910.898	thou³	$1.911 \times 10^{+12}$
kiloliter	0.031	US cup	132.357
kilometer³	3.131×10^{-11}	US fluid ounce	1 058.852
light year³	3.698×10^{-50}	US gallon	8.272
liter	31.314	US pint	66.178
megaliter	3.131×10^{-5}	US quart	33.089
meter³	**0.031**	US tablespoon	2 117.705
metric cup	125.256	US teaspoon	6 353.114
metric tablespoon	2 087.6	yard³	0.041
metric teaspoon	6 262.8		

[*] Surface to volume ratio
$R = SA \div V = 29.167$

Surface area to volume ratio is also known as *surface to volume ratio* and denoted as **sa/vol** or **SA:V**, where SA:V = surface area ÷ volume

Figure 36 – Different measurements and standards to present the volume for the area contained the microalgae suspension for a volume of 30,113L (http://www.aqua-calc.com/calculate/volume-truncated-pyramid, accessed January 2015)



Unit (all)	Surface Area (SA)						Unit
	Base B = a×b = 0.36	Top T = c×d = 0.336	[*]SA(ac) = ½×(a+c)× slant(a) = ½×(a+c)× √¼×(b−d)²+h² = 0.044	[*]SA(bd) = ½×(b+d)× slant(b) = ½×(b+d)× √¼×(a−c)²+h² = 0.064	[*]Lateral L = 2×SA(ac)+ 2×SA(bd) = (a+c)× slant(a)+ (b+d)× slant(b) = (a+c)× √¼×(b−d)²+h² +(b+d)× √¼×(a−c)²+h² = 0.217	[*]Total SA = B+T+L = a×b+c×d+ 2×SA(ac)+ 2×SA(bd) = a×b+c×d+ (a+c)× slant(a)+ (b+d)× slant(b) = a×b+c×d+ (a+c)× √¼×(b−d)²+h² +(b+d)× √¼×(a−c)²+h² = 0.913	
centimeter²	3 600	3 360	443.714	642.932	2 173.292	9 133.292	centimeter²
foot²	3.875	3.617	0.478	0.692	2.339	9.831	foot²
inch²	558.001	520.801	68.776	99.655	336.861	1 415.663	inch²
kilometer²	3.6 × 10⁻⁷	3.36 × 10⁻⁷	4.437 × 10⁻⁸	6.429 × 10⁻⁸	2.173 × 10⁻⁷	9.133 × 10⁻⁷	kilometer²
meter²	**0.36**	**0.336**	**0.044**	**0.064**	**0.217**	**0.913**	**meter²**
mile²	1.39 × 10⁻⁷	1.297 × 10⁻⁷	1.713 × 10⁻⁶	2.482 × 10⁻⁸	8.391 × 10⁻⁸	3.526 × 10⁻⁷	mile²
millimeter²	360 000	336 000	44 371.387	64 293.234	217 329.243	913 329.243	millimeter²

Unit (all)	Length of									
	Base a = 0.5	Base b = 0.72	Base Perimeter p = 2×(a+b) = 2.44	Top c = 0.48	Top d = 0.7	Top Perimeter p = 2×(c+d) = 2.36	height h = 0.09	[*] slant(a) = √¼×(b−d)²+h² = 0.091	[*] slant(b) = √¼×(a−c)²+h² = 0.091	[*]Ed e = √¼×(a−c)²+ = √¼×((a−c)²+ = 0.0
centimeter	50	72	244	48	70	236	9	9.055	9.055	
foot	1.64	2.362	8.005	1.575	2.297	7.743	0.295	0.297	0.297	
inch	19.685	28.346	96.063	18.898	27.559	92.913	3.543	3.565	3.565	
kilometer	0.001	0.001	0.002	0	0.001	0.002	9 × 10⁻⁵	9.055 × 10⁻⁵	9.055 × 10⁻⁵	
meter	**0.5**	**0.72**	**2.44**	**0.48**	**0.7**	**2.36**	**0.09**	**0.091**	**0.091**	
mile	0	0	0.002	0	0	0.001	5.592 × 10⁻⁶	5.627 × 10⁻⁵	5.627 × 10⁻⁵	

Figure 37 – Overall measurements and calculations to find the volume of the area contained the microalgae suspension for a volume of 30,113L (http://www.aqua-calc.com/calculate/volume-truncated-pyramid, accessed January 2015)

11.13 Equation 32

Volumetric productivity (VP), productivity per unit reactor volume (expressed as g/L*d)

$$VP = \frac{P * Vp}{Vo}$$

Where P is productivity in g/L*d, V_p is the volume predicted for the suspension inside the TPBR, here 31,31 L. V_o is the volume observed (remaining culture) in the TPBR.

11.14 Equation 33

Illuminated surface productivity (ISP) is productivity per unit of reactor illuminated surface area (expressed as g/m²*d).

$$ISP = \frac{P * Vo}{ISA}$$

Where P is productivity in g/L*d, V_o is the volume observed (remaining culture) in the TPBR, and ISA is illuminated surface area (the area occupied by the culture in the vessel), here 0,56 m^2.

11.15 Equation 34

Areal productivity (AP), i.e. productivity per unit of ground area occupied by the reactor (expressed as g/ m^2*d)

$$AP = \frac{P * Vo}{TSA}$$

Where P is productivity in g/L*d, V_o is the volume observed (remaining culture) inside the TPBR, in L, TSA is the total surface area or the ground area occupied by the TPBR, here 0,913 m^2.

12 Appendix 3. Calculation of growth rate from two points (Tabs. 15-45 and Figs. 38-67)

Data from TPBR 10 – *Chlorella wild mix Årungen.* Summer 2012. 46 days of semi-continuous cultivation.

In semi-continuous culture of microalgae, the dilutions made in this experiments was of a random pattern character. The most reasonable choice to use the data from days 34 to 37 is based on the longest possible period of exponential growth among the samples. Representing the highest net increase in biomass. It is probably, easier to observe the effect of dilution on the reduction of the value of the calculated growth rate. By means of a pair of values for X1 and Xo, reads off the number of divisions during the interval T1 and T2, and calculate k (explained in the figure legend), The best estimate appears to correspond to the period day 33 to day 39

Table 15 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for fresh water microalgae *Chlorella wild mix Årungen* (TPBR 10), cultivated in semi-continuous culture for 46 days. Summer 2012. (Appendix 1. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (33^{th})	3-4 (36^{th}) [b]	4-5 (37^{th})	5-6 (38^{th})	6-7 (39^{th})
X_1/X_0	1,100	0,943	1,451	1,205	0,757
$\ln(X_1/X_0)$	0,095	-0,058	0,372	0,187	-0,280
$\log_2(X_1/X_0)$	0,137	-0,084	0,538	0,270	-0,401
$\Delta_t = (t_2 - t_1)$	1	3	1	1	1
r (Eq.1)[a]	0,095	-0,019	0,373	0,187	-0,280
k (Eq.2)	0,150	-0,030	0,583	0,292	-0,435
k (Eq.3)	0,137	-0,028	0,538	0,270	-0.401
T_2 (Eq.4)	6,7	-32,853	1,715	3,420	-2,280

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. Eq. = Equations (Appendix, Equations 25, 26, 27 and 28)

[b] Day 34^{th}, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 16 – Growth curve for *Chlorella wild mix Årungen,* TPBR 10

Day	**0**	**1**	**2**	**3**	**4**
TSS (g/L)	**0,969**	**0,914**	**1,327**	**1,6**	**1,211**

S

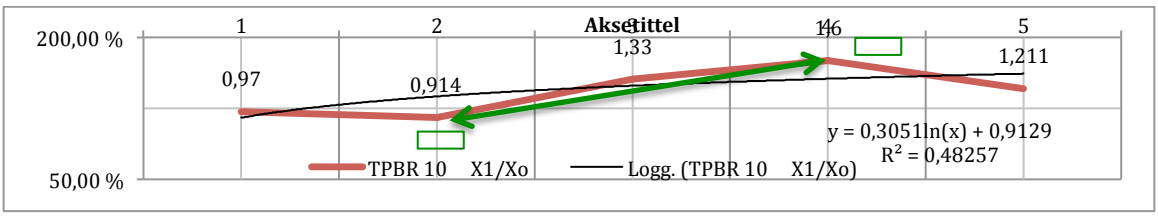


Figure 38 – Specific growth of *Chlorella wild mix Årungen.* Presented as semi-log plot using the data in table 10.1. The black line represents the exponential growth in a log 2.0-scale. The straight green line A-´A" represents a quick observation on growth and fitted by eye.

Figure 15 shows that the biomass did not grow during the weekend, but decreased from Friday (day 0) to Monday (day 1). The population growth was not registered during Saturday and Sunday, yet the plot of the two points registered a 5,7% less production of biomass. There was almost one doubling (45,2% increase) between day 1 and day 2. And similar doubling rate (20,6% increase) between day 2 and day 3. The population again decreased (32%) between day 3 and day 4. If the plot were being strictly maintained daily, this with certainty, have altered the possibility to appreciate the start of exponential phase between day 1 and day 2. Depending just on the basis of these data points, is easy to speculate and fail about more than a doubling time per day. In spite of the severe dilution rate, during the same week, of 14, 18 and 10 L removed culture, the population grew constantly and produced biomass at a rate at almost a half of a doubling time at day 3 and a quarter of a doubling time at day 4.

Table 17 - Computation of population division rate (k) and population doubling time (T_2) for *Chlorella wild mix Årungen,* TPBR 10 based on data from tables 15 and 16

X	**0**	**1**	**2**	**3**	**4**	**log y**
2^X	**1,96**	**1,88**	**2,51**	**3,03**	**2,31**	**y**

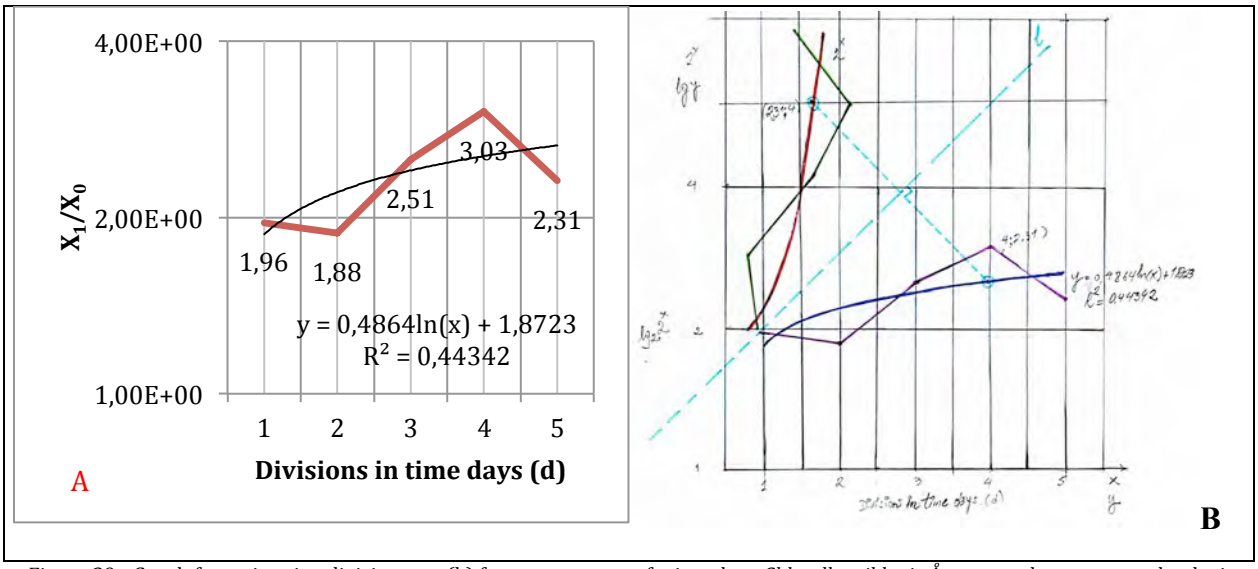


Figure 39 - Graph for estimating division rate (k) from two counts of microalgae *Chlorella wild mix Årungen* culture, assumed to be in exponential growth. A. The ratio X_1/X_0 of the counts at times t_2 and t ($\Delta = t_2 - t_1$, expressed in days) is located on the X-axis. B. The corresponding abscissa then yields the number of divisions in the time interval Δt needed to produce the increments in N. Divide by Δt to obtain k as divisions per day. The function plotted is $y = 2^x$, $x \leq 5$. (Inverse function of y=2^x)

Figure 36B shows that 2^X and lg^X are inverted functions. Usually X is used as argument for the logarithm function. The equivalence between the points plotted on the graph (4; 2,31) and (2,31; 4) demonstrates it (appendix Eq. 30.1 and 30.2). This results shows growth disturbed by the dilution rates. (In this computation, the values of the three dilutions, which happened during this period, has not been included yet)

Table 18 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for fresh water microalgae *Chlorella vulgaris* (TPBR 1), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12). (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (18^{th})	3-4 (19^{th}) [b]	4-5 (20^{th})	5-6 (21^{th})	6-7 (22^{th})
X_1/X_0	1,2153	1,186	1,066	1,043	0,990
$\ln(X_1/X_0)$	0,195	0,170	0,064	0,042	-0,010
$\log_2(X_1/X_0)$	0,281	0,246	0,092	0,061	-0,014
$\Delta_t = (t_2 - t_1)$	3	1	1	1	3
r (Eq.19)[a]	0,065	0,170	0,064	0,042	-0,003
k (Eq.20)	0,102	0,266	0,099	0,066	-0.005
k (Eq.21)	0,031	0,245	0,092	0,061	-0,005
T_2 (Eq.22)	9,834	3,755	10,028	15,186	-198,11

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b] Day 18^{th}, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 19 - TPBR 1 - *Chlorella vulgaris* – Fresh water algae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 18^{th} – 22th, age of culture (10.07.12-14.07.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 1, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Equations 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 18-22 B= (g/L)	Day of culture (days)	Doubling time. Days 18-22	log $y=2^B$
18	0,256	0	1,19416	
19	0,31111	1	1,24066	
20	0,36883	2	1,29130	
21	0,39310	3	1,31321	
22	0,41	4	1,32868	

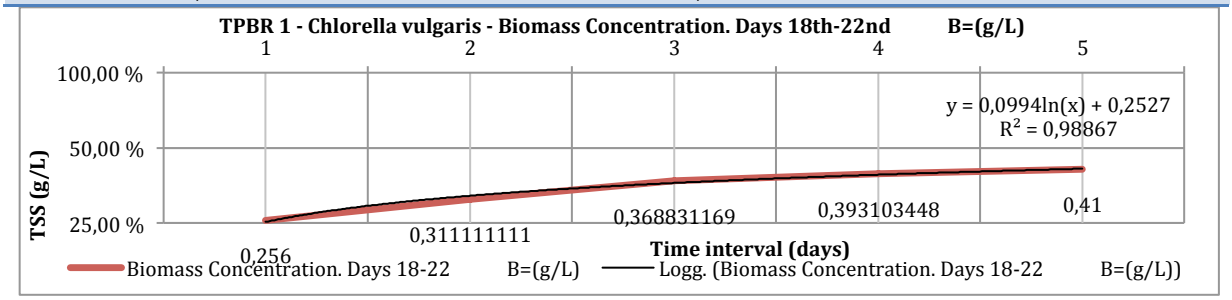


Figure 40 - Biomass concentration sampled the 18^{th}-22^{nd} age of culture of fresh water algae *Chlorella vulgaris*, grown in semi-continuous cultivation system in TPBR 1, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Equations 30.1 and 30.2)

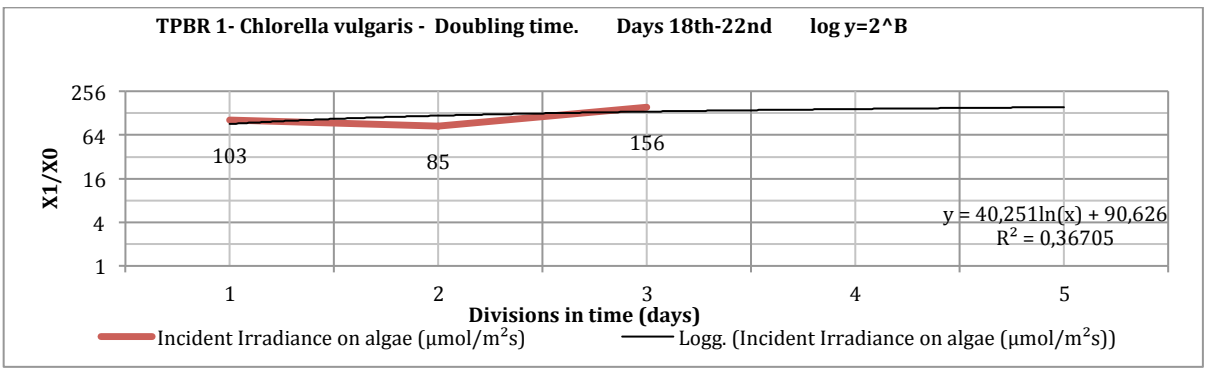


Figure 41 – Doubling time registered 18^{th}-22^{nd} age of culture of fresh water algae *Chlorella vulgaris*, grown in semi-continuous cultivation system in TPBR 1, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Equations 30.1 and 30.2)

Table 20 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for fresh water microalgae *Chlorella vulgaris* (TPBR 1), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12). (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (54th)	3-4 (55th) [b]	4-5 (56th)	5-6 (57th)	
X_1/X_0	0,973	1,16	1,254	1,017	
$\ln(X_1/X_0)$	-0,023	0,147	0,226	0,017	
$\log_2(X_1/X_0)$	-0,04	0,212	0,326	0,024	
$\Delta_t = (t_2 - t_1)$	3	1	1	1	
r (Eq.19)[a]	-0,01	0,147	0,226	0,017	
k (Eq.20)	-0,015	0,23	0,354	0,026	
k (Eq.21)	-0,004	0,212	0,326	0,024	
T_2 (Eq.22)	-69,04	4,35	2,83	38,524	

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b] Day54th, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 21 - TPBR 1 - *Chlorella vulgaris* – Fresh water algae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 54th – 57th, age of culture (14.08.12-17.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 1, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Equations 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 55-58 B=(g/L)	Number days (days)	Doubling time. Days 55-58 log y=2^B
54	0,84117	0	1,79151
55	0,97435	1	1,96476
56	1,22142	2	2,33177
57	1,24186	3	2,36503

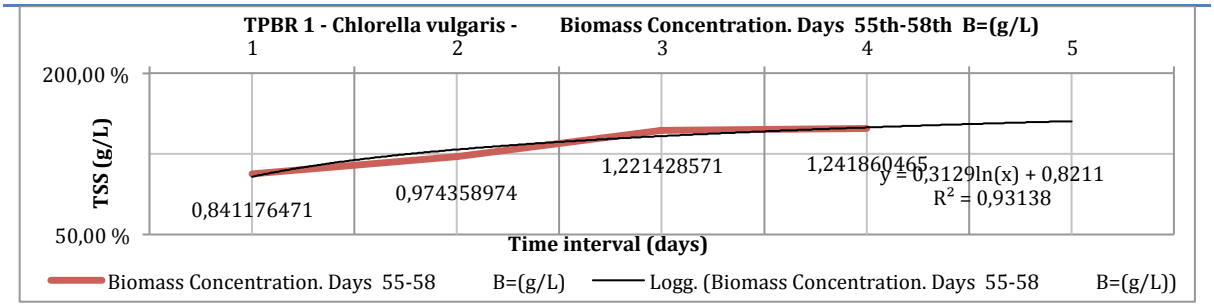


Figure 42 - Biomass concentration sampled the 18th-22nd age of culture of fresh water algae *Chlorella vulgaris*, grown in semi-continuous cultivation system in TPBR 1, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

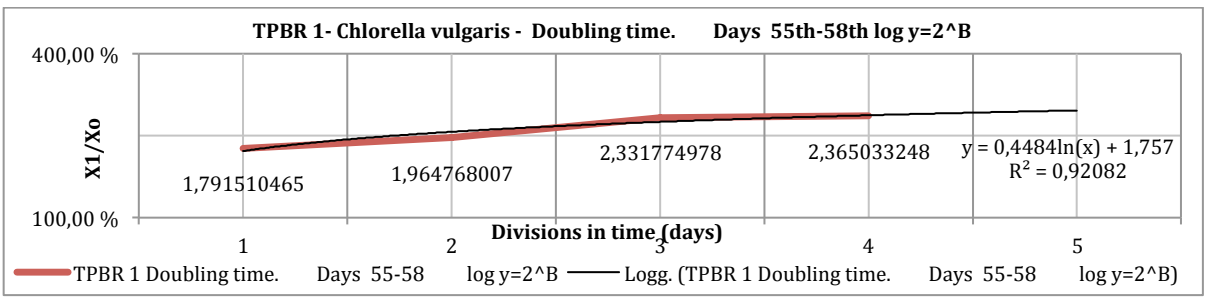


Figure 43 - Doubling time registered 18th-22nd age of culture of fresh water algae *Chlorella vulgaris*, grown in semi-continuous cultivation system in TPBR 1, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 22 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for fresh water microalgae *Chlorella vulgaris* (TPBR 2), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12). (Appendix 2. Eq. 21, 22, 23 and 24)

Table 12.2.1.1 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for fresh water microalgae *Chlorella vulgaris* (TPBR 2), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12). (Appendix 2. Eq. 21, 22, 23 and 24)					
Time interval (days) Age of culture, day number	0-1 (55^{th})	3-4 (56^{th}) [b]	4-5 (57^{th})	5-6 (58^{th})	6-7 (59^{th})
X_1/X_0	1,35	1,052	1,021	1,18	1,44
$\ln(X_1/X_0)$	-0,03	0,05	0,19	0,18	0,29
$\log_2(X_1/X_0)$	0,43	0,07	0,274	0,239	0,412
$\Delta_t = (t_2 - t_1)$	3	1	1	1	3
r (Eq.19)[a]	0,10	0,05	0,19	0,17	0,09
k (Eq.20)	0,16	0,08	0,30	0,26	0,15
k (Eq.21)	0,14	0,07	0,27	0,24	0,14
T_2 (Eq.22)	6,38	12,67	3,37	3,87	6,72
[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28) [b] Day55^{th}, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days					

Table 23 - TPBR 2 - *Chlorella vulgaris* – Fresh water algae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 55^{th} – 59th, age of culture (14.08.12-18.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 2, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 55-59 B=(g/L)	Number of days (days)	Doubling time. Days 55-59 log y=2^B
55	0,80526	0	1,74746
56	1,0875	1	2,12505
57	1,14375	2	2,20954
58	1,38285	3	2,60784
59	1,63125	4	3,09781

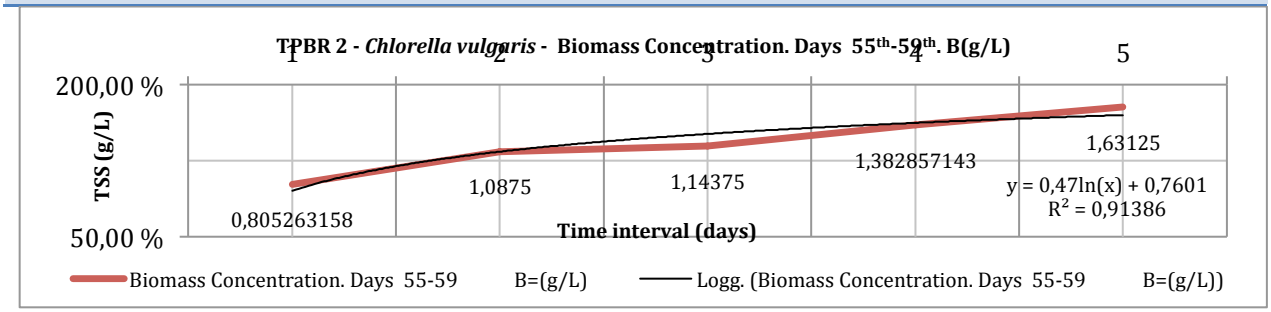


Figure 44 - Biomass concentration sampled the 55^{th}-59^{nd} age of culture of fresh water algae *Chlorella vulgaris*, grown in semi-continuous cultivation system in TPBR 2, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

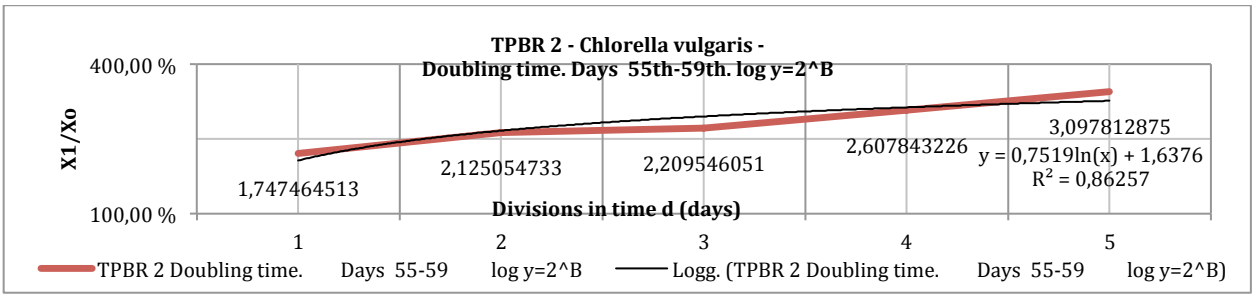


Figure 45 - Doubling time registered 55^{th}-59^{nd} age of culture of fresh water algae *Chlorella vulgaris*, grown in semi-continuous cultivation system in TPBR 2, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 24 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for marine microalgae *Dunaliella salinas* (TPBR 3), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12). (Appendix 1. (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (56th)	1-2 (57th) [b]	2-3 (58th)	3-4 (59th)	
X_1/X_0	0,71	1,05	1,14	1,42	
$\ln(X_1/X_0)$	-0,34	0,05	0,13	0,35	
$\log_2(X_1/X_0)$	-0,49	0,07	0,18	0,51	
$\Delta_t = (t_2 - t_1)$	3	1	1	1	
r (Eq.19)[a]	-0,11	0,05	0,13	0,35	
k (Eq.20)	-0,18	0,08	0,19	0,55	
k (Eq.21)	-0,17	0,07	0,18	0,51	
T_2 (Eq.22)	-5,56	12,86	5,00	1,82	

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

Table 25 - TPBR 3 - *Dunaliella salinas* - marine microalgae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 55th – 59th, age of culture (14.08.12-18.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 3, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 55-59 B=(g/L)	Day of culture (days)	Doubling time. Days 55-59 log y=2^B
56	1,36	0	2,56685
57	1,429268	1	2,69310
58	1,624	2	3,08228
59	2,30625	3	4,94595

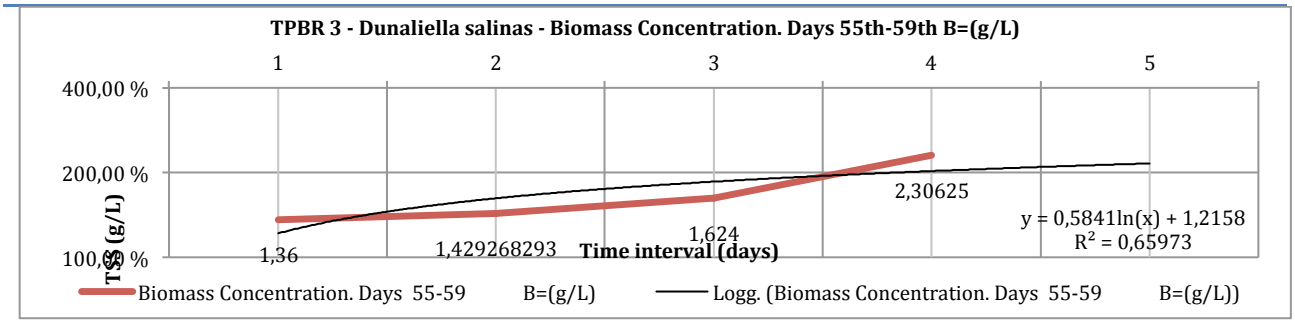


Figure 46 - Biomass concentration sampled the 55th-59th age of culture of marine algae *Dunaliella salinas,* grown in semi-continuous cultivation system in TPBR 3, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

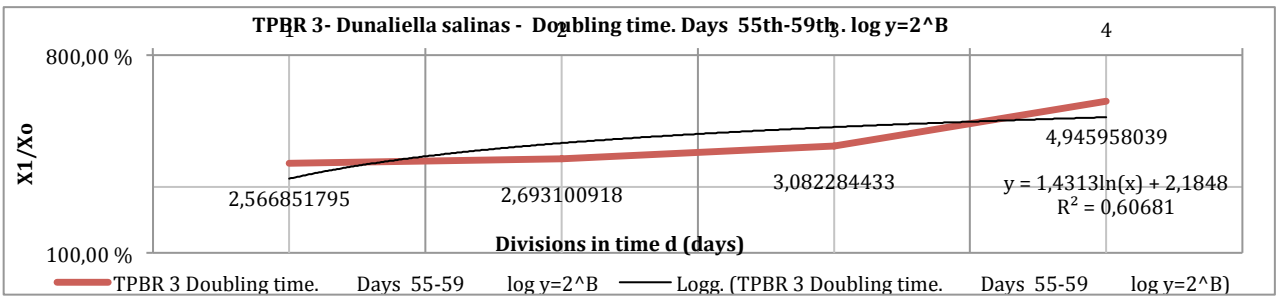


Figure 47 - Doubling time registered the 55th-59th age of culture of marine algae *Dunaliella salinas,* grown in semi-continuous cultivation system in TPBR 3, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 26 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for marine microalgae *Dunaliella salinas* (TPBR 4), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12). (Appendix 1. (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (19^{th})	3-4 (20^{th}) [b]	4-5 (21^{th})	5-6 (22^{th})	
X_1/X_0	0,98	1,14	1,08	1,78	
$\ln(X_1/X_0)$	-0,02	0,13	0,07	0,58	
$\log_2(X_1/X_0)$	-0,03	0,19	0,11	0,83	
$\Delta_t = (t_2 - t_1)$	3	1	1	1	
r (Eq.19)[a]	-0,01	0,13	0,07	0,58	
k (Eq.20)	-0,01	0,20	0,12	0,91	
k (Eq.21)	-001	0,19	0,11	0,83	
T_2 (Eq.22)	-105,06	4,94	8,71	1,10	

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b] Day 19^h, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 27 - TPBR 4 – *Dunaliella salinas* – marine algae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 19^{th} – 22th, age of culture (09.07.12-12.07.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 4, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 19-22 B=(g/L)	Number of days (days)	Doubling time. Days 19-22 log y=2^B
19	0,39677	0	1,31656
20	0,45161	1	1,36756
21	0,486	2	1,40055
22	0,86666	3	1,82344

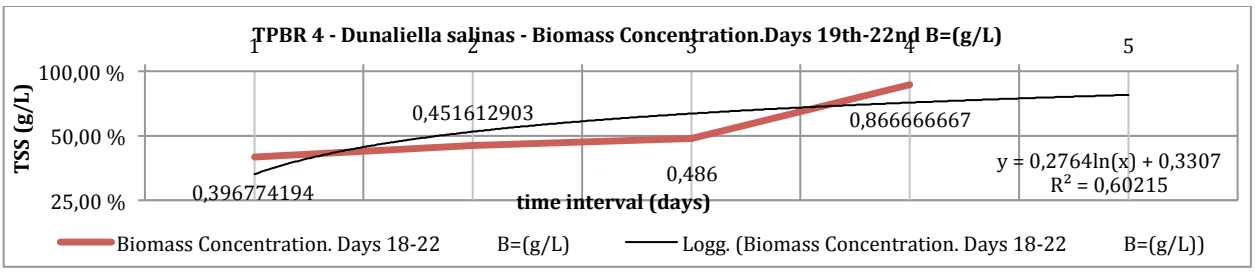


Figure 48 - Biomass concentration sampled the 18^{th}-22^{th} age of culture of marine algae *Dunaliella salinas,* grown in semi-continuous cultivation system in TPBR 4, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

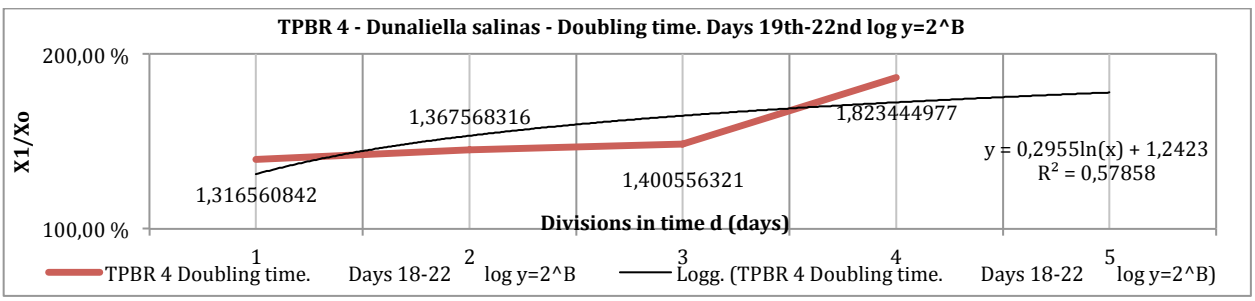


Figure 49 - Doubling time registered the 18th-22^{th} age of culture of marine algae *Dunaliella salinas*, grown in semi-continuous cultivation system in TPBR 4, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 28 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for marine microalgae *Dunaliella salinas* (TPBR 4), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12). (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (55th)	3-4 (56th) [b]	4-5 (57th)	5-6 (58th)	6-7 (59th)
X_1/X_0	1,1	1,07	1,06	1,16	1,43
ln (X_1/X_0)	0,08	0,06	0,06	0,15	0,36
$\log_2(X_1/X_0)$	0,11	0,09	0,9	0,21	0,52
$\Delta_t = (t_2 - t_1)$	3	1	1	1	3
r (Eq.19)[a]	0,03	0,06	0,06	0,15	0,12
k (Eq.20)	0,04	0,1	0,1	0,23	0,19
k (Eq.21)	0,04	0,09	0,09	0,21	0,17
T_2 (Eq.22)	24,66	10,03	10,40	4,38	5,36

[a]ssuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b]ay 55th, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 29 - TPBR 4 – *Dunaliella salinas* - marine microalgae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 55th – 59th, age of culture (14.08.12-18.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 4, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 55-59 B=(g/L)	Number of days (days)	Doubling time. Days 55-59 log y=2^B
55	0,89230	0	1,85614
56	0,96444	1	1,95131
57	1,02790	2	2,03906
58	1,09302	3	2,13320
59	1,26486	4	2,40304

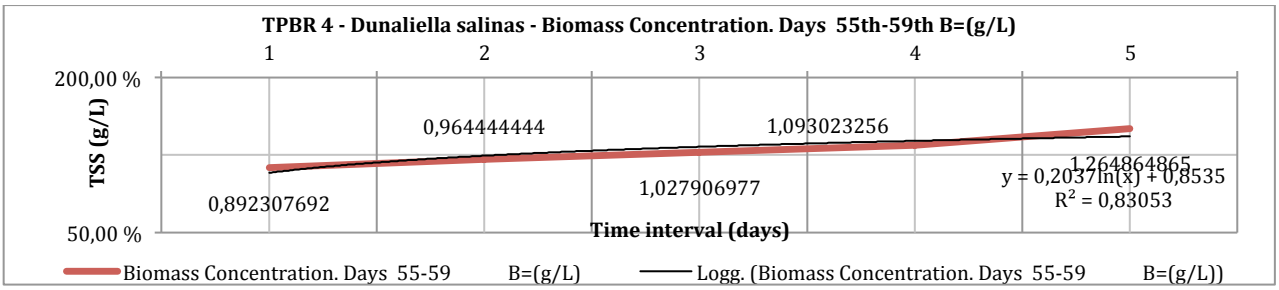


Figure 50 - Biomass concentration sampled the 55th-59th age of culture of marine algae *Dunaliella salinas,* grown in semi-continuous cultivation system in TPBR 4, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

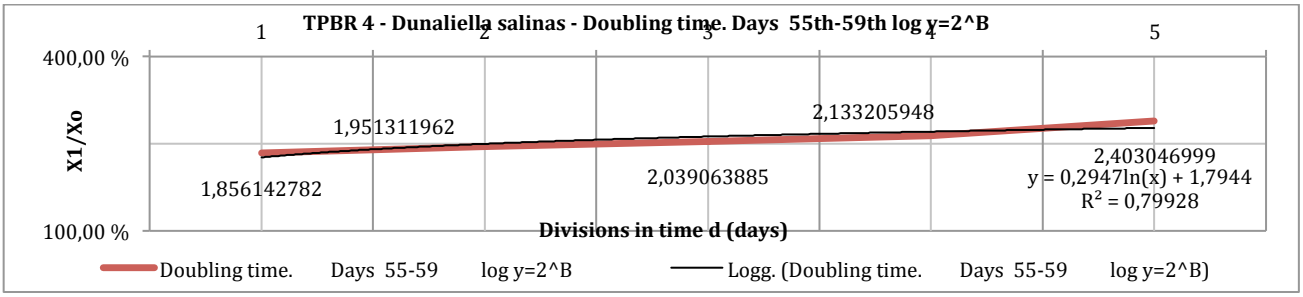


Figure 51 - Doubling time registered the 55th-59th age of culture of marine algae *Dunaliella salinas,* grown in semi-continuous cultivation system in TPBR 4, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 30 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for marine microalgae *Nannochloropsis oculata* (TPBR 5), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (4^{th})	3-4 (5^{th}) [b]	4-5 (6^{th})	5-6 (7^{th})	
X_1/X_0	1,26	1,21	1,20	0,81	
$\ln(X_1/X_0)$	0,23	0,19	0,18	´0,21	
$\log_2(X_1/X_0)$	0,33	0,28	0,26	-030	
$\Delta_t = (t_2 - t_1)$	3	1	1	1	
r (Eq.19)[a]	0,08	0,19	0,18	-0,21	
k (Eq.20)	0,12	0,30	0,28	-0,32	
k (Eq.21)	0,11	0,28	0,26	-0,30	
T_2 (Eq.22)	8,24	3,33	3,53	-3,09	

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b] Day 25^{th}, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 31 - TPBR 5 - *Nannochloropsis oculata* – marine microalgae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 4^{th} – 7^{th}, age of culture (25.06.12-28.06.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 5, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 4-7 B=(g/L)	Number of days (days)	Doubling time. Days 4-7 log y=2^B
4	0,37123	0	1,29345
5	0,46716	1	1,38238
6	0,566	2	1,48041
7	0,67826	3	1,60020

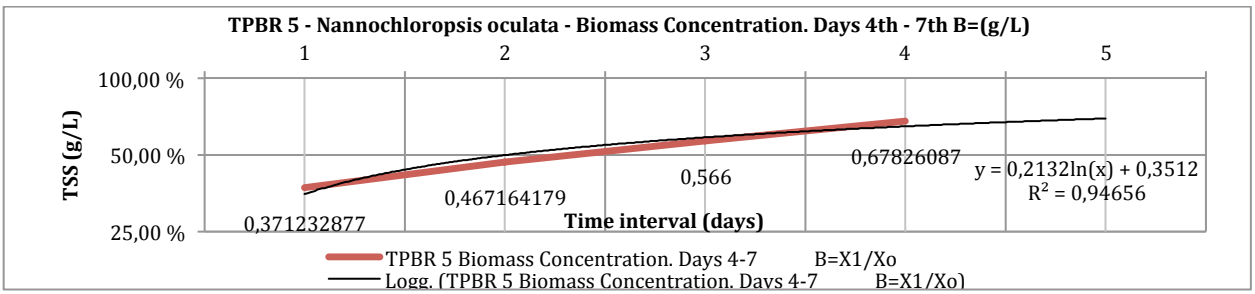


Figure 52 - Biomass concentration sampled the 4^{th}-7^{th} age of culture of marine algae *Nannochloropsis oculata*, grown in semi-continuous cultivation system in TPBR 5, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

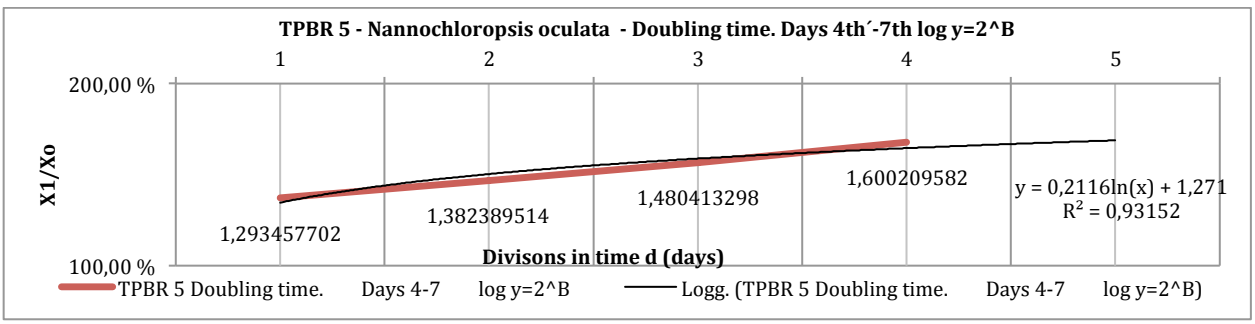


Figure 53 - Doubling time registered the 4th-7^{th} age of culture of marine algae Nannochloropsis oculata, grown in semi-continuous cultivation system in TPBR 5, for a time interval of 63 days (20.06.12-23.08.12 (Appendix 2. Eq. 30.1 and 30.2)

Table 32 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for marine microalgae *Nannochloropsis oculata* (TPBR 5), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12). (Eq. = Equation) (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (56^{th})	1-2 (57^{th})	2-3 (58^{th})	3-4 (59^{th})	
X_1/X_0	0,67	1,15	1,06	1,25	
$\ln(X_1/X_0)$	-0,40	0,14	0,06	0,23	
$\log_2(X_1/X_0)$	-0,58	0,20	0,09	0,33	
$\Delta_t = (t_2 - t_1)$	3	1	1	1	
r (Eq.19)[a]	-0,13	0,14	0,06	0,23	
k (Eq.20)	-0,21	0,22	0,93	0,36	
k (Eq.21)	-0,19	0,20	0,09	0,33	
T_2 (Eq.22)	-4,75	4,69	10,75	2,81	

[a]ssuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

Table 33 - TPBR 5 - *Nannochloropsis oculata* – marine microalgae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 55^{th} – 59th, age of culture (14.08.12-17.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 5, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Days 56-59	Concentration. B=(g/L)	Number of days (days)	Doubling time.	Days 56-59	log y=2^B
56	1,378		0	2,59907		
57	1,57894		1	2,98751		
58	1,67567		2	3,19468		
59	2,10285		3	4,29559		

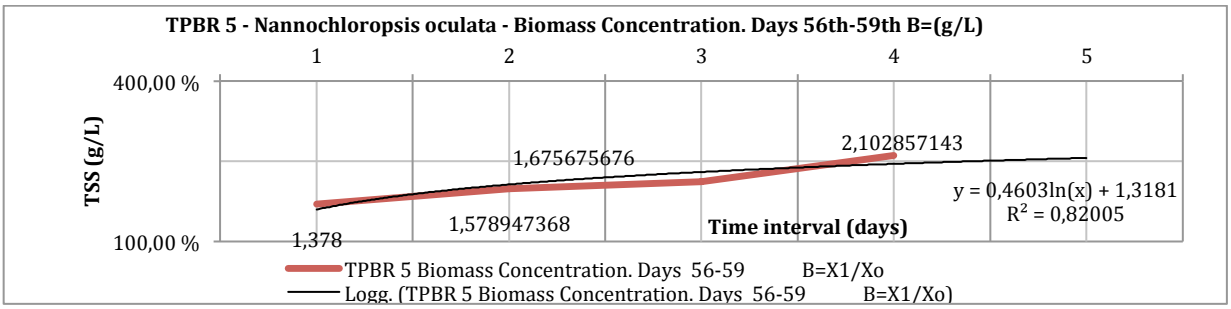


Figure 54 - Biomass concentration sampled the 56^{th}-59^{th} age of culture of marine algae *Nannochloropsis oculata*, grown in semi-continuous cultivation system in TPBR 5, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

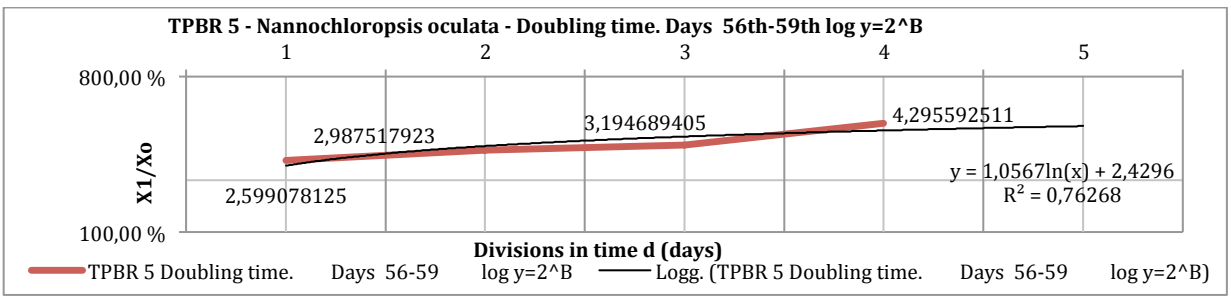


Figure 55 - Doubling time registered the 56th-59^{th} age of culture of marine algae Nannochloropsis oculata, grown in semi-continuous cultivation system in TPBR 5, for a time interval of 63 days (20.06.12-23.08.1) (Appendix 2. Eq. 30.1 and 30.2)

Table 34 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for marine microalgae *Nannochloropsis oculata* (TPBR 6), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (19th)	3-4 (20th)	4-5 (21th)	5-6 (22nd)	
X_1/X_0	0,51	1,07	1,31	1,16	
$\ln(X_1/X_0)$	-0,68	0,06	0,27	0,15	
$\log_2(X_1/X_0)$	-0,98	0,09	0,39	0,21	
$\Delta_t = (t_2 - t_1)$	3	1	1	1	
r (Eq.19)[a]	-0,23	0,06	0,27	0,15	
k (Eq.20)	-0,35	0,10	0,42	0,23	
k (Eq.21)	-0,33	0,09	0,39	0,21	
T_2 (Eq.22)	-2,82	9,96	2,37	4,36	

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b] Day 19th, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 35 - TPBR 6 – *Nannochloropsis oculata* – marine microalgae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 19th – 22th, age of culture (09.07.12-12.07.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 6, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration Days 19-22 B = (g/L)	Number of days (days)	Doubling time. Days 19-22 log y = 2^B
19	0,505	0	1,41912
20	0,53846	1	1,45242
21	0,70540	2	1,63060
22	0,81666	3	1,76133

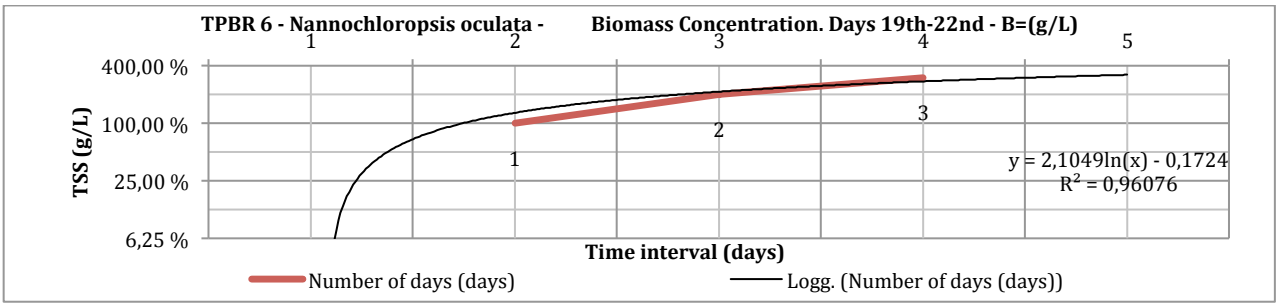


Figure 56 - Biomass concentration sampled the 19th-22nd age of culture of marine algae *Nannochloropsis oculata*, grown in semi-continuous cultivation system in TPBR 6, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

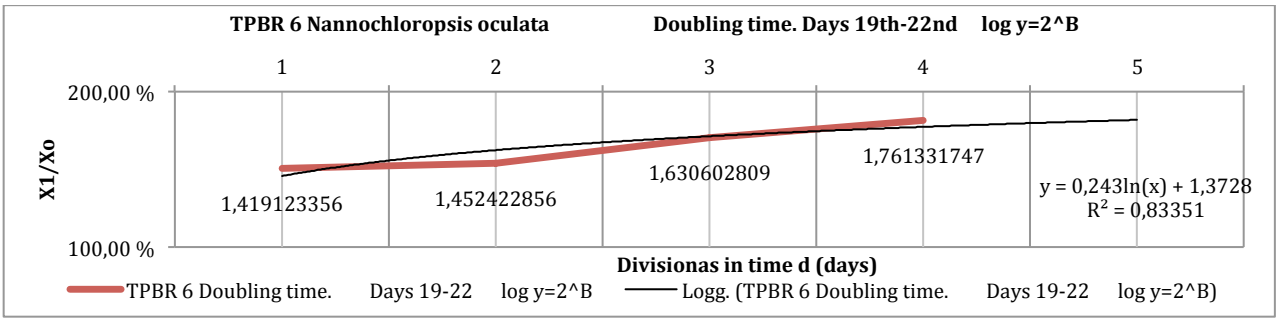


Figure 57 - Doubling time registered the 19th-22nd age of culture of marine algae Nannochloropsis oculata, grown in semi-continuous cultivation system in TPBR 6, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 36 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for marine microalgae *Nannochloropsis oculata* (TPBR 6), cultivated in semi-continuous production system for 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (55th)	3-4 (56th) [b]	4-5 (57th)	5-6 (58th)	6-7 (59th)
X_1/X_0	0,97	1,16	1,23	1,03	1,44
$\ln(X_1/X_0)$	-0,03	0,15	0,21	0,03	0,36
$\log_2(X_1/X_0)$	-0,04	0,21	0,30	0,05	0,52
$\Delta_t = (t_2 - t_1)$	3	1	1	1	3
r (Eq.19)[a]	-0.01	0,15	0,21	0,03	0,12
k (Eq.20)	-0,01	0,23	0,36	0,05	0,19
k (Eq.21)	-0,01	0,21	0,31	0,05	0,17
T_2 (Eq.22)	-69,04	4,35	3,07	18,64	5,29

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b] Day 34th, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 37 - TPBR 6 – *Nannochloropsis oculata* – marine microalgae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 56th – 59th, age of culture (14.08.12-17.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 6, for a total time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration Days 56-59 B = (g/L)	Number of days (days)	Doubling time. Days 56-59. log y = 2^B
56	0,84117	0	1,79151
57	0,97435	1	1,96476
58	1,2	2	2,29739
59	1,24186	3	2,36503

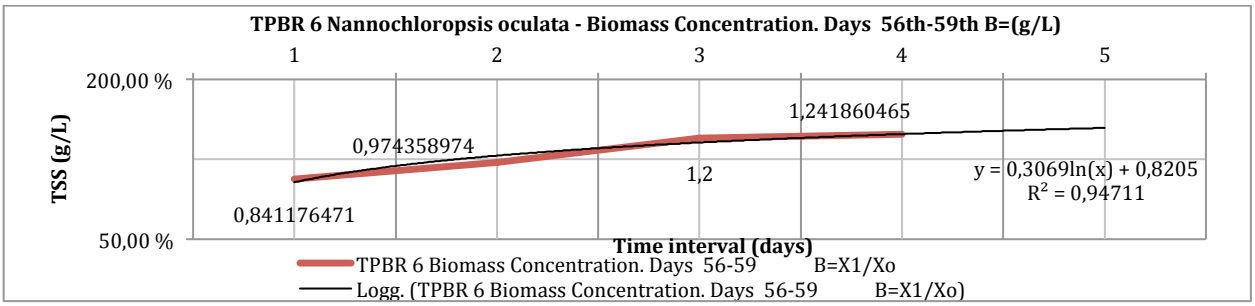


Figure 58 - Biomass concentration sampled the 56th-59nd age of culture of marine algae *Nannochloropsis oculata*, grown in semi-continuous cultivation system in TPBR 6, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

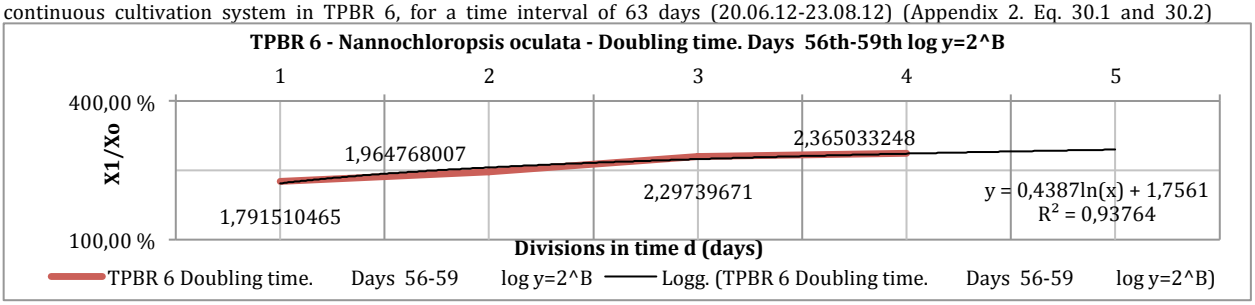


Figure 59 - Doubling time registered the 56th-59th age of culture of marine algae *Nannochloropsis oculata*, grown in semi-continuous cultivation system in TPBR 6, for a time interval of 63 days (20.06.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 38 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for fresh water microalgae *Scenedesmus sp* (TPBR 7), cultivated in semi-continuous production system for 24 days (11.07.12-23.08.12) (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (34^{th})	3-4 (35^{th}) [b]	4-5 (36^{th})	5-6 (37^{th})	
X_1/X_0	3,36	3,86	1,19	1,4	
$\ln(X_1/X_0)$	1,21	1,34	0,17	0,33	
$\log_2(X_1/X_0)$	1,75	1,94	0,25	0,48	
$\Delta_t = (t_2 - t_1)$	3	1	1	1	
r (Eq.19)[a]	0,40	1,34	0,17	0,33	
k (Eq.20)	0,63	2,10	0,27	0,52	
k (Eq.21)	0,6	1,94	0,25	048	
T_2 (Eq.22)	1,58	0,48	3,72	1,94	

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b] Day 34^{th}, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 39 - TPBR 7 - *Scenedesmus sp.* – Fresh water algae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 34^{th} – 37th, age of culture (14.08.12-17.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 7, for a total time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 34-37 B=(g/L)	Number of days (days)	Doubling time. Days 34-37 log y=2^B
34	0,336	0	1,26225
35	1,28888	1	2,44339
36	1,53043	2	2,88872
37	2,128	3	4,37111

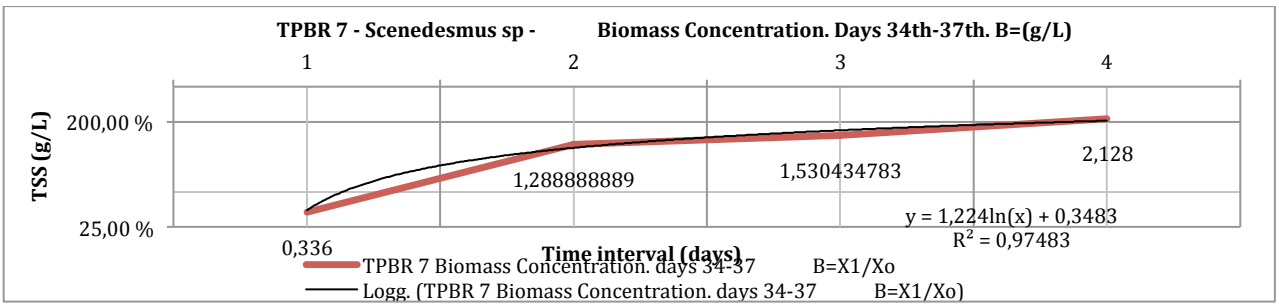


Figure 60 - Biomass concentration sampled the 34^{th}-37^{th} age of culture (14.08.12-17.08.12) of fresh water *Scenedesmus sp.,* grown in semi-continuous cultivation system in TPBR 7, for a time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

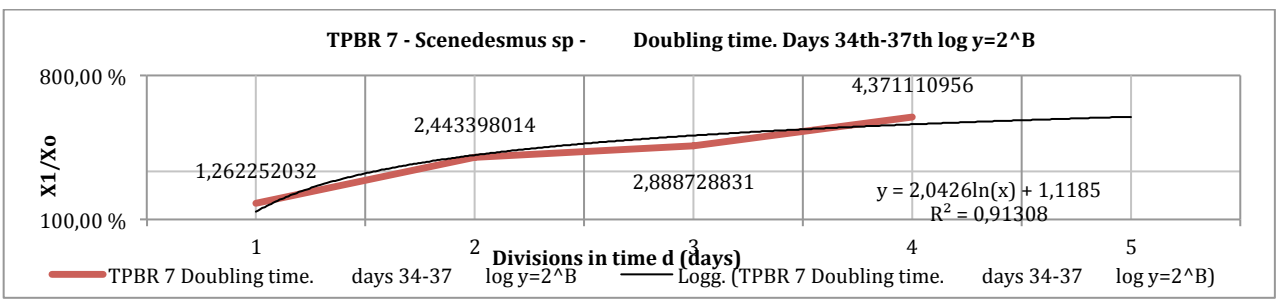


Figure 61 - Doubling time registered 34^{th}-37^{nd}, age of culture (14.08.12-17.08.12) of fresh water algae *Scenedesmus sp.,* grown in semi-continuous cultivation system in TPBR 7, for a total time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 40 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for fresh water microalgae *Scenedesmus sp* (TPBR 8), cultivated in semi-continuous production system for 24 days (11.07.12-23.08.12) (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (40th)	3-4 (41nd) [b]	4-5 (42th)		
X_1/X_0	1,51	1,96	1,10		
$\ln(X_1/X_0)$	0,41	0,67	0,10		
$\log_2(X_1/X_0)$	0,59	0,97	0,14		
$\Delta_t = (t_2 - t_1)$	1	3	1		
r (Eq.19)[a]	0,41	0,22	0,10		
k (Eq.20)	0,64	0,35	0,15		
k (Eq.21)	0,59	0,32	0,14		
T_2 (Eq.22)	1,56	2,85	6,70		

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b] Day 40th, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 41 - TPBR 8 - *Scenedesmus sp.* – Fresh water algae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 40th – 42th, age of culture (21.08.12-23.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 8, for a total time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 40-42 B =(g/L)	Number of days (days)	Doubling time. Days 40-42 log $y=2^B$
40	0,30270	0	1,09951
41	0,59285	1	2,19902
42	0,65217	2	4,39805

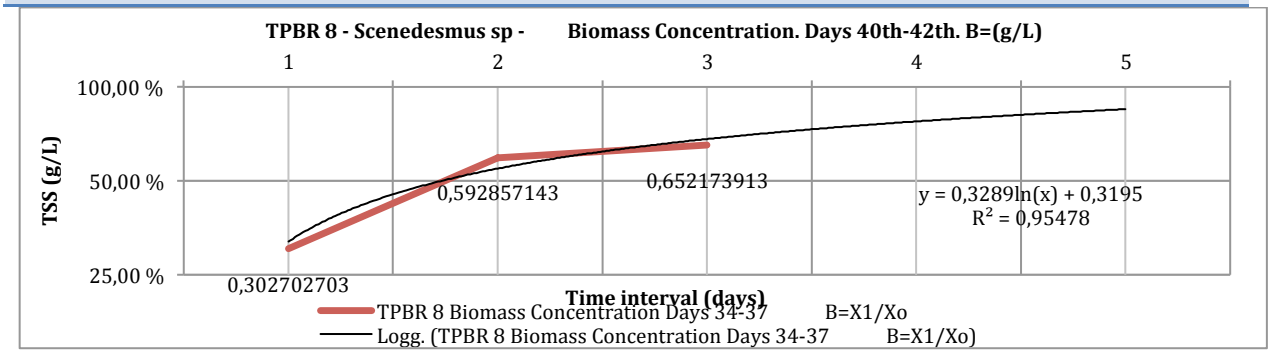


Figure 62 - Biomass concentration sampled the 40th-42nd, age of culture (21.08.12-23.08.12) of fresh water *Scenedesmus sp.,* grown in semi-continuous cultivation system in TPBR 8, for a time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

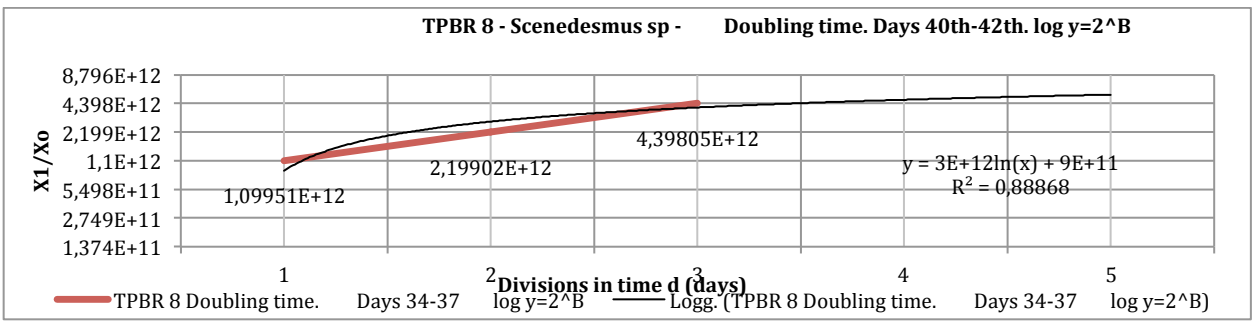


Figure 63 - Doubling time registered 40th-42nd, age of culture (21.08.12-23.08.12) of fresh water algae *Scenedesmus sp.,* grown in semi-continuous cultivation system in TPBR 8, for a total time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 42 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for fresh water microalgae *Chlorella wild mix Årungen* (TPBR 9), cultivated in semi-continuous production system for 24 days (11.07.12-23.08.12) (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (34^{th})	1-2 (35^{th})	2-3 (36^{th})	3-4 (37^{th})	
X_1/X_0	1,01	1,65	1,02	1,05	
$\ln(X_1/X_0)$	0,01	0,51	0,02	0,05	
$\log_2(X_1/X_0)$	0,01	0,72	0,03	0,07	
$\Delta_t = (t_2 - t_1)$	3	1	1	1	
r (Eq.19)[a]	0,002	0,50	0,02	0,05	
k (Eq.20)	0,003	0,78	0,03	0,08	
k (Eq.21)	0,003	0,72	0,03	0,07	
T_2 (Eq.22)	327,67	1,27	32,62	12,95	

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

Table 43 - TPBR 9 – *Chlorella wild mix Årungen* – Fresh water algae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 34^{th} – 37th, age of culture (14.08.12-17.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 9, for a total time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration Days 34-37 B=(g/L)	Number of days (days)	Doubling time. Days 34-37 log y=2^B
34	0,857	0	1,81126
35	1,415	1	2,66659
36	1,443	2	2,71885
37	1,516	3	2,85996

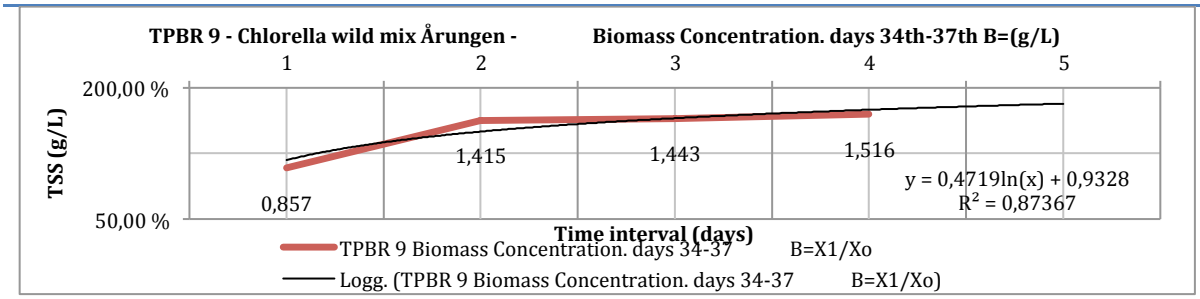


Figure 64 - Biomass concentration sampled the 34^{th}-37^{nd}, age of culture (14.08.12-17.08.12) of fresh water algae *Chlorella wild mix Årungen*, grown in semi-continuous cultivation system in TPBR 9, for a total time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

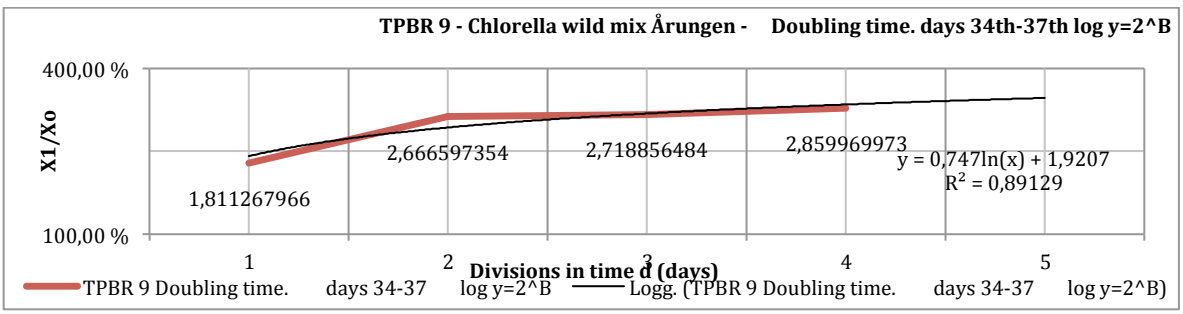


Figure 65 - Doubling time registered 34^{th}-37^{nd}, age of culture (14.08.12-17.08.12) of fresh water algae *Chlorella wild mix Årungen*, grown in semi-continuous cultivation system in TPBR 9, for a total time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Table 44 - Computation of population growth rate (r), divisions per day (k), and population doubling time (T_2) for fresh water microalgae *Chlorella wild mix Årungen* (TPBR 10), cultivated in semi-continuous production system for 24 days (11.07.12-23.08.12) (Appendix 2. Eq. 21, 22, 23 and 24)

Time interval (days) Age of culture, day number	0-1 (33^{th})	3-4 (36^{th}) [b]	4-5 (37^{th})	5-6 (38^{th})	6-7 (39^{th})
X_1/X_0	1,100	0,943	1,451	1,205	0,757
$\ln(X_1/X_0)$	0,095	-0,058	0,372	0,187	-0,280
$\log_2(X_1/X_0)$	0,137	-0,084	0,538	0,270	-0,401
$\Delta_t = (t_2 - t_1)$	1	3	1	1	1
r (Eq.19)[a]	0,095	-0,019	0,373	0,187	-0,280
k (Eq.20)	0,150	-0,030	0,583	0,292	-0,435
k (Eq.21)	0,137	-0,028	0,538	0,270	-0.401
T_2 (Eq.22)	6,7	-32,853	1,715	3,420	-2,280

[a] Assuming there is exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population. (Appendix 2. Eq. 25, 26, 27 and 28)

[b] Day 34^{th}, was a Monday, the TSS of that day comprehends the growth during the weekend, i.e. Friday-Saturday-Sunday. 3 days

Table 45 - TPBR 10 – *Chlorella wild mix Årungen* – Fresh water algae. Biomass concentration (B=g/L) for time and doubling time (log $y=2^B$) for interval 34^{th} – 37th, age of culture (14.08.12-17.08.12), data extracted from the register of the algae growth in semi-continuous cultivation system in TPBR 10, for a total time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

Age of culture (days)	Biomass Concentration. Days 34-37 B=(g/L)	Number of days (days)	Doubling time. Days 34-37 log y=2^B
34	0,91428	0	1,88463
35	1,32727	1	2,50927
36	1,6	2	3,03143
37	1,21142	3	2,31566

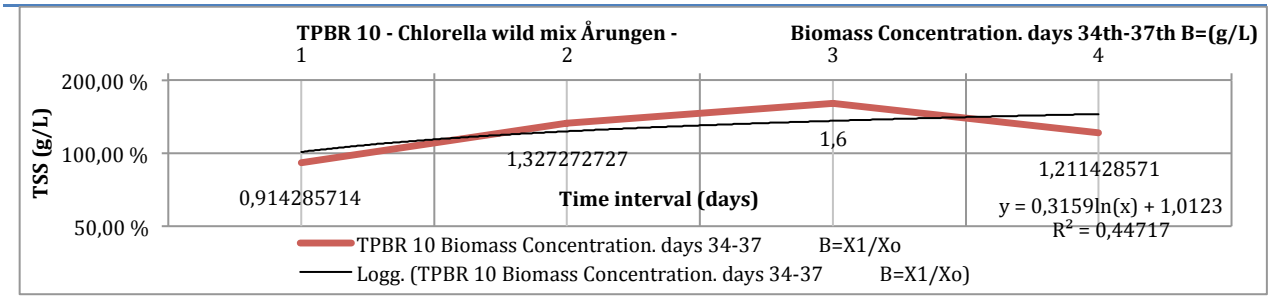


Figure 66 - Biomass concentration sampled the 34^{th}-37^{nd}, age of culture (14.08.12-17.08.12) of fresh water algae *Chlorella wild mix Årungen*, grown in semi-continuous cultivation system in TPBR 10, for a total time interval of 42 days (11.07.12-23.08.12) (Appendix 2. Eq. 30.1 and 30.2)

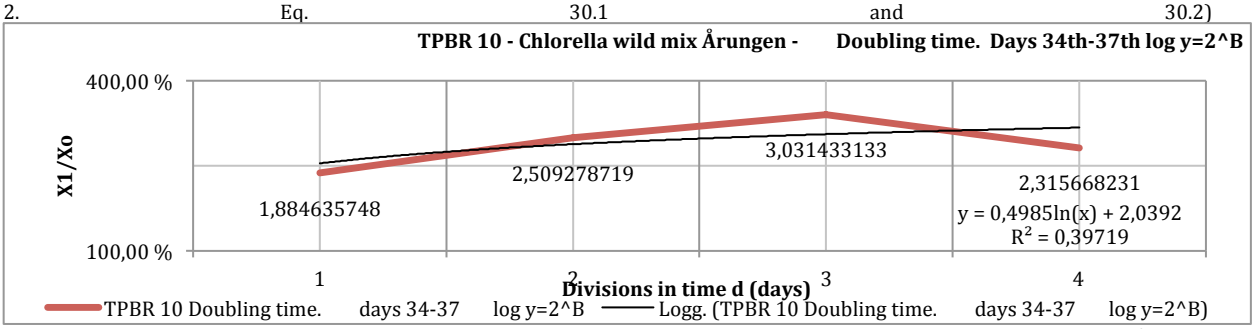


Figure 67- Doubling time registered 34^{th}-37^{nd}, age of culture (14.08.12-17.08.12) of fresh water algae *Chlorella wild mix Årungen*, grown in semi-continuous cultivation system in TPBR 10, for a total time interval of 42 days (11.07.12-23.08.12). (Appendix 2. Eq. 30.1 and 30.2)

13 Appendix 4. Semicontinuous production system (Figs. 68-77)

13.1 Semi-continuous production system for five microalgae strains in duplicate in TPBR 1 to TPBR 10. The diagrams represent the complete cultivation periods. For explanation about the effects of temperature and irradiance on biomass productivity (*P*), see sections 3.1, 3.2, 3.3, 3.5, 3.6 and 3.7

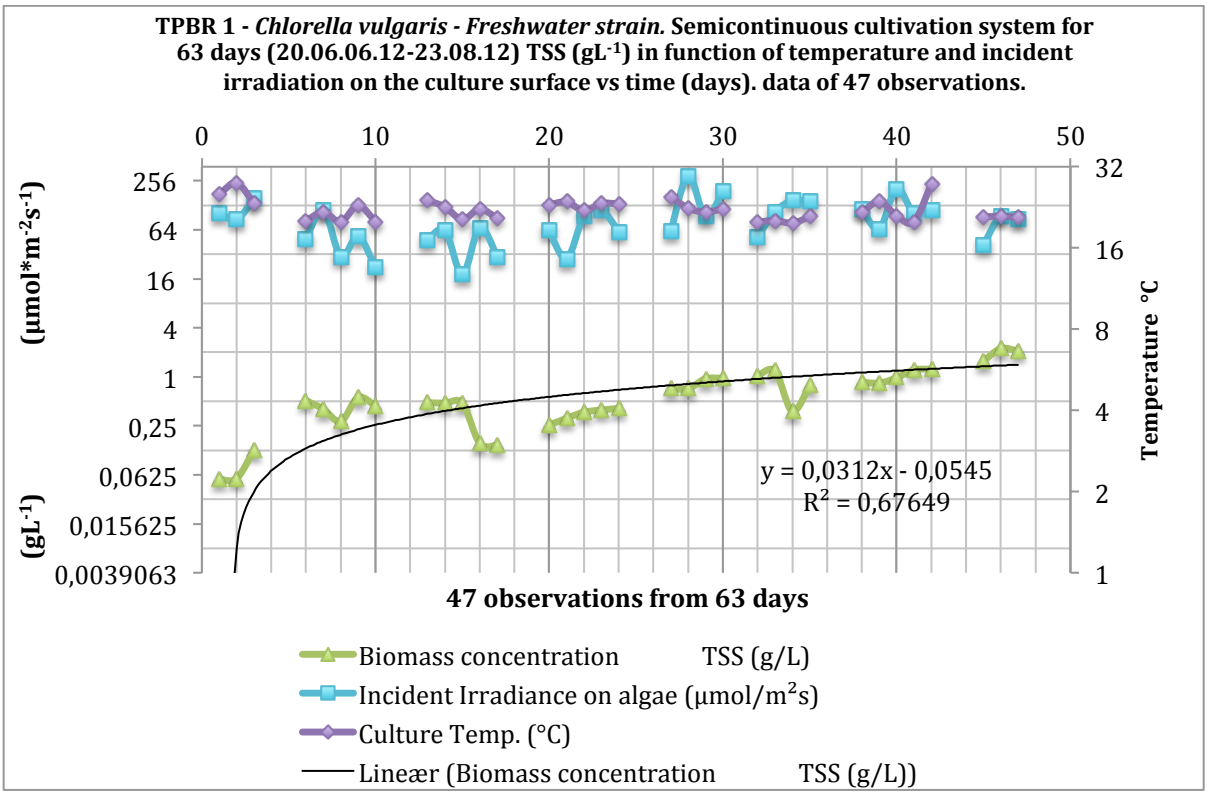


Figure 68 - TPBR 1 - *Chlorella vulgaris* – fresh water algae cultivated in semi-continuous production system in greenhouse and natural solar irradiation conditions, for 63 days. (20.06.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

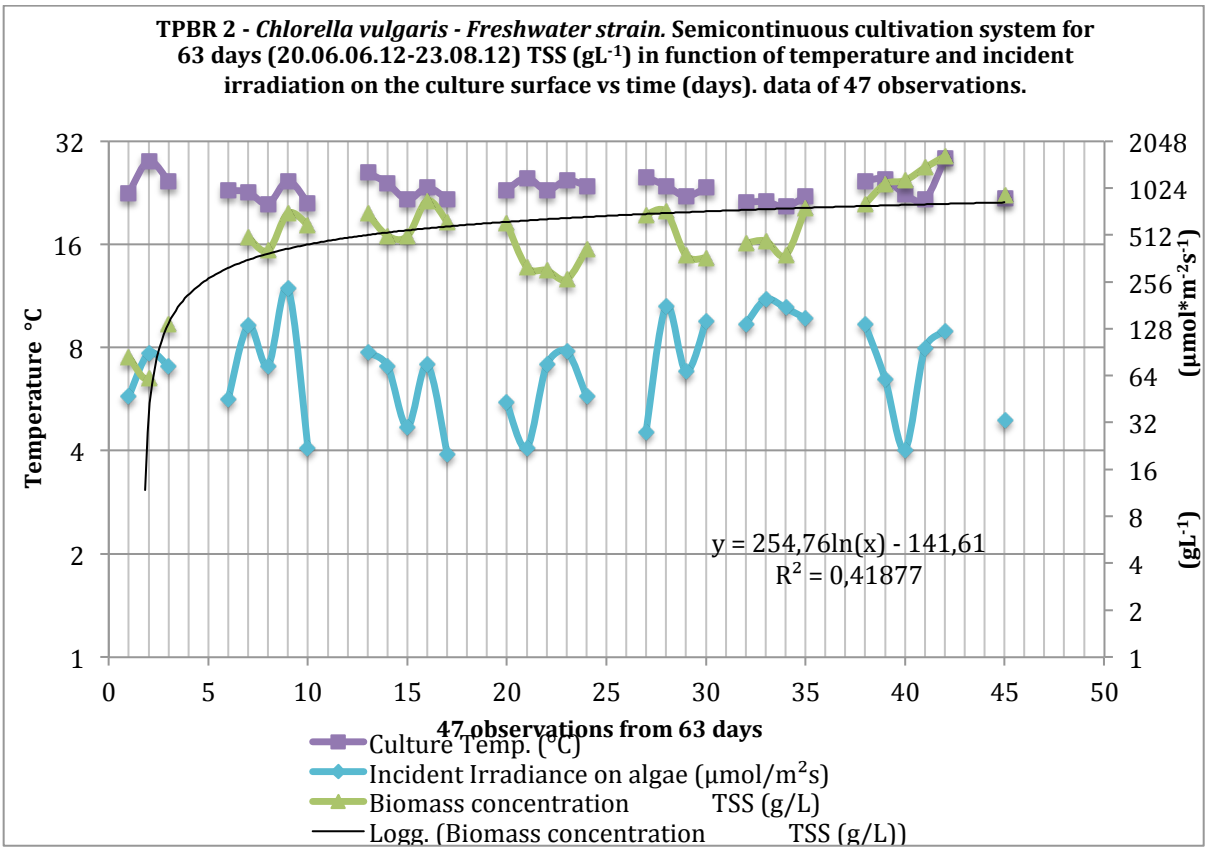


Figure 69 – TPBR 2 - *Chlorella vulgaris* – fresh water algae cultivated in semi-continuous production system in greenhouse and natural solar irradiation conditions, for 63 days. (20.06.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

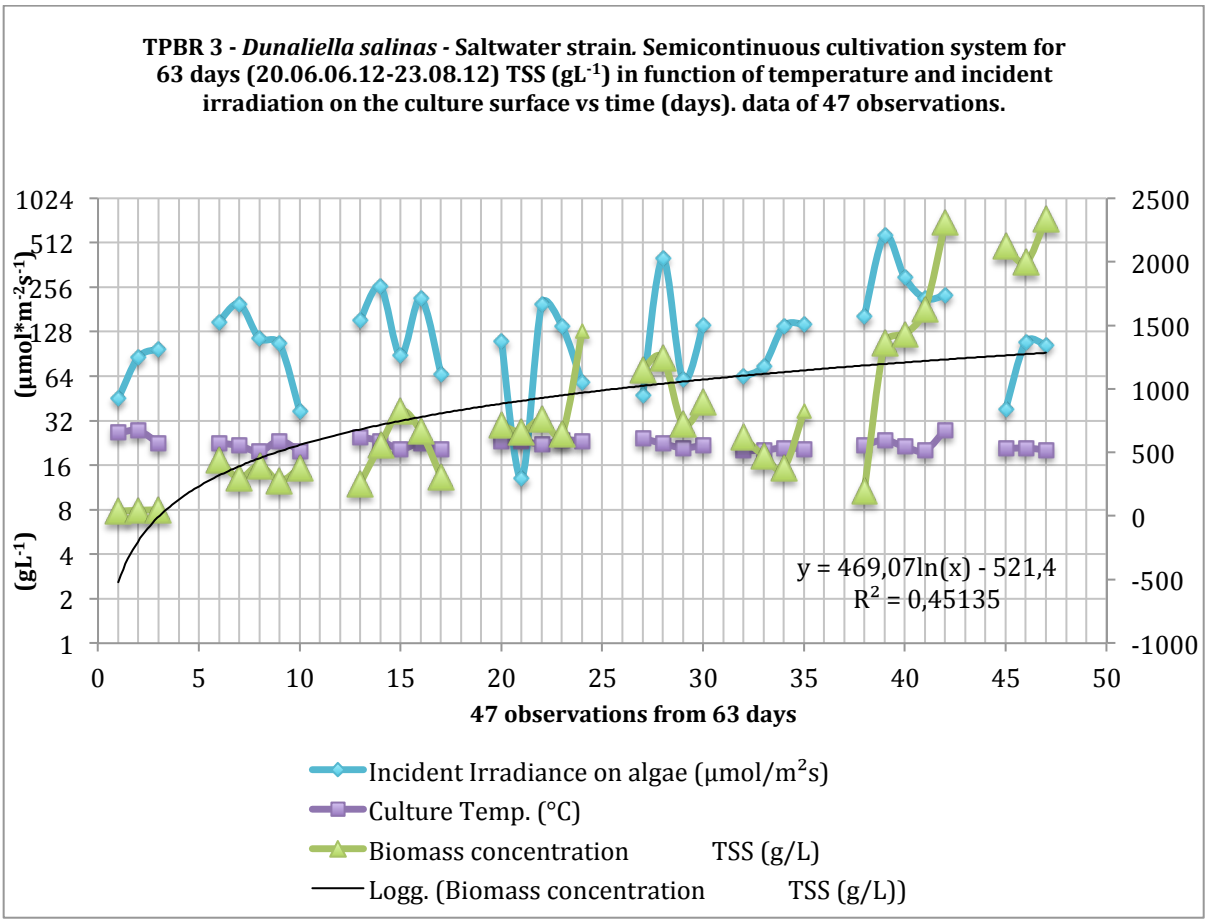


Figure 70 – TPBR 3 - *Dunaliella salinas* – marine algae cultivated in semi-continuous production system at greenhouse and natural solar irradiation conditions, for 63 days. (20.06.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

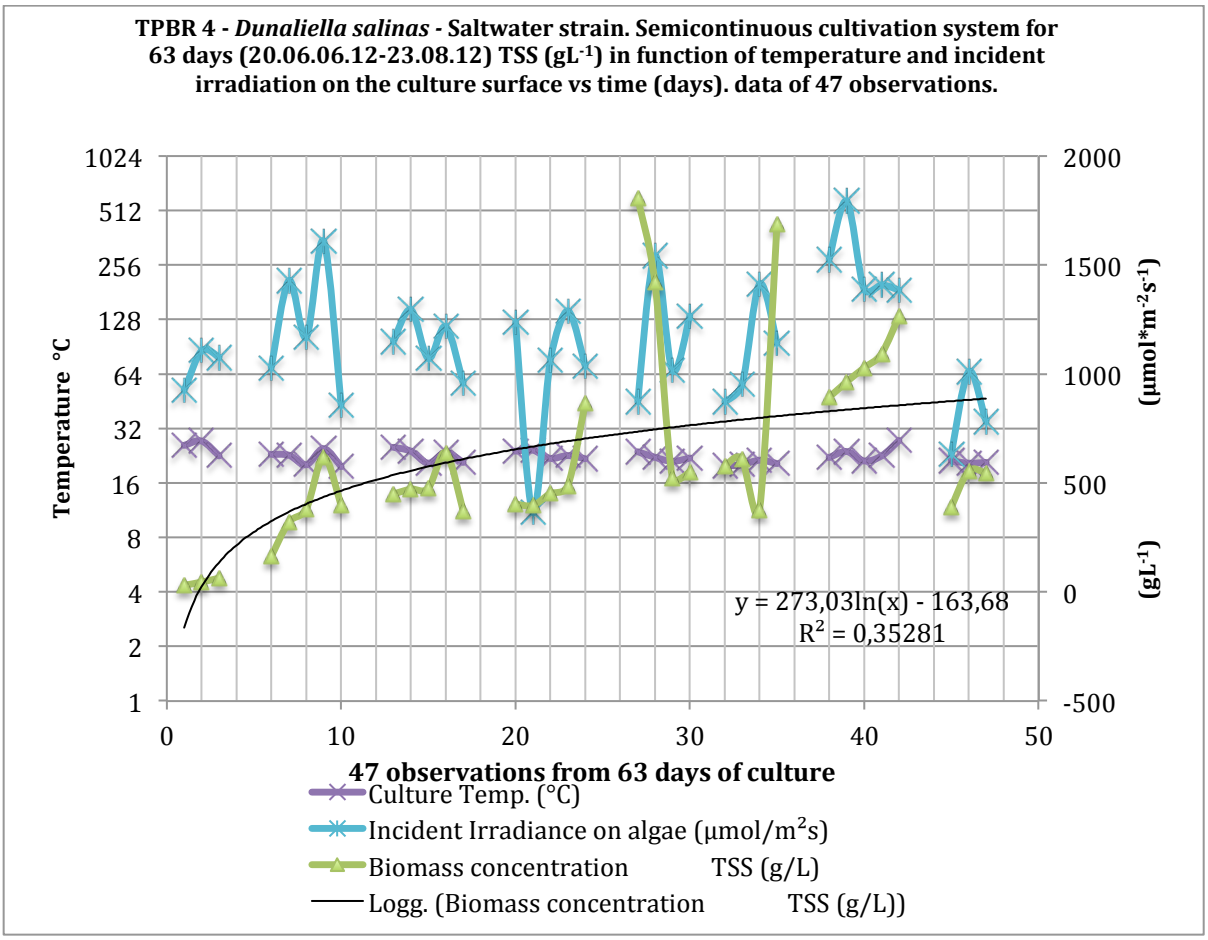


Figure 71 - TPBR 4 - *Dunaliella salinas* – marine algae cultivated in semi-continuous production system at greenhouse and natural solar irradiation conditions, for 63 days. (20.06.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

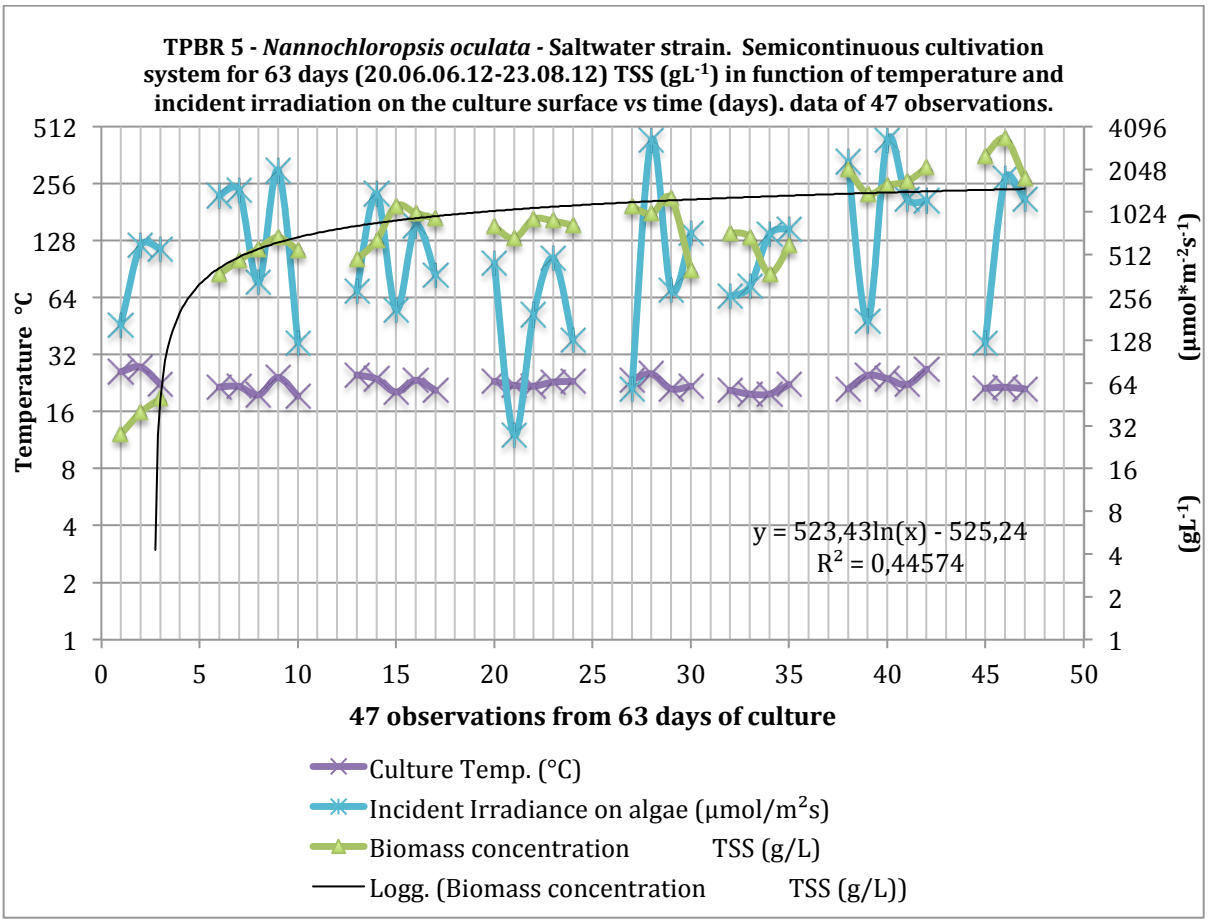


Figure 72 - TPBR 5 - *Nannochloropsis oculata* – marine algae cultivated in semi-continuous production system at greenhouse and natural solar irradiation conditions, for 63 days. (20.06.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

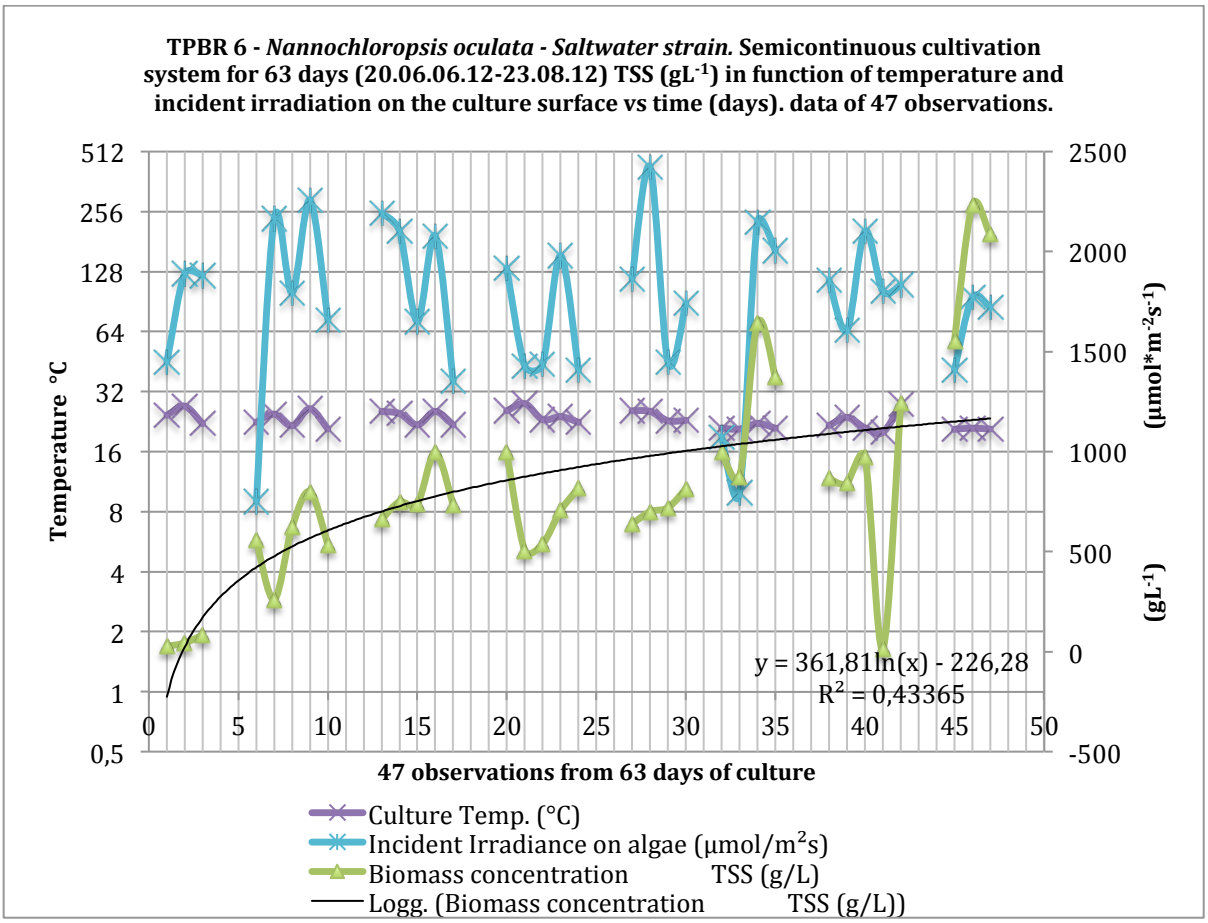


Figure 73 - TPBR 6 - *Nannochloropsis oculata* – marine algae cultivated in semi-continuous production system at greenhouse and natural solar irradiation conditions, for 63 days. (20.06.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

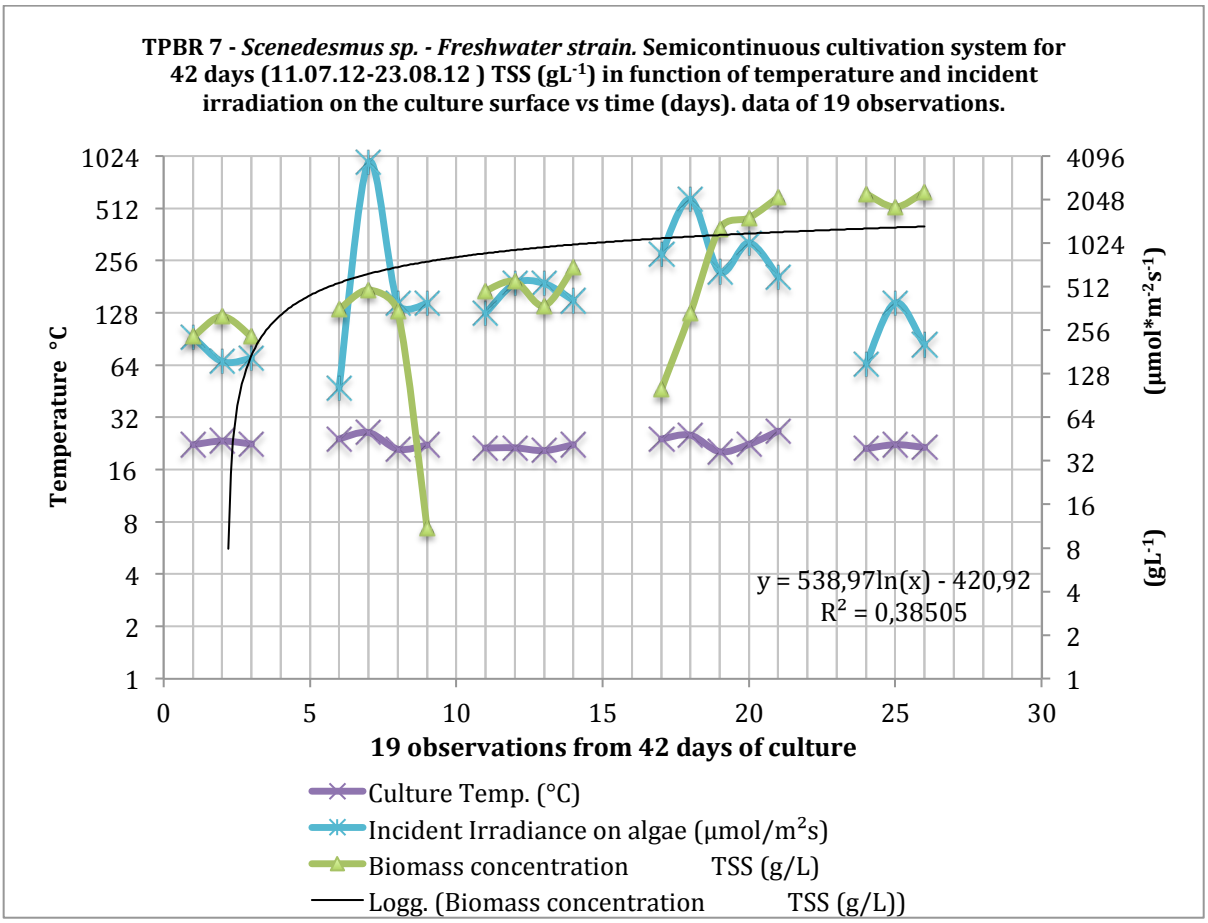


Figure 74- TPBR 7 - *Scenedesmus sp.* – fresh water algae cultivated in semi-continuous production system at greenhouse and natural solar irradiation conditions, for 42 days. (11.07.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

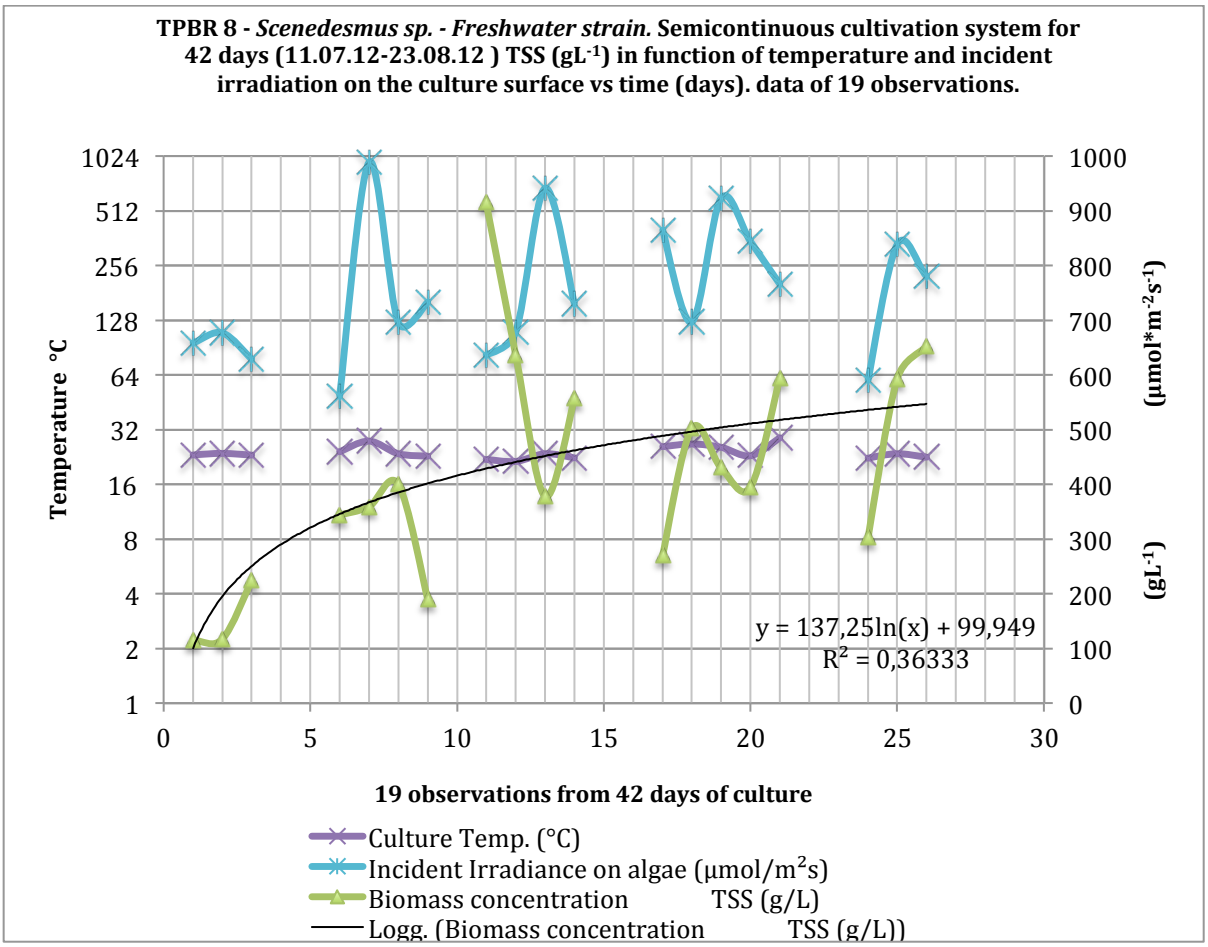


Figure 75 - TPBR 78- *Scenedesmus sp.* – fresh water algae cultivated in semi-continuous production system at greenhouse and natural solar irradiation conditions, for 42 days. (11.07.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

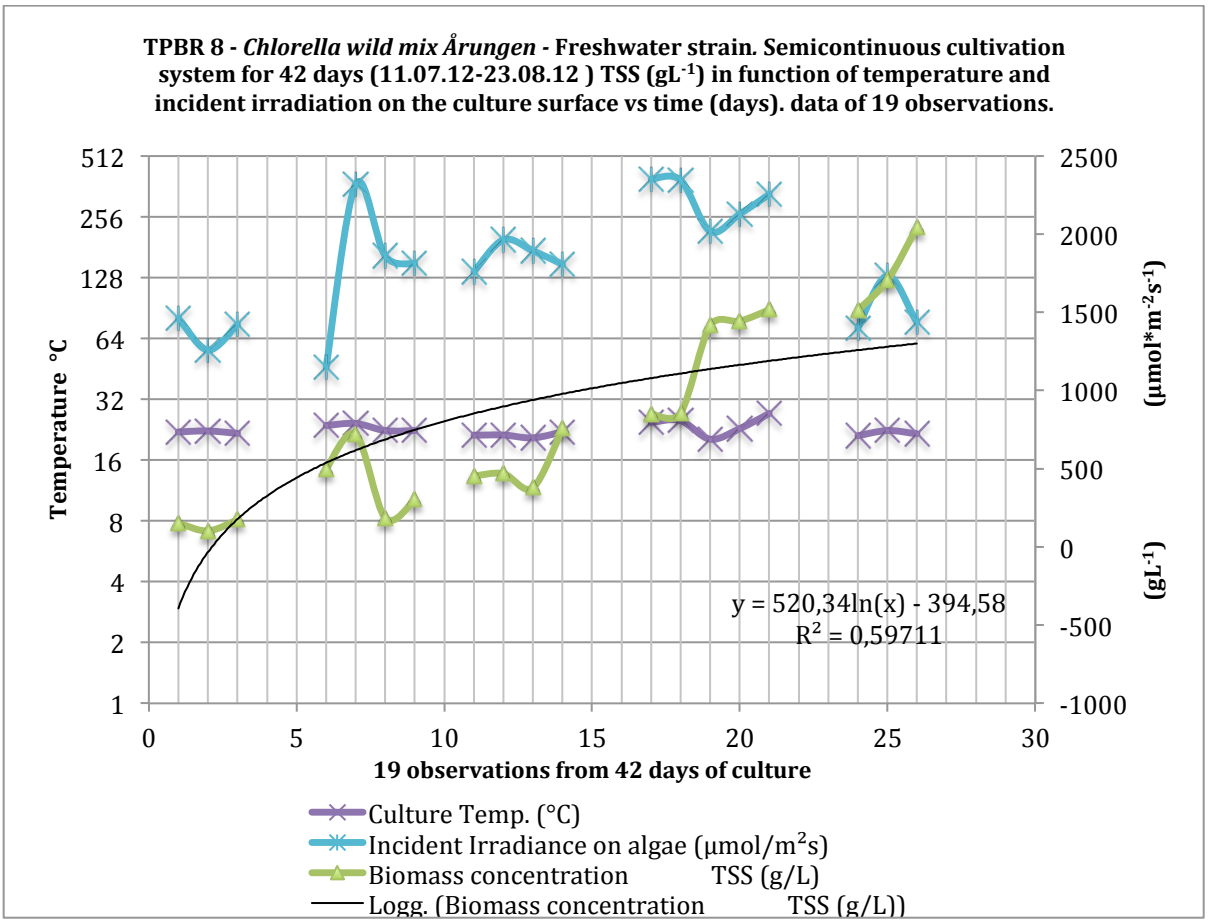


Figure 76 - TPBR 9 - *Chlorella wild mix Årungen* – fresh water algae cultivated in semi-continuous production system at greenhouse and natural solar irradiation conditions, for 42 days. (11.07.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

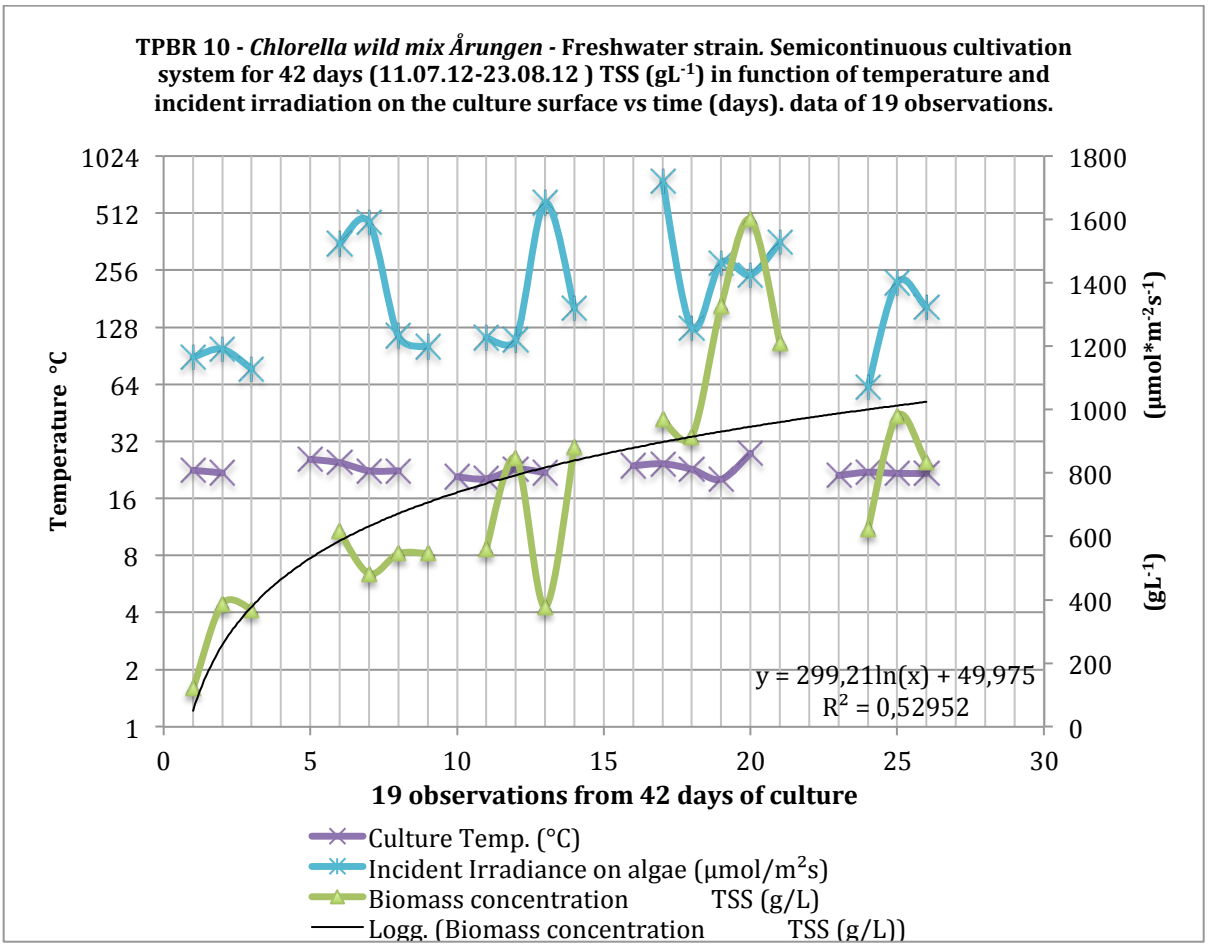


Figure 77 - TPBR 10 - *Chlorella wild mix Årungen* – fresh water algae cultivated in semi-continuous production system at greenhouse and natural solar irradiation conditions, for 42 days. (11.07.12-23.08.12). Location: Senter for klimaregulert planteforskning (SKP) at the NMBU university campus in Ås (59°40´05.44" N 10°46´12.80" E, elevation 103 meters above sea level). Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance (µmol*m^{-2}*s^{-1}), temperature (°C) and biomass concentration (g/L). The line represent an abscissa, indicating the logarithmic growth pattern with it´s respective function and R^2 value noted in the graph.

14 Appendix 5. Specific growth rate diagrams (Tabs. 46-59 and Figs. 78-91)

All data registered on TPBR 1 to TPBR 10, in this appendix 5 is based on the data from appendix 3.

All meteorological data of the outside environment to the greenhouse, used to do the computation in these experiments, is based on the data registered at the meteorological station at Ås (N 59° 39' 37'', Ø 10 ° 46' 54'', 93.3 meters above sea level). It was possible to calculate the incident solar irradiance at the roof level of the green house, and this value of irradiance was given in Wm^{-2}, then the conversion factor for intensity towards solar irradiance, the approximate conversion factor for sunlight is 1 Wm^{-2} equals about 4,5 µmol photon m^{-2} s^{1} (Richmond 2004).

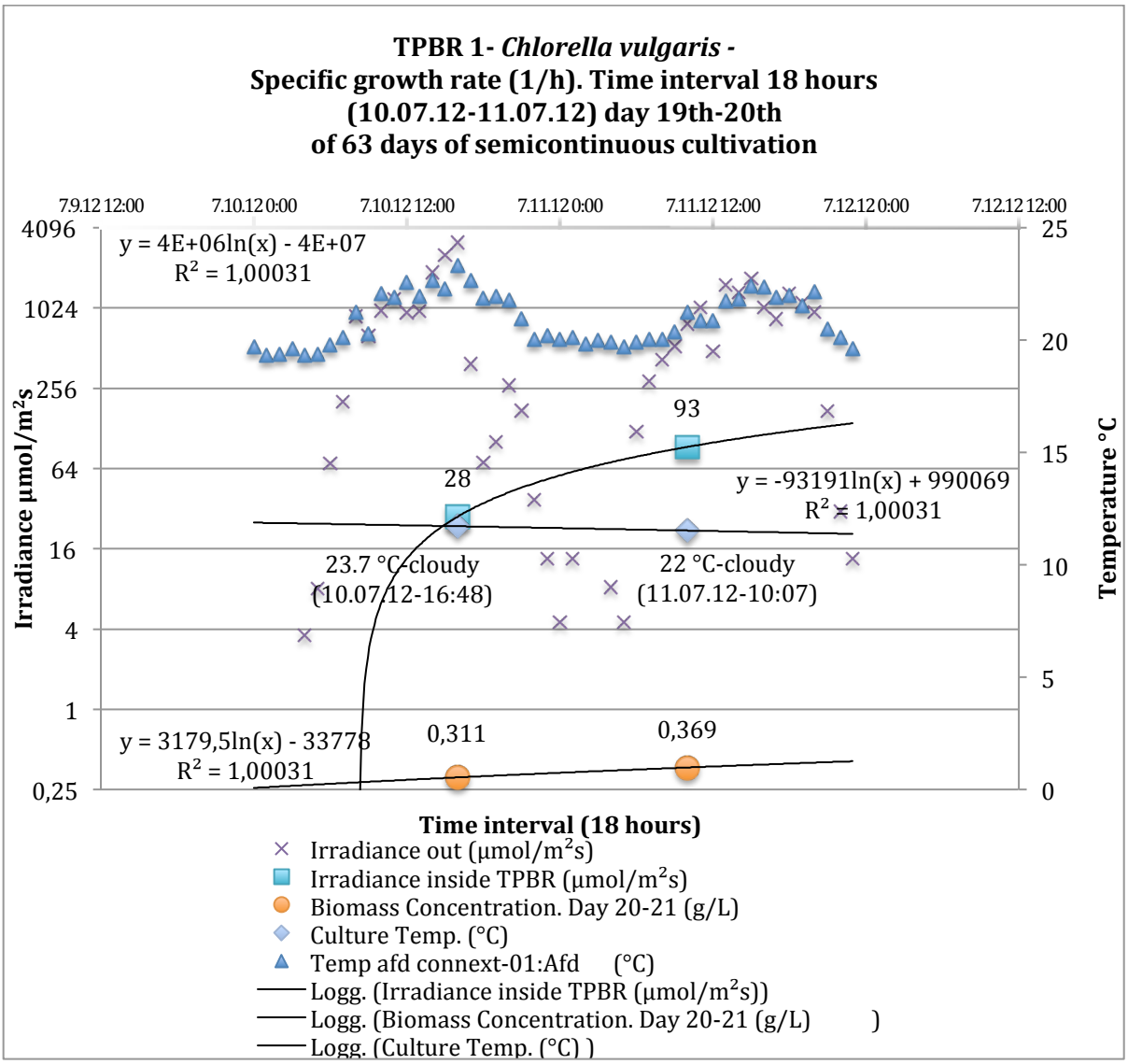


Figure 78 - The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for fresh water microalgae *Chlorella vulgaris.* Maximum biomass concentration sampled 10.07.12 and 11.07.12 (day 19th and 20th age of culture) from algae culture operated in semi-continuous system production (TPBR 1) in greenhouse conditions and natural solar irradiation for 63 days. . Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of the same shape and colour represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 46 - Computation of population specific growth rate (µ) observed 10.07.12-11.07.12 (day 19h-20th, age of culture), for fresh water microalgae *Chlorella vulgaris* (TPBR 1), cultivated in semicontinuous system production for 63 days. 20.06.12 – 23.08.12 (see table 12.1)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
19	0,311	23,29	3162,915	100712	16:48	Cloudy	23,7	28	99,11
20	0,369	21,26	775,89	110712	10:07	Cloudy	22	93	88,02

Figure 78 shows the highest values for growth rate per hour corresponding to the day 10.07.12 and 11.07.12 (day 19th and 20th, age of culture) with a biomass concentration (TSS) of 0,311 g/L and 0,369 g/L, respectively. During this time interval of 18 hours, the algae had a specific growth rate of 0,00322h^{-1}, ((0,369 - 0,311)/18 h).

The value registered for irradiance was low, 28 and 93 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 1, above the suspension but under the lid of the reactor. The culture grew very poorly.

The data registered by the meteorological station for 10.07.12 and 11.07.12, was 702,87 W*m^{-2} and 172,42 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 3162,915 µmol photon m^{-2} s^{1} and 775,42 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for these days, at the time of sampling, were both cloudy, and it was some significant difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 1, and the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was +0,41 °C lower (10.07.12 – 16:48), and 0,74 °C higher (11.07.12 – 10:07) than the outside temperature. The differences in temperature for those days were +1,72% and +3,36% for 10.07.12 and 11.07.12, respectively. The TPBR 1 was accumulating some heat in the algae suspension, both days. The second day of the measure, accumulated almost twice heat than the first day.

Analysing the irradiances registered, it´s possible to calculate the percentage of incident light irradiance that reached the algae culture. (28*100%/3162,915)= 0,89 % and (93*100%/775,89)= 11,98% of the solar irradiance reached the algae culture. This means a loss of 99,11% and 88,02% of the incident solar irradiation on the greenhouse registered at the time of sampling for 10.07.12 and 11.07.12, respectively. This loss of irradiation can be explained as a sunscreen was applied under the roof to prevent overheating by direct solar exposure. The culture was too cold.

The unstable weather and the sunscreen affected directly the algae culture, lowering the registered levels of incident irradiation on the algae culture, as well as lowering the temperature recorded in the suspension, affecting negatively the algae biomass production. This phenomenon of low irradiation on and temperature levels in the algae culture did affect, negatively, the photosynthetic production.

From the graph can we conclude that the TPBR 1 did not offered optimal growth conditions and the irradiation reaching the algae suspension should be increased above 300 µmol*$m^{-2}$$s^{-1}$ as well as the temperature towards a level of 25-27 °C. The specific growth rate was poor, 0,00322h^{-1}.

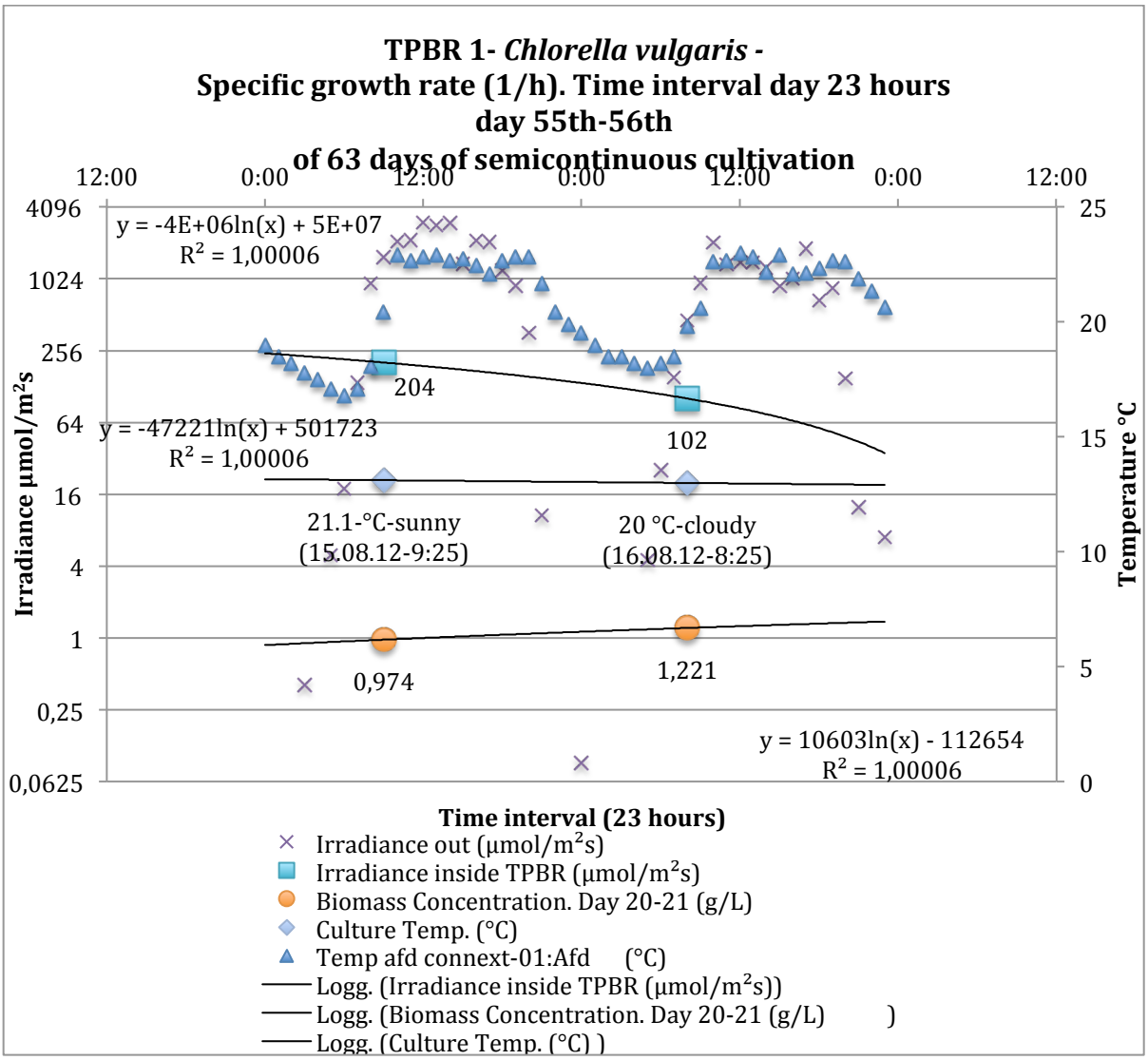


Figure 79 - The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for fresh water microalgae *Chlorella vulgaris.* Maximum biomass concentration sampled 15.08.12 and 16.08.12 (day 55th and 56th, age of culture) from algae culture operated in semi-continuous system production (TPBR 1) in greenhouse conditions for 63 days. Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of the same shape and colour represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 47 - Computation of population specific growth rate (µ) observed 15.08.12-16.08.12 (day 55h-56th, age of culture), for fresh water microalgae *Chlorella vulgaris* (TPBR 1), cultivated in semicontinuous system production for 63 days. 20.06.12 – 23.08.12 (see table 12.1.1)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
55	0,974	20,44	1552,14	150812	9:25	Sunny	21,1	204	86,86
56	1,221	19,81	406,08	160812	8:25	Cloudy	20	102	77,83

Figure 79 shows the highest values for growth rate per hour corresponding to the day 15.08.12 and 16.08.12 (day 55^{th} and 56^{th}, age of culture), with a biomass concentration (TSS) of 0,974 g/L and 1,221g/L, respectively. During this time interval of 23 hours, the algae had a specific growth rate of 0,01074h^{-1}, ((1,221 - 0,974)/23 h).

The value registered for irradiance was low, 204 and 102 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 1, above the suspension but under the lid of the reactor. The culture grew poorly.

The data registered by the meteorological station for 15.08.12 and 16.08.12, was 344,92 W*m^{-2} and 102,24 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 1552,14 µmol photon m^{-2} s^{1} and 460,08 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for these days, at the time of sampling, were sunny and cloudy, and it was some significant difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 1, and the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was +0,66 °C higher (15.08.12 – 9:25), and +0,19 °C higher (17.08.12 – 14:54) than the outside temperature. The differences in temperature for those days were +3,13% and +0,95% for 15.08.12 and 16.08.12, respectively. The TPBR 1 was accumulating some heat in the algae suspension, both days. The first day of the measure, accumulated 3,3 times more than the second day.

Analysing the irradiances registered, it´s possible to calculate the percentage of incident light irradiance that reached the algae culture. (204*100%/1552,14)= 13,14 % and (102*100%/460,08)= 22,17% of the solar irradiance reached the algae culture. This means a loss of 86,86% and 77,83% of the incident solar irradiation on the greenhouse registered at the time of sampling for 15.08.12 and 16.08.12, respectively.

The fluctuating weather conditions, appears to have an indirect influence on the low levels of incident irradiation registered, and together with the low temperature recorded in the suspension, affected negatively the algae biomass production. This phenomenon of low irradiation level and low culture did affect, negatively, the photosynthetic production.

From the graph can we conclude that the TPBR 1 did not offered good growth conditions and have to be readjust to an optimal level. The parameters as the irradiation reaching the algae suspension should be increased above 300 µmol*$m^{-2}$$s^{-1}$ as well as the temperature should be increased to a level of 25-27 °C. The specific growth rate was rather poor, 0,01074h^{-1}.

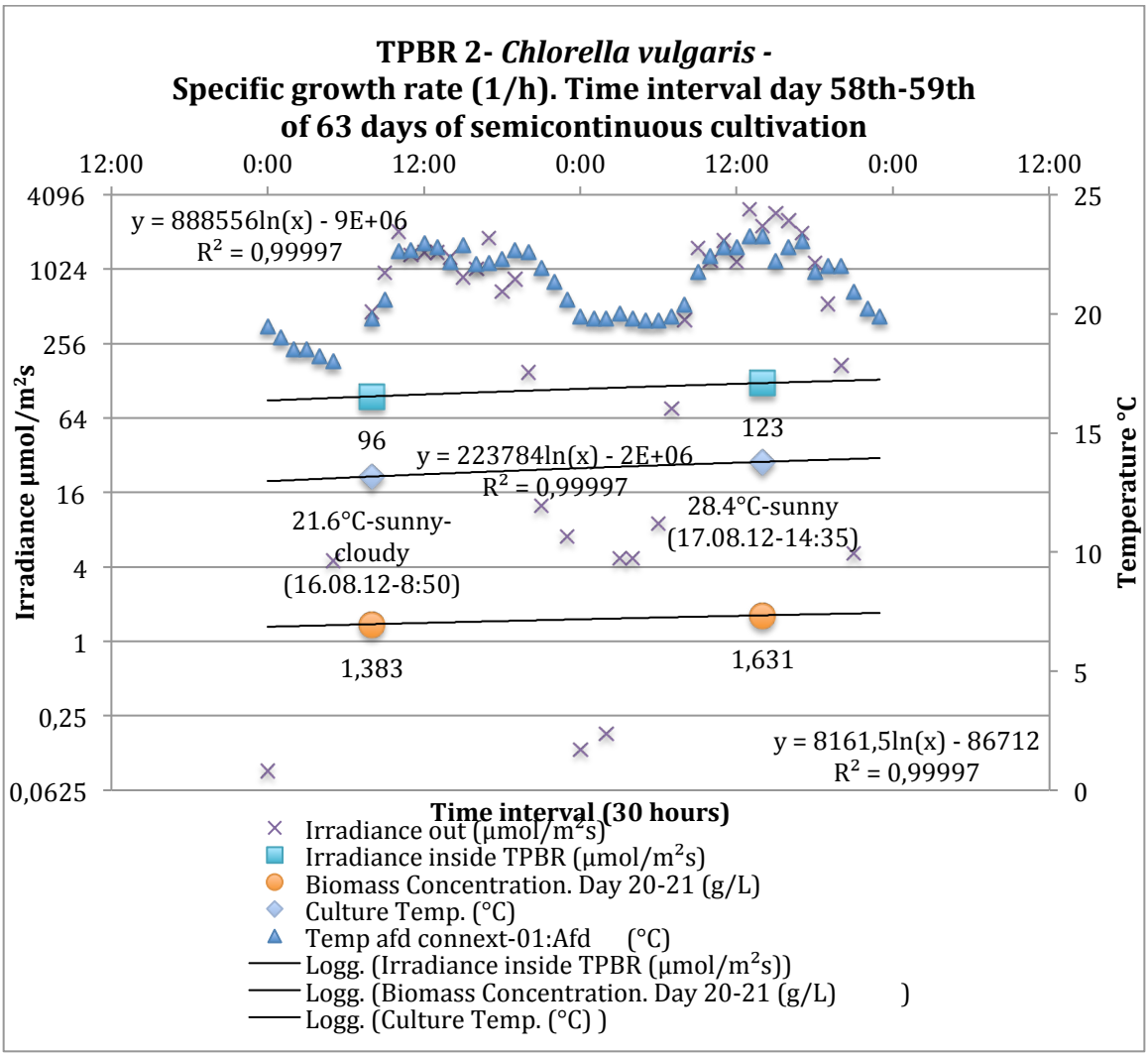


Figure 80 - The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for fresh water microalgae *Chlorella vulgaris.* Maximum biomass concentration sampled 16.08.12 and 17.08.12 (day 58th and 59th, age of culture) from algae culture operated in semi-continuous system production (TPBR 2) in greenhouse conditions for 63 days. . Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of the same shape and colour represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 48 Computation of population specific growth rate (µ) observed 16.08.12-17.08.12 (day 58h-59th, age of culture), for fresh water microalgae *Chlorella vulgaris* (TPBR 2), cultivated in semicontinuous system production for 63 days. 20.06.12–23.08.12 (see table 12.2)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
58	1,383	19,81	460,08	160812	8:50	Cloudy-Sunny	21,6	96	79,13
59	1,631	23,26	2269,305	170812	14:35	Sunny	28,4	123	94,6

Figure 80 shows the highest values for growth rate per hour corresponding to the day 16.08.12 and 17.08.12 (day 58th and 59th, age of culture) with a biomass concentration (TSS) of 1,383 g/L and 1,631 g/L, respectively. During this time interval of 29 hours, the algae had a specific growth rate of 0,00827h^{-1}, ((1,265 - 1,093)/30 h).

The value registered for irradiance was low, 96 and 123 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 2, above the suspension but under the lid of the reactor. The culture grew poorly.

The data registered by the meteorological station for 16.08.12 and 17.08.12, was 102,24 W*m^{-2} and 504,29 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 460,08 µmol photon m^{-2} s^{1} and 2269,305 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for these days, at the time of sampling, were cloudy- sunny and sunny, and it was some significant difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 2, and the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was +1,25 °C lower (16.08.12 – 10:17), and +5,14 °C higher (17.08.12 – 14:54) than the outside temperature. The differences in temperature for those days were +5,94% and +18,1% for 16.08.12 and 17.08.12, respectively. The TPBR 2 was accumulating heat in the algae suspension, both days. The second day of the measure, accumulated three times more than the first day.

Analysing the irradiances registered, it´s possible to calculate the percentage of incident light irradiance that reached the algae culture. (96*100%/460,08)= 20,87 % and (123*100%/2269,31)= 5,42% of the solar irradiance reached the algae culture. This means a loss of 79,13% and 94,6% of the incident solar irradiation on the greenhouse registered at the time of sampling for 16.08.12 and 17.08.12, respectively.

In spite of the sunny weather, the low levels of incident irradiation observed together with the higher temperature recorded in the suspension, affected negatively the algae biomass production. This phenomenon of low irradiation level and warmer culture did affect, negatively, the photosynthetic production.

From the graph can we conclude that the TPBR 2 did not offered good growth conditions and the irradiation reaching the algae suspension should be increased above 200 µmol*$m^{-2}$$s^{-1}$ and the temperature should be lowered under 27 °C. The specific growth rate was poor, 0,00827h^{-1}.

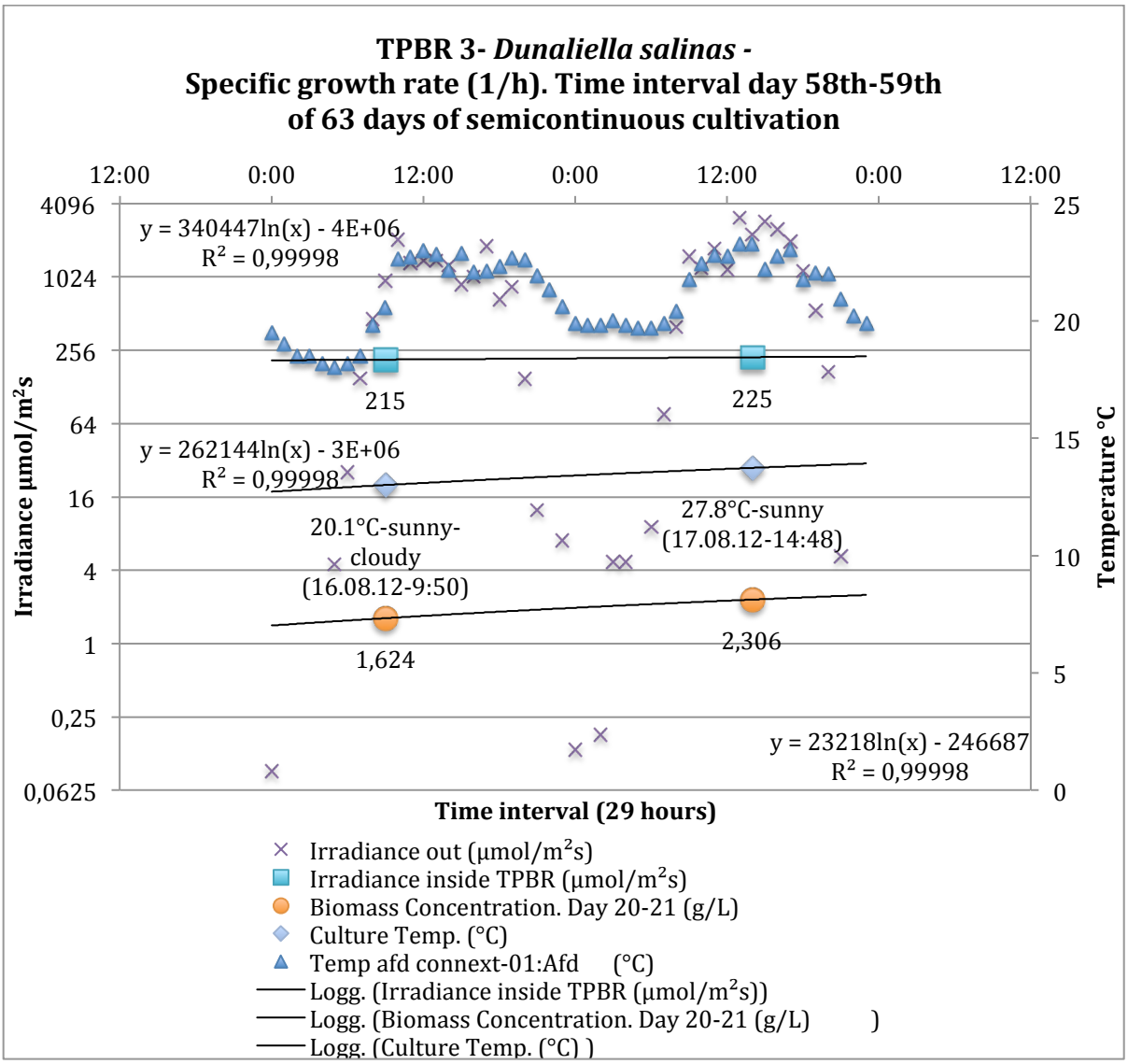


Figure 81 - The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for marine microalgae *Dunaliella salinas.* Maximum biomass concentration sampled 16.08.12 and 17.08.12 (day 58th and 59th, age of culture) from algae culture operated in semi-continuous system production (TPBR 3) in greenhouse conditions for 63 days. . Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of the same shape and colour represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 49 - Computation of population specific growth rate (µ) observed 16.08.12-17.08.12 (day 58h-59th, age of culture), for marine microalgae *Dunaliella salinas* (TPBR 3), cultivated in semicontinuous system production for 63 days. 20.06.12 – 23.08.12 (see table 12.3)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
58	1,624	20,59	953,8	160812	9:05	Sunny-cloudy	20,1	215	77,46
59	2,306	23,26	2269,305	170812	14:48	Sunny	27,8	225	90,9

Figure 81 shows the highest values for growth rate per hour corresponding to the day 16.08.12 and 17.08.12 (day 58th and 59th, age of culture) with a biomass concentration (TSS) of 1,624 g/L and 2,306 g/L, respectively. During this time interval of 29 hours, the algae had a specific growth rate of 0,02352h^{-1}, ((1,265 - 1,093)/28 h).

The value registered for irradiance was low, 215 and 225 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 3, above the suspension but under the lid of the reactor. The culture grew normally.

The data registered by the meteorological station for 16.08.12 and 17.08.12, was 211,8 W*m^{-2} and 504,29 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 953,1 µmol photon m^{-2} s^{1} and 2269,305 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for these days, at the time of sampling, were sunny-cloudy and sunny, and it was some significant difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 3, and the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was -0,49 °C lower (16.08.12 – 10:17), and +4,24 °C higher (17.08.12 – 14:54) than the outside temperature. The differences in temperature for those days were -2,38% and +15,42% for 16.08.12 and 17.08.12, respectively. The TPBR 3 was accumulating heat, the second day of the measure, in the algae suspension.

Analysing the irradiances registered, it´s possible to calculate the percentage of incident light irradiance that reached the algae culture. (215*100%/953,8)= 22,54 % and (225*100%/2269,31)= 9,91% of the solar irradiance reached the algae culture. This means a loss of 77,46% and 90,9% of the incident solar irradiation on the greenhouse registered at the time of sampling for 16.08.12 and 17.08.12, respectively.

In spite of the sunny weather, the middle level of incident irradiation observed together with the higher temperature recorded in the suspension, affected positively the algae biomass production. This phenomenon of middle irradiation level and warmer culture did affect, positively, the photosynthetic production.

From the graph can we conclude that the TPBR 3 did offered good growth conditions and the irradiation reaching the algae suspension could be maintained at the same values, or increase above 200 µmol*$m^{-2}$$s^{-1}$ and the temperature could as well be maintained at the registered values of the interval of 20-28 °C. The specific growth rate was 0,02352h^{-1}.

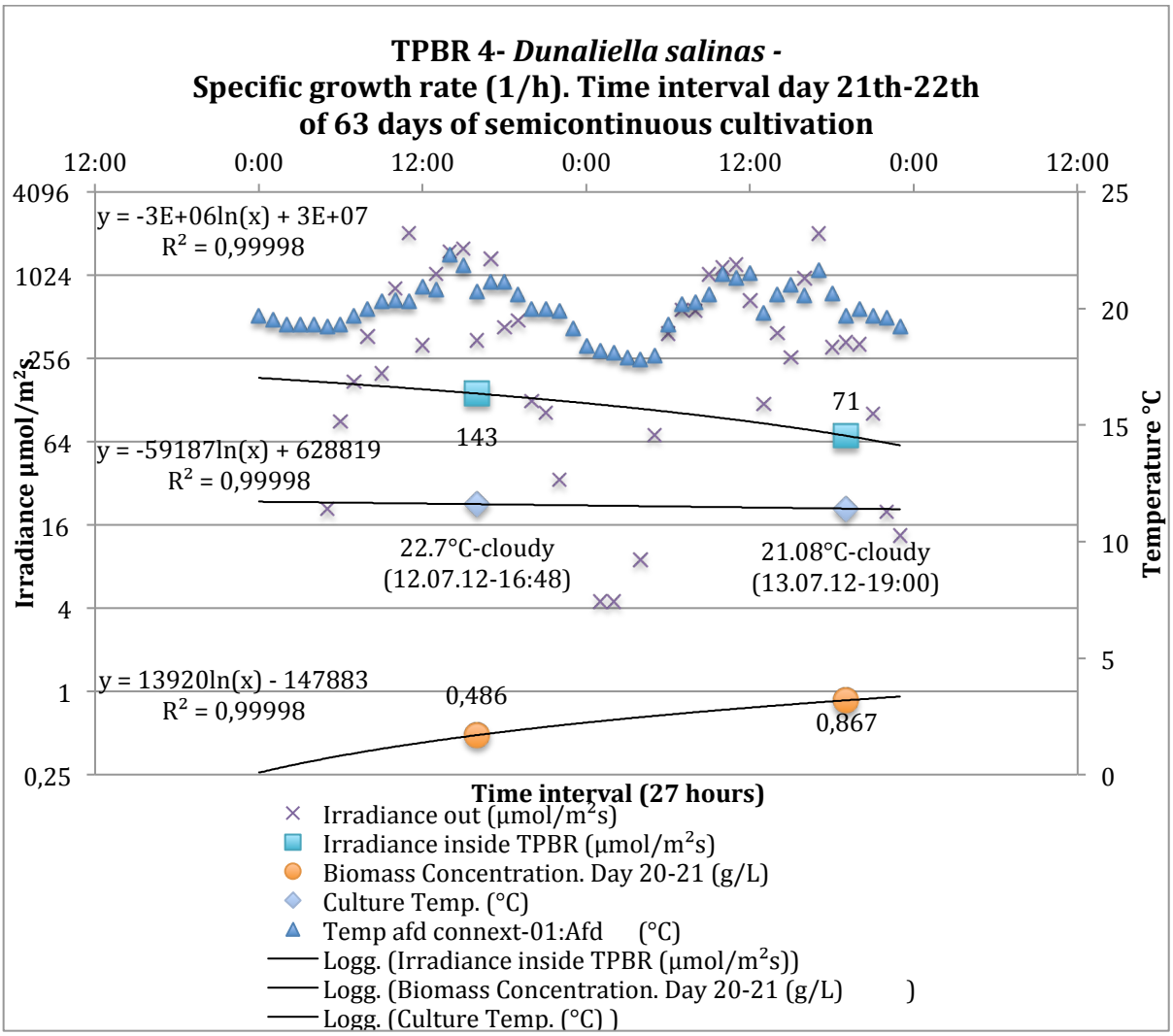


Figure 82 - The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for marine microalgae *Dunaliella salinas.* Maximum biomass concentration sampled 12.07.12 and 13.07.12 (day 21th and 22nd, age of culture) from algae culture operated in semi-continuous system production (TPBR 4) in greenhouse conditions for 63 days. . Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of the same shape and colour represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 50 - Computation of population specific growth rate (µ) observed 12.07.12-13.07.12 (day 21th-22th, age of culture), for marine microalgae *Dunaliella salinas* (TPBR 4), cultivated in semicontinuous system production for 63 days. 20.06.12–23.08.12 (See table 12.4)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
21	0,486	20,73	344,7	120712	16:48	Cloudy	22,7	143	58,51
22	0,867	19,7	336,96	130712	19:00	Cloudy	21,8	71	78,93

Figure 82 shows the highest values for growth rate per hour corresponding to the day 12.07.12 and 13.07.12 (day 21th and 22th, age of culture) with a biomass concentration (TSS) of 0,486 g/L and 0,867 g/L, respectively. During this time interval of 27 hours, the algae had a specific growth rate of 0,01411h^{-1}, ((0,867 - 0,486)/27 h).

The value registered for irradiance was low, 143 and 71 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 4, above the suspension but under the lid of the reactor. The culture grew normally.

The data registered by the meteorological station for 12.07.12 and 13.07.12, was 76,6 W*m^{-2} and 74,88 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 344,7 µmol photon m^{-2} s^{1} and 336,96 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for these days, at the time of sampling, were both cloudy, and it was some significant difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 4, and the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was +1,97 °C higher (12.07.12 – 16:48), and +2,1 °C higher (13.07.12 – 19:00) than the outside temperature. The differences in temperature for those days were +8,67% and +9,63% for 12.07.12 and 13.07.12, respectively. The TPBR 4 was accumulating some heat in the algae suspension, in spite of the cloudy weather. The TPBR 4 was located almost at the middle of the room but closest to the polycarbonate wall and perhaps the vicinity with the big corridor that run across the center of the greenhouse, could have disturbing effect on the entire environment of the greenhouse. The TPBR 4 was placed in a corner closest to the entrance to the room, facing on to three TPBRs on two of it´s walls.

Analysing the irradiances registered, it´s possible to calculate the percentage of incident light irradiance that reached the algae culture. (143*100%/344,7)= 41,49 % and (71*100%/336,96)= 21,07% of the solar irradiance reached the algae culture. This means a loss of 58,51% and 78,93% of the incident solar irradiation on the greenhouse registered at the time of sampling for 12.07.12 and 13.07.12, respectively.

In spite of the cloudy weather, the average incident irradiation together with the higher temperature recorded in the suspension, affected positively the algae biomass production. This phenomenon of average irradiation and warmer culture did not affect, apparently, the photosynthetic production.

From the graph can we conclude that the TPBR 4 did offered an adequate growth conditions and the irradiation reaching the algae suspension has to increase from the registered value of 336,96 µmol*$m^{-2}$$s^{-1}$ and the temperature could be maintained at the registered values of the interval of 22,2-26,08 °C. The specific growth rate was 0,01411h^{-1}.

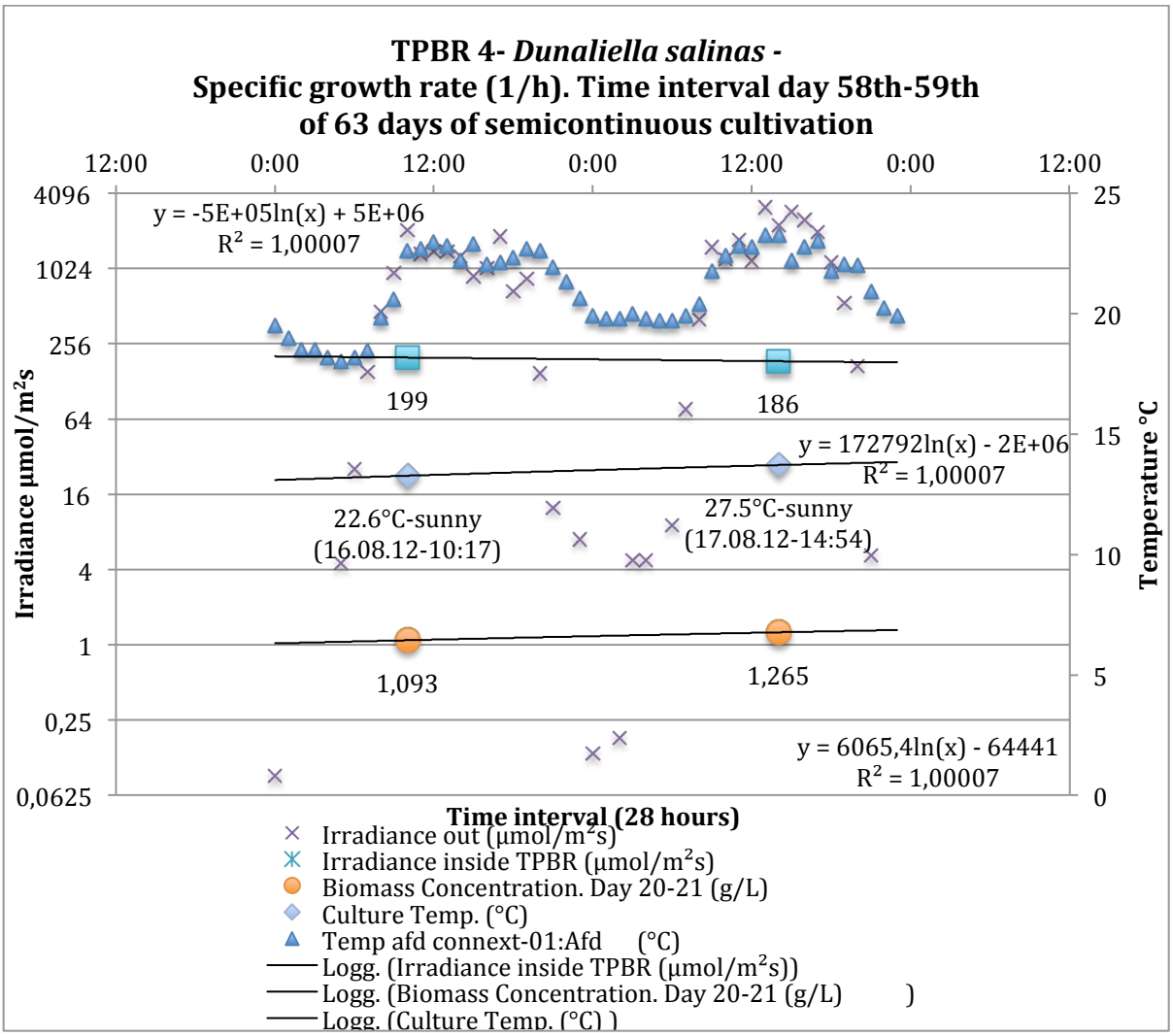


Figure 83 - The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for marine microalgae *Dunaliella salinas.* Maximum biomass concentration sampled 16.08.12 and 17.08.12 (day 58th and 59th, age of culture) from algae culture operated in semi-continuous system production (TPBR 4) in greenhouse conditions for 63 days. . Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of the same shape and colour represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 51 Computation of population specific growth rate (µ) observed 16.08.12-17.08.12 (day 58h-59th, age of culture), for marine microalgae *Dunaliella salinas* (TPBR 4), cultivated in semicontinuous system production for 63 days. 20.06.12 – 23.08.12 (see table 12.4.1)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
58	1,093	22,64	2063,07	160812	10:17	Sunny	22,6	199	90,35
59	1,265	23,26	2269,305	170812	14:54	Sunny	27,5	186	91,80

Figure 83 shows the highest values for growth rate per hour corresponding to the day 16.08.12 and 17.08.12 (day 58th and 59th, age of culture) with a biomass concentration (TSS) of 1,093 g/L and 1,265 g/L, respectively. During this time interval of 28 hours, the algae had a specific growth rate of 0,01411h^{-1}, ((1,265 - 1,093)/28 h).

The value registered for irradiance was low, 199 and 186 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 4, above the suspension but under the lid of the reactor. The culture grew normally.

The data registered by the meteorological station for 16.08.12 and 17.08.12, was 458,46 W*m^{-2} and 504,29 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 2063,07 µmol photon m^{-2} s^{1} and 2269,305 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for these days, at the time of sampling, were both sunny, and it was some significant difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 4, and the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was -0,04 °C lower (16.08.12 – 10:17), and +4,24 °C higher (17.08.12 – 14:54) than the outside temperature. The differences in temperature for those days were -0,18% and +15,42% for 16.08.12 and 17.08.12, respectively. The TPBR 4 was accumulating heat, the second day of the measure, in the algae suspension.

Analysing the irradiances registered, it´s possible to calculate the percentage of incident light irradiance that reached the algae culture. (199*100%/2063,07)= 9,65 % and (186*100%/2269,305)= 8,2% of the solar irradiance reached the algae culture. This means a loss of 90,35% and 91,8% of the incident solar irradiation on the greenhouse registered at the time of sampling for 16.08.12 and 17.08.12, respectively.

In spite of the sunny weather, the middle level of incident irradiation observed together with the higher temperature recorded in the suspension, affected positively the algae biomass production. This phenomenon of middle irradiation level and warmer culture did not affect, apparently, the photosynthetic production.

From the graph can we conclude that the TPBR 4 did offered an appropriate growth conditions and the irradiation reaching the algae suspension could be maintained at the same values, 186-199 µmol*$m^{-2}$$s^{-1}$ and the temperature could as well be maintained at the registered values of the interval of 22,6-27,5 °C. The specific growth rate was 0,01411h^{-1}.

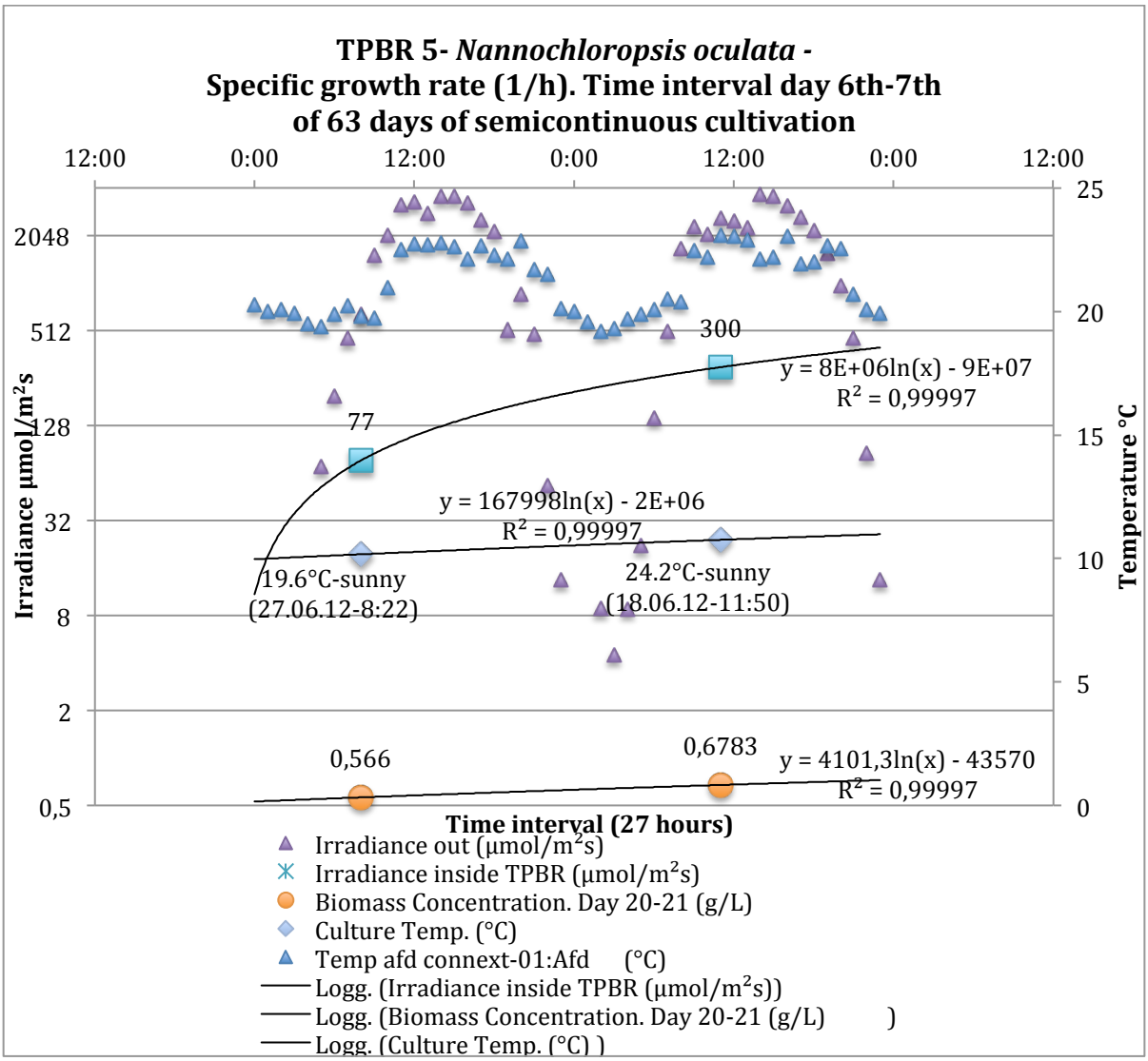


Figure 84 - The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for marine microalgae *Nannochloropsis oculata.* Maximum biomass concentration sampled 27.06.12 and 28.06.12 (day 6th and 7th, age of culture) from age culture operated in semi-continuous system production (TPBR 5) in greenhouse conditions for 63 days. . Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of the same shape and colour represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 52 - Computation of population specific growth rate (µ) observed 27.06.12-28.06.12 (day 6th and 7th, age of culture), for marine microalgae *Nannochloropsis oculata* (TPBR 5), cultivated in semicontinuous system production for 63 days. 20.06.12 – 23.08.12 (see table 12.5)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
6	0,566	19,81	644,265	270612	8:22	Sunny	19,6	77	88,05
7	0,6783	23,07	2645,01	280612	11:50	Sunny	24,2	300	88,66

Figure 84 shows the highest values for growth rate per hour corresponding to the days 27.06.12 and 28.06.12 (day 6th and 7th, age of culture) with a biomass concentration (TSS) of 0,566 g/L and 0,6783 g/L, respectively. During this time interval of 27 hours, the algae had a specific growth rate of 0,00416h^{-1}, ((0,6783-0,566)/27 h).

The value registered for irradiance was low, 77 and 300 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 5, above the suspension but under the lid. The culture grew poorly.

The data registered by the meteorological station for 27.06.12 and 28.06.12, was 143,17 W*m^{-2} and 687,78 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 644,265 µmol photon m^{-2} s^{1} and 2645,01 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for those days, at the time of sampling, were both sunny, and it was not significant difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 5, and the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was -0,21 °C lower (27.06.12 – 8:22), and 1,13 °C higher (28.06.12 – 11:50) than the outside temperature. The differences in temperature for those days were -1,06% and +4,67% for 27.06.12 and 28.06.12, respectively. The TPBR 5 was accumulating some heat in the algae suspension. Probably the location of the TPBR 5 had some indirect effect on this heat accumulation. Surrounded on three sides by other TPBRs but facing the refracted irradiation from the plastic wall of the room, that was heated by the incident solar irradiation. The weather was sunny both days.

Analysing the irradiances registered, it´s possible to calculate the percentage of incident light irradiance that reached the algae culture. (77*100%/644,265)=11,95 % and (300*100%/2645,01)=11,34% of the solar irradiance reached the algae culture. This means a loss of 88,05% and 88,66% (almost identical values) of the incident solar irradiation on the greenhouse registered at the time of sampling for 27.06.12 and 28.06.12, respectively.

In spite of the good weather, the low incident irradiation together with the higher temperature recorded in the suspension (recorded the second day), affected the algae biomass production. This phenomenon affected the photosynthetic production, inhibiting it.

From the graph can we conclude that the TPBR 5 did not offered optimal microalgal grow conditions and the irradiation reaching the algae suspension has to increase from the registered value of 208 µmol*$m^{-2}$$s^{-1}$ and the temperature has to be at the middle value of the interval of 22,2-26,08 °C. The specific growth rate was 0,00416h^{-1}.

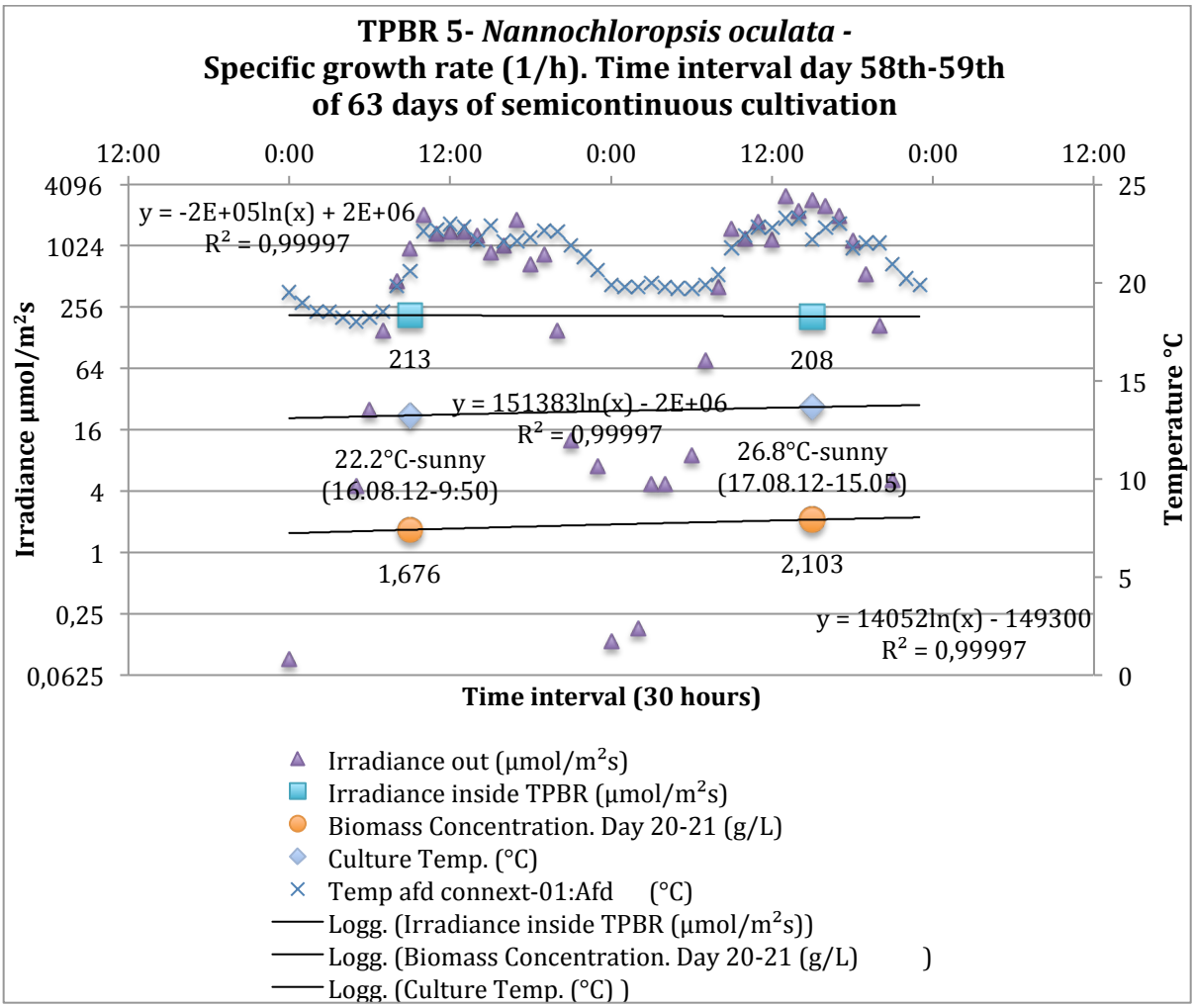


Figure 85 - The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for marine microalgae *Nannochloropsis oculata.* Maximum biomass concentration sampled 16.08.12 and 17.08.12 (day 58th and 59th, age of culture) from algae culture operated in semi-continuous system production (TPBR 5) in greenhouse conditions for 63 days. Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbol of the same shape and colour, represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 53 - Computation of population specific growth rate (µ) observed 16.08.12-17.08.12 (day 58th and 59th, age of culture), for marine microalgae *Nannochloropsis oculata* (TPBR 5), cultivated in semicontinuous system production for 63 days. 20.06.12 – 23.08.12 (see table 12.5.1)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
58	1,676	20,59	953,1	160812	9:50	Sunny	22,2	213	77,65
59	2,103	22,2	2897,28	170812	15:05	Sunny	26,8	208	92,82

Figure 85 shows the highest values for growth rate per hour corresponding to the day 16.08.12 and 17.08.12 (day 58th and 59th, age of culture) with a biomass concentration (TSS) of 1,676 g/L and 2,103 g/L, respectively. During this time interval of 30 hours, the algae had a specific rate, 0,01423h^{-1}, ((2,103-1,676)/30 h).

The value registered for irradiance was low, 213 and 208 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 5, above the suspension but under the lid. The culture grew but not so fast as expected.

The data registered by the meteorological station for 16.08.12 and 17.08.12, was 211,8 W*m^{-2} and 643,84 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 953,1 µmol photon m^{-2} s^{1} and 2897,28 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for those days, at the time of sampling, were both sunny, and it was some grades of difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 5, the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was 1,61 °C higher (16.08.12 – 9:50), and 4,6 °C higher (17.08.12 – 15:05) than the outside temperature. The differences in temperature for those days were +7,25% and +17,16% for 16.08.12 and 17.08.12, respectively. The TPBR 5 was accumulating heat in the algae suspension. Could that be affecting the specific growth rate of the algae? The TPBR 5 was located on a bench, at the most center position of the room at the greenhouse. The weather was sunny and shiny.

Analysing the irradiances registered, it´s interesting to see the percentage of incident light irradiance reached the algae culture. (213*100%/953,1)=22,35 % and (208*100%/2897,28)= 7,18% of the solar irradiance reached the algae culture. This means a loss of 77,65% and 92,82% of the incident solar irradiation on the greenhouse registered at the time of sampling for 16.08.12 and 17.08.12, respectively.

In spite of the good weather, the low incident irradiation together with the higher temperature recorded in the suspension, affected the algae biomass production. This phenomenon affected the photosynthetic production, inhibiting it.

From the graph can we conclude that the TPBR 5 did not offered optimal microalgal grow conditions and the irradiation reaching the algae suspension was between 208-213 µmol*$m^{-2}$$s^{-1}$ and the temperature was between 22,2-26,08 °C. The specific growth rate was 0,01423h^{-1}.

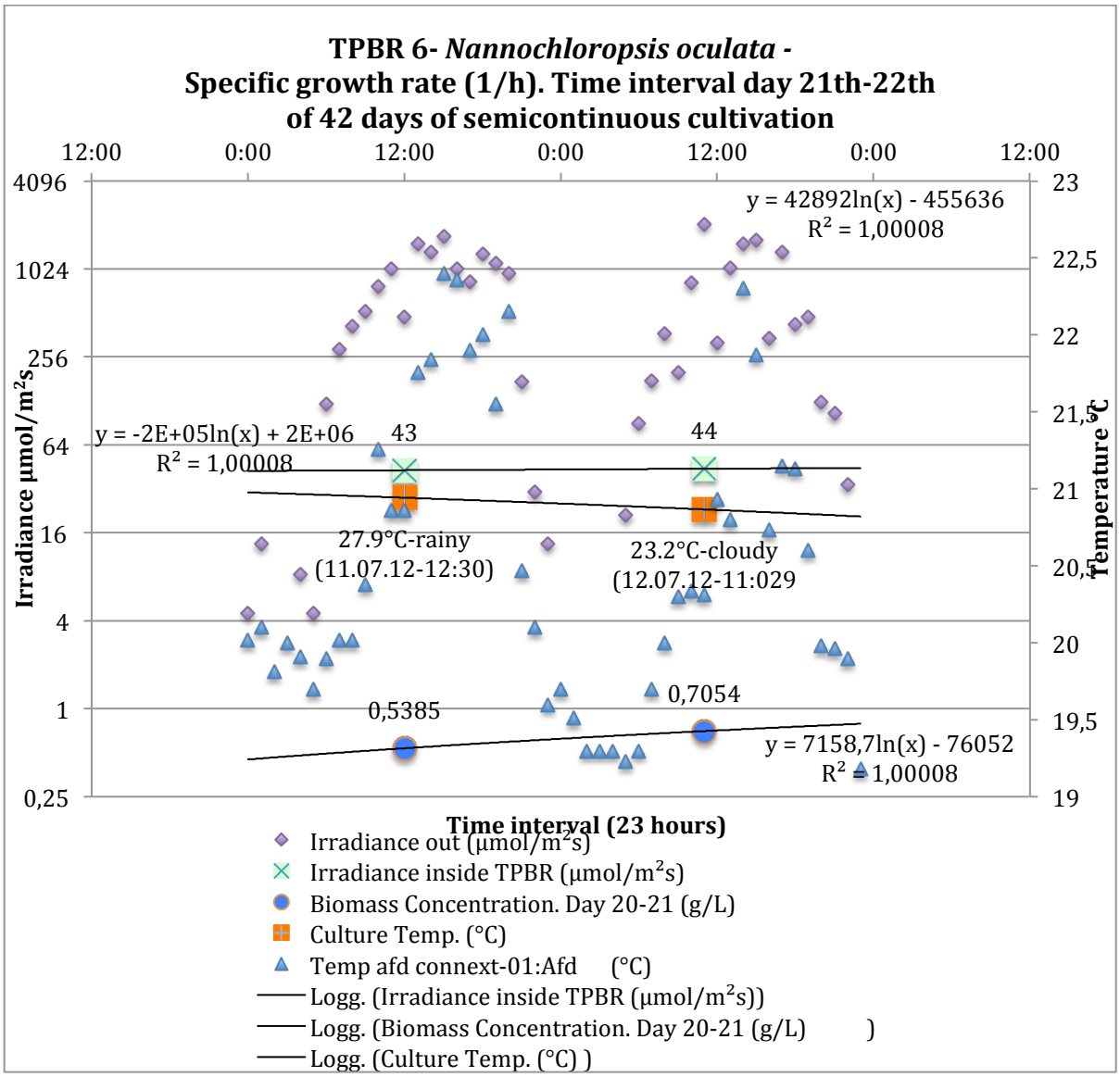


Figure 86 - The effect of solar irradiation and temperature on specific growth rate (h^{-1}) for marine microalgae *Nannochloropsis oculata.* Maximum biomass concentration sampled 11.07.12 and 12.07.12 (days 21th and 22nd, age of culture) from algae culture operated in semi-continuous system production (TPBR 6) in greenhouse conditions for 63 days. Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of the same colour and shape represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 54 - Computation of population specific growth rate (µ), observed 11.07.12-12.07.12 (days 21th and 22nd, age of culture), for marine microalgae *Nannochloropsis oculata* (TPBR 6), cultivated in semicontinuous system production for 63 days. 20.06.12 – 23.08.12 (see table 12.6)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
21	0,5385	20,86	481,77	110712	12:30	Rainy	27,9	43	91,07
22	0,7054	20,31	2075,72	120712	11:02	Cloudy	23,02	44	97,88

Figure 86 shows the highest values for maximal growth rate per hour corresponding to the day 11.07.12 and 12.07.12 (day 21th and 22th, age of culture) with a biomass concentration (TSS) of 0,5385 g/L and 0,7054 g/L, respectively. During this time interval of 23 hours, the algae had a specific rate, 0,00726h^{-1}, ((0,7054-0,5385)/23 h).
The value registered for irradiance was very low, 43 and 44 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 6, above the suspension but under the lid. The culture grew but not so fast as expected.

The data registered by the meteorological station for 11.07.12 and 12.07.12, was 107,06 W*m^{-2} and 461,27 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 481,77 µmol photon m^{-2} s^{1} and 2075,715 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for those days, at the time of sampling, were rainy and sunny, and it was some degree of difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 6, the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was 7,04 °C higher (11.07.12 – 12:30), and 2,71 °C higher (12.07.12 – 9:15) than the outside temperature. The differences in temperature for those days were +27,09% and +13,34% for 11.07.12 and 12.07.12, respectively. The TPBR 6 was accumulating heat in the algae suspension. Could that be affecting the specific growth rate of the algae? The TPBR 6 was located on a bench, at the most center position of the room at the greenhouse. Probably the TPBR 6 was receiving more direct solar irradiation from previous days, therefore in spite the day was rainy, the suspension retained the heat still.

Analysing the irradiances registered, it´s interesting to see the percentage of incident light irradiance reached the algae culture. (43*100%/481,77)= 8,92 % and (44*100%/2075,72)= 2,11% of the solar irradiance got into the algae culture. This means a loss of 91,07% and 97,88% of the incident solar irradiation on the greenhouse registered at the time of sampling for 11.07.12 and 12.07.12, respectively.

The rainy weather of one day affected the biomass production and the algae had a deficit of incident irradiation and the temperature was higher in the suspension that the outside temperature. This phenomenon affected the photosynthetic production, inhibiting it.

From the graph can we conclude that the TPBR 6 did not offered optimal microalgal grow conditions and the irradiation reaching the algae suspension was between 43-44 µmol*m^{-2}s^{-1} and the temperature was between 23,2-27,09 °C. The specific growth rate was 0,00726h^{-1}.

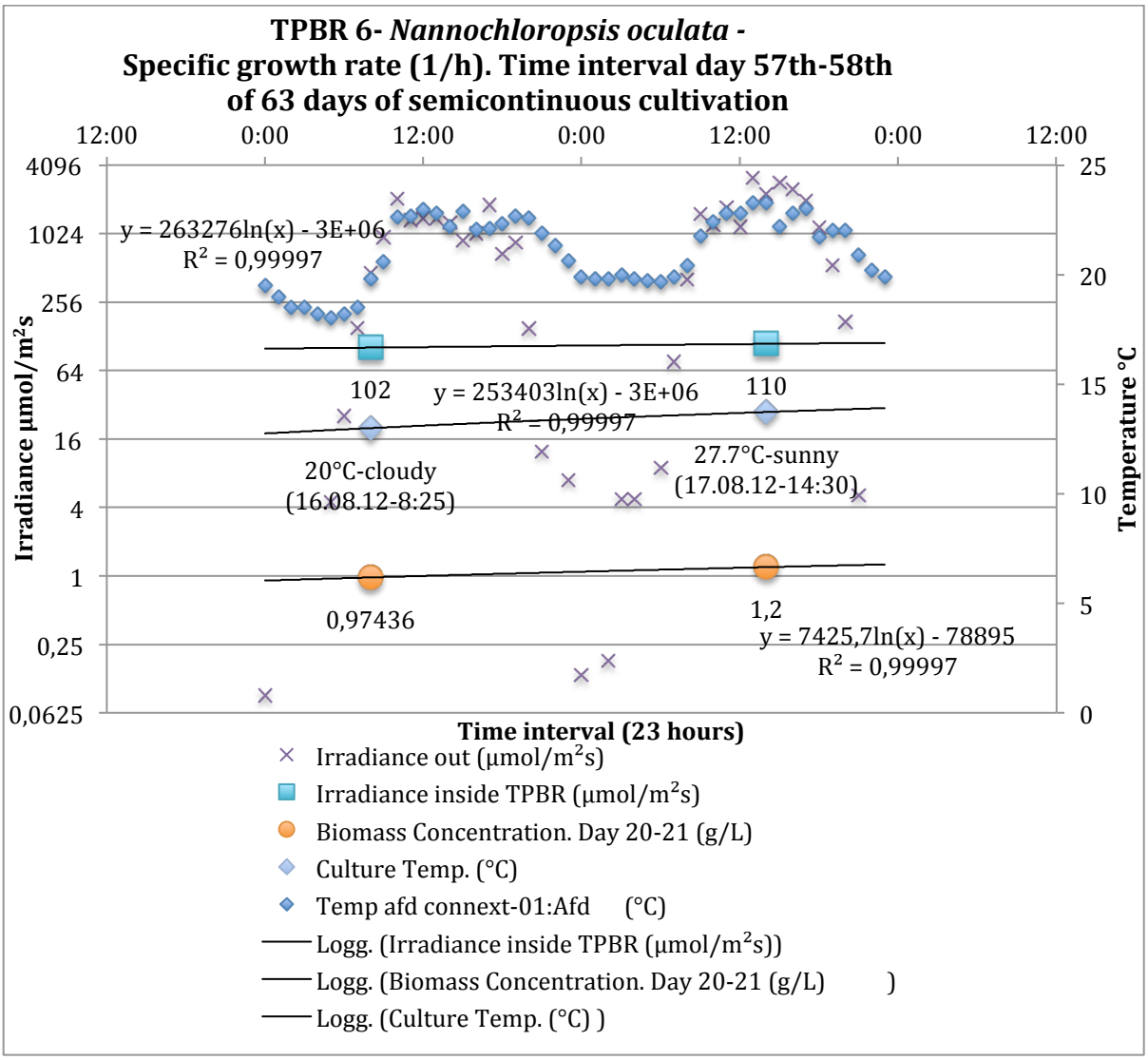


Figure 87 - The effect of solar irradiation and temperature on specific growth rate (h^{-1}) for marine microalgae *Nannochloropsis oculata.* Maximum biomass concentration sampled 16.08.12 and 17.08.12 (days 57th and 58th, age of culture) from algae culture operated in semi-continuous system production (TPBR 6) in greenhouse conditions for 63 days. Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of the same colour and shape represents abscissas with theirs respective function and R^2 values noted in the graph.

Table 55 - Computation of population specific growth rate (µ), observed 16.08.12-17.08.12 (days 57th and 58th, age of culture), for marine microalgae *Nannochloropsis oculata* (TPBR 5), cultivated in semicontinuous system production for 63 days. 20.06.12 – 23.08.12 (see table 12.6.1)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
57	0,97436	19,81	460,08	160812	8:25	Cloudy	20	102	77,83
58	1,2	23,26	2269,31	170812	14:30	Sunny	27,7	110	95,15

Figure 87 shows the highest values for maximal growth rate per hour corresponding to the day 16.08.12 and 17.08.12 (day 57th and 58th, age of culture) with a biomass concentration (TSS) of 0,97436 g/L and 1,2 g/L, respectively. During this time interval of 23 hours, the algae had a specific rate, 0,0098h^{-1}, ((1,2-0,97436)/23 h).

The value registered for irradiance was low, 102 and 110 µmol*m^{-2}*s^{-1}. This measure was registered inside the TPBR 7, above the suspension but under the lid. The culture grew but not so fast as expected.

The data registered by the meteorological station for 16.08.12 and 17.08.12, was 102,24 W*m^{-2} and 504,29 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 460,08 µmol photon m^{-2} s^{1} and 2269,31 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for those days, at the time of sampling, were cloudy and sunny, and it was some degree of difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 6, the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was 0,19 °C higher (16.08.12 – 8:25), and 4,34 °C higher (17.08.12 – 14:30) than the outside temperature. The differences in temperature for those days were +0,96% and +19,1% for 16.08.12 and 17.08.12, respectively. The TPBR 6 was accumulating heat in the algae suspension. Could that be affecting the specific growth rate of the algae? The TPBR 6 was located on a bench, at the most center position of the room at the greenhouse. Probably the TPBR 6 was receiving more direct solar irradiation from previous days, therefore in spite the day was cloudy, the suspension retained the heat still.

Analysing the irradiances registered, it´s interesting to see the percentage of incident light irradiance reached the algae culture. (102*100%/460,08)= 22,17 % and (110*100%/2269,31)= 4,85% of the solar irradiance got into the algae culture. This means a loss of 77,83% and 95,15% of the incident solar irradiation on the greenhouse registered at the time of sampling for 16.08.12 and 17.08.12, respectively.

The rainy weather of one day affected the biomass production and the algae had a deficit of incident irradiation and the temperature was higher in the suspension that the outside temperature. This phenomenon affected the photosynthetic production, inhibiting it.

From the graph can we conclude that the TPBR 6 did not offered optimal microalgal grow conditions and the irradiation reaching the algae suspension was too low, between 102-110 µmol*$m^{-2}$$s^{-1}$ and the temperature was too high, between 20-27,7 °C. The specific growth rate was 0,0098h^{-1}.

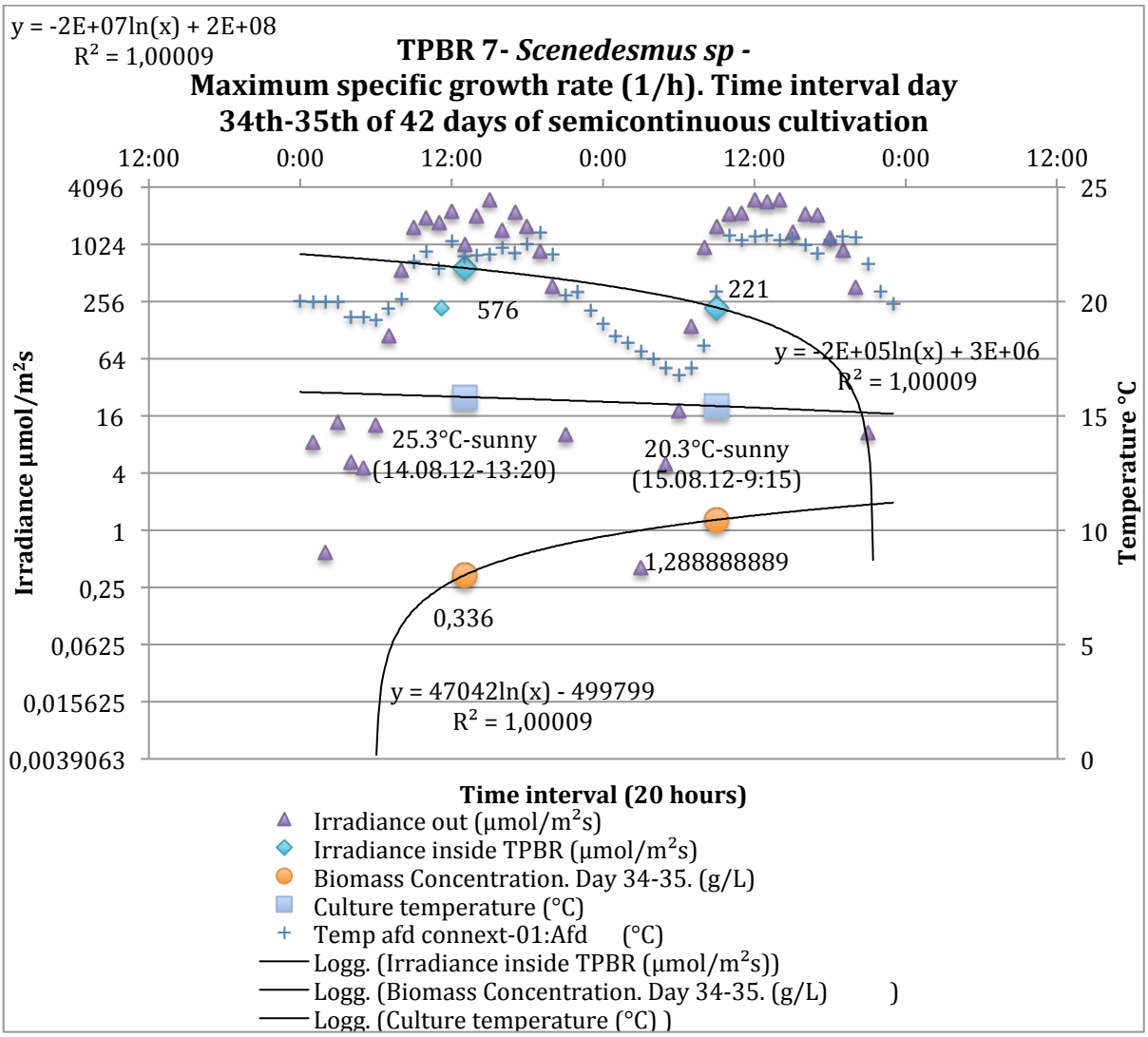


Figure 88 - The effect of solar irradiation and temperature on specific growth rate (h^{-1}) for microalgae *Scenedesmus sp.* Maximum biomass concentration sampled 14.08.12 and 15.08.12 (days 34th and 35th, age of culture) from algae culture operated in semi-continuous system production (TPBR 7) in greenhouse conditions for 42 days. Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The three lines crossing the two symbols of it colour and shape, represent abscissas with it´s respective function and R^2 values noted in the graph.

Table 56 Computation of population specific growth rate (µ), observed 14.08.12-15.08.12 (days 34th and 35th, age of culture) for fresh water microalgae *Scenedesmus sp.* (TPBR 7), cultivated in semicontinuous system production for 42 days. 11.07.12 – 23.08.12 (see table 12.7)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
34	0,336	21.98	1021,5	140812	13:20	Sunny	25,3	576	43,61
35	1,289	20,44	1552,14	150812	09:15	Sunny	20,3	221	85,76

Figure 88 shows the highest values for maximal growth rate per hour corresponding to day 14.08.12 and 15.08.12 (day 34th and 35th of culture) with a biomass concentration of 0,336 g/L and 1,289 g/L (TSS) respectively. During this time interval of 20 hours, the algae grew at a specific rate of 0, 04765 h^{-1}, ((1,289 -0,336)/20 h).

In spite of the low irradiance 576 and 221 µmol*m^{-2}*s^{-1}, which is the value registered inside the TPBR 7, under the lid but over the algae suspension, the culture grow successfully.

Based on the data registered at the meteorological station at Ås (N 59° 39' 37'', Ø 10 ° 46' 54'', 93.3 meters above sea level), can be calculated the solar irradiance on the roof of the green house, and the value of irradiance was given in Wm^{-2}, then the conversion factor for intensity towards solar irradiance, the approximate conversion factor for sunlight is 1 Wm^{-2} equals about 4,5 µmol photon m^{-2} s^{1} (Richmond 2004).

The data given for 14.08.12 and 15.08.12, was 227 W*m^{-2} and 344,92 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 1021,5 µmol photon m^{-2} s^{1} and 1552,14 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for those days, at the time of sampling, were sunny and sunny, and it was some degree of difference of temperatures between outside the green house and the temperature measured inside the algae suspension collected from the TPBR 7, the temperature of the culture was measured with a handheld multi-meter. The temperature in the algae suspension was 3,32 °C higher (14.08.12 – 13:20), and 0,140 °C lower (15.08.12 – 9:15) than the outside temperature. The differences in temperature for those days were +15,10% and -0,69% for 14.08.12 and 15.08.12, respectively. Probably, the reason for this thermic inequality could easily be explained, by the location of the TPBR 7, surrounded by other TPBRs in the vicinity. The TPBR 7 was located on a bench, surrounded by three TPBR on three of it´s surface walls and it´s fourth wall surface, facing the corridor of empty space to the neighbour bench. Could this corridor affect the thermic environment of the TPBR 7?

Analysing the irradiances registered, it´s interesting to see the percentage of incident light irradiance reached the algae culture. (576*100%/1021,5)= 56,39 % and (221*100%/1552,14)= 14,24% of the solar irradiance got into the algae culture. This means a loss of 43,61% and 85,76% of the incident solar irradiation on the greenhouse registered at the time of sampling for 21.08.12 and 22.08.12, respectively.

The algae grew best when the incident irradiation and the temperature were lowering.
From the graph can we conclude that the TPBR 7 offered optimal microalgal grow conditions when the irradiation reaching the algae suspension was between 220-576 µmol*$m^{-2}$$s^{-1}$ and the temperature of the algae culture was between 20,3-25,3 °C. The specific growth rate was 0, 04765h^{-1}.

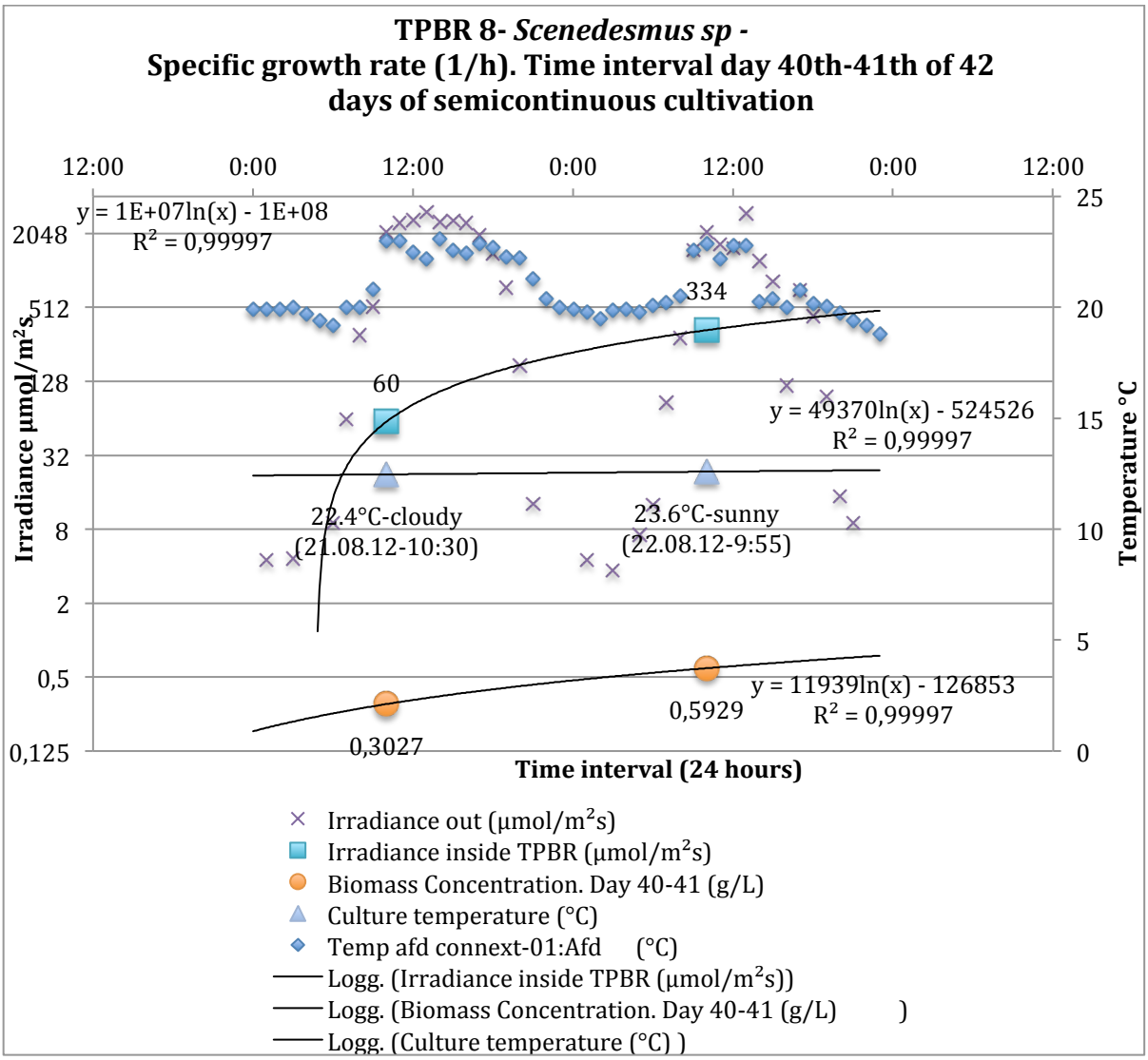


Figure 89 - The effect of solar irradiation and temperature on specific growth rate (h^{-1}) for microalgae *Scenedesmus sp.* Maximum biomass concentration sampled 21.08.12 and 22.08.12 (days 40th and 41th, age of culture) from algae culture operated in semi-continuous system production (TPBR 8) in greenhouse conditions for 42 days. Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The line crossing the two symbols of same shape and colour, represent abscissas with it´s respective function and R^2 values noted in the graph.

Table 57 - Computation of population specific growth rate (µ), observed 21.08.12-22.0812 (days 40th and 41th, age of culture), for fresh water microalgae *Scenedesmus sp.* (TPBR 8), cultivated in semicontinuous system production for 42 days. 11.07.12 – 23.08.12. (See table 12.8)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Light Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
40	0,3027	22.9	2082,915	210812	10:30	Cloudy	22,4	60	97,12
41	0,5928	20,59	2096,28	220812	09:55	Sunny	23,6	334	84,07

Figure 89 shows the highest values for maximal growth rate per hour corresponding to the day 21.08.12 and 22.08.12 (day 40th and 41th of culture) with a biomass concentration of 0,3027 g/L and 0,5928 g/L (TSS) respectively. During this time interval of 24 hours, the algae grew at a specific rate of 0, 01209 h^{-1}, ((0,5928 -0,3027)/24 h).

In spite of the low irradiance 60 and 334 µmol*m^{-2}*s^{-1}, which is the value registered under the lid but over the algae culture, the culture grow successfully.

The data given for 14.08.12 and 15.08.12, was 462,87 W*m^{-2} and 465,84 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 2082,915 µmol photon m^{-2} s^{1} and 2096,28 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for those days, at the time of sampling, were cloudy and sunny, it was some degree of difference of temperatures between outside the green house and the temperature measured in the algae culture. The temperature in the algae suspension was 0,5 °C lower (21.08.12 – 10:30), and 3,5 °C higher (22.08.12 – 9:56) than the outside temperature. The differences in temperature for those days were -7,18% and +14,62% for 21.08.12 and 22.08.12, respectively.

The reason for this thermic inequality cannot easily be explained, it was expected the algae culture should show approximately the same thermic stability inside the TPBR 8, as the other TPBR in the vicinity. Probably there were another thermic conditions in the locality where the TPBR 8 was placed. The TPBR 8 was located in the periphery surrounded by three TPBR on three of it´s surface walls and a corridor of empty space on it´s fourth wall surface, facing the wall of the room. Analysing the irradiances registered, it´s interesting to see the percentage of incident light irradiance reached the algae culture. (60*100%/2082,915)= 2.88 % and (334*100%/2096,28)=15,93% of the solar irradiance got into the algae culture. This means a loss of 97,12% and 84,07% of the incident solar irradiation on the greenhouse registered at the time of sampling for 21.08.12 and 22.08.12, respectively.

The algae grew best when the incident irradiation and the temperature were under 350 µmolm^{-2} s^{1} and under 25 °C, respectively.

From the graph can we conclude that the TPBR 8 offered optimal microalgal grow conditions when the irradiation reaching the algae suspension was closest 334 µmol*$m^{-2}$$s^{-1}$ and the temperature of the algae culture was between 22,4-23,6 °C. The specific growth rate was 0, 01209 h^{-1}.

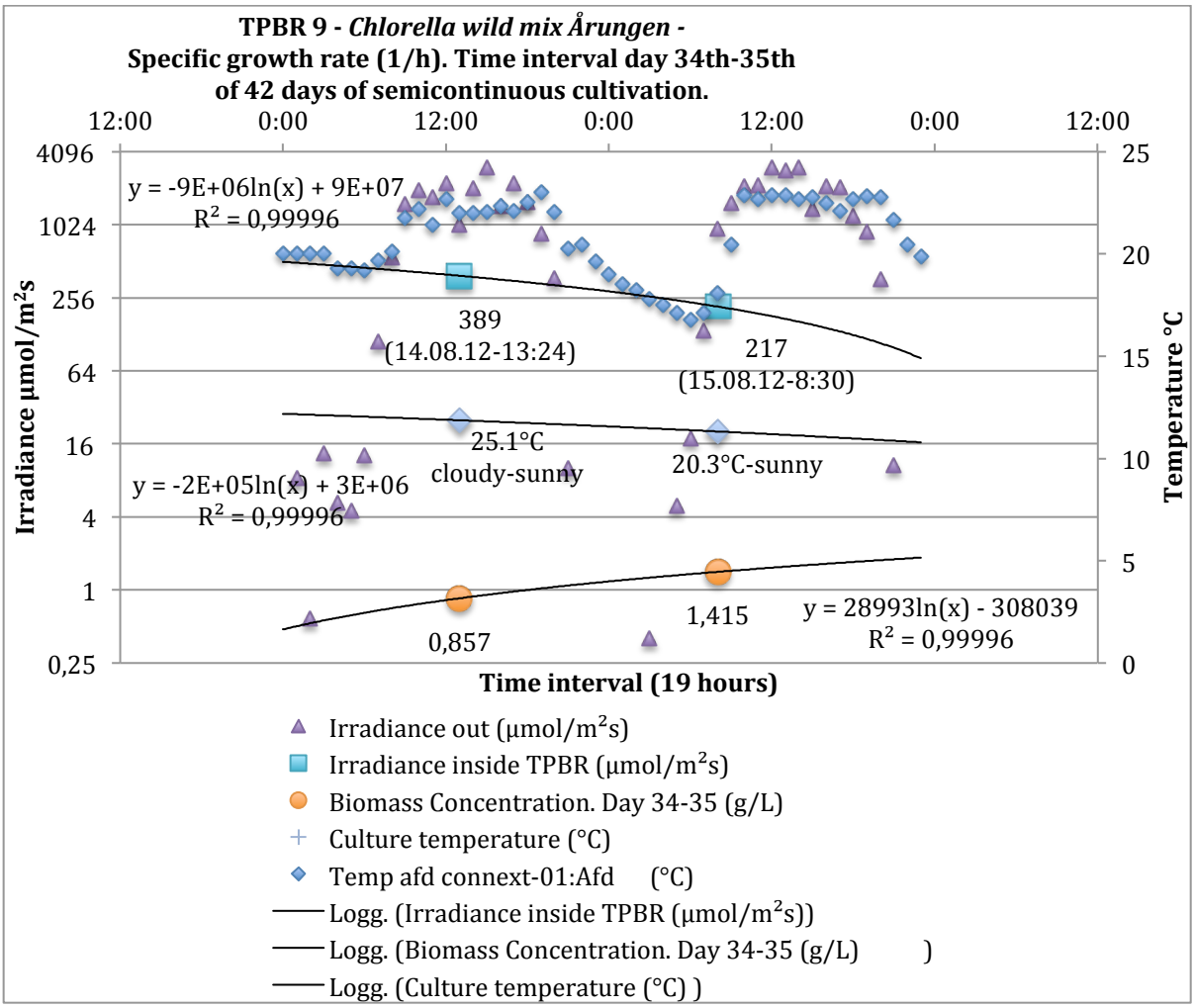


Figure 90 - The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for microalgae *Chlorella wild mix Årungen.* Maximum biomass concentration sampled 14.08.12 and 15.08.12 (days 34th and 35th, age of culture) from algae culture operated in semi-continuous system production (TPBR 10) in greenhouse conditions for 42 days. Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The lines crossing the two symbols of same shape and colour represent abscissas with it´s respective function noted in the graph.

Table 58 - Computation of population specific growth rate (µ), observed 14.08.12-15.08.12 (days 34th and 35th, age of culture) for fresh water microalgae *Chlorella wild mix Årungen* (TPBR 9), cultivated in semi-continuous system production for 46 days. 11.07.12 – 23.08.12 (see table 12.9)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp. Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture Temp. (°C)	Light Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
34	0,857	21.98	1021,5	140812	13:24	Sunny-cloudy	25,1	389	61,92
35	1,415	18,09	940,05	150812	08:30	Sunny	20,3	217	76,92

Figure 90 shows the highest values for maximal growth rate per hour corresponding to the day 14.08.12 and 15.08.12 (day 34th and 35th of culture) with a biomass concentration of 0,857 g/L and 1,415 g/L (TSS) respectively. During this time interval of 19 hours, the algae grew at a specific rate of 0, 02937 h^{-1}, ((0,857-1,415)/19).

In spite of the low irradiance 389 and 217 $\mu mol*m^{-2}*s^{-1}$, which is the value registered under the lid but over the algae culture, the culture grow successfully.

The data given for 14.08.12 and 15.08.12, was 227 $W*m^{-2}$ and 208,9 $W*m^{-2}$, respectively. Applying the 4,5 conversion rate, the values will be 1021,5 µmol photon m^{-2} s^{1} and 940,05 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for those days were sunny-cloudy and sunny, it was remarkable difference of temperatures between outside the green house and the temperature measured inside the empty space of the TPBR 9, between the lid and the algae culture. The temperature in the algae suspension was higher than the outside temperature. The difference was 12,43% and 10,89% higher for 14.08.12 and 15.08.12, respectively.

The reason for this inequality can be explained, that the physical barriers between the outside environment and the algae culture, maintained thermo-stability inside the TPBR 9. Analysing the irradiances registered, it´s interesting to see the percentage of incident light irradiance reached the algae culture. (389*100%/1021,5)= 38,08 % and (217*100%/940,05)=23,08% of the solar irradiance got into the algae culture. This means a loss of 61,92% and 76,92% for the two samples collected 14.08.12 and 15.08.12, respectively.

The algae grew best when the incident irradiation and the temperature were under 400 $\mu molm^{-2}$ s^{1} and under 25,5 °C, respectively.

From the graph can we conclude that the TPBR 9 offered optimal microalgal grow conditions when the irradiation reaching the algae suspension was between 217-389 $\mu mol*m^{-2}s^{-1}$ and the temperature of the algae culture was between 20,3-25,1 °C. The specific growth rate was 0, 02937h^{-1}.

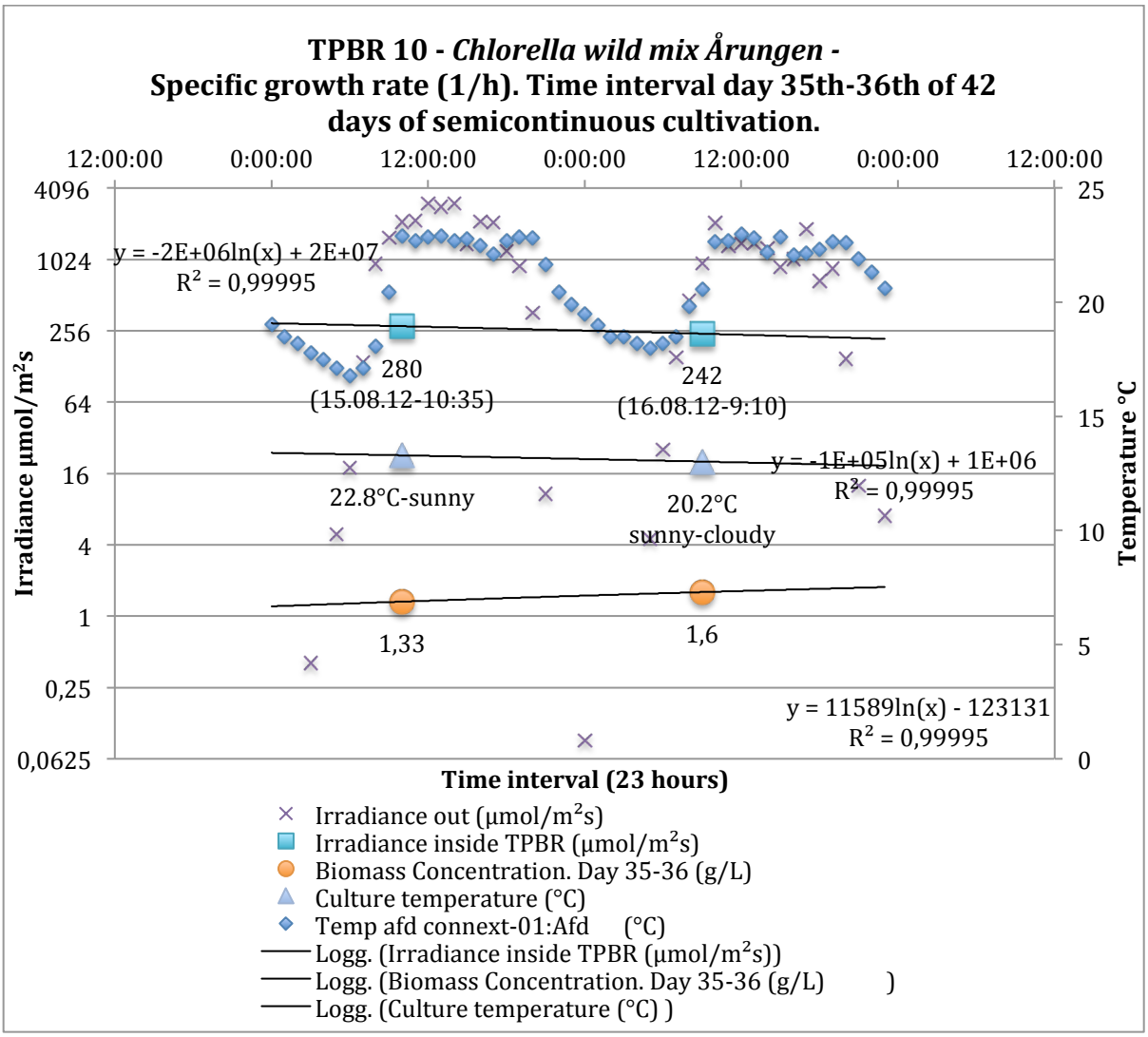


Figure 91 –The effects of solar irradiation and temperature on specific growth rate (h^{-1}) for microalgae *Chlorella wild mix Årungen.* Maximum biomass concentration sampled 15.08.12 and 16.08.12 (days 35th and 36th, age of culture) from algae culture operated in semi-continuous system production (TPBR 10) in greenhouse conditions for 42 days. Diagram plotted as $\log_{2.0}$ scale to emphasize differences between the data and fits low irradiance, temperature and biomass concentration (g/L). The lines crossing the two symbols of same shape and colour represent abscissas with it´s respective function noted in the graph.

Table 59 - Computation of population maximum specific growth rate (µ), observed 15.08.12-16.08.12 (days 35th and 36th, age of culture) for fresh water microalgae *Chlorella wild mix Årungen* (TPBR 10), cultivated in semi-continuous system production for 46 days. 11.07.12 – 23.08.12 (see table 12.10)

Age of culture (day)	Biomass Concentration TSS (g/L)	Temp Out (°C)	Irradiance out (µmol/m²s)	Date (days)	Time of sample (hours)	Weather condition	Culture temp. (°C)	Irradiance inside TPBR (µmol/m²s)	Irradiation energy loss (%)
35	1,327	22.9	2118,30	150812	10:35	Sunny	22,8	280	86,78
36	1,6	20,59	953,10	160812	09:10	Sunny-cloudy	20,2	242	74,61

Figure 91 shows the highest values for maximal growth rate per hour corresponding to the days 15.08.12 and 16.08.12 (day 35th and 36th of culture) with a biomass concentration of 1,33 g/L and 1,6 g/L (TSS) respectively. During this time interval of 23 hours, the algae grew at a specific rate of 0, 01187 h^{-1}, ((1,6-1,33)/23).

In spite of the low irradiance 282 and 240 µmol*m^{-2}*s^{-1}, which is the value registered under the lid but over the algae culture, the culture grow successfully.

The data given for 15.08.12 and 16.08.12, was 470,73 W*m^{-2} and 211,80 W*m^{-2}, respectively. Applying the 4,5 conversion rate, the values will be 2118,30 µmol photon m^{-2} s^{1} and 953,10 µmol photon m^{-2} s^{1}, respectively.

The weather conditions for those days were sunny and sunny-cloudy, it´s remarkable the almost no difference of temperature outside the green house and the temperature measured inside the empty space of the TPBR 10, between the lid and the algae culture.
The reason for this equality can be understood if we make count the physical barriers between the outside environment and the algae culture. The room in the green house was coated with a white paint on the outside face, the almost white polypropylene plastic on the walls and lid of the TPBR, filtered out most of the solar irradiance. Analysing the irradiances registered, it´s interesting to see the percentage of incident light irradiance reached the algae culture. (280*100%/2118,285)= 13,218 % and (242*100%/953,1)=25,39% of the solar irradiance got into the algae culture. This means a loss of 86,782% and 74,609% for the two samples collected 15.08.12 and 16.08.12, respectively.

The algae grew best when the incident irradiation and the temperature were under 300 µmolm^{-2} s^{1} and under 25 °C, respectively.

From the graph can we conclude that the TPBR 10 offered optimal microalgal grow conditions when the irradiation reaching the algae suspension was between 242-280 µmol*$m^{-2}$$s^{-1}$ and the temperature of the algae culture was between 20,2-22,8 °C. The specific growth rate was 0, 01187h^{-1}.

15 Appendix 6. All data registered on all TPBRs (Tab. 60-79)

The algae cultures were distributed as follow:
Group 1 and cultivated for a period of 63 days (20.06.12-23.08,12)
TPBR 1 and TPBR 2 – *Chlorella vulgaris*
TPBR 3 and TPBR 4 – *Dunaliella salinas*
TPBR 5 and TPBR 6 - *Nannochloropsis oculata*
Group 2 and cultivated for a period of 42 days (20.07.12-23.08.12)
TPBR 7 and TPBR 8 - *Scenedesmus sp.,*
TPBR 9 and TPBR 10 - *Chlorella wild mix Årungen*

The data registered for five microalgae strains in duplicate were: age of culture, culture date, time of sampling, outside weather condition, algae temperature, irradiation on surface culture, pH-values, dissolved oxygen, conductivity, salinity, remaining culture volume, fresh volume added, dilution rate, biomass concentration (TSS) and the calculations performed were: growth rate, specific growth rate, doublings per day, doubling time, biomass productivity, illuminated area productivity, volumetric productivity, areal productivity, biomass output energy, PAR irradiance input energy, photosynthetic energy, areal CO_2 fixation rate.

Table 60 - Data registered on TPBR 1 – *Chlorella vulgaris* – freshwater microalgae for 63 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Age of culture (days)	Culture Date	Time of Sampling	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture ($\mu mol\ m^{-2}\ s^{-1}$)	pH	Dissolved Oxygen (DO) ($mg\ L^{-1}$)	Conductivity ($mS\ cm^{-1}$)	Salinity ($g\ L^{-1}$) or ppt	Remaining culture volume V_o ($g\ L^{-1}$)	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) ($g\ L^{-1}$)
0	200612	10:45	Sunny	25,30	103	8,12	8,56	1,56	0,75671	25,599				0,05497
1	210612	16:10	Sunny	27,90	85	6,851	8,68	1,604	0,77996	25,456				0,05664
2	220612	08:50	Sunny	23,4	156	6,786	10,93	1,559	0,75618	25,356				0,126
5	250612	10:12	Cloudy	20,2	49	7,395	11,68	1,537	0,74458	19,305	6			0,51176
6	260612	08:45	Sunny	21,7	112	7,41	9,42	1,248	0,59362	25,274		6	0,23739	0,40322
7	270612	07:45	Sunny	20	30	7,419	10,87	1,242	0,59052	19,249	6			0,284
8	280612	11:08	Cloudy	23	53	7,696	12,63	1,241	0,59000	25,228		6	0,23783	0,56190
9	290612	07:37	Cloudy/rainy	20	22	7,422	9,88	1,314	0,62785	25,211				0,43636
12	20712	14:00	Cloudy	24	48	7,682	10,74	1,292	0,61642	25,197				0,48275
13	30712	09:38	Sunny	22,7	63	7,675	12,03	1,31	0,62577	25,18				0,47647
14	40712	08:25	Cloudy	20,4	18	7,725	15,13	1,32	0,63097	7,163	18			0,47647
15	50712	09:10	Sunny	22,3	68	7,756	14,04	1,326	0,63409	25,146		18	0,71581	0,15294
16	60712	08:32	Cloudy	20,6	30	7,201	10,36	1,498	0,72405	25,102				0,14545
19	90712	13:05	Cloudy	23,1	63	7,377	10,22	1,512	0,73142	25,077				0,256
20	100712	16:48	Cloudy	23,7	28	7,556	11,27	1,52	0,73563	15,0275	10			0,31111
21	110712	10:07	Cloudy	22	93	7,483	11,79	1,512	0,73142	24,989		10	0,40017	0,36883
22	120712	16:25	Cloudy	23,4	112	7,336	11,6	1,523	0,73721	24,96				0,39310
23	130712	17:58	Cloudy	23,3	61	7,556	13,48	1,528	0,73984	24,94				0,41
26	160712	19:45	Cloudy	24,7	62	7,65	10,48	1,495	0,72248	24,9125				0,72363
27	170712	09:36	Sunny	22,5	294	7,836	15,52	1,493	0,72142	24,8945				0,71666
28	180712	10:30	Cloudy	21,7	93	7,674	10,04	1,503	0,72668	24,873				0,94883
29	190712	11:30	Cloudy	22,4	192	7,92	16,78	1,508	0,72931	24,8545				0,96216
48	70812	07:55	Cloudy	20	52	7,647	10,51	1,721	0,84204	24,842				1,024
49	80812	08:50	Cloudy	20,2	106	7,606	9,85	1,725	0,84417	21,8285	3			1,18518
50	90812	09:00	Sunny	19,8	146	7,526	12,48	1,625	0,79107	24,8085		3	0,12092	0,37778
51	100812	10:00	Cloudy	20,9	142	7,652	14,64	1,619	0,78789	24,791				0,79428
52	130812	10:30	Patchy-cloudy	21,6	116	7,945	15,65	1,586	0,77044	24,7725				0,86486
55	140812	10:30	Sunny	23,8	65	7,928	16,83	1,587	0,77097	24,7555				0,84117
56	150812	09:25	Sunny	21,1	204	7,704	11,8	1,611	0,78366	24,736				0,97435
57	160812	08:25	Cloudy	20	102	7,67	12,26	1,631	0,79425	14,722	10			1,22142
58	170812	14:30	Sunny	27,7	110	7,768	11,75	1,654	0,80644	24,7005		10	0,40485	1,24186
61	200812	09:25	Cloudy	20,8	41	7,614	8,63	1,702	0,83193	24,6835				1,55294
62	210812	08:50	Cloudy	21	95	7,617	5,8	1,712	0,83725	0,774	23,9			2,23157
63	220812	08:00	Cloudy	20,8	85	7,63	5,55	0,227	0,09296	0,7635				2,08571
Average				22,24	91,147	7,583	11,53	1,4719	0,7117	21,938			0,35283	0,69572
Total											76,9	53		23,6545

Table 61 - Biomass productivity and CO_2 fixation rate for the TPBR 1- *Chlorella vulgaris* – freshwater microalgae for 63 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1-LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931/ r	Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1})	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1})	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1})	Biomass Output Energy (E_{out}) (MJ $m^{-2}d^{-1}$)	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$)	Photosynthetic Efficiency (PE) (100*E_{out}/E_{in})	Areal CO_2 Fixation rate (0,45P*(44/12) (g CO_2 m^{-2} d^{-1})
200612	0-1	Sunny	0,02998	0,04692	21,31247	0,00167	0,07649	0,00204	0,04691	0,00175	40,685	0,00431	0,12621
210612	1-2	Sunny	0,79950	1,25098	0,79936	0,06935	3,15275	0,08531	1,93378	0,07232	33,575	0,21541	5,20204
250612	5-6	Sunny	-0,07945	-0,12432	-8,04343	-0,03617	-1,24722	-0,05868	-0,76500	-0,02861	19,355	-0,14782	-2,05792
260612	6-7	Sunny	-0,23836	-0,37297	-2,68114	-0,11922	-5,38091	-0,14771	-3,30045	-0,12343	44,24	-0,27901	-8,87851
270612	7-8	Cloudy	-0,35052	-0,54846	-1,82327	0,27790	9,55247	0,45209	5,85913	0,21913	11,85	1,84923	15,76159
280612	8-9	Cloudy/rain	-0,25285	-0,39564	-2,52752	-0,12554	-5,65562	-0,15582	-3,46895	-0,12974	20,935	-0,61972	-9,33178
20712	12-13	Sunny	-0,00437	-0,00683	-146,23841	-0,00209	-0,09430	-0,00260	-0,05784	-0,00216	18,96	-0,01141	-0,15561
30712	13-14	Cloudy	0	0	0	0	0	0	0	0	24,885	0	0
40712	14-15	Sunny	-1,13635	-1,77805	-0,56241	-0,32352	-4,13828	-1,41435	-2,53827	-0,09493	7,11	-1,33519	-6,82817
50712	15-16	Cloudy	-0,05018	-0,07853	-12,73367	-0,00748	-0,33617	-0,00932	-0,20619	-0,00771	26,86	-0,02871	-0,55469
90712	19-20	Cloudy	0,06499	0,10169	9,83368	0,01837	0,82263	0,02293	0,50457	0,01887	24,885	0,07583	1,35734
100712	20-21	Cloudy	0,17018	0,26629	3,75523	0,05772	1,54890	0,12027	0,95004	0,03553	11,06	0,32126	2,55569
110712	21-22	Cloudy	0,06373	0,09972	10,02764	0,02427	1,08310	0,03041	0,66433	0,02484	36,735	0,06763	1,78712
120712	22-23	Cloudy	0,04208	0,06584	15,18616	0,01689	0,75310	0,02119	0,46192	0,01727	44,24	0,03905	1,24262
160712	26-27	Sunny	-0,00325	-0,00504	-198,10556	-0,00232	-0,10335	-0,00292	-0,06339	-0,00237	24,49	-0,00968	-0,17053
170712	27-28	Cloudy	0,28062	0,43909	2,27740	0,23217	10,32101	0,29203	6,33052	0,23676	116,13	0,20387	17,02967
180712	28-29	Cloudy	0,01394	0,02182	45,82755	0,01332	0,59184	0,01677	0,36301	0,01357	36,735	0,03695	0,97653
80812	49-50	Cloudy	0,00769	0,01203	83,06670	0,00848	0,37633	0,01069	0,23082	0,00863	20,54	0,04203	0,62094
90812	50-51	Sunny	-1,14333	-1,78898	-0,55897	-0,80740	-31,47222	-1,15826	-19,30388	-0,72197	41,87	-1,72431	-51,92916
100812	51-52	Cloudy	0,74313	1,16277	0,860009	0,41650	18,45157	0,52572	11,31750	0,42327	57,67	0,73396	30,44509
140812	55-56	Sunny	-0,00925	-0,01448	-69,03766	-0,00789	-0,34929	-0,00998	-0,21424	-0,00801	45,82	-0,01748	-0,57634
150812	56-57	Sunny	0,14697	0,22997	4,34826	0,13318	5,88749	0,16846	3,61117	0,13505	25,675	0,52603	9,71437
160812	57-58	Cloudy	0,22599	0,35361	2,82791	0,24706	10,91341	0,31277	6,69388	0,25035	80,58	0,31068	18,00713
170812	58-59	Sunny	0,01658	0,02595	38,52437	0,02043	0,53713	0,04345	0,32946	0,01232	40,29	0,03058	0,88628
210812	61-62	Cloudy	0,12085	0,18909	5,28824	0,22621	9,97092	0,28697	6,11579	0,22873	16,195	1,41236	16,45202
220812	62-63	Cloudy	-0,06759	-0,10577	-9,45442		-0,20160	-5,90129	-0,12365	-0,00462	37,525	-0,01232	-0,33264
Average			-0,02343	-0,03666	-7,99351	0,01327	0,96385	-0,24883	0,59119	0,02211	34,9575	0,06475	1,59035
Total						0,34516	26,02405	-6,71861	15,96218	0,59699	943,8525	1,74830	42,93969

Table 62 - Data registered on TPBR 2 – *Chlorella vulgaris* – freshwater microalgae for 63 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media: 3N-BBM+Vitamins

Age of culture (days)	Culture Date	Time of Sampling	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture ($\mu mol\ m^{-2}\ s^{-1}$)	pH	Dissolved Oxygen (DO) ($mg\ L^{-1}$)	Conductivity ($mS\ cm^{-1}$)	Salinity ($g\ L^{-1}$) or ppt	Remaining culture volume V_o ($g\ L^{-1}$)	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) ($g\ L^{-1}$)
0	20612	18:40	Sunny	22,60	47	7,066	9,14	1,547	0,74985	25,55				0,084
1	210612	16:55	Sunny	28,00	89	7,049	9,25	1,58	0,76727	25,408				0,06197
2	220612	08:30	Sunny	24,4	73	6,975	12,93	1,537	0,74458	25,343				0,13692
5	250612	08:55	Cloudy	23	45	7,473	11,93	1,531	0,74142	19,2985	6			0,43146
6	260612	08:52	Sunny	22,7	134	7,485	12,93	1,361	0,65232	25,2985		6	0,23716	0,49655
7	270612	07:56	Sunny	21	74	7,477	11,45	1,363	0,65336	15,2465	10			0,40434
8	280612	11_20	Sunny	24,5	232	7,756	9,69	1,352	0,64763	25,2305		10	0,39634	0,70625
9	290612	07:50	Cloudy-rainy	21,1	22	7,483	11,32	1,416	0,68104	25,2165				0,59285
12	20712	10:30	Sunny	25,9	90	8,669	15,93	1,403	0,67425	16,2165	9			0,70370
13	30712	09:45	Cloudy	24,1	74	7,812	15,25	1,456	0,70200	25,203		9	0,35710	0,50555
14	40712	08:55	Cloudy	21,6	30	7,598	13,13	1,461	0,70462	18,1645	7			0,50243
15	50712	09:18	Sunny	23,4	75	7,914	13,79	1,453	0,70042	25,145		7	0,27838	0,85128
16	60712	08:37	Cloudy	21,7	20	7,577	10,42	1,493	0,72142	25,1205				0,62040
19	90712	08:55	Cloudy	23,1	43	7,59	7,65	1,532	0,74195	12,0955	13			0,608
20	100712	17:00	Cloudy	25	22	7,378	9,36	1,542	0,74722	25,0705		13	0,51853	0,31777
21	110712	10:14	Cloudy	23	75	7,434	13,85	1,563	0,75829	17,0255	8			0,30312
22	120712	16:34	Cloudy	24,6	92	7,286	10,09	1,561	0,75724	24,9935		8	0,32008	0,26506
23	130712	18:20	Cloudy	23,7	47	7,412	9,72	1,572	0,76304	24,9365				0,41290
26	160712	19:50	Cloudy	25,1	28	7,538	8,28	1,602	0,77890	9,9365	15			0,68947
27	170712	09:44	Sunny	23,7	179	7,635	10,38	1,615	0,78578	24,9175		15	0,60198	0,72105
28	180712	10:41	Cloudy	22,1	68	7,28	9,26	1,583	0,76885	24,8715				0,37778
29	190712	11:48	Cloudy	23,4	143	7,428	11,08	1,587	0,77097					0,36551
48	70812	11:00	Cloudy	21,2	137	7,676	9,25	1,551	0,75196	24,8425				0,45217
49	80812	11:15	Cloudy	21,3	197	7,777	9,25	1,568	0,76093	24,8195	20			0,46829
50	90812	10:00	Cloudy	20,6	174	7,801	9,08	1,567	0,76040	4,799				0,37778
51	100812	11:40	Cloudy	22,1	149	8,4	15,72	1,486	0,71775					0,756
52	110812	11:30	Sunny	24,5	137	8,494	16,79	1,591	0,77308	34,746		30	0,86340	0,80526
55	140812	11:45	Patchy-cloud	24,8	61	7,697	13,55	1,58	0,76727	34,73				1,0875
56	150812	09:45	Sunny	22,4	21,5	7,788	15,7	1,581	0,76780	34,714				1,14375
57	160812	08:50	Patchy-cloud	21,6	96	7,854	16,35	1,59	0,77255	24,6965	10			1,38285
58	170812	14:35	Sunny	28,4	123	7,838	12,05	1,612	0,78419					1,63125
61	200812	09:35	Cloudy	21,8	33	7,65	10,03	1,582	0,76833	34,6555		10	0,28855	0,924
62	210812	08:55	Cloudy	22	72	7,725	11,45	1,596	0,77573	9,635	25			1,22926
63	220812	08:00	Cloudy	20,8	85	7,63	5,55	0,227	0,09296					
Average				23,21	86,64285	7,631	11,53	1,48702	0,71942	22,47882			0,4099	0,6091
Total											123	108		21,31747

Table 63 - Biomass productivity and CO_2 fixation rate for the TPBR 2 – *Chlorella vulgaris* – freshwater microalgae strain for 63 days of semi-continuous production system, under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Culture Date (days)	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1-LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931 /r	Productivity (P) (g DW *L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1})	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1})	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1})	Biomass Output Energy (E_{out}) (MJ $m^{-2}d^{-1}$)	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$)	Photosynthetic Efficiency (PE) (100*E_{out}/E_{in})	Areal CO_2 Fixation rate (0,45P*) (44/12) (g CO_2 m^{-2} d^{-1})
200612	0-1	Sunny	-0,30413	-0,47588	-2,10135	-0,022028169	-1,005035211	-0,026997655	-0,616450951	-0,02305	18,565	-0,12418	-1,65830
210612	1-2	Sunny	0,79273	1,24039	0,80619	0,074951246	3,400645101	0,092373399	2,085828321	0,07801	35,155	0,22190	5,61106
250612	5-6	Cloudy	0,04683	0,07328	13,64516	0,021697017	0,747714064	0,035205865	0,458619798	0,01715	17,775	0,09649	1,23372
260612	7-8	Sunny	-0,20541	-0,32140	-3,11130	-0,092203898	-4,165393419	-0,114128224	-2,554896292	-0,09555	52,93	-0,18052	-6,87289
270612	8-9	Sunny	0,55769	0,87262	1,14596	0,301902174	8,21955624	0,620061304	5,041567902	0,18855	29,23	0,64507	13,56226
280612	12-13	Sunny	-0,17501	-0,27384	-3,65166	-0,113392857	-5,108854432	-0,140733792	-3,133579937	-0,11719	91,64	0,08681	5,72221
40712	13-14	Sunny	-0,11023	-0,17248	-5,79771	-0,066049383	-1,912660384	-0,127541108	-1,173154233	-0,04387	35,55	-0,12342	-3,15588
50712	14-15	Cloudy	-0,00618	-0,00967	-103,35325	-0,003116531	-0,140260598	-0,0038722	-0,086030597	-0,00321	29,23	-0,01100	-0,23142
60712	15-16	Cloudy	0,52726	0,82501	1,21209	0,348843027	11,31528422	0,601374689	6,940371481	0,25957	11,85	1,59119	13,56226
70712	19-20	Sunny	-0,31636	-0,49501	-2,02012	-0,230873888	-10,36664985	-0,287515806	-6,358514692	-0,23781	29,625	-0,39560	-8,42960
110712	21-22	Cloudy	-0,21627	-0,33840	-2,95504	-0,096740741	-2,089513624	-0,250451784	-1,281629386	-0,04793	16,985	-0,28221	-3,44769
120712	21-22	Cloudy	-0,04720	-0,07386	-13,53822	-0,014652778	-0,655986545	-0,018301872	-0,402357574	-0,01504	8,69	-0,17316	-1,08237
130712	22-23	Cloudy	-0,13418	-0,20996	-4,76271	-0,038064759	-1,157270634	-0,070010271	-0,709826457	-0,02654	29,625	-0,08961	-1,90949
170712	26-27	Cloudy	0,44325	0,69356	1,44183	0,147842985	6,598417217	0,185230369	4,047221951	0,15136	36,34	0,41653	10,88738
140712	27-28	Cloudy	0,01492	0,02335	42,81254	0,010526316	0,186776316	0,033172752	0,114561596	0,00428	11,06	0,03874	0,30818
180812	28-29	Sunny	-0,64640	-1,01142	-0,98870	-0,343272632	-15,27409964	-0,431393165	-9,368560567	-0,35038	70,705	-0,49556	-25,20226
190812	48-49	Cloudy	-0,03299	-0,05163	-19,367508	-0,012262759	-0,544630716	-0,015439198	-0,33405608	-0,01249	26,86	-0,04651	-0,89864
80812	49-50	Cloudy	0,00184	0,00288	346,67630	0,000848356	0,037634449	0,001069354	0,023083561	0,00086	54,115	0,00159	0,06209
90812	50-51	Cloudy	-0,21478	-0,33606	-2,97558	-0,090512683	-4,011570596	-0,114197069	-2,460547135	-0,09202	77,815	0,09555	5,34799
100812	51-52	Cloudy	0,69372	1,08547	0,92125	0,37822	3,241210321	2,467926876	1,988036999	0,07435	68,73	0,49471	24,45643
140812	55-56	Sunny	0,10015	0,15671	6,38105	0,094078947	5,837262688	0,084786397	3,580358275	0,13390	54,115	0,24744	9,63148
150812	56-57	Patchy-cloud	0,05043	0,07890	12,67279	0,05625	3,488504464	0,050717319	2,139717963	0,08002	24,095	0,33212	5,75603
160812	57-58	Sunny	0,18983	0,29704	3,36652	0,239107143	14,82208099	0,215688226	9,091309263	0,34001	8,4925	4,00375	24,45643
170812	58-59	Patchy-cloud	0,16519	0,25848	3,86876	0,248392857	10,95434678	0,314950456	6,718985976	0,25129	37,92	0,66269	18,07467
210812	62-63	Cloudy	0,09515	0,14888	6,71647	0,101756098	34,6555	0,09194	3,86244	0,14445	13,035	1,10822	10,3903
Average			0,06007	0,09400	10,18808	0,041146051	1,213660295	0,13703158	0,744413762	0,02784	37,444	0,32521	4,10012519
Total						1,152089415	33,98248825	3,836884251	20,84358535	0,77955	1048,447	9,10601	114,8035053

Table 64 - Data registered on TPBR 3 – *Dunaliella salinas* - marine microalgae for 63 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media: 3N-BBM+Vitamins

Age of culture (days)	Culture Date	Time of Sampling	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture ($\mu mol\ m^{-2}\ s^{-1}$)	pH	Dissolved Oxygen (DO) ($mg\ L^{-1}$)	Conductivity ($mS\ cm^{-1}$)	Salinity ($g\ L^{-1}$) or ppt	Remaining culture volume V_o ($g\ L^{-1}$)	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) ($g\ L^{-1}$)
0	200612	19:10	Sunny	26,50	45	7,165	8,58	32,2	20,37498348	25,55				0,038
1	210612	16:20	Sunny	27,40	86	6,504	8,90	29,7	18,66019224	25,353				0,044670051
2	220612	09:00	Sunny	22,5	97	6,501	10,18	30,2	19,00217007	25,159				0,048453608
5	250612	16:00	Cloudy	22,6	149	7,819	16,83	34	21,6169498	19,094	6			0,441538462
6	260612	09:05	Sunny	21,7	197	7,665	14,63	31,6	19,96233119	25,044		6	0,239578342	0,298
7	270612	08:04	Sunny	19,8	115	7,342	12,08	32,1	20,30616086	20,007	5			0,397297297
8	280612	11:25	Variable	23,4	106	8,252	15,35	32,8	20,78831147	24,9765		5	0,200188177	0,275409836
9	290612	08:00	Cloudy-rainy	19,9	37	7,188	10:22	32,5	20,58156373	24,9475				0,379310345
12	20712	14:10	Cloudy	24,8	152	7,452	12,68	33,1	20,99522531	24,9205				0,255555556
13	30712	09:50	Sunny	23,4	258	7,461	12,57	33,4	21,20230388	24,9				0,56097561
14	40712	09:06	Cloudy	20,6	88	7,353	10,26	34	21,6169498	24,878				0,827272727
15	50712	09:25	Sunny	22,6	215	7,659	15,13	34,5	21,96297968	24,858				0,665
16	60712	08:47	Cloudy	20,6	66	7,184	10,93	31,7	20,03105916	24,8195				0,309090909
19	90712	13:10	Cloudy	23,2	111	7,47	14,34	32,4	20,51268497	24,793				0,716981132
20	100712	17:10	Cloudy	23,4	13	7,207	8,33	32,8	20,78831147	24,757				0,661111111
21	110712	10:26	Cloudy	22,2	196	7,441	15,08	32,9	20,85726437	24,725				0,7609375
22	120712	16:40	Cloudy	23,7	140	7,108	10,26	32,8	20,78831147	24,685				0,6425
23	130712	18:30	Cloudy	23,4	58	7,224	9,72	33,2	21,06423327	24,67				1,453333333
26	160712	19:58	Cloudy	24,4	48	7,298	7,96	34	21,6169498	24,6525				1,148571429
27	170712	09:50	Sunny	22,4	400	7,405	12,42	34	21,6169498	24,635				1,245714286
28	180712	10:50	Cloudy	21	61	7,304	9,47	34,8	22,17080946	24,591				0,722727273
29	190712	11:55	Cloudy	21,8	142	7,413	11,46	35,2	22,44816043	24,552				0,897435897
48	70812	08:15	Cloudy	20,1	64	7,366	9,73	35,3	22,51754154	4,5215	20			0,62295082
49	80812	09:03	Cloudy	20,2	75	7,319	10,3	35,7	22,79523814	20,4815		16	0,781192784	0,4725
50	90812	10:42	Cloudy	21	140	7,502	10,55	36,3	23,21229465	20,4615				0,37778
51	100812	10:25	Cloudy	20,6	143	7,47	10,19	36,6	23,42105062	20,4365				0,824
54	130812	09:30	Sunny	21,8	162	8,26	16,96	37,4	23,97846494	4,365	20			1,92
55	140812	10:55	Sunny	23,7	574	7,213	9,05	35	22,30945016	14,4265		14	0,97043635	1,36
56	150812	09:55	Sunny	21,3	298	7,344	11,55	35,8	22,8647051	14,3885				1,429268293
57	160812	09:05	Sunny-cloudy	20,1	215	7,314	10	36,5	23,35144854	14,3635				1,624
58	170812	14:48	Sunny	27,8	225	7,449	10,25	37,5	24,04821584	14,3475				2,306,25
61	200812	09:50	Cloudy	20,8	38	7,221	8,4	38,1	24,4670628	14,328				2,123076923
62	210812	09:10	Cloudy	20,9	109	7,356	9,6	38,7	24,88648931	14,311				1,994117647
63	220812	09:15	Cloudy	20,3	104	7,408	8,72	39,1	25,1664249	14,295				2,33125
Average				22,35	144,9117	7,371	10,996	34,291	21,82303654	20,80275			0,547848913	0,8445
Total											51	41		27,86883004

Table 65 - Biomass productivity and CO_2 fixation rate for the TPBR 3 – *Dunaliella salinas* - marine microalgae strain for 63 days of semi-continuous production system, under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1-LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931 /r	Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1})	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1})	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1})	Biomass Output Energy (E_{out}) (MJ $m^{-2}d^{-1}$)	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$)	Photosynthetic Efficiency (PE) (100*E_{out}/E_{in})	Areal CO_2 Fixation rate (0,45P*(44/12) (g CO_2 m^{-2} d^{-1})
200612	0-1	Sunny	0,16171	0,25303	3,95196	0,00667	0,30432	0,00817	0,18665	0,00698	17,775	0,03927	0,50212
210612	1-2	Sunny	0,08130	0,12721	7,86066	0,00378	0,17129	0,00467	0,10506	0,00392	33,97	0,01156	0,28263
250612	5-6	Cloudy	-0,13	-0,20506	-4,87649	-0,04784	-1,63138	-0,07846	-1,00062	-0,03742	58,855	-0,06358	-2,69178
260612	6-7	Sunny	0,28759	0,44999	2,22225	0,09929	4,44071	0,12415	2,72376	0,10187	77,815	0,13091	7,32718
270612	7-8	Sunny	-0,36642	-0,57334	-1,74415	-0,12188	-4,35464	-0,19077	-2,67097	-0,09989	45,425	-0,21991	-7,18516
280612	8-9	Variable	0,32009	0,50085	1,99659	0,10390	4,63405	0,13026	2,84235	0,10630	41,87	0,25389	7,64619
20712	12-13	Cloudy	0,26207	0,41007	2,43857	0,10180	4,53048	0,12792	2,77883	0,10392	60,04	0,17310	7,47530
30712	13-14	Sunny	0,38845	0,60781	1,64522	0,26629	11,84071	0,33489	7,26264	0,27162	101,91	0,26653	19,53717
40712	14-15	Cloudy	-0,21834	-0,34164	-2,92698	-0,16227	-7,20896	-0,20425	-4,42170	-0,16537	34,76	-0,47575	-11,89479
50712	15-16	Sunny	-0,76615	-1,19879	-0,83416	-0,35590	-15,79855	-0,44834	-9,69023	-0,36241	84,925	-0,42675	-26,06760
90712	19-20	Cloudy	-0,02704	-0,04231	-23,63313	-0,01862	-0,82451	-0,02352	-0,50572	-0,01891	43,845	-0,04313	-1,36044
100712	20-21	Cloudy	0,14062	0,22004	4,54457	0,09982	4,41321	0,12626	2,70690	0,10123	5,135	1,97155	7,28180
110712	21-22	Cloudy	-0,16918	-0,26472	-3,77753	-0,11843	-5,22922	-0,15000	-3,20741	-0,11995	77,42	-0,15494	-8,62822
120712	22-23	Cloudy	0,81624	1,27718	0,7829726	0,81083	35,74182	1,02857	21,92269	0,81991	55,3	1,48267	58,97400
160712	26-27	Cloudy	0,02706	0,04234	23,61493	0,03238	1,42548	0,04113	0,87433	0,03270	18,96	0,17247	2,35204
170712	27-28	Sunny	-0,54443	-0,85187	-1,17388	-0,52298	-23,00675	-0,66477	-14,11148	-0,52777	158	-0,33403	-37,96115
180712	28-29	Cloudy	0,21650	0,33877	2,95182	0,17470	7,67189	0,22247	4,70565	0,17599	24,095	0,73041	12,65862
80812	49-50	Cloudy	-0,01454	-0,02276	-43,92760	-0,00791	-0,06393	-0,05483	-0,03921	-0,00146	25,28	-0,00580	-0,10549
90812	50-51	Cloudy	-0,22372	-0,35006	-2,85662	-0,09472	-3,46429	-0,14481	-2,12487	-0,07947	29,625	-0,26825	-5,71609
100812	51-52	Cloudy	0,77985	1,22024	0,81950	0,44622	16,30416	0,68288	10,00036	0,37401	55,3	0,67634	26,90186
140812	55-56	Sunny	-0,11494	-0,17985	-5,55996	-0,18666	-4,80883	-0,40517	-2,94955	-0,11031	63,99	-0,17239	-7,93457
150812	56-57	Sunny	0,04967	0,07773	12,86486	0,06926	1,78229	0,15053	1,09319	0,04088	226,73	0,01803	2,94079
160812	57-58	Sunny	0,12772	0,19985	5,00353	0,19473	5,00338	0,42379	3,06889	0,11477	117,71	0,09750	8,25558
170812	58-59	Patchy-cloud	0,35073	0,54878	1,82219	0,68225	17,49910	1,48737	10,73329	0,40142	84,925	0,47268	28,87352
210812	62-63	Cloudy	-0,02088	-0,03268	-30,59615	-0,04298	-1,09983	-0,09394	-0,67459	-0,02523	15,01	-0,16808	-1,81473
220812	63-64	Cloudy	0,15620	0,24441	4,09147	0,33713	8,61553	0,73768	5,28444	0,19764	43,055	0,45904	14,21563
Average			0,06035	0,09443	-1,74213	0,06726	2,18798	0,12199	1,34202	0,05019	61,60480	0,17782	3,61017
Total						1,81611	59,07552	3,29389	36,23471	1,35519	1663,32980	4,80115	97,47461

Table 66 - Data registered on TPBR 4- *Dunaliella salinas* –marine microalgae for 63 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Age of culture (days)	Culture Date	Time of Sampling (hhmm)	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture ($\mu mol\ m^{-2}\ s^{-1}$)	pH	Dissolved Oxygen (DO) ($mg\ L^{-1}$)	Conductivity ($mS\ cm^{-1}$)	Salinity ($g\ L^{-1}$) or ppt	Remaining culture volume V_o ($g\ L^{-1}$)	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) ($g\ L^{-1}$)
0	200612	19:25	Sunny	25,90	52	7,21	8,25	32,2	20,37498348	25,55				0,03
1	210612	16:30	Sunny	27,30	86	6,474	8,76	29,9	18,79692319	25,353				0,045177665
2	220612	09:10	Sunny	22,6	79	6,52	9,52	30,3	19,07062557	25,223				0,063076923
5	250612	18:00	Cloudy	23	69	7,244	10,99	31,8	20,09980616	18,148	7			0,162666667
6	260612	09:11	Sunny	22,8	210	7,394	14,72	31,5	19,89362231	25,089		7	0,279006736	0,320338983
7	270612	08:12	Sunny	20,2	102	7,184	10,88	32	20,23735707	9,543	15,5			0,380434783
8	280612	11:42	Sunny	24,8	346	7,607	13,34	32	20,23735707	25,008		15,5	0,619801663	0,62
9	290612	08:11	Cloudy rainy	19,7	43	7,044	10,54	31,6	19,96233119	24,9825				0,396078431
12	20712	13:40	Cloudy rainy	25,2	97	7,779	15,93	32,5	20,58156373	24,96				0,448888889
13	30712	11:00	Sunny	24	145	8,003	16,13	32,7	20,71937703	24,939				0,471428571
14	40712	09:26	Cloudy	20,6	78	7,375	12,3	33,5	21,27136644	17,9165	7			0,475555556
15	50712	09:38	Sunny	23,6	117	7,654	15,29	22,7	13,92953361	24,896		7	0,281169666	0,63902439
16	60712	08:55	Cloudy	20,8	57	7,205	10,42	31,8	20,09980616	24,8575				0,371428571
19	90712	11:00	Cloudy	24	123	8,765	16,03	32,3	20,44382487	12,3575	12,5			0,404081633
20	100712	17:25	Cloudy	24,3	11	7,071	7,97	31,7	20,03105916	24,833		12,5	0,503362461	0,396774194
21	110712	10:55	Cloudy	21,9	76	7,148	11,78	31,3	19,75626207	18,771	6			0,451612903
22	120712	16:48	Cloudy	22,7	143	7,102	9,12	31,9	20,16857215	24,7245		6	0,24267427	0,486
23	130712	19:00	Cloudy	21,8	71	7,254	12,28	32,2	20,37498348	24,6745				0,866666667
26	160712	20:10	Cloudy	23,9	45	7,317	8,47	33,7	21,40954582	24,6505				1,805555556
27	170712	10:00	Sunny	22,2	287	7,585	16,75	34,2	21,7553085	7,6325	17			1,421428571
28	180712	11:00	Cloudy	21,1	67	7,11	9,49	32,5	20,58156373	24,6185		17	0,690537604	0,524
29	190712	12:05	Cloudy	22	134	7,203	11,5	32,9	20,85726437	24,5685				0,551020408
48	70812	08:25	Cloudy	19,9	45	7,368	10,3	34,4	21,89373827	24,5195				0,575
49	80812	02:24	Cloudy	20,2	56	7,318	10,28	34,5	21,96297968	4,4875	20			0,611111111
50	90812	10:55	Sunny	21,4	201	7,306	10,25	35,2	22,44816043	18,4515		14	0,758745901	0,37778
51	100812	09:15	Cloudy	20,5	95	8,13	17	35,2	22,44816043	18,424				1,684848485
54	130812	11:15	Sunny	22,1	273	7,425	9,63	36,9	23,62995688	18,4075				0,892307692
55	140812	11:05	Patchy-cloud	23,9	577	7,562	13,33	37	23,69962551	8,4075	10			0,964444444
56	150812	10:00	Sunny	21,2	188	7,416	11,25	37,5	24,04821584	23,388		15	0,641354541	1,027906977
57	160812	10:17	Sunny	22,6	199	7,499	14,15	37,9	24,32738234	23,3655				1,093023256
58	170812	14:54	Sunny	27,5	186	7,604	13,09	38,3	24,60680765	0,304	23			1,264864865
61	200812	09:50	Cloudy	21,2	23	7,351	8,81	38,2	24,53692719	25,254		25	0,989942187	0,388
62	210812	09:15	Cloudy	20,6	66	7,19	9,39	34,5	21,96297968	25,2185	3			0,554929577
63	220812	08:25	Sunny	20,8	35	7,197	9,28	34,6	22,03223871	22,1795				0,546153846
Average				22,54	128,8823	7,371	11,68	33,27647059	21,12500617	20,46185			0,556288337	0,63
Total											121	119		21,31

Table 67 - Biomass productivity and CO_2 fixation rate for the TPBR 4- *Dunaliella salinas* – marine microalgae strain for 63 days of semi-continuous production system, under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1-LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931/r	Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1})	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1})	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1})	Biomass Output Energy (E_{out}) (MJ $m^{-2}d^{-1}$)	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$)	Photosynthetic Efficiency (PE) (100*E_{out}/E_{in})	Areal CO_2 Fixation rate (0,45P*(44/12) (g CO_2 m^{-2} d^{-1})
200612	0-1	Sunny	0,40940	0,64059	1,56104	0,01517	0,692480964	0,018601699	0,424741884	0,01588	20,54	0,077339403	1,14259
210612	1-2	Sunny	0,33375	0,52222	1,91489	0,01789	0,810356948	0,022107734	0,497042597	0,01858	33,97	0,054723545	1,33708
250612	5-6	Cloudy	0,22589	0,35345	2,82922	0,05255	1,703236427	0,090686778	1,044701423	0,03907	82,95	0,047103368	2,81034
260612	6-7	Sunny	0,17193	0,26902	3,71710	0,06009	2,692399134	0,075006571	1,651416774	0,06176	40,29	0,153297682	4,44245
270612	7-8	Sunny	0,48840	0,76420	1,30854	0,23956	4,082447981	0,786099258	2,504020668	0,09365	136,67	0,068523712	6,73603
280612	8-9	Sunny	-0,44810	-0,70115	-1,42622	-0,22392	-9,999697479	-0,280385477	-6,133439856	-0,22939	16,985	-1,350562615	-16,49950
20712	12-13	Cloudy rainy	0,01633	0,02555	39,13479	0,00751	0,334875283	0,00942585	0,205399955	0,00768	57,275	0,013412552	0,55254
30712	13-14	Sunny	0,00871	0,01363	73,32397	0,00412	0,183790816	0,005181939	0,112730402	0,00421	30,81	0,013684392	0,30325
40712	14-15	Cloudy	0,29545	0,46230	2,16307	0,16346	5,22998103	0,285706644	3,207874454	0,11997	46,215	0,259603516	8,62946
50712	15-16	Sunny	-0,54258	-0,84898	-1,17787	-0,26759	-11,89654555	-0,336579992	-7,296895406	-0,27290	22,515	-1,212110836	-19,62930
90712	19-20	Cloudy	-0,00608	-0,00951	-105,05994	-0,00243	-0,108015259	-0,00307152	-0,066252514	-0,00247	4,345	-0,057028079	-0,17822
100712	20-21	Cloudy	0,12945	0,20256	4,93673	0,05483	1,838173963	0,091482572	1,127467053	0,04216	30,02	0,140465392	3,03298
110712	21-22	Cloudy	0,07338322	0,11482	8,70907	0,03438	1,518221025	0,043551843	0,931219906	0,03482	56,485	0,06165883	2,50506
120712	22-23	Cloudy	0,57844	0,90509	1,10485	0,38066	16,77278512	0,483097773	10,2877981	0,38476	28,045	1,371965379	27,67509
160712	26-27	Cloudy	-0,07973	-0,12476	-8,01526	-0,12804	-1,745148337	-0,525321646	-1,070408619	-0,04003	17,775	-0,22522477	-2,87949
170712	27-28	Sunny	-0,99792	-1,56145	-0,64042	-0,89742	-39,45240	-1,14150	-24,19862	-0,90503	113,365	-0,798339971	-65,09646
180712	28-29	Cloudy	0,050280163	0,07867	12,71077	0,027020408	1,185448032	0,034439101	0,727109417	0,02719	26,465	0,102755254	1,95598
80812	49-50	Cloudy	0,003205724	0,00501	199,36215	0,001900585	0,015230133	0,013262376	0,009341593	0,00034	17,775	0,001965565	0,02512
90812	50-51	Sunny	-0,480966778	-0,75256	-1,32878	-0,233331111	-7,68805178	-0,395985715	-4,715562976	-0,17636	22,12	-0,797305189	-12,68528
100812	51-52	Cloudy	1,495118903	2,33941	0,42745	1,307068485	43,00255315	2,221534006	26,37615527	0,98647	79,395	1,242494577	70,95421
140812	55-56	Sunny-cloudy	0,025913737	0,04054	24,66259	0,024045584	1,004246642	0,032194434	0,615967272	0,02303	107,835	0,021363581	1,65700
150812	56-57	Sunny	0,063727722	0,09971	10,02860	0,063462532	2,647917497	0,085051282	1,624133405	0,06074	227,915	0,026651702	4,36906
160812	57-58	Sunny	0,061422813	0,09610	10,40492	0,065116279	2,714418605	0,087347976	1,664922693	0,06226	74,26	0,08385236	4,47879
170812	58-59	Sunny	0,146017804	0,22847	4,37686	0,171841609	7,156742727	0,230723471	4,389677905	0,16417	78,605	0,208861622	11,80862
210812	62-63	Cloudy	0,119278626	0,18663	5,35804	0,055643192	2,509309256	0,068995443	1,539116301	0,05756	26,07	0,220803814	4,14036
220812	63-64	Sunny	-0,015940513	-0,02494	-40,09281	-0,008775731	-0,395197822	-0,010896891	-0,24239954	-0,00906	13,825	-0,065575682	-0,65207
Average			0,081723185	0,12787	9,62666	0,037879409	0,954213701	0,076567425	0,58527894	0,02188	54,32769	-0,012908496	1,57445
Total						1,02274404	25,76376992	2,067320477	15,80253138	0,56913122	1412,52	-0,348529391	42,51022

Table 68 - Data registered on TPBR 5 – *Nannochloropsis oculata* – marine microalgae for 63 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media: 3N-BBM+Vitamins.

Culture Date	Age of culture (days)	Time of Sampling	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture ($\mu mol\, m^{-2} s^{-1}$)	pH	Dissolved Oxygen (DO) ($mg\, L^{-1}$)	Conductivity ($mS\, cm^{-1}$)	Salinity ($g\, L^{-1}$) or ppt	Remaining culture volume V_o ($g\, L^{-1}$)	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) ($g\, L^{-1}$)
0	200612	19:30	Sunny	26	46	7,107	8,34	31	19,55036	25,499				0,02788
1	210612	16:38	Sunny	27,5	121	6,415	8,64	29,9	18,79692	25,257				0,03966
2	220612	09:20	Sunny	22,3	115	6,433	9,38	30,1	18,93373	25,007				0,0496
5	250612	17:00	Cloudy	21,6	221	7,225	16,22	34,5	21,96297	18,934	6			0,37123
6	260612	09:25	Sunny	21,7	237	7,134	12,53	32,2	20,37498	24,867		6	0,241283629	0,46716
7	270612	08:22	Sunny	19,6	77	7,203	11,69	32,7	20,71937	17,817	7			0,566
8	280612	11:50	Sunny	24,2	300	74,94	12,84	33,4	21,20230	24,771		7	0,282588511	0,67826
9	290612	08:21	Cloudy-sunny	19,3	37	7,211	10,32	33,2	21,06423	24,736				0,55142
12	020712	13:20	Cloudy	24,9	69	7,629	14,25	34,3	21,82451	24,704				0,475
13	030712	10:30	Cloudy	23,8	226	8,128	15,25	34,7	22,10151	16,579	8,1			0,644
14	040712	09:15	Cloudy	20,3	55	7,481	10,32	35,6	22,72578	24,657		8,1	0,328507118	1,13181
15	050712	09:50	Sunny	23,4	153	7,833	13,93	36,1	23,07320	9,6365	15			1,00487
16	060712	09:19	Cloudy	20,7	84	7,498	12,65	34,3	21,82451	24,6075		15	0,609570253	0,92413
19	090712	13:25	Cloudy	23,1	97	7,521	13,33	32,6	20,65046	24,5775				0,81333
20	100712	17:34	Rainy	22	12	7,294	8,51	33,4	21,20230	24,5295				0,66458
21	110712	11:05	Cloudy	21,8	52	7,52	12,48	33,5	21,27136	24,498				0,91111
22	120712	16:55	Cloudy	22,9	103	7,34	11,07	33,2	21,06423	24,463				0,88571
23	130712	19:47	Cloudy	23,1	38	7,356	12,4	33,6	21,34044	24,438				0,824
26	160712	20:15	Cloudy	23,5	21	7,483	8,87	35	22,30945	8,438	16			1,12380
27	170712	11:35	Sunny	25,3	434	7,714	19,26	35,4	22,58693	24,417		16	0,655281157	0,99302
28	180712	10:00	Cloudy	21,1	70	7,621	17,12	33,4	21,20230	7,374	17			1,27631
29	190712	12:22	Cloudy	21,8	140	8,082	17,7	31,2	19,68761	24,336		17	0,698553583	0,39444
48	70812	09:39	Cloudy	20,7	65	7,778	17,89	38	24,39721	24,246				0,72978
49	80812	09:20	Cloudy	19,8	73	7,495	11,28	38,1	24,46706	18,199	6			0,68
50	90812	09:30	Cloudy	19,8	138	7,858	14,04	38,5	24,74661	24,149		6	0,248457493	0,37778
51	100812	13:15	Cloudy	22,2	147	7,52	15,25	36,7	23,49066	14,103	10			0,59145
54	130812	10:45	Cloudy-sunny	21	333	7,728	11,05	38,2	24,53692	24,0445		10	0,415895527	2,064
55	140812	12:00	Cloudy-sunny	24,8	48	7,502	10,15	37,4	23,97846	4,0445	20			1,378
56	150812	11:15	Sunny	23,9	433	7,567	13,74	37,6	24,11798	4,0195				1,57894
57	160812	09:50	Sunny	22,2	213	7,606	16,46	38,4	24,67670	3,9695				1,67567
58	170812	15:05	Sunny	26,8	208	7,653	11,15	38,5	24,74661	3,9505				2,10285
61	200812	10:10	Cloudy	21,3	37	7,45	8,92	39,3	25,30648	3,932				2,53488
62	210812	09:25	Sunny	21,5	273	7,566	17,03	39,7	25,58679	3,9145				3,37142
63	220812	08:40	Sunny	21,1	210	7,504	12,15	40,1	25,86735	3,893				1,76585
	Average			22,5	143,71	9,452	12,83	35,11176	22,39377	22,5			0,43502	0,990
	Total										105,1	85,1		33,66808

Table 69 - Biomass productivity and CO_2 fixation rate for the TPBR 5- *Nannochloropsis oculata* – marine microalgae strain for 63 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media: 3N-BBM+Vitamins.

Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1-LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931/ r	Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1})	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1})	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1})	Biomass Output Energy (E_{out}) (MJ $m^{-2}d^{-1}$)	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$)	Photosynthetic Efficiency (PE) (100*E_{out}/E_{in})	Areal CO_2 Fixation rate (0,45P*(44/12) (g CO_2 m^{-2} d^{-1})
200612	0-1	Sunny	0,35236	0,55135	1,81372	0,01178	0,53643	0,01446	0,32902	0,01230	18,17	0,06772	0,88511
210612	1-2	Sunny	0,22341	0,34956	2,86065	0,00993	0,44788	0,01231	0,27471	0,01027	47,795	0,02149	0,73901
250612	5-6	Cloudy	0,07661	0,11988	8,34148	0,03197	1,08116	0,05288	0,66314	0,02480	87,295	0,02841	1,78392
260612	6-7	Sunny	0,19191	0,30028	3,33014	0,09883	4,38883	0,12445	2,69195	0,10067	93,615	0,10754	7,24158
270612	7-8	Sunny	0,18093	0,28311	3,53215	0,11226	3,57169	0,19730	2,19074	0,08193	30,415	0,26938	5,89330
280612	8-9	Sunny	-0,20701	-0,32392	-3,08714	-0,12683	-5,61029	-0,16033	-3,44114	-0,12870	118,5	-0,10860	-9,25697
020712	12-13	Cloudy	0,10146	0,15875	6,29895	0,05633	2,48510	0,07140	1,52427	0,05700	27,255	0,20916	4,10042
030712	13-14	Cloudy	0,56388	0,88230	1,13339	0,48781	14,44203	0,92137	8,85820	0,33130	89,27	0,37112	23,82935
040712	14-15	Cloudy	-0,11895	-0,18613	-5,37243	-0,12694	-5,58921	-0,16121	-3,42821	-0,12821	21,725	-0,59018	-9,22221
050712	15-16	Sunny	-0,08376	-0,13105	-7,63012	-0,08074	-1,38937	-0,26236	-0,85219	-0,03187	60,435	-0,05273	-2,29247
090712	19-20	Cloudy	-0,06732	-0,10534	-9,49248	-0,04958	-2,17613	-0,06317	-1,33475	-0,04992	38,315	-0,13028	-3,59061
100712	20-21	Rainy	0,31550	0,49367	2,02564	0,24652	10,79857	0,31471	6,62344	0,24771	4,74	5,22614	17,81765
110712	21-22	Cloudy	-0,02827	-0,04423	-22,60665	-0,02539	-1,11102	-0,03246	-0,68145	-0,02548	20,54	-0,12408	-1,83318
120712	22-23	Cloudy	-0,07222	-0,11300	-8,84887	-0,06171	-2,69592	-0,07899	-1,65357	-0,06184	40,685	-0,15200	-4,44827
160712	26-27	Cloudy	-0,04124	-0,06453	-15,49640	-0,04359	-1,90083	-0,05590	-1,16590	-0,04360	8,295	-0,52568	-3,13638
170712	27-28	Sunny	0,25097	0,392706	2,54642	0,28329	3,73035	1,20301	2,28806	0,08557	171,43	0,04991	6,15508
180712	28-29	Cloudy	-1,17425	-1,83735	-0,54426	-0,88187	-38,32360	-1,13473	-23,50626	-0,87914	27,65	-3,17954	-63,23395
070812	49-50	Cloudy	-0,00371	-0,00581	-171,84913	-0,00262	-0,08515	-0,00450	-0,05223	-0,00195	25,675	-0,00760	-0,14051
080812	51-52	Cloudy	-0,58778	-0,91970	-1,08731	-0,30222	-13,03269	-0,39188	-7,99376	-0,29897	28,835	-1,03683	-21,50395
090812	52-53	Cloudy	0,44827	0,70140	1,42570	0,21367	5,38112	0,47443	3,300580	0,12344	54,51	0,22645	8,87885
140812	56-57	Patchy-cloud	-0,13467	-0,21071	-4,74564	-0,22866	-1,64129	-1,78143	-1,00670	-0,03765	18,96	-0,19858	-2,70813
150812	57-58	Sunny	0,13612	0,21299	4,69494	0,20094	1,42439	1,58520	0,87366	0,03267	171,035	0,01910	2,35024
160812	58-59	Sunny	0,05945	0,09303	10,74875	0,09672	0,68236	0,76672	0,41853	0,01565	84,135	0,01860	1,12590
170812	59-60	Sunny	0,22708	0,35531	2,81442	0,42718	2,99942	3,40202	1,83973	0,06880	82,16	0,08374	4,94904
210812	62-63	Sunny	0,09506	0,14874	6,72291	0,27884	1,93849	2,24296	1,18899	0,04446	107,835	0,04123	3,19851
220812	63-64	Sunny	-0,64670	-1,01189	-0,98824	-1,60557	-11,12147	-12,96132	-6,82149	-0,25512	82,95	-0,30756	-18,35043
Average			0,00219	0,00343	-7,44074	-0,03767	-1,18342	-0,21942	-0,72587	-0,02714	60,08557	0,01255	-1,95265
Total						-0,97962	-30,76914	-5,70505	-19,57136	-0,70585	1562,225	0,32636	-50,76907

Table 70 - Data registered on TPBR 6 – *Nannochloropsis oculata* –marine microalgae for 63 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media: 3N-BBM+Vitamins.

Age of culture (days)	Culture Date	Time of Sampling (hhmm)	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture (µmol m^{-2} s^{-1})	pH	Dissolved Oxygen (DO) (mg L^{-1})	Conductivity (mS cm^{-1})	Salinity (g L^{-1}) or ppt	Remaining culture volume V_o (g L^{-1})	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) (g L^{-1})
0	200612	19:45	Sunny	24,4	45	7,104	8,52	28,6	17,90963021	25,458				0,02602
1	210612	16:47	Sunny	27,1	125	6,412	8,86	30	18,86531881	25,176				0,04326
2	220612	09:30	Sunny	22,1	122	6,478	9,7	30,7	19,34464539	24,894				0,08333
5	250612	18:30	Cloudy	22,5	9	7,044	10:56	32,2	20,37498348	18,831	6			0,55555
6	260612	09:35	Sunny	24,7	236	7,549	14,07	32,2	20,37498348	24,772		6	0,24220	0,25932
7	270612	08:30	Sunny	21,6	100	7,184	9,74	32,6	20,6504611	7,2305	17,5			0,62168
8	280612	11:57	Sunny	26,3	293	7,882	14:08	31,8	20,09980616	24,6925		17,5	0,70871	0,8
9	290612	08:30	Cloudy	20,8	73	7,177	10:56	32,6	20,6504611	24,6625				0,53333
12	020712	11:30	Sunny	25,4	251	8,409	15,97	33,2	21,06423327	20,6625	4			0,66071
13	030712	10:15	Sunny	24,9	205	7,719	12,55	33,3	21,13325947	24,6345		4	0,16237	0,74893
14	040712	09:35	Cloudy	21,8	72	7,426	14,15	34	21,6169498	24,611				0,73877
15	050712	09:55	Sunny	25,5	193	8,123	13,25	34,2	21,7553085	16,4865	8,1		0,32850	0,99534
16	060712	09:00	Cloudy	21,8	36	7,394	10,16	33,2	21,06423327	24,565		8,1	0,32973	0,73333
19	090712	14:00	Cloudy	25,6	133	8,57	15,99	32,6	20,6504611	24,5365			0,60957	0,99692
20	100712	12:30	Rainy	27,9	43	8,601	16,03	31,2	19,68761081	14,5365	10			0,505
21	110712	11:02	Cloudy	23,2	44	7,168	11,28	31,1	19,61897886	27,504		13	0,47265	0,53846
22	120712	15:00	Cloudy	24	154	7,15	10,12	31,6	19,96233119	17,464	10			0,70540
23	130712	19:15	Cloudy	22,5	41	7,357	10,5	32,2	20,37498348	27,425		10	0,36463	0,81666
26	160712	13:00	Cloudy	25,7	118	8,561	15,86	34,1	21,68612025	27,388				0,63870
27	170712	11:45	Sunny	25,5	428	7,282	11,79	31,9	20,16857215	27,364				0,7
28	180/12	10:12	Cloudy	22,9	45	7,415	12,16	32,6	20,6504611	25,364	2			0,71969
29	190712	12:10	Cloudy	23	89	7,218	10,53	32,1	20,30616086	27,333		2	0,07317	0,81391
48	070812	09:10	Cloudy	21	19	7,553	11,35	36,8	23,56030483	5,293	22			0,99534
49	080812	09:25	Cloudy	20,8	10	7,263	9,22	33,9	21,54779722	13,227		8	0,60482	0,87254
50	090812	11:05	Sunny	22,2	227	7,405	10,65	34,5	21,96297968	8,1265	5			1,644
51	100812	10:30	Cloudy	21	163	7,365	10,35	34,9	22,24012109	33,1695		25	3,07635	1,372
54	130812	10:30	Patchy-cloud	21,6	116	7,945	15,65	34,6	22,03223871	8,1265	25			0,86486
55	140812	10:30	Sunny	23,8	65	7,928	16,83	38,3	24,60680765	33,0755		25	0,75584	0,84117
56	150812	09:25	Sunny	21,1	204	7,704	11,8	38,2	24,53692719	27,0255	6			0,97435
57	160812	08:25	Cloudy	20	102	7,67	12,26	38,3	24,60680765	36,9755		10	0,27044	1,2
58	170812	14:30	Sunny	27,7	110	7,768	11,75	37,9	24,32738234	36,957				1,24186
61	200812	09:25	Cloudy	20,8	41	7,614	8,63	37,5	24,04821584	36,94				1,55294
62	210812	08:50	Cloudy	21	95	7,617	5,8	37	23,69962551	36,9205				2,23157
63	220812	08:00	Cloudy	20,8	85	7,63	5,55	36,9	23,62995688	36,9065				2,08571
	Average			23,264	120,352	7,549	10,927	33,729	21,43556231	24,06864			0,615311558	0,856
	Total				40919,9996		109,27				115,6	128,6		29,11079808

Table 71- Biomass productivity and CO_2 fixation rate for the TPBR 6- *Nannochloropsis oculata* – marine microalgae strain for 63 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 20.06.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1- LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931 /r	Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1})	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1})	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1})	Biomass Output Energy (E_{out}) (MJ $m^{-2}d^{-1}$)	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$)	Photosynthetic Efficiency (PE) (100*E_{out}/E_{in})	Areal CO_2 Fixation rate (0,45P*(44/12) (g CO_2 m^{-2} d^{-1})
200612	0-1	Sunny	0,50813	0,79507	1,25773	0,01723	0,78351	0,02119	0,48057	0,01797	17,775	0,10111	1,29280
210612	1-2	Sunny	0,65556	1,02576	0,97488	0,04007	1,80147	0,04984	1,10495	0,04132	49,375	0,08369	2,97243
250612	5-6	Cloudy	-0,25396	-0,39738	-2,51647	-0,09874	-3,32046	-0,16420	-2,03664	-0,07617	3,555	-2,14265	-5,47875
260612	6-7	Sunny	0,87436	1,368120	0,73092	0,36236	16,02946	0,45806	9,83187	0,36771	93,22	0,39446	26,44861
270612	7-8	Sunny	0,25217	0,39457	2,53434	0,17831	2,30231	0,77224	1,41215	0,05281	39,5	0,13370	3,79881
280612	8-9	Sunny	-0,40546	-0,63443	-1,57621	-0,26666	-11,75833	-0,33817	-7,21212	-0,26973	115,735	-0,23306	-19,40125
020712	12-13	Sunny	0,04177	0,06536	15,29773	0,02940	1,29363	0,03738	0,79346	0,02967	99,145	0,02993	2,13449
030712	13-14	Sunny	-0,01365	-0,02137	-46,78740	-0,01016	-0,44654	-0,01292	-0,27389	-0,01024	80,975	-0,01265	-0,73679
040712	14-15	Cloudy	0,29809	0,46643	2,14391	0,25657	7,55356	0,48732	4,63307	0,17327	28,44	0,60927	12,46338
050712	15-16	Sunny	-0,30549	-0,47800	-2,09202	-0,26201	-11,49359	-0,33400	-7,04973	-0,26366	76,235	-0,34585	-18,96442
090712	19-20	Cloudy	-0,22670	-0,35472	-2,81908	-0,16397	-8,05348	-0,18668	-4,93970	-0,18474	52,535	-0,35166	-13,28824
100712	20-21	Rainy	0,06415	0,10038	9,96140	0,03346	1,04352	0,05999	0,64005	0,02393	16,985	0,14093	1,72181
110712	21-22	Cloudy	0,27005	0,42255	2,36654	0,16694	8,17577	0,19061	5,01471	0,18755	17,38	1,07912	13,49003
120712	22-23	Cloudy	0,14645	0,22916	4,36369	0,11126	5,44147	0,12721	3,33759	0,12482	60,83	0,20520	8,97842
160712	26-27	Cloudy	0,03054	0,04779	20,92429	0,02043	0,99717	0,02340	0,61162	0,02287	46,61	0,04907	1,64533
170712	27-28	Sunny	0,02774	0,04342	23,03070	0,01969	0,18617	0,11652	0,11419	0,00427	169,06	0,00252	0,30718
180712	28-29	Cloudy	0,12302	0,19249	5,19495	0,09421	2,22535	0,22304	1,36494	0,05104	17,775	0,28719	3,67182
070812	49-50	Cloudy	-0,00693	-0,01084	-92,21911	-0,00646	-0,09379	-0,02490	-0,05752	-0,00215	3,95	-0,05446	-0,15475
080812	51-52	Cloudy	0,63346	0,99118	1,00888	0,77145	45,56451	0,73036	27,94756	1,04524	89,665	1,16572	75,18144
090812	52-53	Patchy-cloud	-0,180862	-0,28299	-3,53361	-0,272	-13,12667	-0,31516	-8,05140	-0,30112	64,385	-0,46769	-21,65900
140812	56-57	Sunny	-0,009257	-0,01448	-69,03766	-0,00789	-0,52110	-0,00669	-0,31962	-0,01195	25,675	-0,04655	-0,85981
150812	57-58	Cloudy	0,146978	0,22997	4,34826	0,13318	8,78528	0,11289	5,38856	0,20153	80,58	0,25010	14,49572
160812	58-59	Sunny	0,208297	0,32592	3,06821	0,22564	14,87639	0,19137	9,12462	0,34126	40,29	0,84702	24,54604
170812	59-60	Cloudy	0,034289	0,05365	18,63859	0,04186	2,75879	0,03551	1,69213	0,06328	43,45	0,14565	4,55200
210812	62-63	Cloudy	0,120852	0,18909	5,28824	0,22621	14,89286	0,19213	9,13472	0,34164	37,525	0,91043	24,57323
220812	63-64	Cloudy	-0,067598	-0,10577	-9,45442	-0,14586	-9,60062	-0,12392	-5,88866	-0,22023	33,575	-0,65595	-15,84103
Average			0,114079	0,17849	-4,18856	0,05748	2,93448	0,08932	1,79990	0,06731	54,008	0,08171	4,84190
Total						1,43795	73,36218	2,23316	44,9952	1,175025	1404,225	2,18765	125,8895

Table 72 - Data registered on TPBR 7 – *Scenedesmus sp* - freshwater microalgae for 42 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level) (cultivation period: 11.07.12-23.08.12). Nutrition media: 3N-BBM+Vitamins

Age of culture (days)	Culture Date	Time of Sampling (hh.mm)	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture ($\mu mol\ m^{-2}\ s^{-1}$)	pH	Dissolved Oxygen (DO) ($mg\ L^{-1}$)	Conductivity ($mS\ cm^{-1}$)	Salinity ($g\ L^{-1}$) or ppt	Remaining culture volume V_o ($g\ L^{-1}$)	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) ($g\ L^{-1}$)
0	110712	11:25	Cloudy	22,3	93	7,266	11,87	1,664	0,81174	25,652	6	6	0,23389	0,231372
1	120712	17:05	Cloudy	23,4	67	7,261	11,99	1,65	0,80432	20,113	20	20	0,99438	0,319148
2	130712	20:05	Cloudy	22,5	70	7,013	10,09	1,55	0,75144	25,613	5,5	5,5	0,21473	0,230769
5	160712	20:28	Cloudy	24,1	47	7,36	8,94	1,546	0,74933	5,578	20			0,357142
6	170712	11:20	Sunny	26,2	940	8,003	16,45	1,546	0,74933	25,541		20	0,78305	0,486486
7	180712	11:06	Cloudy	21	145	8,205	16,56	1,548	0,75038	25,31				0,343661
8	190712	13:07	Cloudy	22,2	145	7,18	11,9	1,535	0,74353	25,31				0,11
27	70812	10:55	Cloudy	21,3	127	7,908	11,83	1,584	0,76938	22,26	3			0,48
28	80812	11:10	Cloudy	21,4	189	7,982	13,49	1,586	0,77044	25,235		3	0,11888	0,556
29	90812	10:15	Cloudy	20,6	189	8,1	14,39	1,591	0,77308	25,214				0,37778
30	100812	12:15	Cloudy	22,3	151	7,862	11,84	1,597	0,77625	5,214	20			0,695238
33	130812	12:40	Sunny	24	278	7,846	11,65	1,656	0,80750	19,162		14	0,73061	0,1
34	140812	13:20	Sunny	25,3	576	7,813	9,29	1,674	0,81705	19,119				0,336
35	150812	09:15	Sunny	20,3	221	7,721	10,8	1,68	0,82024	9,105	10			1,288888
36	160812	10:30	Sunny	22,4	324	7,8	12,06	1,689	0,82502	24,095		15	0,62253	1,530434
37	170812	15:25	Sunny	26,7	206	7,787	12,36	1,691	0,82608	24,087				2,128
40	200812	10:50	Cloudy	21,1	65	7,628	8,05	1,758	0,86175	24,079				2,25625
41	210812	10:00	Sunny	22,3	146	7,712	10,89	1,753	0,85908	24,063				1,81875
42	220812	09:05	Cloudy	21,5	84	7,667	10,33	1,783	0,87509	24,051				2,308333
Average				22,678	213,842	7,690	11,830	1,635	0,79690	20,9895			0,52830	0,839697
Total					4063		224,78				84,5	83,5		15,95425

Table 73 - Biomass productivity and CO_2 fixation rate for the TPBR 7 – *Scenedesmus sp* - freshwater microalgae strain for 42 days of semi-continuous production system, under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 11.07.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1-LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931 /r	Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1}) [a]	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1}) [b]	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1}) [c]	Biomass Output Energy (E_{out}) (IAP*HVV) (MJ $m^{-2}d^{-1}$)[d]	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$) [e]	Photosynthetic Efficiency (PE) (100* E_{out}/E_{in})	Areal CO_2 Fixation rate (ACO_2Fr) (0,45P* (44/12) (g CO_2 m^{-2} d^{-1}) [f]
110712	0-1	Cloudy	0,321628701	0,50325	1,98707	0,087776387	4,020785506	0,10715	2,466199215	0,09223	36,735	0,251087027	6,63429
120712	1-2	Cloudy	-0,324239668	-0,50733	-1,97107	-0,088379705	-3,174251812	-0,1375	-1,946967157	-0,07281	26,465	-0,275145802	-5,23751
160712	5-6	Cloudy	0,103024421	0,16120	6,20338	0,043114543	1,966408117	0,05285	1,20612108	0,04510	18,565	0,24298089	3,24457
170712	6-7	Sunny	-0,34755059	-0,54381	-1,83886	-0,142824515	-6,495964979	-0,17559	-3,984381586	-0,14901	371,3	-0,040133972	-10,71834
180712	7-8	Cloudy	-1,139178168	-1,78247	-0,56101	-0,233661972	-10,56068662	-0,28909	-6,477529581	-0,24226	57,275	-0,422980622	-17,42513
70812	27-28	Cloudy	0,007735905	0,01210	82,61477	0,004	0,159	0,00562	0,097524644	0,00364	50,165	0,007270926	0,26235
80812	28-29	Cloudy	-0,386456279	-0,60468	-1,65374	-0,17822	-8,03103875	-0,22115	-4,925938335	-0,18423	74,655	-0,246777883	-13,25121
90812	29-30	Cloudy	0,609942354	0,95437	1,04780	0,317458095	14,29355074	0,39426	8,767128602	0,32789	74,655	0,439212449	23,58435
130812	33-34	Sunny	0,403980325	0,63210	1,58200	0,078666667	2,688292857	0,12872	1,648898138	0,06166	109,81	0,056160129	4,43568
140812	34-35	Sunny	1,34442464	2,10362	0,47537	0,952888889	32,53264762	1,56068	19,95430741	0,74629	227,52	0,328014652	53,67886
150812	35-36	Sunny	0,171771346	0,26877	3,72064	0,241545894	3,927277433	0,83072	2,408844866	0,09009	87,295	0,103203785	6,48000
160812	36-37	Sunny	0,329630705	0,51577	1,93883	0,597565217	25,71131056	0,77659	15,77035478	0,58981	127,98	0,460866904	42,42366
210812	41-42	Sunny	-0,071851564	-0,11242	-8,89472	-0,145833333	-6,26640625	-0,18977	-3,843578861	-0,14375	57,67	-0,249265406	-10,33957
220812	42-43	Cloudy	0,238376311	0,3729875	2,68105	0,489583333	21,02672991	0,63742	12,89700849	0,48235	57,67	0,836402261	34,69410
Average			0,09008846	0,14096	6,23796	0,144548536	5,12840	0,24863	3,145570836	0,11764	98,41142	0,106492524	8,46186
Total			1,261238438	1,97346	87,33151	2,02367	71,79765	3,48084	44,03799	1,64703	1377,76	1,49089534	118,46612

[a] Illuminated area productivity (*IAP*) was calculated according to *(P/IAS*V_o)*, where *P* is daily productivity (g day^{-1}), *IAS* is illuminated area surface of the algae culture in the TPBR (0,56 m^2) and V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *IAP* is expressed in (g m^{-2} d^{-1}).

[b] Volumetric productivity (*VP*) was calculated according to *(P/V_o)*V_p)*, where *P* is daily productivity (g day^{-1}), V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L) and V_p is the predicted volume for the illuminated area of 0,56 m^2, and equal to 31,314 L. *VP* is expressed in (g L^{-1} d^{-1}).

[c] Areal Productivity (AP) was calculated according to *[(P/TSA)*V_o]*, where *P* is daily productivity (g day^{-1}), *TSA* is the total surface area of the TPBR included the empty area over the algae suspension (0,913 m^2) and V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *AP* is expressed in (g m^{-2} d^{-1}).

[d] Biomass output energy (E_{out}) was calculated according to *(HHV*IAP)*, where *HHV* is the higher heating value registered by combusting approximately 1 g (dry weight) algae samples in a Parr 1341 oxygen bomb calorimeter, i.e. the higher heating value *(HHV)* produced by 1 g DW m^{-2} d^{-1} was 22,94 kJ g^{-1} and referred as biomass output energy (0,02294 MJ m^{-2} d^{-1}), (Hulatt and Thomas, 2011). *IAP* is the illuminated area productivity. E_{OUT} is expressed in (MJ/m2d1). (Hulatt and Thomas, 2010).

[e] PAR irradiance input energy (E_{in}) was calculated according to *(I_o* CF)*, where I_o is irradiation on registered above the surface of algae culture but under the lid of the TPBR (µmol $m^{-2}s^{-1}$) and *CF* is the conversion factor from PAR to $Jm^{-2}s^{-1}$, and is equal to 4,57 µmol m^{-2} s^{-1} per 1 J m^{-2} s^{-1} (Thimijan & Heins, 1983; Zhang et al., 2001), and expressed as the equivalent daily energy input MJ m^{-2} d^{-1}, [(4,57 * 10^{-6} MJ/86,4*10^{-6} d)= 0,395 MJ m^{-2} d^{-1}. (E_{in}) is expressed in (MJ/m2d1).

[f] Areal CO_2 Fixation rate (ACO_2Fr) was calculated according to (0,45 *IAP* * (44/12), where 0,45 the carbon content of dried cells (g carbon g $biomass^{-1}$) (Hulatt and Thomas, 2011), *P* is the IAP, the illuminated area productivity (g biomass m^{-2} d^{-1}), and 44 and 12 the molecular weights of carbon dioxide and carbon, respectively. (Zhang K et al 2001). (ACO_2Fr) is expressed in (g CO_2 m^{-2} d^{-1}).

Table 74 - Data registered on TPBR 8 – *Scenedesmus sp* - freshwater microalgae for 42 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94'' N, 10 ° 46' 15,52'', elevation 107 meters above sea level) (cultivation period: 11.07.12-23.08.12). Nutrition media: 3N-BBM+Vitamins

Age of culture (days)	Culture Date	Time of Sampling (hh.mm)	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture (μmol m^{-2} s^{-1})	pH	Dissolved Oxygen (DO) (mg L^{-1})	Conductivity (mS cm^{-1})	Salinity (g L^{-1}) or ppt	Remaining culture volume V_o (g L^{-1})	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) (g L^{-1})
0	110712	11:35	Cloudy	23,3	96	7,307	10,24	1,624	0,790545964	25,75	6	6	0,233009709	0,114666667
1	120712	17:20	Cloudy	23,7	109	7,206	9,03	1,61	0,783135364	25,675	20	20	0,778967868	0,116923077
2	130712	19.47	Cloudy	23,2	78	7,242	8,56	1,51	0,73036974	25,585				0,224
5	160712	20:25	Cloudy	24,2	49	7,338	7,94	1,505	0,72773934	0,56	25			0,344
6	170712	11:23	Sunny	27,7	946	7,692	8,64	1,506	0,728265359	25,5355		25	0,979029195	0,359183673
7	180712	11:30	Cloudy	23,6	124	7,48	9,35	1,508	0,729317488	10,5105	15			0,4
8	190712	12:30	Cloudy	22,9	161	7,22	12,05	1,496	0,723006556	25,453		15	0,589321495	0,189565217
27	70812	10:18	Cloudy	22	82	7,677	11	1,621	0,788957505	0,433				0,915
28	80812	09:43	Cloudy	21,4	110	7,621	10,36	1,636	0,796902375	25,408		25	0,983942065	0,636
29	90812	11:25	Sunny	23,6	681	7,68	11,49	1,19	0,563678711	22,3935	3			0,37778
30	100812	12:49	Cloudy	22,4	157	7,572	9,25	1,248	0,593627312	2,3775	20			0,55625
33	130812	12:18	Sunny	25,8	400	7,687	10,46	1,303	0,622140084	16,3505		14	0,856242928	0,27037037
34	140812	13:00	Sunny -cloudy	26,7	125	7,585	7,65	1,352	0,647631692	16,33				0,502439024
35	150812	11:08	Sunny	25,6	600	7,554	8,42	1,385	0,664845466	16,31				0,43
36	160812	09:35	Sunny	23	347	7,584	8,76	1,431	0,688900408	6,2915	10			0,394594595
37	170812	15:15	Sunny	28,9	202	7,67	8,1	1,465	0,706723961	21,273		15	0,705119165	0,594594595
40	200812	10:30	Cloudy	22,4	60	7,351	9,25	1,471	0,70987309	21,2545				0,302702703
41	210812	09:55	Sunny	23,6	334	7,431	10,95	1,475	0,711973137	21,2405				0,592857143
42	220812	08:48	Sunny	22,6	223	7,55	13,46	1,478	0,7135485	21,229				0,652173913
Average				23,892	257,571	7,547	10,03928	1,432785714	0,690080449	16,20389			0,783656413	0,486737683
Total					3606		140,55				99	120		6,81432756

Table 75 - Biomass productivity and CO_2 fixation rate for the TPBR 8 – *Scenedesmus sp* - freshwater microalgae strain for 42 days of semi-continuous production system, under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 11.07.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1-LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931 /r	Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1}) [g]	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1}) [h]	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1}) [i]	Biomass Output Energy (E_{out}) (IAP*HVV) (MJ $m^{-2}d^{-1}$)[j]	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$) [k]	Photosynthetic Efficiency (PE) (100* E_{out}/E_{in})	Areal CO_2 Fixation rate (ACO_2Fr) (0,45P* (44/12) (g CO_2 m^{-2} d^{-1}) [l]
110712	0-1	Cloudy	0,01948	0,03049	32,79641	0,00225	0,10375	0,00274	0,06363	0,00238	37,92	0,006276714	0,171195055
120712	1-2	Cloudy	0,65012	1,01725	0,98303	0,10707	4,90928	0,13059	3,01117	0,11261	43,055	0,261570118	8,100321429
160712	5-6	Cloudy	0,01439	0,02252	44,38993	0,00506	0,00506	0,28301	0,00310	0,00011	19,355	0,000599868	0,00835102
170712	6-7	Sunny	0,10763	0,16840	5,93789	0,04081	1,86118	0,05005	1,14158	0,04269	373,67	0,011426032	3,070960277
180712	7-8	Cloudy	-0,74673	-1,16841	-0,85586	-0,21043	-3,94959	-0,62694	-2,42253	-0,09060	48,98	-0,184981164	-6,516836413
070812	28-29	Cloudy	-0,01914	-0,02995	-33,38479	-0,01468	-0,01135	-1,06194	-0,00696	-0,00026	43,45	-0,000599452	-0,018734168
80812	29-30	Cloudy	-0,52088	-0,81503	-1,22694	-0,25822	-11,71581	-0,31824	-7,18603	-0,26876	268,995	-0,099912894	-19,33108697
90812	30-31	Sunny	0,38690	0,60539	1,65182	0,17847	7,13672	0,24956	4,37740	0,16371	62,015	0,263995084	11,77560198
130812	34-35	Sunny	0,20656	0,32320	3,09400	0,07735	2,25859	0,14815	1,38533	0,05181	49,375	0,104936009	3,726680697
140812	35-36	Patchy-cloud	-0,15568	-0,24360	-4,10497	-0,07243	-2,11237	-0,13890	-1,29565	-0,04845	237	-0,020446351	-3,485416594
150812	36-37	Sunny	-0,08592	-0,13444	-7,43776	-0,03540	-1,03118	-0,06797	-0,63248	-0,02365	137,065	-0,017258472	-1,701451014
160812	37-38	Sunny	0,41002	0,64155	1,55870	0,2	2,24696	0,99543	1,37820	0,05154	79,79	0,064601279	3,707491071
210812	41-42	Cloudy	0,22406	0,35059	2,85226	0,09671	3,67088	0,14249	2,25158	0,08421	131,93	0,063829388	6,056960985
220812	42-43	Sunny	0,09535	0,14920	6,70212	0,05931	2,24985	0,08744	1,37997	0,05161	88,085	0,05859299	3,712257972
Average			0,04187	0,06551	3,78256	0,01256	0,40157	-0,00889	0,24630	0,00921	115,763	0,036616368	0,662592523
Total			0,58618	0,91719	52,95585	0,175888596	5,621997167	-0,12451	3,44832	0,12896	1620,685	0,51262915	9,276295326

[g] Illuminated area productivity (*IAP*) was calculated according to *(P/IAS*V_o)*, where *P* is daily productivity (g day^{-1}), *IAS* is illuminated area surface of the algae culture in the TPBR (0,56 m^2) and V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *IAP* is expressed in (g m^{-2} d^{-1}).

[h] Volumetric productivity (*VP*) was calculated according to *(P/V_o)*V_p)*, where *P* is daily productivity (g day^{-1}), V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L) and V_p is the predicted volume for the illuminated area of 0,56 m^2, and equal to 31,314 L. *VP* is expressed in (g L^{-1} d^{-1}).

[i] Areal Productivity (AP) was calculated according to *[(P/TSA)*V_o]*, where *P* is daily productivity (g day^{-1}), *TSA* is the total surface area of the TPBR included the empty area over the algae suspension (0,913 m^2) and V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *AP* is expressed in (g m^{-2} d^{-1}).

[j] Biomass output energy (E_{out}) was calculated according to *(HHV*IAP)*, where *HHV* is the higher heating value registered by combusting approximately 1 g (dry weight) algae samples in a Parr 1341 oxygen bomb calorimeter, i.e. the higher heating value *(HHV)* produced by 1 g DW m^{-2} d^{-1} was 22,94 kJ g^{-1} and referred as biomass output energy (0,02294 MJ m^{-2} d^{-1}), (Hulatt and Thomas, 2011). *IAP* is the illuminated area productivity. E_{OUT} is expressed in (MJ/m2d1). (Hulatt and Thomas, 2010).

[k] PAR irradiance input energy (E_{in}) was calculated according to *(I_o* CF)*, where I_o is irradiation on registered above the surface of algae culture but under the lid of the TPBR *(*µmol $m^{-2}s^{-1}$) and *CF* is the conversion factor from PAR to $Jm^{-2}s^{-1}$, and is equal to 4,57 µmol m^{-2} s^{-1} per 1 J m^{-2} s^{-1} (Thimijan & Heins, 1983; Zhang et al., 2001), and expressed as the equivalent daily energy input MJ m^{-2} d^{-1}, [(4,57 * 10^{-6} MJ/86,4*10^{-6} d)= 0,395 MJ m^{-2} d^{-1}. (E_{in}) is expressed in (MJ/m2d1).

[l] Areal CO_2 Fixation rate (ACO_2Fr) was calculated according to (0,45 *IAP* * (44/12), where 0,45 the carbon content of dried cells (g carbon g $biomass^{-1}$) (Hulatt and Thomas, 2011), *P* is the IAP, the illuminated area productivity (g biomass m^{-2} d^{-1}), and 44 and 12 the molecular weights of carbon dioxide and carbon, respectively. (Zhang K et al 2001). (ACO_2Fr) is expressed in (g CO_2 m^{-2} d^{-1}).

Table 76- Data registered on TPBR 9 – *Chlorella wild mix Årungen* - freshwater microalgae for 42 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 11.07.12-23.08.12). Nutrition media: 3N-BBM+Vitamins

Age of culture (days)	Culture Date	Time of Sampling (hh.mm)	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture ($\mu mol\ m^{-2}\ s^{-1}$)	pH	Dissolved Oxygen (DO) ($mg\ L^{-1}$)	Conductivity ($mS\ cm^{-1}$)	Salinity ($g\ L^{-1}$) or ppt	Remaining culture volume V_o ($g\ L^{-1}$)	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) ($g\ L^{-1}$)
0	110712	11:45	Rainy	22,1	80	7,287	10,55	1,578	0,766218161	25,675	22	22	0,856864654	0,151
1	120712	17:14	Cloudy	22,3	56	7,084	10,12	1,567	0,760409795	25,5415	13	13	0,508975589	0,102
2	130712	19:52	Cloudy	21,7	75	7,186	10,2	1,532	0,741952552	19,5415	6	6	0,307038866	0,173
5	160712	20:35	Cloudy	23,7	46	7,516	8,56	1,478	0,7135485	4,5135	21			0,5
6	170712	10:45	Sunny	24,3	371	8,939	16,08	1,472	0,710398055	25,496		21	0,823658613	0,72
7	180712	11:20	Cloudy	22,4	164	7,176	12,42	1,513	0,731948346	20,424	5			0,185
8	190712	13:00	Cloudy	22,4	150	7,328	12,9	1,506	0,728265359	25,3785		5	0,19701716	0,301
27	70812	11:00	Cloudy	21,2	137	7,676	9,25	1,551	0,751967657	25,3555				0,452
28	80812	11:15	Cloudy	21,3	197	7,777	9,25	1,568	0,760937681	0,335	25			0,468
29	90812	10:00	Cloudy	20,6	174	7,801	9,08	1,567	0,760409795	30,326		30	0,989250148	0,378
30	100812	11:40	Cloudy	22,1	149	8,4	15,72	1,486	0,717750839	10,301	20			0,756
33	130812	12:50	Sunny	24,6	393	7,795	13,17	1,476	0,712498227	24,2875		14	0,576428204	0,852
34	140812	13:24	Patchy-cloud	25,1	389	7,722	11,2	1,489	0,719327229	6,268	18			0,857
35	150812	08:30	Sunny	20,3	217	7,89	15,17	1,506	0,728265359	21,2545		15	0,705732904	1,415
36	160812	10:30	Sunny	22,9	264	7,84	13,86	1,516	0,733527228	11,243	10			1,443
37	170812	15:40	Sunny	27,2	331	7,812	10,11	1,535	0,743533163	23,7275		12,5	0,526814877	1,516
40	200812	11:00	Cloudy	21,1	72	7,636	9,07	1,59	0,772558615	23,7145				1,515
41	210812	10:10	Sunny	22,4	131	7,695	12,5	1,601	0,778374391	23,705				1,705
42	220812	09:15	Cloudy	21,6	77	7,686	12,26	1,634	0,795842688	23,6955				2,042
Average				22,594	182,789	7,697	11,656	1,535	0,743564928	19,514			0,610197891	0,817
Total					3473		221,47			370,783	140	138,5		15,531

Table 77 - Biomass productivity and CO_2 fixation rate for the TPBR 9 – *Chlorella wild mix Årungen* - freshwater microalgae strain for 42 days of semi-continuous production system, under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 11.07.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX_1-LnX_o)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T_2=0,6931 /r	Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V_o) (g m^{-2} d^{-1}) [m]	Volumetric Productivity [VP= (P/V_o)*V_p)] (g L^{-1} d^{-1}) [n]	Areal Productivity [AP= (P/TSA)*V_o] (g m^{-2} d^{-1}) [o]	Biomass Output Energy (E_{out}) (IAP*HVV) (MJ $m^{-2}d^{-1}$)[p]	PAR Irradiance Input Energy (E_{in}) (MJ $m^{-2}d^{-1}$) [q]	Photosynthetic Efficiency (PE) (100* E_{out}/E_{in})	Areal CO_2 Fixation rate (ACO_2Fr) (0,45P* (44/12) (g CO_2 m^{-2} d^{-1}) [r]
110712	0-1	Rainy	-0,39230	-0,61384	-1,62908	0,00225641	0,10345	0,002751986	0,063453815	0,00237	31,6	0,007510119	0,17069
120712	1-2	Cloudy	0,52831	0,82666	1,20968	0,107076923	4,88375	0,131276815	2,995515039	0,11203	22,12	0,506480286	8,05820
160712	5-6	Cloudy	0,12154	0,19018	5,25801	0,005061224	0,04079	0,035114032	0,025020632	0,00093	18,17	0,005150146	0,06730
170712	6-7	Sunny	-1,35884	-2,12626	-0,47030	0,040816327	1,85830	0,050130313	1,139817154	0,04262	146,545	0,029089774	3,06620
180712	7-8	Cloudy	0,48675	0,76162	1,31298	-0,210434783	-7,67485	-0,322637817	-4,70746988	-0,17606	64,78	-0,271783302	-12,66351
070812	28-29	Cloudy	0,00183	0,00286	349,07317	-0,014684211	-0,66486	-0,018134975	-0,407804491	-0,01525	77,815	-0,019600396	-1,09703
080812	29-30	Cloudy	-0,21357	-0,33417	-2,99240	-0,25822	-0,15447	-24,13701815	-0,094746659	-0,00354	68,73	-0,005155772	-0,25487
090812	30-31	Cloudy	0,69314	1,08456	0,92202	0,17847	9,66478	0,184284429	5,928018861	0,22171	58,855	0,376705861	15,94690
130812	34-35	Sunny	0,00195	0,00305	327,66563	0,077356218	3,35498	0,099735774	2,057819436	0,07696	153,655	0,050088351	5,53571
140812	35-36	Patchy-cloud	0,50144	0,78461	1,27451	-0,072439024	-0,81079	-0,361894641	-0,497314135	-0,01859	85,715	-0,021699521	-1,33781
150812	36-37	Sunny	0,01959	0,03065	32,61588	-0,035405405	-1,34379	-0,052162359	-0,824232409	-0,03082	104,28	-0,029561388	-2,21725
160812	37-38	Sunny	0,04935	0,07721	12,95009	0,2	4,01535	0,557039936	2,46286966	0,09211	130,745	0,070451867	6,62533
210812	41-42	Cloudy	0,03938	0,06162	16,22772	0,096718147	4,09575	0,127712246	2,512182355	0,09395	28,44	0,330367817	6,75799
220812	42-43	Sunny	0,18036	0,28221	3,54337	0,05931677	2,51090	0,07835669	1,540092045	0,05760	51,745	0,111315195	4,14298
Average			0,04706	0,07364	53,3543795	0,012563471	1,419950403	-1,687531837	0,870944387	0,03257	74,51392	0,081382788	2,34291
Total							19,87930564	-23,62544572	12,19322142	0,45603	1043,195	1,139359035	32,80085

[m] Illuminated area productivity (*IAP*) was calculated according to *(P/IAS*V_o)*, where *P* is daily productivity (g day^{-1}), *IAS* is illuminated area surface of the algae culture in the TPBR (0,56 m^2) and V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *IAP* is expressed in (g m^{-2} d^{-1}).

[n] Volumetric productivity (*VP*) was calculated according to *(P/V_o)*V_p)*, where *P* is daily productivity (g day^{-1}), V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L) and V_p is the predicted volume for the illuminated area of 0,56 m^2, and equal to 31,314 L. *VP* is expressed in (g L^{-1} d^{-1}).

[o] Areal Productivity (AP) was calculated according to *[(P/TSA)*V_o]*, where *P* is daily productivity (g day^{-1}), *TSA* is the total surface area of the TPBR included the empty area over the algae suspension (0,913 m^2) and V_o is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *AP* is expressed in (g m^{-2} d^{-1}).

[p] Biomass output energy (E_{out}) was calculated according to *(HHV*IAP)*, where *HHV* is the higher heating value registered by combusting approximately 1 g (dry weight) algae samples in a Parr 1341 oxygen bomb calorimeter, i.e. the higher heating value *(HHV)* produced by 1 g DW m^{-2} d^{-1} was 22,94 kJ g^{-1} and referred as biomass output energy (0,02294 MJ m^{-2} d^{-1}), (Hulatt and Thomas, 2011). *IAP* is the illuminated area productivity. E_{OUT} is expressed in (MJ/m2d1). (Hulatt and Thomas, 2010).

[q] PAR irradiance input energy (E_{in}) was calculated according to *(I_o* CF)*, where I_o is irradiation on registered above the surface of algae culture but under the lid of the TPBR (μmol $m^{-2}s^{-1}$) and *CF* is the conversion factor from PAR to $Jm^{-2}s^{-1}$, and is equal to 4,57 μmol m^{-2} s^{-1} per 1 J m^{-2} s^{-1} (Thimijan & Heins, 1983; Zhang et al., 2001), and expressed as the equivalent daily energy input MJ m^{-2} d^{-1}, [(4,57 * 10^{-6} MJ/86,4*10^{-6} d)= 0,395 MJ m^{-2} d^{-1}. (E_{in}) is expressed in (MJ/m2d1).

[r] Areal CO_2 Fixation rate (ACO_2Fr) was calculated according to (0,45 *IAP* * (44/12), where 0,45 the carbon content of dried cells (g carbon g $biomass^{-1}$) (Hulatt and Thomas, 2011), *P* is the IAP, the illuminated area productivity (g biomass m^{-2} d^{-1}), and 44 and 12 the molecular weights of carbon dioxide and carbon, respectively. (Zhang K et al 2001). (ACO_2Fr) is expressed in (g CO_2 m^{-2} d^{-1}).

Table 78 - Data registered on TPBR 10 – *Chlorella wild mix Årungen* - freshwater microalgae for 42 days of semi-continuous production system under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 11.07.12-23.08.12). Nutrition media: 3N-BBM+Vitamins

Age of culture (days)	Culture Date	Time of Sampling	Outside weather condition	Algae culture temp (°C)	Irradiation on surface culture ($\mu mol\ m^{-2}\ s^{-}$)	pH	Dissolved Oxygen (DO) ($mg\ L^{-1}$)	Conductivity ($mS\ cm^{-1}$)	Salinity ($g\ L^{-1}$) or ppt	Remaining culture volume V_o ($g\ L^{-1}$)	Removed Volume (L)	Fresh volume added (F) (L)	Dilution rate ($D=F/V_o$) (d^{-1})	Biomass Concentration (TSS) ($g\ L^{-1}$)
0	110712	11:55	Rainy	21,4	89	7,128	10,38	1,564	0,758826315	25,65	22	22	0,857699805	0,121
1	120712	17:35	Cloudy	22,6	98	7,219	10,27	1,544	0,748276621	25,596	13	13	0,507891858	0,387037037
2	130712	20:00	Cloudy	21,8	77	7,22	9,36	1,548	0,750385605	25,596	5	5	0,195343022	0,369491525
5	160712	14:38	Sunny	25,8	354	8,909	15,63	1,483	0,716174729	15,5665	10			0,614634146
6	170712	11:30	Sunny	24,7	458	7,949	16,48	1,48	0,714598898	25,546		10	0,391450716	0,482051282
7	180712	11:48	Cloudy	22,4	115	7,303	13,34	1,483	0,716174729	25,5265				0,545454545
8	190712	12:44	Cloudy	22,4	101	7,463	10,2	1,489	0,719327229	25,5045				0,545833333
27	70812	10:30	Cloudy	20,8	113	7,688	11,06	1,512	0,731422114	25,468				0,56
28	80812	09:50	Cloudy	20,3	110	7,642	10,9	1,533	0,742479392	17,451	8			0,847058824
29	90812	11:40	Sunny	22,8	579	7,912	11,5	1,539	0,745641066	17,441				0,37778
30	100812	12:25	Cloudy	21,9	161	7,698	15,07	1,543	0,74774945	17,4225				0,881081081
33	130812	12:30	Sunny	23,8	755	7,784	13,8	1,555	0,754077478	3,4225	14			0,969230769
34	140812	13:10	Patchy-cloud	24,4	127	7,722	11,21	1,567	0,760409795	23,3955		20	0,854865252	0,914285714
35	150812	10:35	Sunny	22,8	280	7,817	13,5	1,578	0,766218161	5,3845	18			1,327272727
36	160812	09:10	Patchy-cloud	20,2	242	7,665	11,85	1,583	0,768859504	20,375		15	0,736196319	1,6
37	170812	15:19	Sunny	27,7	359	7,78	13,82	1,524	0,737738919	10,3575	10			1,211428571
40	200812	10:35	Cloudy	21,2	62	7,499	11,14	1,501	0,725635573	22,8575		12,5	0,546866455	0,622857143
41	210812	10:00	Sunny	22	220	7,596	16,02	1,493	0,721429516	22,82				0,98
42	220812	08:55	Patchy-cloud	21,7	163	7,616	13,89	1,481	0,715124144	22,8				0,835
Average				22,67	234,89473	7,663	12,601	1,526	0,738976276	19,9042			0,58433049	0,746920879
Total					4463		239,42				100	97,5		14,1914967

Table 79 - Biomass productivity and CO_2 fixation rate for the TPBR 10 – *Chlorella wild mix Årungen* - freshwater microalgae strain for 42 days of semi-continuous production system, under green house conditions at NMBU campus, Ås, Norway (59° 40' 06.94" N, 10 ° 46' 15,52", elevation 107 meters above sea level) (cultivation period: 11.07.12-23.08.12). Nutrition media 3N-BBM+Vitamins

Culture Date	Time Interval (days)	Outside Weather Condition	Growth rate [r= (LnX$_1$-LnX$_o$)/ Δt]	Doublings per day (k= r/0,6931)	Doubling Time T$_2$=0,6931 /r	Productivity (P) (g DW L^{-1} d^{-1})	Illuminated Area Productivity (IAP= P/IAS*V$_o$) (g m^{-2} d^{-1}) [s]	Volumetric Productivity [VP= (P/V$_o$)*V$_p$)] (g L^{-1} d^{-1}) [t]	Areal Productivity [AP= (P/TSA)*V$_o$] (g m^{-2} d^{-1}) [u]	Biomass Output Energy (E$_{out}$) (IAP*HVV) (MJ m^{-2}d^{-1})[v]	PAR Irradiance Input Energy (E$_{in}$) (MJ m^{-2}d^{-1}) [w]	Photosynthetic Efficiency (PE) (100* E$_{out}$/E$_{in}$)	Areal CO_2 Fixation rate (ACO_2Fr) (0,45P* (44/12) (g CO_2 m^{-2} d^{-1}) [x]
110712	0-1	Rainy	3,19865	1,16272	1,67746	0,266037037	12,18544	0,32478	7,47409	0,27953	35,155	0,795147607	20,10598
120712	1-2	Cloudy	0,95466	-0,04639	-0,06693	-0,017545512	-0,80195	-0,02146	-0,49188	-0,01839	38,71	-0,047524806	-1,32322
160712	5-6	Sunny	0,78428	-0,24297	-0,35054	-0,044194288	-1,22848	-0,08890	-0,75350	-0,02818	139,83	-0,020154041	-2,02699
170712	6-7	Sunny	1,13152	0,12356	0,17827	0,063403263	2,89232	0,07771	1,77404	0,06634	180,91	0,036675609	4,77232
180712	7-8	Cloudy	1,00069	0,00069	0,00100	0,000378788	0,01726	0,00046	0,01059	0,00034	45,425	0,000871962	0,02848
070812	28-29	Cloudy	1,51260	0,41383	0,59703	0,015108359	0,68710	0,01857	0,42144	0,01576	44,635	0,0353136	1,13372
80812	29-30	Cloudy	0,44599	-0,80745	-1,16491	-0,469278824	-14,62390	-0,84207	-8,96975	-0,33547	43,45	-0,77208814	-24,12943
90812	30-31	Sunny	2,33225	0,84683	1,22172	0,503301081	15,67513	0,90363	9,61453	0,35958	228,705	0,157227668	25,86396
130812	34-35	Sunny	0,94331	-0,05835	-0,08419	-0,018315018	-0,11193	-0,16757	-0,06865	-0,00256	50,165	-0,005118649	-0,18469
140812	35-36	Patchy-cloud	1,45170	0,37273	0,53774	0,412987013	17,25363	0,55276	10,58273	0,39579	110,6	0,357864802	28,46850
150812	36-37	Sunny	1,20547	0,18687	0,26960	0,272727273	2,62232	1,58606	1,60843	0,06015	95,59	0,062931325	4,32683
160812	37-38	Sunny-cloudy	0,75714	-0,27820	-0,40136	-0,388571429	-14,13775	-0,59718	-8,67156	-0,32432	141,805	-0,22870851	-23,32729
210812	41-42	Cloudy	1,57339	0,45323	0,65388	0,119047619	4,85544	0,16321	2,97814	0,11138	86,9	0,128174734	8,01147
220812	42-43	Sunny	0,85204	-0,16012	-0,23100	-0,145	-5,90875	-0,19897	-3,62420	-0,13554	64,385	-0,210525316	-9,74943
Average			1,29598	0,14050	0,20269	0,040720383	1,38399	0,12221	0,84888	0,03174	93,30464	0,02072056	2,28358
Total			18,14376	1,96700	2,83778	0,570085363	19,37589	1,711061	11,88444	0,44448	1306,265	0,290087844	31,970229

[s] Illuminated area productivity (*IAP*) was calculated according to *(P/IAS*V$_o$)*, where *P* is daily productivity (g day^{-1}), *IAS* is illuminated area surface of the algae culture in the TPBR (0,56 m^2) and *V$_o$* is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *IAP* is expressed in (g m^{-2} d^{-1}).

[t] Volumetric productivity (*VP*) was calculated according to *(P/V$_o$)*V$_p$)*, where *P* is daily productivity (g day^{-1}), *V$_o$* is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L) and *V$_p$* is the predicted volume for the illuminated area of 0,56 m^2, and equal to 31,314 L. *VP* is expressed in (g L^{-1} d^{-1}).

[u] Areal Productivity (AP) was calculated according to *[(P/TSA)*V$_o$]*, where *P* is daily productivity (g day^{-1}), *TSA* is the total surface area of the TPBR included the empty area over the algae suspension (0,913 m^2) and *V$_o$* is the remaining algae culture volume in the TPBR at the moment of the sample was collected (L). *AP* is expressed in (g m^{-2} d^{-1}).

[v] Biomass output energy (*E$_{out}$*) was calculated according to *(HHV*IAP)*, where *HHV* is the higher heating value registered by combusting approximately 1 g (dry weight) algae samples in a Parr 1341 oxygen bomb calorimeter, i.e. the higher heating value *(HHV)* produced by 1 g DW m^{-2} d^{-1} was 22,94 kJ g^{-1} and referred as biomass output energy (0,02294 MJ m^{-2} d^{-1}), (Hulatt and Thomas, 2011). *IAP* is the illuminated area productivity. *E$_{OUT}$* is expressed in (MJ/m2d1). (Hulatt and Thomas, 2010).

[w] PAR irradiance input energy (*E$_{in}$*) was calculated according to *(I$_o$* CF)*, where *I$_o$* is irradiation on registered above the surface of algae culture but under the lid of the TPBR *(*µmol m^{-2}s^{-1}) and *CF* is the conversion factor from PAR to Jm^{-2}s^{-1}, and is equal to 4,57 µmol m^{-2} s^{-1} per 1 J m^{-2} s^{-1}(Thimijan & Heins, 1983; Zhang et al., 2001), and expressed as the equivalent daily energy input MJ m^{-2} d^{-1}, [(4,57 * 10^{-6} MJ/86,4*10^{-6} d)= 0,395 MJ m^{-2} d^{-1}. (*E$_{in}$*) is expressed in (MJ/m2d1).

[f] Areal CO_2 Fixation rate (ACO_2Fr) was calculated according to (0,45 *IAP* * (44/12), where 0,45 the carbon content of dried cells (g carbon g biomass^{-1}) (Hulatt and Thomas, 2011), *P* is the IAP, the illuminated area productivity (g biomass m^{-2} d^{-1}), and 44 and 12 the molecular weights of carbon dioxide and carbon, respectively. (Zhang K et al 2001). (ACO_2Fr) is expressed in (g CO_2 m^{-2} d^{-1}).

16 Appendix 7. ANOVA Statistical analysis

Two-Sample T-Test and CI par-wise for the two TPBR with the same algae strain culture was used to corroborate the hypothesis H: $\mu_1 \geq \mu_2$, i.e. the biomass production of algae was greater in TPBR1 than in TPBR 2.
To compare the data from all TPBRs intrinsic and among themselves was used ANOVA one-way (unstacked) HSB best choice among families.
One-way ANOVA, Hsu's multiple comparisons with the best (MCB) and z-test using Minitab 15.1.30.0 (Minitab, Inc., State College, PA) were conducted to evaluate the impact of temperature, irradiation and outside weather conditions on pH, dissolved oxygen, conductivity, salinity, dilution rate on algae growth rate, doublings/day, doubling time, biomass productivity, illuminated area productivity, volumetric productivity, areal productivity, biomass output energy, PAR irradiance input energy, photosynthetic efficiency and areal CO_2 fixation rate for all responses.

An extract from the analysis is presented here.

Two-Sample T-Test and CI: T1 Productivity (g/L*d); T2 Productivity (g/L*d)

```
Two-sample T for T1 Productivity (g/L*d) vs T2 Productivity (g/L*d)

                          N    Mean   StDev SE Mean
T1 Productivity (g/L*d)   7  0,0417  0,0262   0,0099
T2 Productivity (g/L*d)   9  0,0536  0,0296   0,0099


Difference = mu (T1 Productivity (g/L*d)) - mu (T2 Productivity (g/L*d))
Estimate for difference:  -0,0119
95% lower bound for difference:  -0,0366
T-Test of difference = 0 (vs >): T-Value = -0,85 P-Value = 0,795 DF = 13
```

Two-Sample T-Test and CI: T3 Productivity (g/L*d); T4 Productivity (g/L*d)

```
Two-sample T for T3 Productivity (g/L*d) vs T4 Productivity (g/L*d)

                          N    Mean   StDev SE Mean
T3 Productivity (g/L*d)   4  0,0369  0,0174   0,0087
T4 Productivity (g/L*d)   9  0,0372  0,0211   0,0070


Difference = mu (T3 Productivity (g/L*d)) - mu (T4 Productivity (g/L*d))
Estimate for difference:  -0,0003
95% lower bound for difference:  -0,0215
T-Test of difference = 0 (vs >): T-Value = -0,02 P-Value = 0,509 DF = 7
```

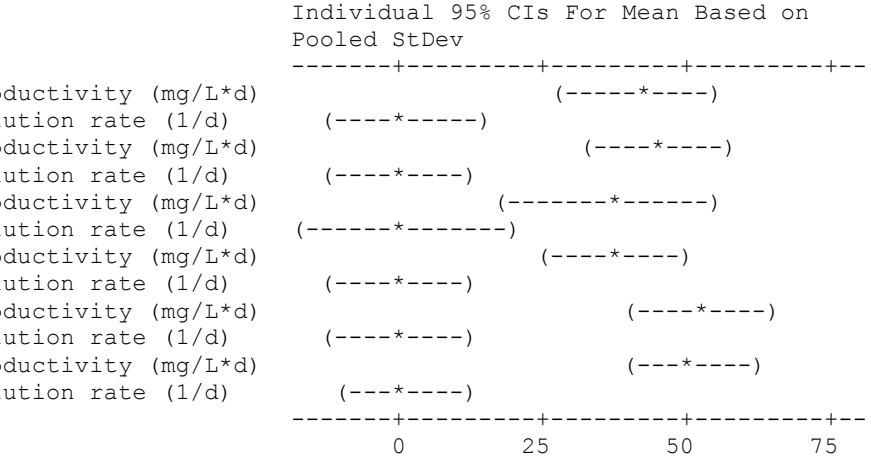
Two-Sample T-Test and CI: T5 Productivity (g/L*d); T6 Productivity (g/L*d)

```
Two-sample T for T5 Productivity (g/L*d) vs T6 Productivity (g/L*d)

                          N    Mean   StDev SE Mean
T5 Productivity (g/L*d)   8  0,0527  0,0288    0,010
T6 Productivity (g/L*d)  10  0,0509  0,0281   0,0089


Difference = mu (T5 Productivity (g/L*d)) - mu (T6 Productivity (g/L*d))
Estimate for difference:  0,0018
95% lower bound for difference:  -0,0220
T-Test of difference = 0 (vs >): T-Value = 0,13 P-Value = 0,449 DF = 14
```

One-way ANOVA: T1 Productiv; T1 Dilution ; T2 Productiv; T2 Dilution ; ...

```
Source  DF     SS    MS      F      P
Factor  11  47852  4350  12,70  0,000
Error   82  28079   342
Total   93  75931

S = 18,50   R-Sq = 63,02%   R-Sq(adj) = 58,06%
```

Level	N	Mean	StDev
T1 Productivity (mg/L*d)	7	41,71	26,17
T1 Dilution rate (1/d)	7	0,36	0,19
T2 Productivity (mg/L*d)	9	44,25	28,63
T2 Dilution rate (1/d)	9	0,43	0,20
T3 Productivity (mg/L*d)	4	36,90	17,44
T3 Dilution rate (1/d)	4	0,55	0,39
T4 Productivity (mg/L*d)	9	37,17	21,14
T4 Dilution rate (1/d)	9	0,50	0,29
T5 Productivity (mg/L*d)	8	52,70	28,83
T5 Dilution rate (1/d)	8	0,44	0,19
T6 Productivity (mg/L*d)	10	50,92	28,09
T6 Dilution rate (1/d)	10	0,41	0,24




```
Pooled StDev = 18,50
```

One-way ANOVA: T1 Age cultu; T1 TSS (mg/L; T1 Dilution ; T1 Prod. rat; ...

```
Source   DF        SS      MS      F      P
Factor   59  55292143  937155  51,10  0,000
Error   410   7519890   18341
Total   469  62812033

S = 135,4   R-Sq = 88,03%   R-Sq(adj) = 86,31%
```

Level	N	Mean	StDev
T1 Age culture (d)	7	26,6	20,3
T1 TSS (mg/L)	7	754,4	355,9
T1 Dilution rate (1/d)	7	0,4	0,2
T1 Prod. rate (mg/L*d)	7	14,0	8,9
T1 Productivity (mg/L*d)	7	41,7	26,2
T1 Temp water (°C)	7	22,6	2,5

T1 pH	7	7,6	0,1
T1 DO (mg/L)	7	11,7	1,6
T1 L Int.(µmol/m2s1)	7	96,4	30,6
T1 Conductivity (µS/cm)	7	1444,1	172,5
T2 Age culture (d)	9	23,9	18,3
T2 TSS (mg/L)	9	720,6	376,5
T2 Dilution rate (1/d)	9	0,4	0,2
T2 Prod. rate (mg/L)	9	16,1	9,0
T2 Productivity (mg/L*d)	9	44,2	28,6
T2 Temp water (°C)	9	24,1	2,2
T2 pH	9	7,8	0,4
T2 DO (mg/L)	9	11,5	2,9
T2 L. Int. (µmol/m2s1)	9	108,2	65,0
T2 Conductivity (µS/cm)	9	1493,9	103,0
T3 Age culture (d)	4	29,3	25,8
T3 TSS (mg/L)	4	845,4	723,0
T3 Dilution rate (1/d)	4	0,5	0,4
T3 Prod. rate (mg/L*d)	4	18,0	11,6
T3 Productivity (mg/L*d)	4	36,9	17,4
T3 Temp water (°C)	4	21,8	1,3
T3 pH	4	7,9	0,5
T3 DO (mg/L)	4	14,3	2,8
T3 L. Int. (µmol/m2s1)	4	135,0	54,8
T3 Conductivity (µS/cm)	4	34,4	2,7
T4 Age culture (d)	9	28,4	20,0
T4 TSS (gm/L)	9	818,1	503,2
T4 Dilution rate (1/d)	9	0,5	0,3
T4 Prod. rate (mg/L*d)	9	20,1	13,8
T4 Productivity (mg/L*d)	9	37,2	21,1
T4 Temp water (°C)	9	23,5	2,1
T4 pH	9	7,6	0,5
T4 DO (mg/L)	9	13,0	3,1
T4 L. Int. (µmol/m2s1)	9	178,8	111,7
T4 Conductivity (µS/cm)	9	32,7	4,4
T5 Age culture (d)	8	24,8	17,4
T5 TSS (mg/L)	8	875,5	295,8
T5 Dilution rate (1/d)	8	0,4	0,2
T5 Prod. rate (mg/L*d)	8	22,0	12,3
T5 Productivity (mg/L*d)	8	52,7	28,8
T5 Temp water (°C)	8	21,7	1,5
T5 pH	8	15,9	23,9
T5 DO (mg/L)	8	12,6	2,6
T5 L. Int. (µmol/m2s1)	8	123,4	97,7
T5 Conductivity (µS/cm)	8	34,8	1,9
T6 Age culture (d)	10	23,7	15,6
T6 TSS (gm/L)	10	895,5	301,4
T6 Dilution rate (1/d)	10	0,4	0,2
T6 Prod. rate (mg/L*d)	10	20,5	19,1
T6 Productivity (mg/L*d)	10	50,9	28,1
T6 Temp water (°C)	10	24,1	2,0
T6 pH	10	7,7	0,5
T6 DO (mg/L)	10	11,4	4,4
T6 L. Int. (µmol/m2s1)	10	148,4	87,4
T6 Conductivity (µS/cm)	10	33,0	1,2

17 Appendix 8. SEM protocol for microalgae suspensions (24 microphotos)

Microalgae suspensions cryopreserved at 4°C for 100 days

Henry Bedoya

Preparation of microorganisms in microalgal suspensions for study with SEM (Scanning electron microscopy)

Abstract
A simple and straith forward method of analysing freshwater and marine phytoplankton by means of scanning electron microscope is described here. This first step of the procedure can be performed in field. This three-steps procol starts with fixation of the sample with 2,5 % glutaraldehyd on to glassbed lenses, followed by dehydration of the glass lenses by means of rinsing with a serie of increasing concentrations of ethyl alkohol. Step two, Critical point drying (CPD) performed in the laboratory is followed by a third and finally step: coating of the samples.

I *thank Hilde Kolstad and Elin Ørmen, Senior Engineers*, for their instructions and advice during the procedures and preparations of the samples, in their microscopy lab (Imagine Center) at IPM-UMB. The appendix was kindly provided by Elin Ørmen.

Methods and Materials
The three steps protocol was: fixation, CPD, coating (gold-palladium) procedures by Elin Ørmen and Hilde at imagine center at IPM (Institute for plante- og miljøvitenskap) at the UMB university campus. Kindled the glasslens with lenspaper to clean from impurities before immersion for 20s ec, in Poly-l-lysine, beforehand liquified at room temperature.

For fixation, the round glass lens (8 mm diameter) were immersed for 20 sec in a 2.5% v/v glutaraldehyde solution (GA 25%, 500 µL) buffered with 0,05M sodium cacodylate (caco, final concentration). It was essential to use buffer with freshwater samples because sodium cacodylate do not precipitate in lake water.

The marine microalgae (2 species) were buffered with the supernatant from the same sample, this procedure was necessary to substitute the sodium cacodylate. The reason was to avoid differences in the osmotic pressure leading to collapse of the algae cell walls. The seawater samples were transferred trough decreasing concentrations of filtered seawater after fixation to eliminate salts (100%, 75%, 50%, 25%, and distilled water for 5 min each).

After 22 hours fixation the samples were dehydrated by transfer through a series of vials of increasing concentrations of ethyl alcohol (10%, 30%, 50%, 70%, 90%, 96% and

100% for 10 min each). The critical point drying (CPD) was done shortly after the last step.
For CPD, the samples were transferred from 100% etyl alcohol to a cassette and kept immersed in 100% etOH, which was immediately put into a CPD chamber. In this pressure bomb apparatus, the etOH was replaced with carbon dioxide.
The aim of substituting the alcohol by CO2 (liquid phase) is to avoid destruction of the cellular walls and organelles of the microalgae. The CO2 is bled under pressure of 4 bar for 30 min at a temperature of 10 C. At this temperature the gas is liquid and flow into the cell chambers, substituting the alcohol. The pressure bomb is closed at about 72 atm of CO2 (critical pressure). The liquid CO2 inside the pressure chamber is now heated slightly above the critical temperature (sublimation phase). The liquid CO2 is converted to gas. This method of slowly bleding off, by means of critical point bring the samples to dryness with no surface stress. The overflow alcohol is discarded by manipulating the gas in and out buttons in the control panel and the alcohol is released through a hose on the back of the CPD machine. The procedure is completed when the dry ice formed from the hose sprout is totally dry. The instrument was a Critical Point Dryer BAL-TEC CPD 030.

The glass lenses were mounted directly on SEM sample stubs beforehand glued with silver based double-coated tape. All samples were coated in vacuum with 2 layers of gold-palladium atoms with tickness of under 100Å. The instrument used for coating was a Sputter Coater, type Polaron SC 7640.

The samples were observed with a Zeiss EVO - 50 - EP Scanning Electron Microscope (SEM)

Results and Discussion

This protocol pretends to cast light on a posteriori experiments perfor.med at the green house SKP (center for plant- and environmental sciences) at the UMB (The Norwegian University of Life Sciences). The need of understanding and see what kind of microorganisms were present under the cultivation of microalgae under continuous conditions and natural light was the aim of this paper. The microalga were breed up in axenic conditions for batch culture and the inoculum was dispersed into tap water (eventually prepared sea water based on tap water) under non-axenic conditions. The new microflora and microfauna present in the tap water just exploded. In light microscope was perceptible the living microorganisms in action. Fixation with glutaraldehyde at a concentration of 2,5% stopped the bioprocesses of bacteria and phytoplankton but left the structures intact after drying procedures.
In the different photos this constellation of microbiota is beautifully captured.

Chlorella vulgaris is a spherical shaped microalgae. Here is the algae got invaded, after inoculation and during cryo-stored period, by *microcystis aureginosa*, some *scenedesmus sp* and bacteria (figure 1, 2, 3, 4, 5)
Dunaliella salina is a marine microalga with no cellular wall of 2 μm diameter. The bacteria did overrun the culture, probably promoted by the cryoperiod in darkness (figure 6, 7, 8). There are other types of microalgae, which were present in the tap water probably collapsed in the salt medium. It´s possible to appreciate rests of anabaena, and diatomee microalgar. The photo shows biofilm probably from bacteria origins (Figure 9, 10, 11).
Nannochloropsis oculata, another marine microalgae was a more homogeneous and almost pure monoculture. (figure 12, 13, 14)
Scenedesmus sp, a freshwater microalgae in this inoculum, shows a more homogeneous, but not pure monoculture, (figure 15, 16). The sampled analysed was infected by *microcystis aureginosa* and some bacteria which presented traces of biofilm (17, 18, 19, 20)
The wild type species from Årungen Lake at UMB Campus was a micro-cosmos of many shapes. The sample analysed got a poor representative value, due to the low microalgal concentration of the inoculum at the moment of the start of the cultivation on the TPBR.

Need further studies among them genetical sequencing of the microorganisms in the microalgal suspension storaged at SKP laboratory refrigerator room.

This procedure aims to investigate plankton and other microorganisms present in not fresh microalgal suspensions. This protocol was applied on old cultures stored in darkness at 4 C, for approximately 100 days. The poor results of fenotypical (visual) analysis found in chlorella vulgaris, Dunaliella Salinas and wild type, reflects the uncontrolled axenic conditions in the non-treated tap water used to disperse the inoculum in the tray photo-bioreactor. In spite of those poor results, this protocol is enough to investigate mikrobiota smaller than 5 μm.

This study needs further analysis. One method to corroborate and systemise the founds in this paper is the 27 ribosomal DNA (27 rDNA) gen sequencing.

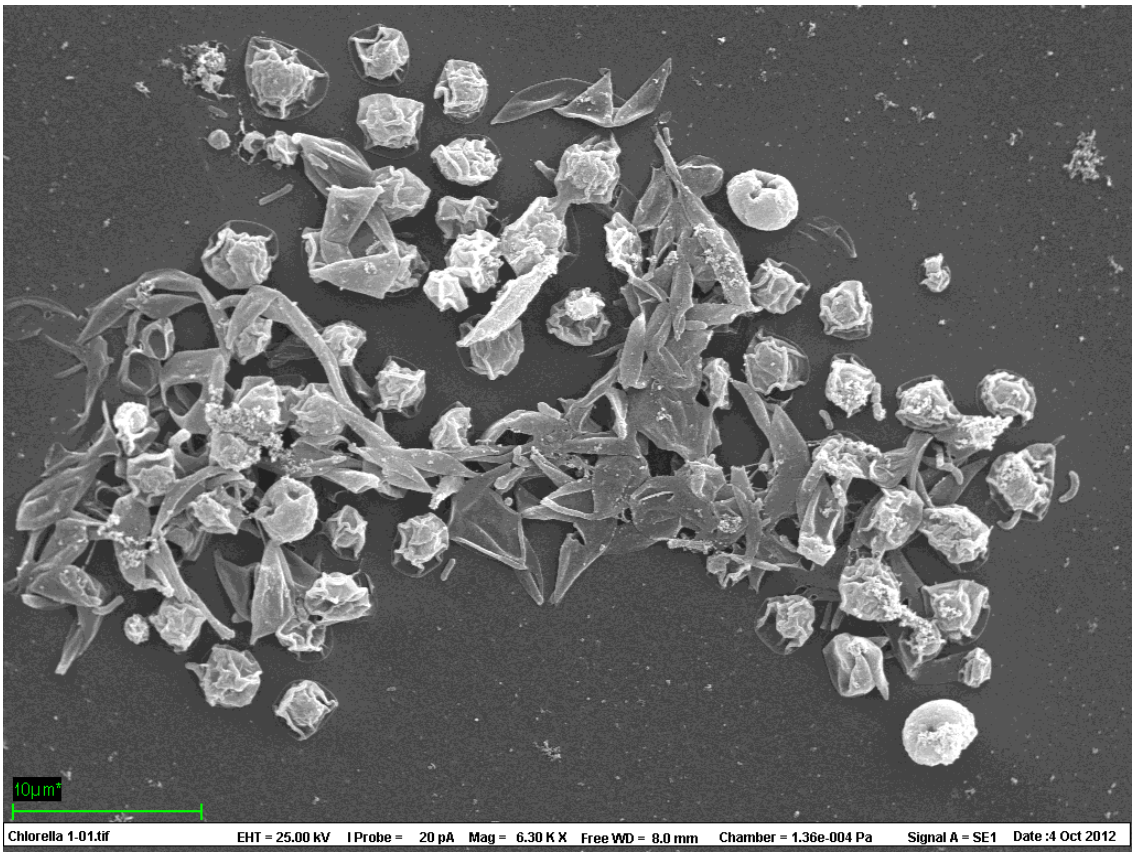


Figure3 *Chlorella vulgaris* inoculum 180612 in tap water. Cryopreserved at 4°C. SEM 041012 Photo: Elin Ørmen

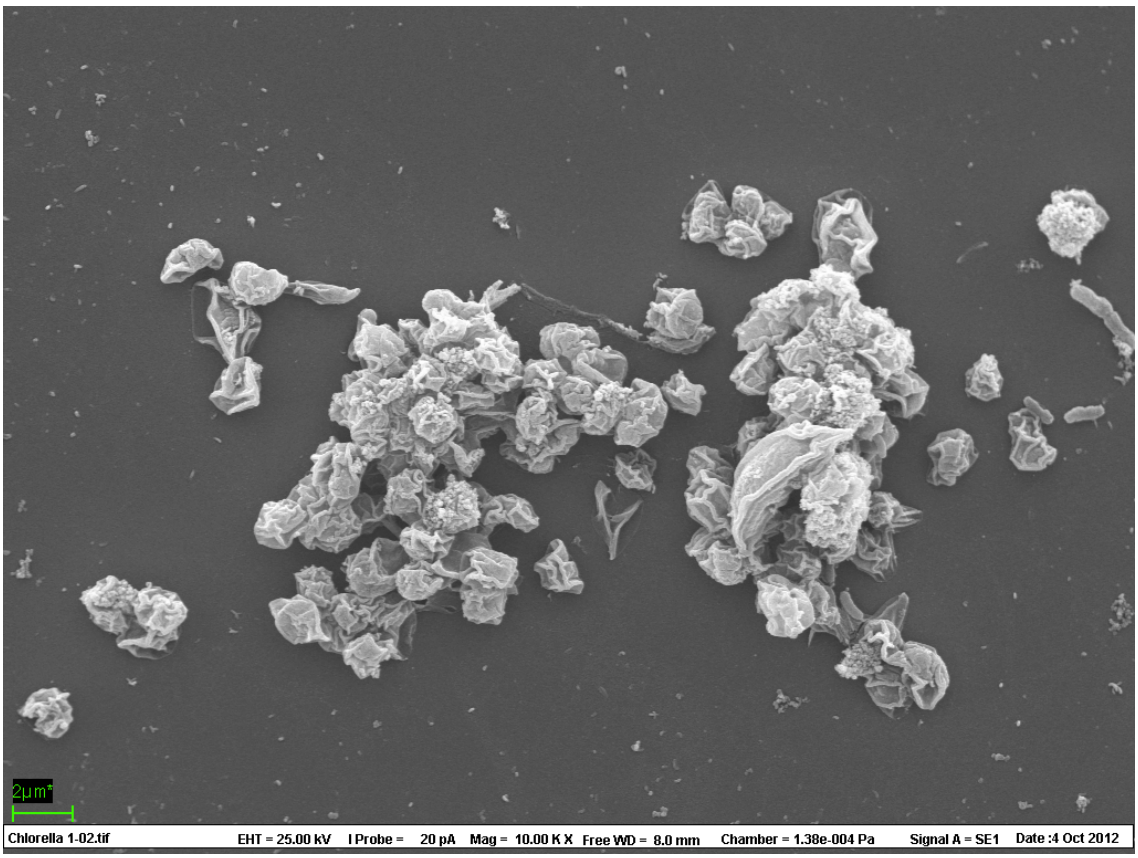


Figure 4 *Chlorella vulgaris* inoculum 180612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

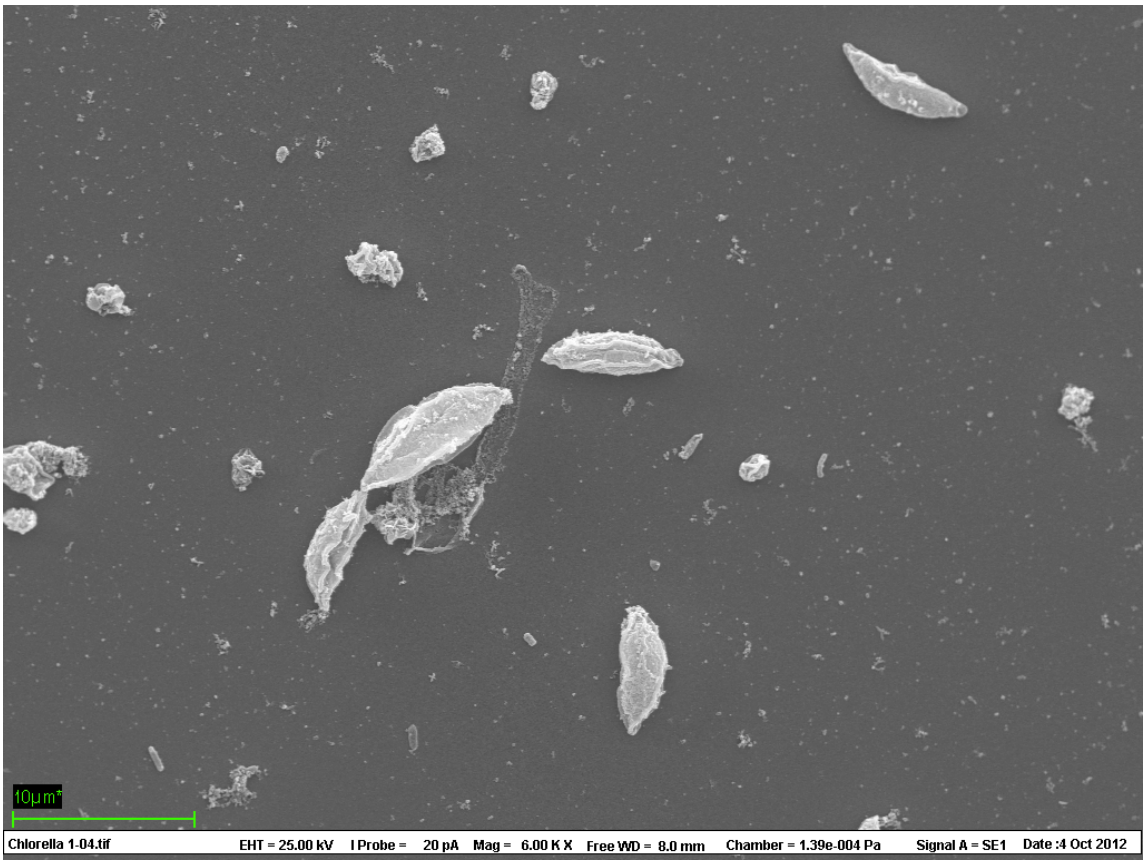


Figure 5 *Chlorella vulgaris* inoculum 180612 in tap water. Cryopreserved at 4 C. SEM 041012. Photo: Elin Ørmen

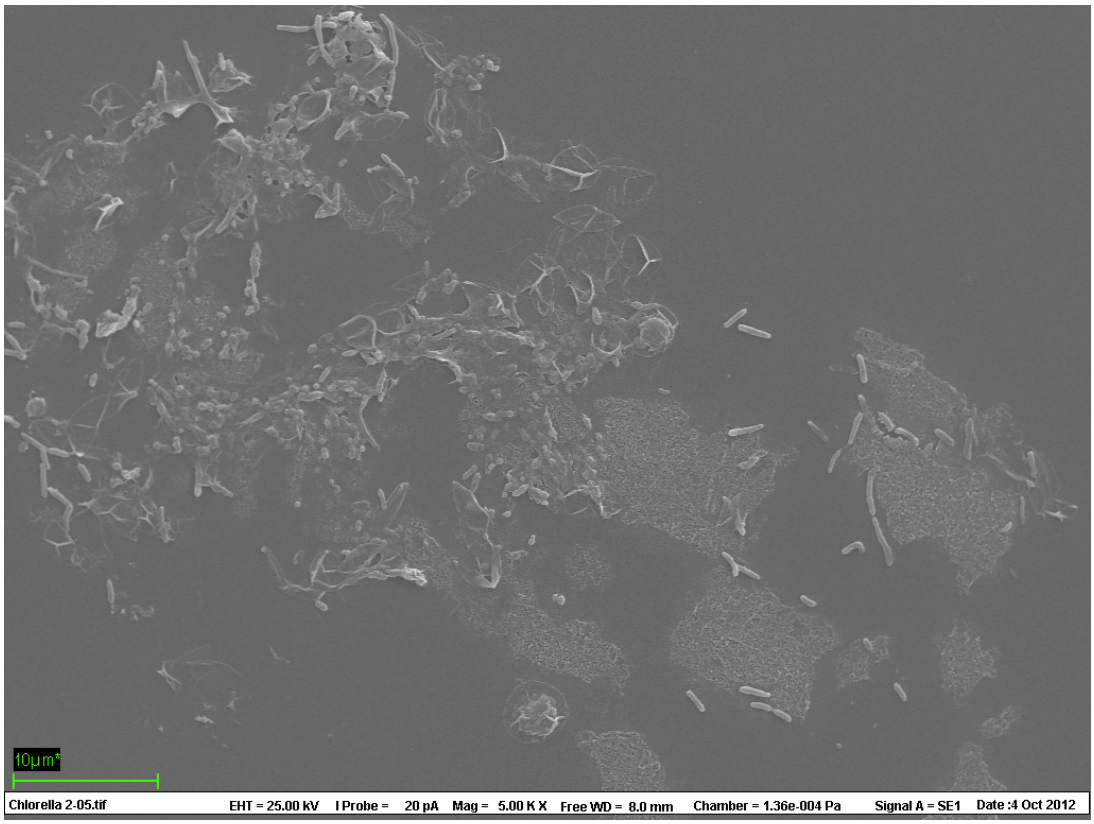


Figure 6 *Chlorella vulgaris* inoculum 180612 in tap water. Cryopreserved at 4 C. SEM 041012. Photo: Elin Ørmen

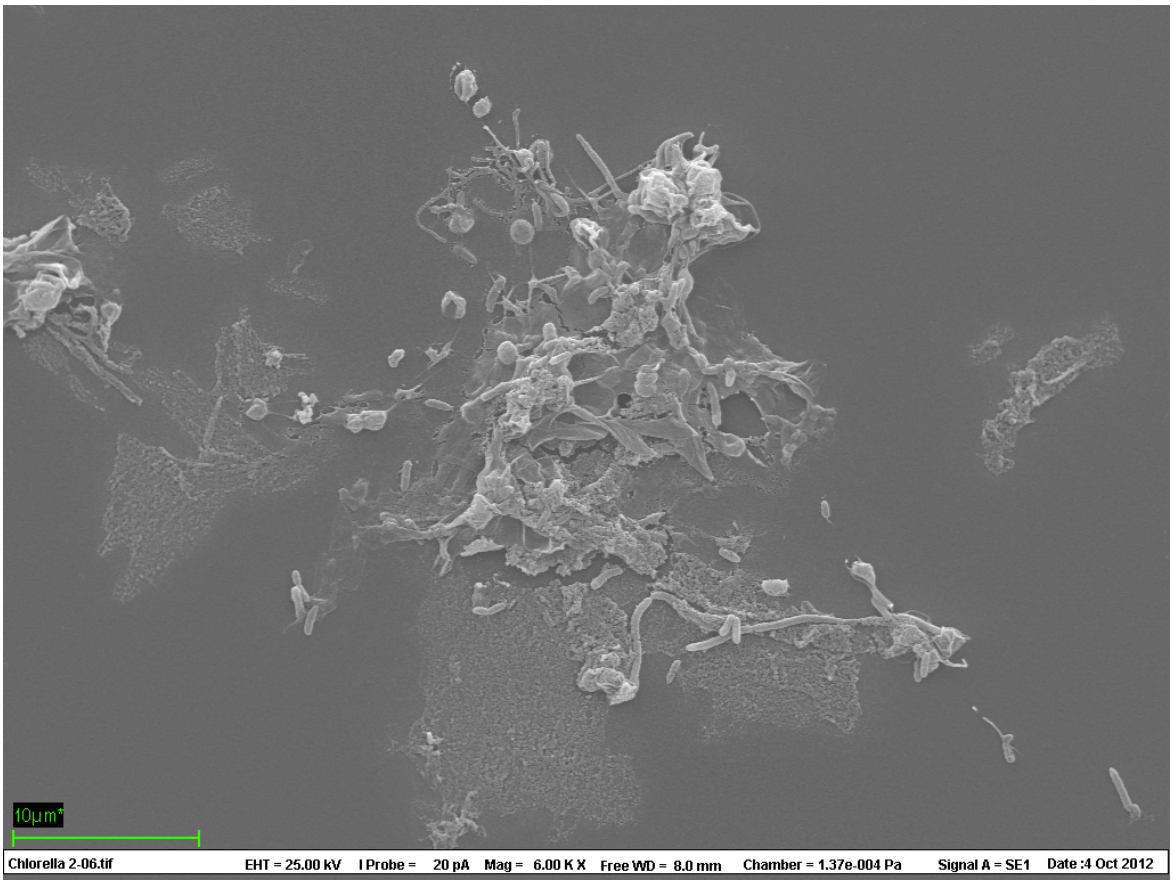


Figure 7 *Chlorella vulgaris* inoculum 180612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

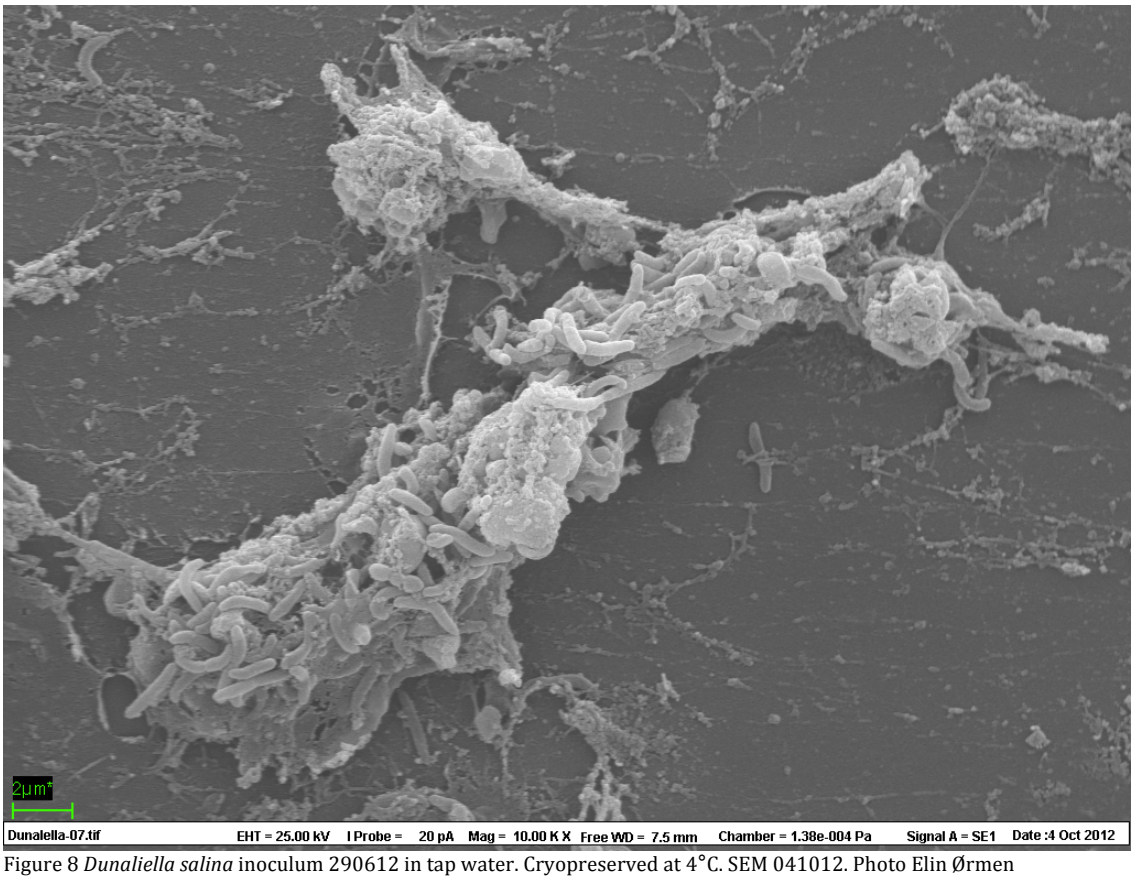


Figure 8 *Dunaliella salina* inoculum 290612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

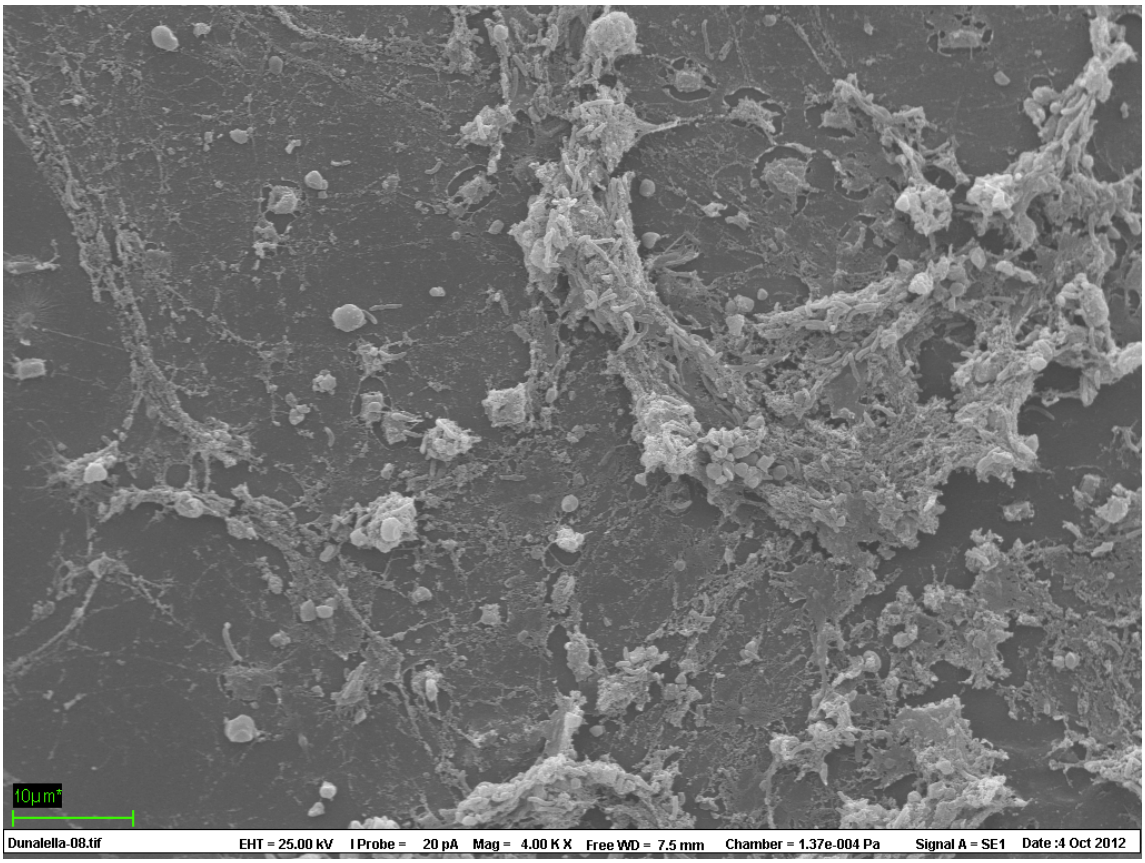


Figure 9 *Dunaliella salina* inoculum 290612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

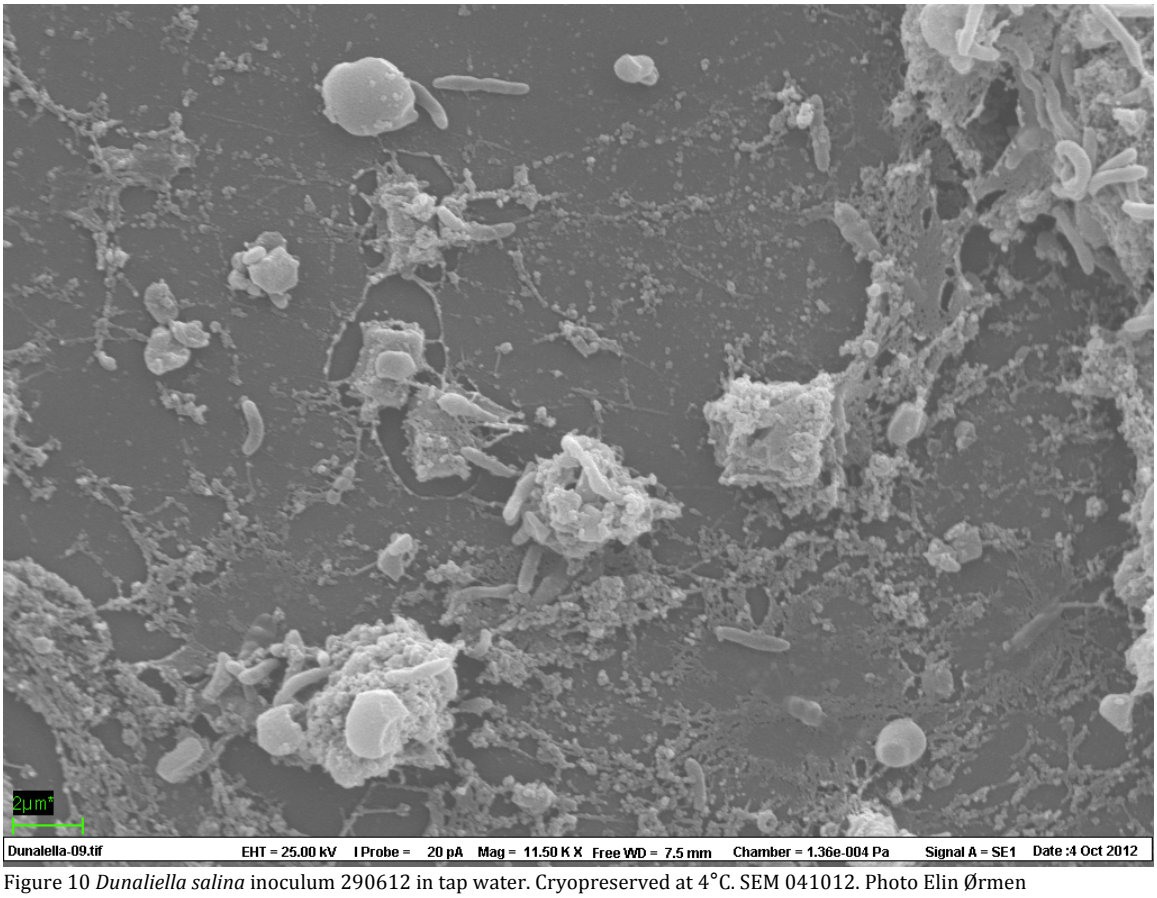


Figure 10 *Dunaliella salina* inoculum 290612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

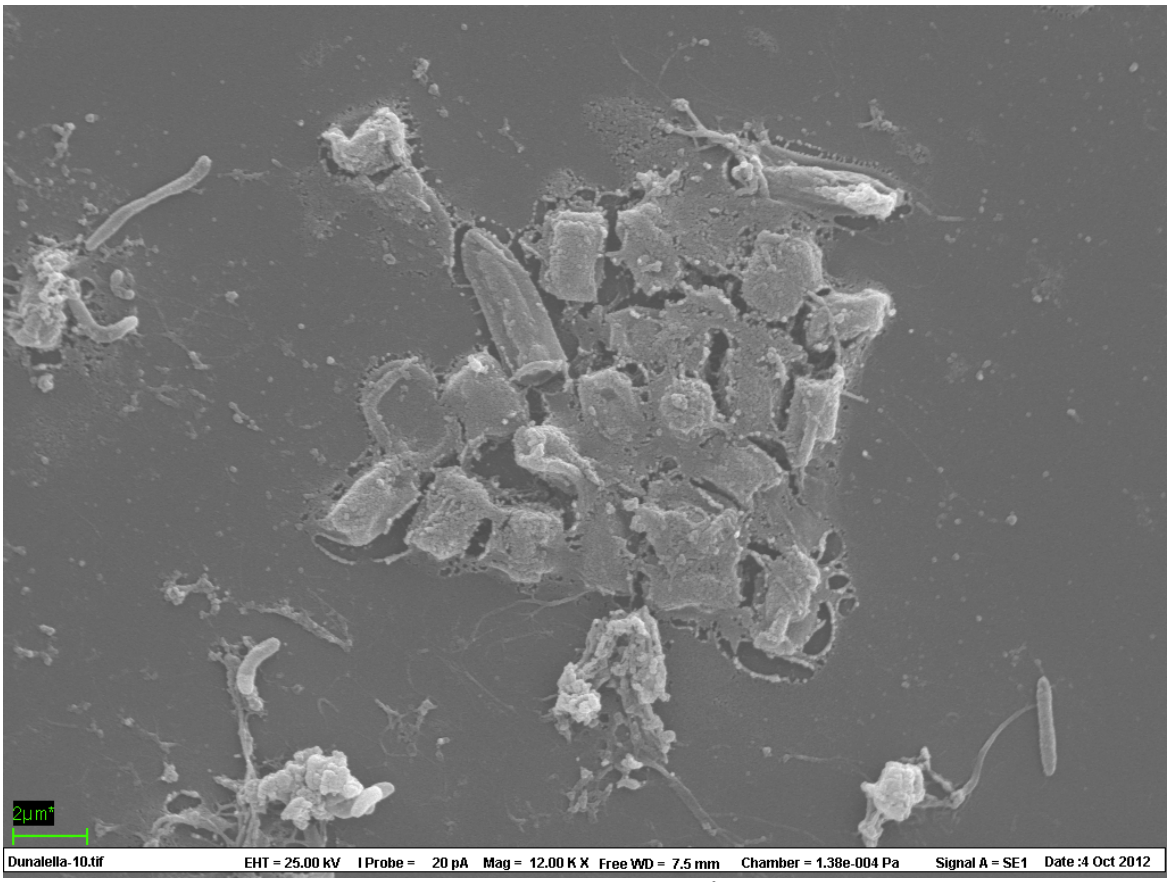


Figure 11 *Dunaliella salina* inoculum 290612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

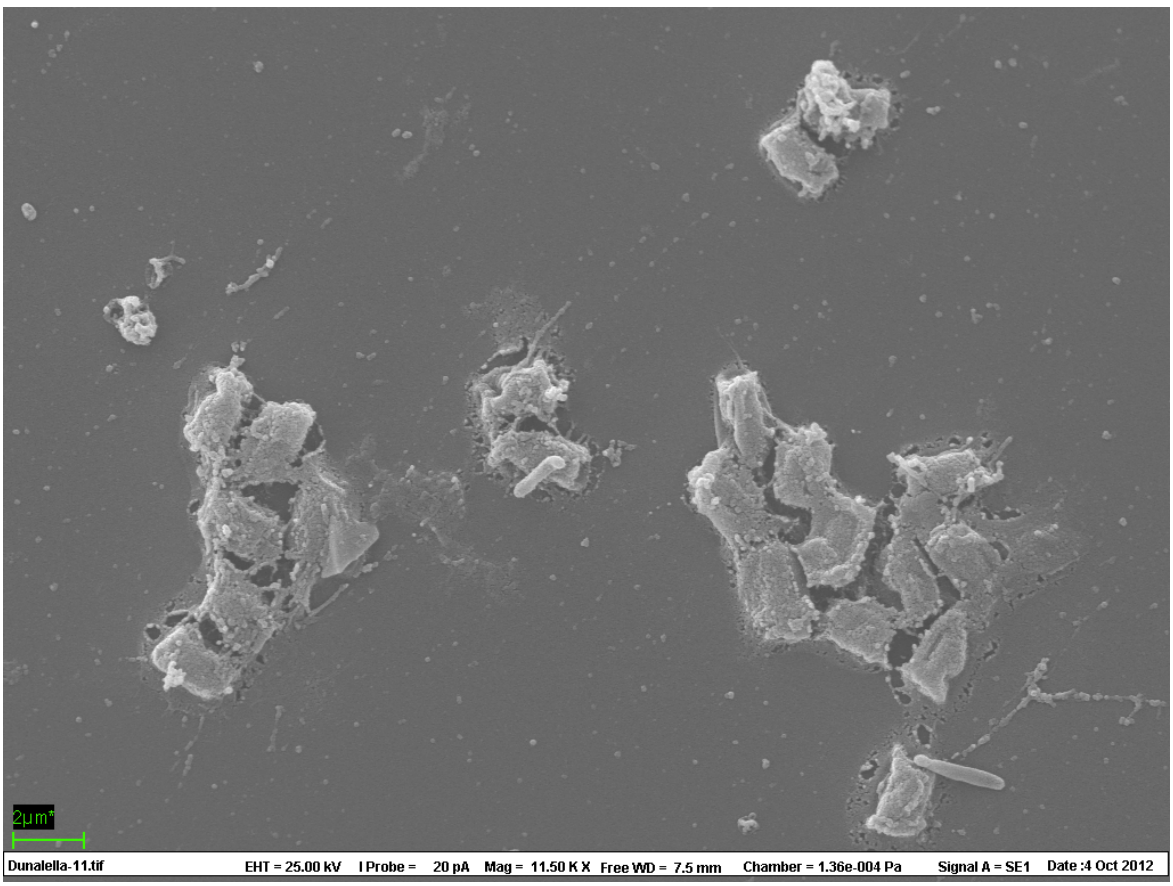


Figure 12 *Dunaliella salina* inoculum 290612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo: Elin Ørmen

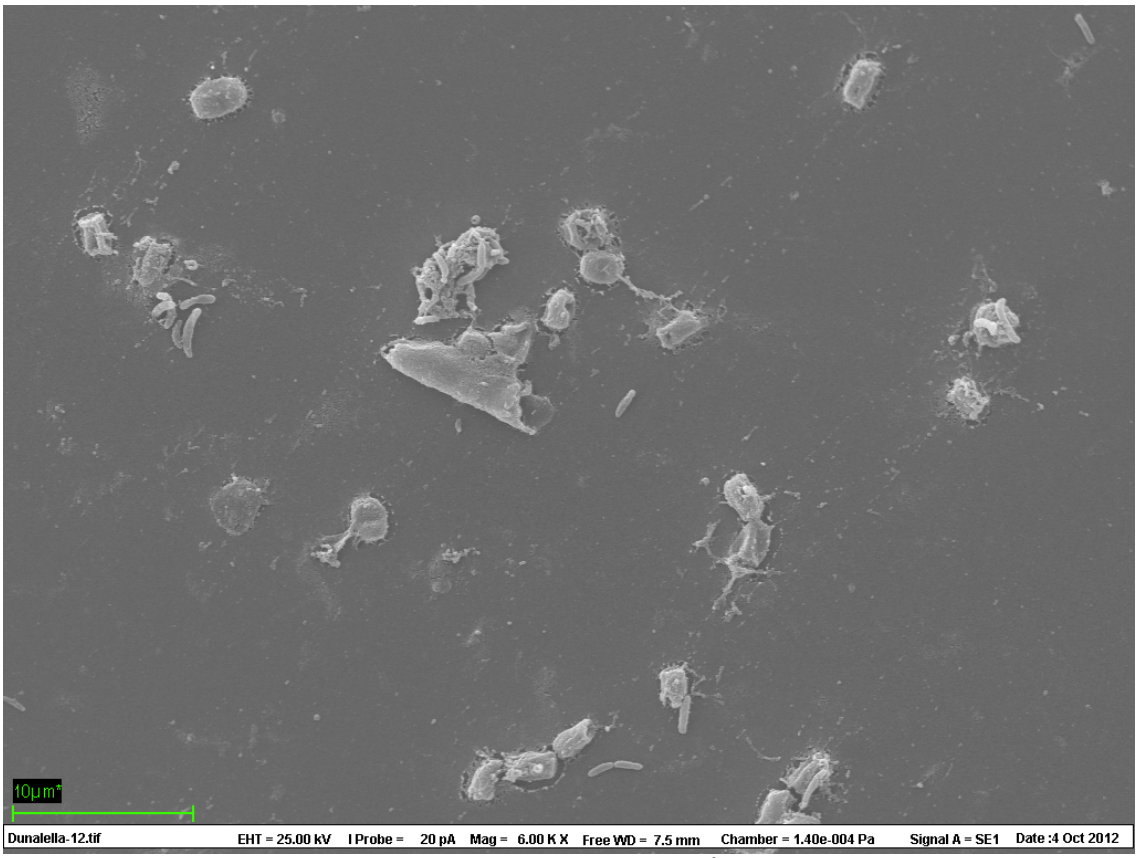


Figure 13 *Dunaliella salina* inoculum 290612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

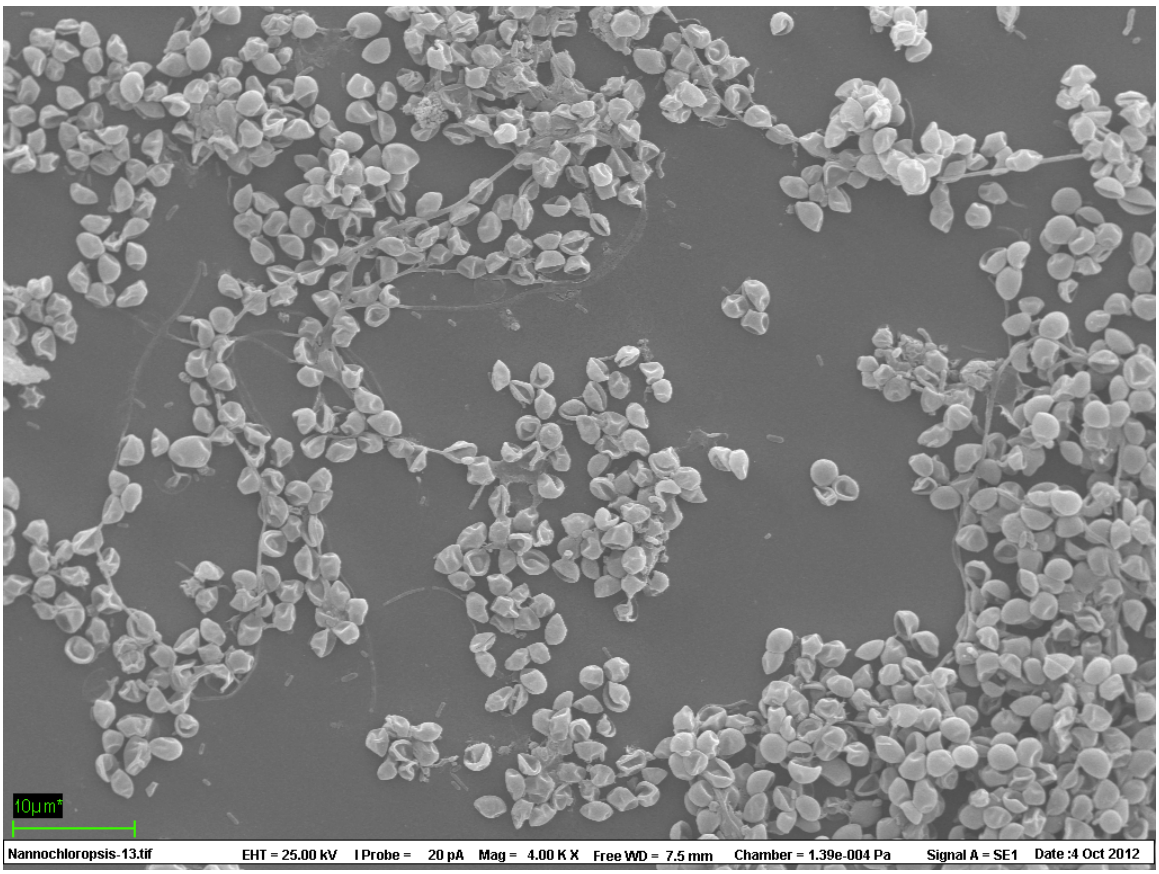


Figure 14 *Nannochloropsis oculata* inoculum 290612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

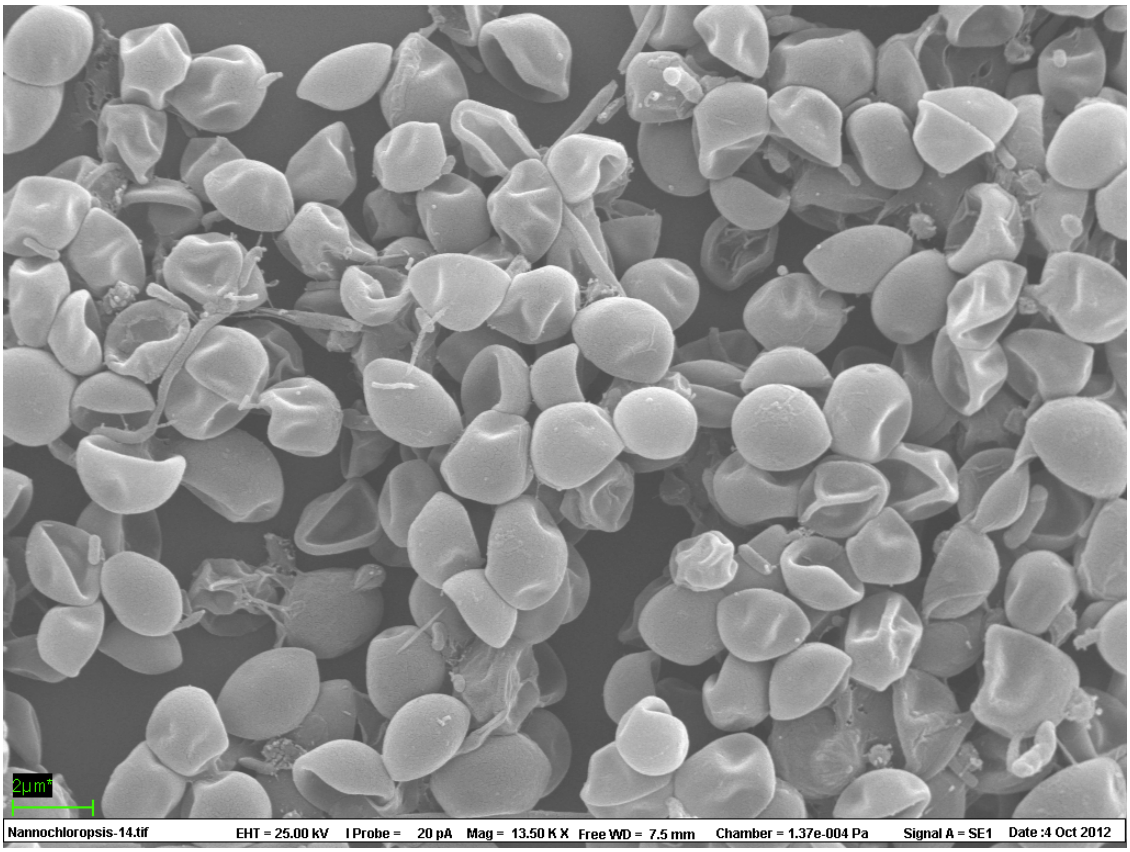


Figure 15 *Nannochloropsis oculata* inoculum in tap water 290612. Cryopreserved at 4°C. SEM 041012. Photo: Elin Ørmen

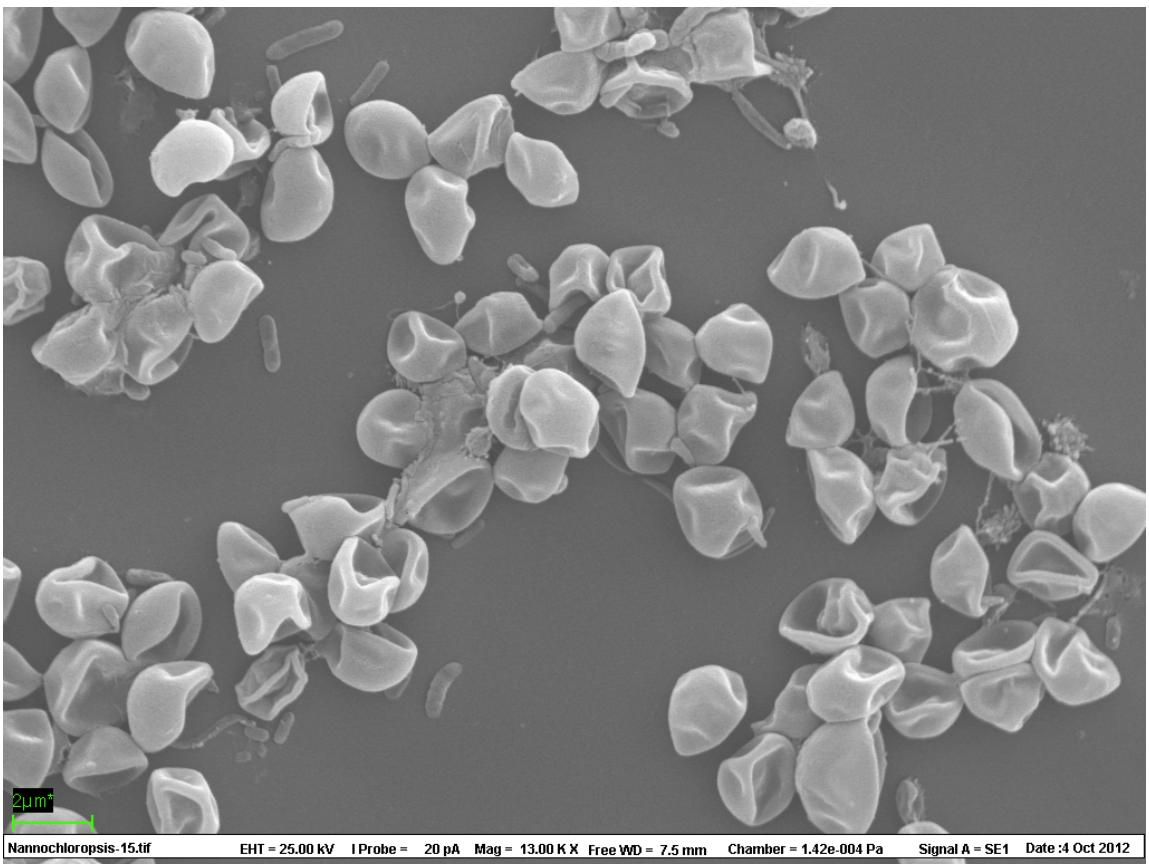


Figure 16 *Nannochloropsis oculata* inoculum 290612 in tap water. Cryopreserved at 4°C. SEM 041012. Photo: Elin Ørmen

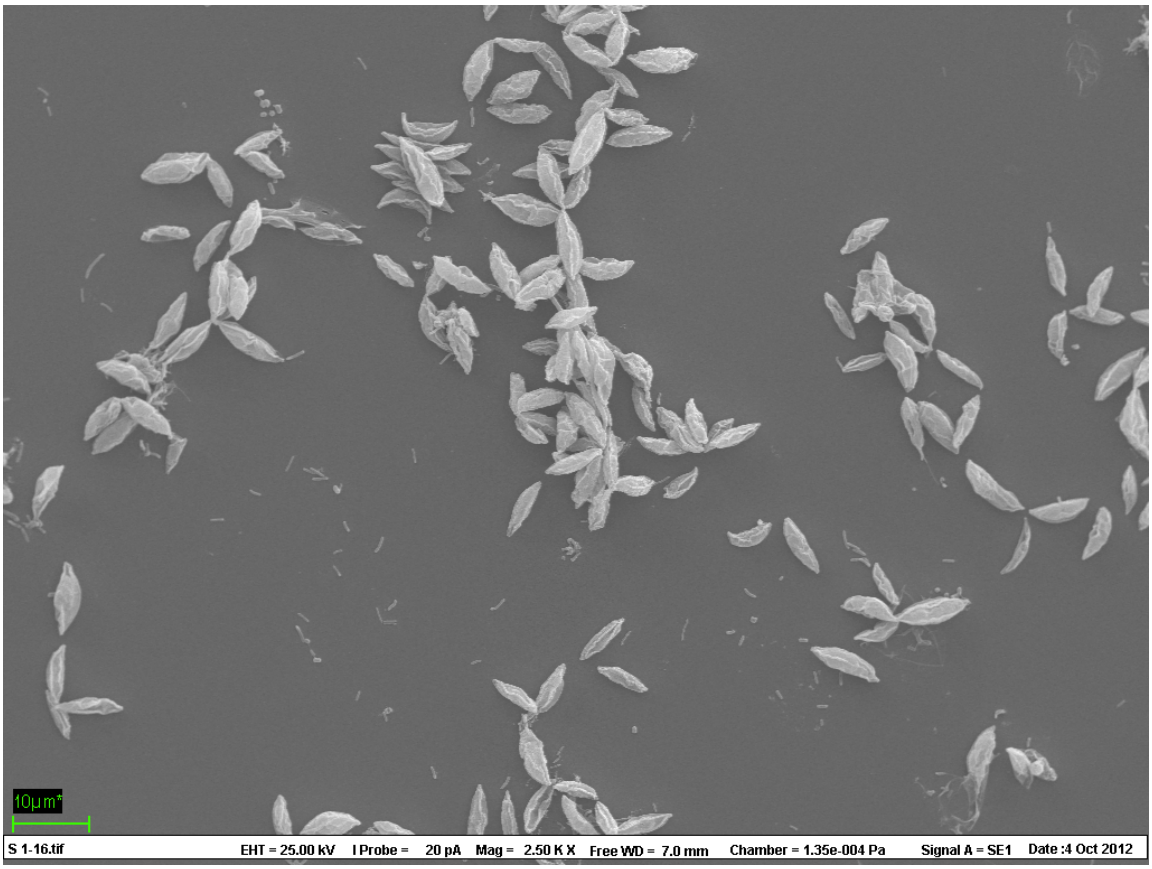


Figure 17 *Scenedesmus sp.* inoculum 060712 in tap water. Cryopreserved at 4°C. SEM 041012. Photo: Elin Ørmen

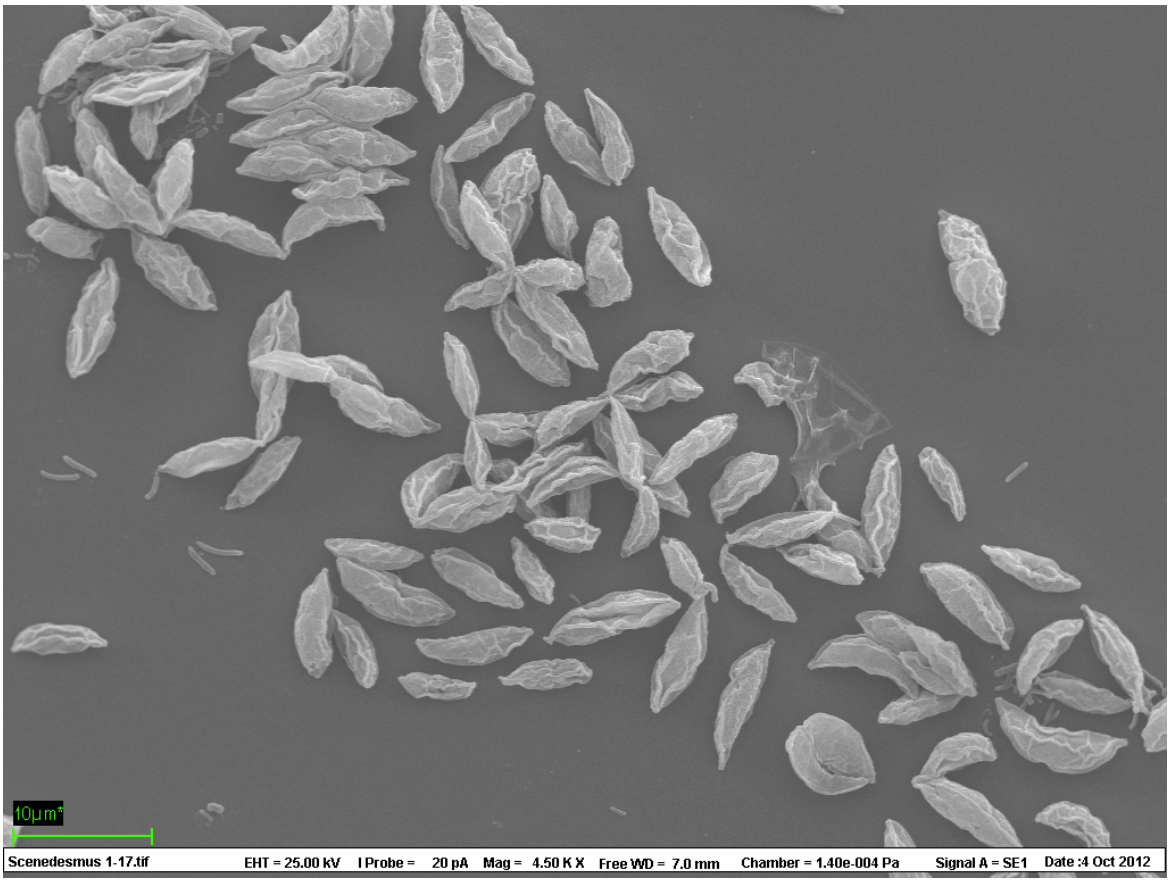


Figure 18 *Scenedesmus sp.* inoculum 060712 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

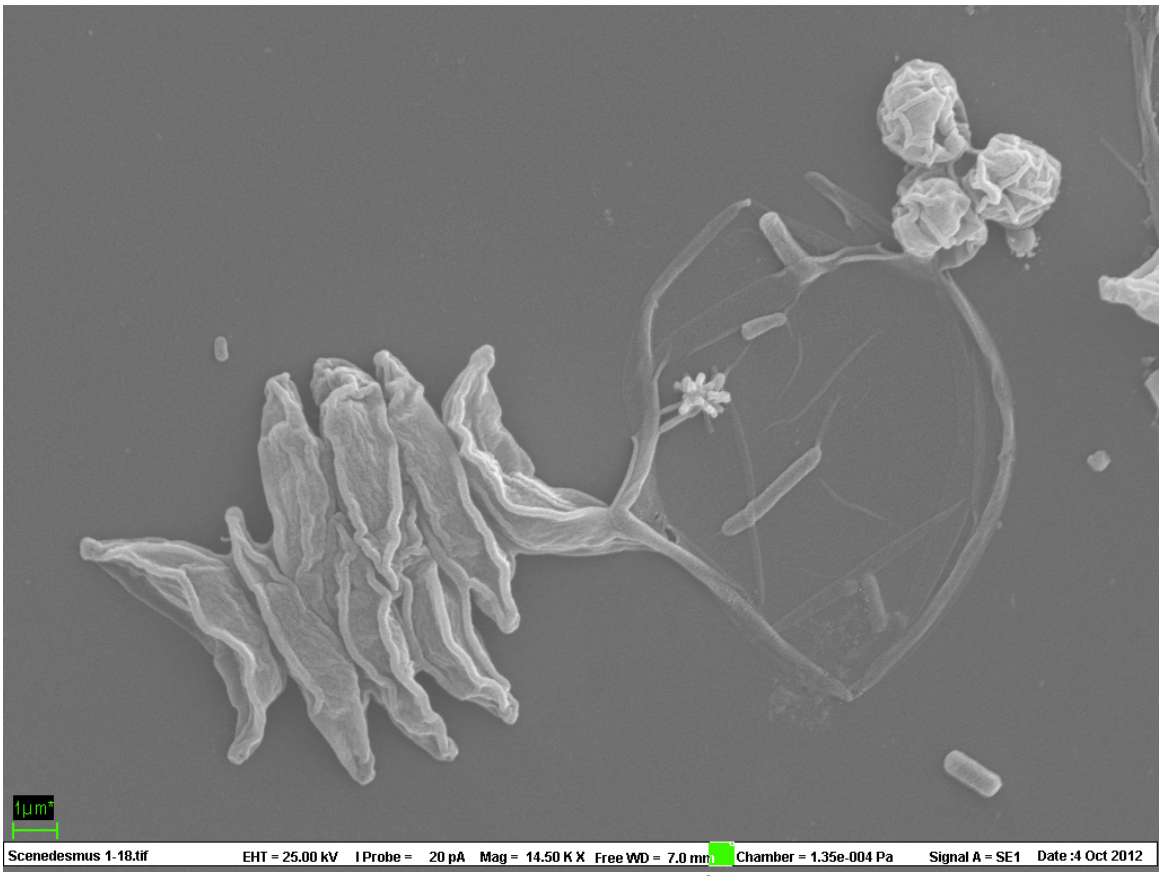


Figure 19 *Scenedesmus sp.* inoculum 060712 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

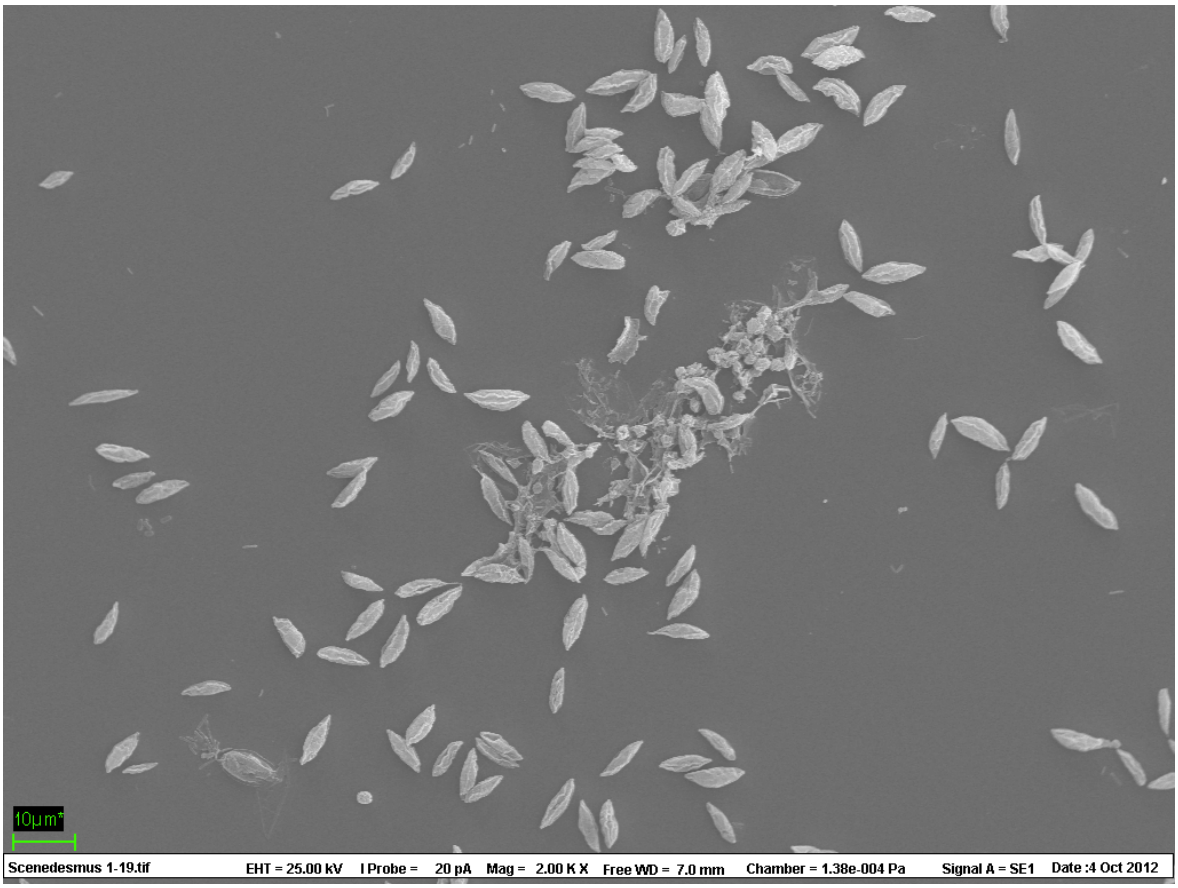


Figure 20 *Scenedesmus sp.* inoculum 060712 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

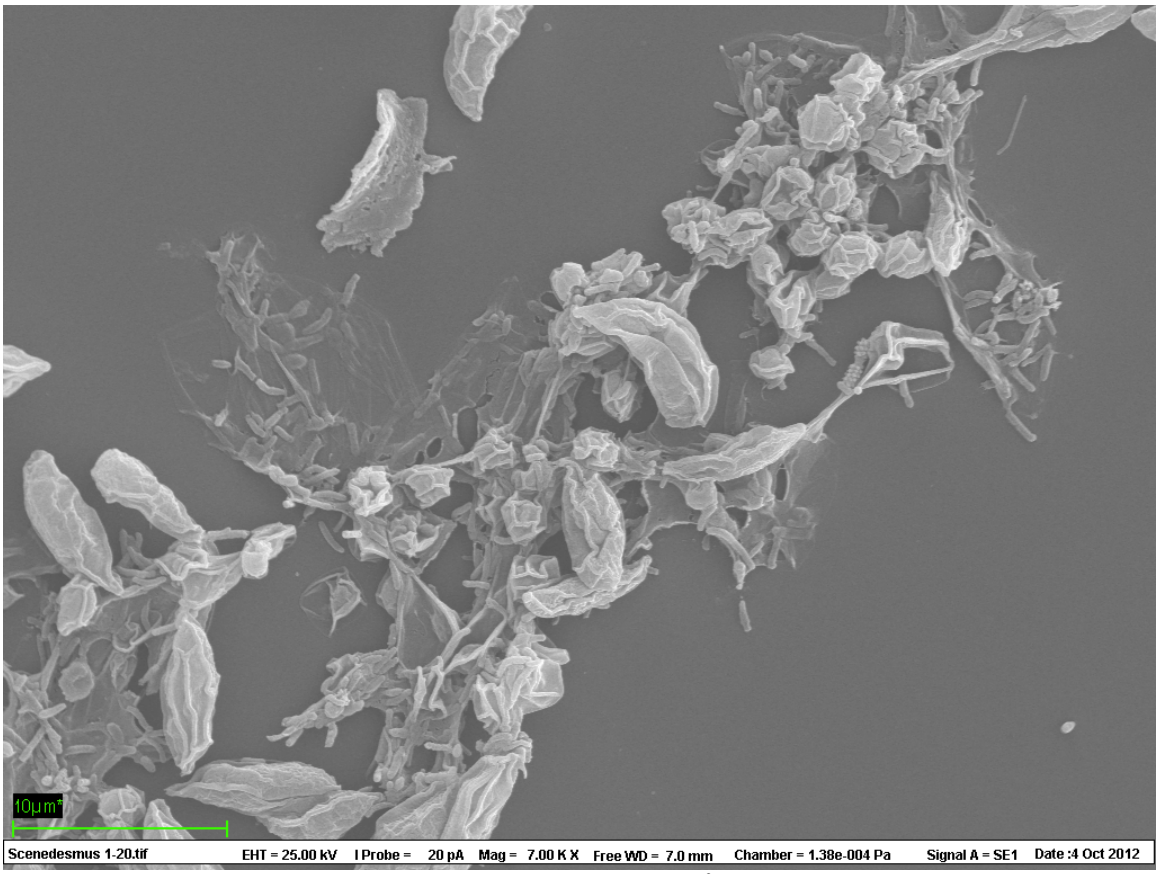


Figure 21 *Scenedesmus sp.* inoculum 060712 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

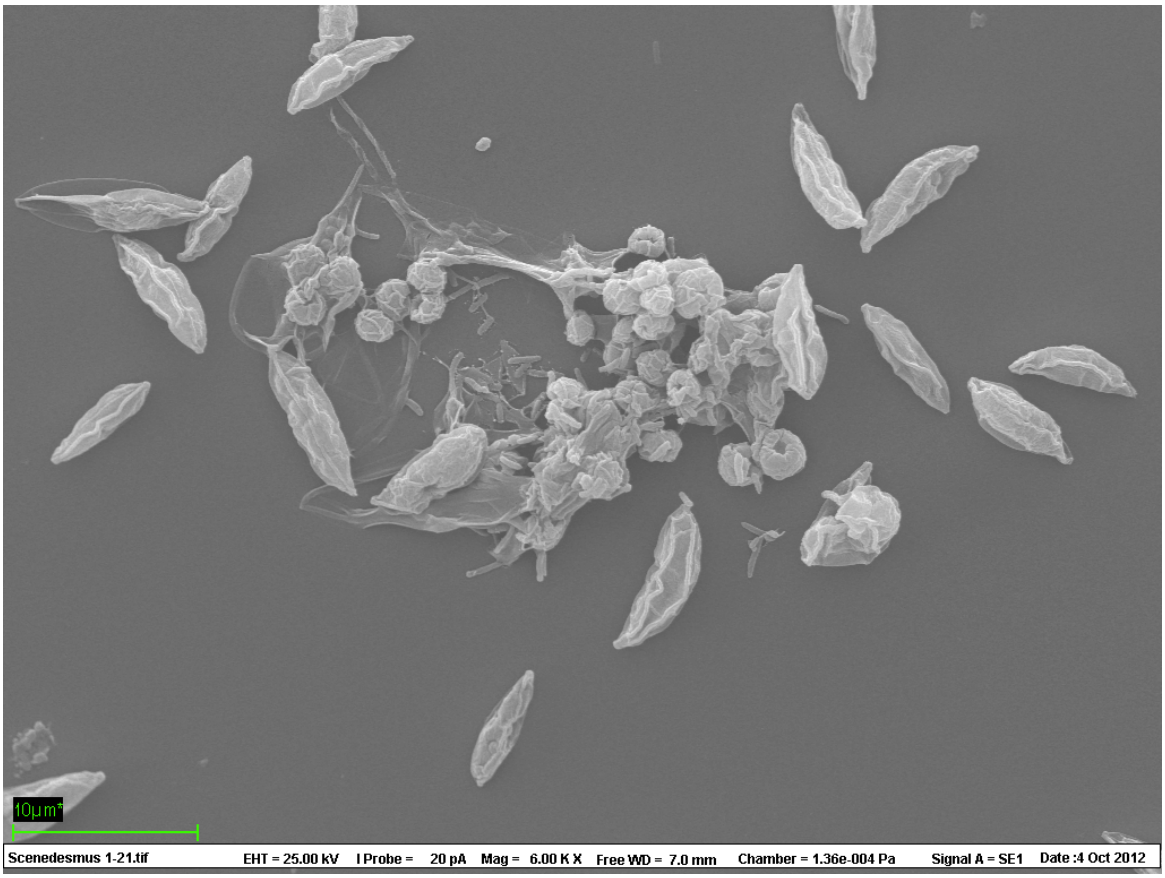


Figure 22 *Scenedesmus sp.* inoculum 060712 in tap water. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

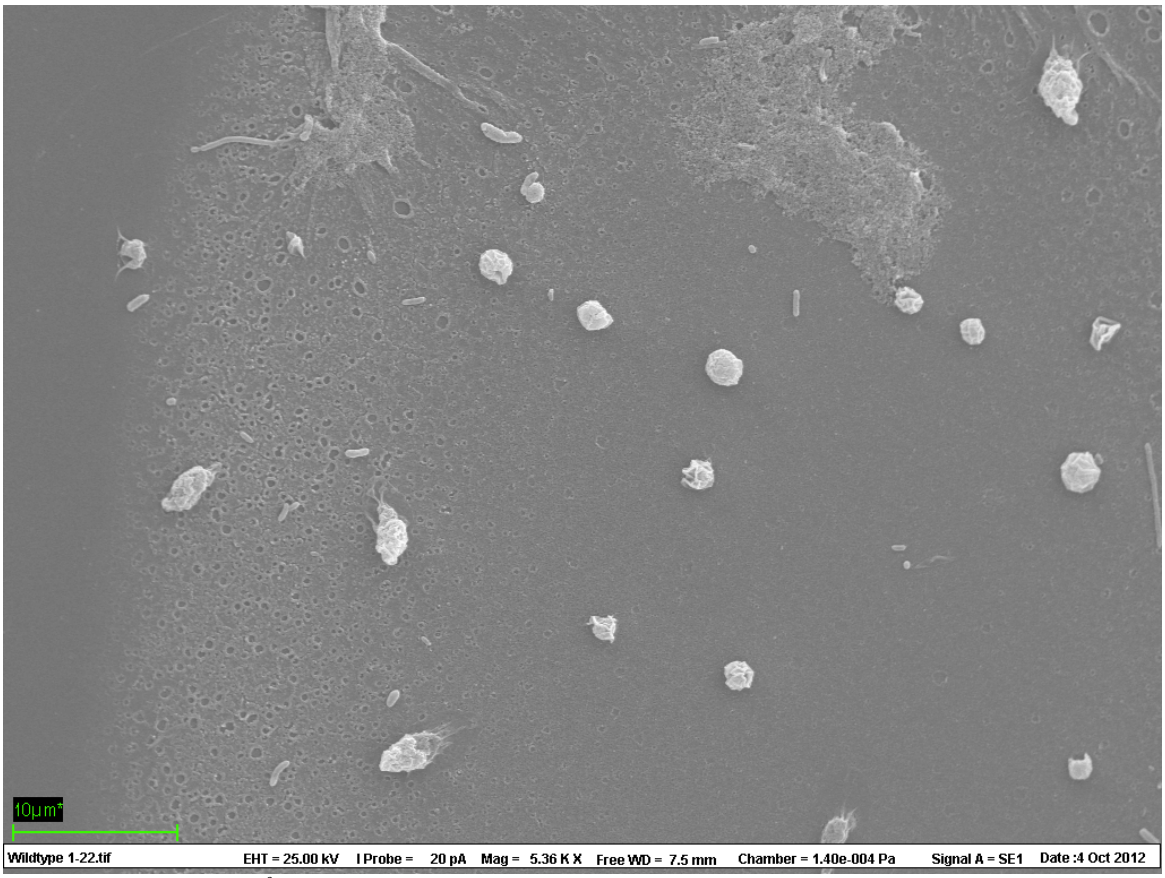


Figure 23 *Wild type sp. Lake Årungen* inoculum 060712. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

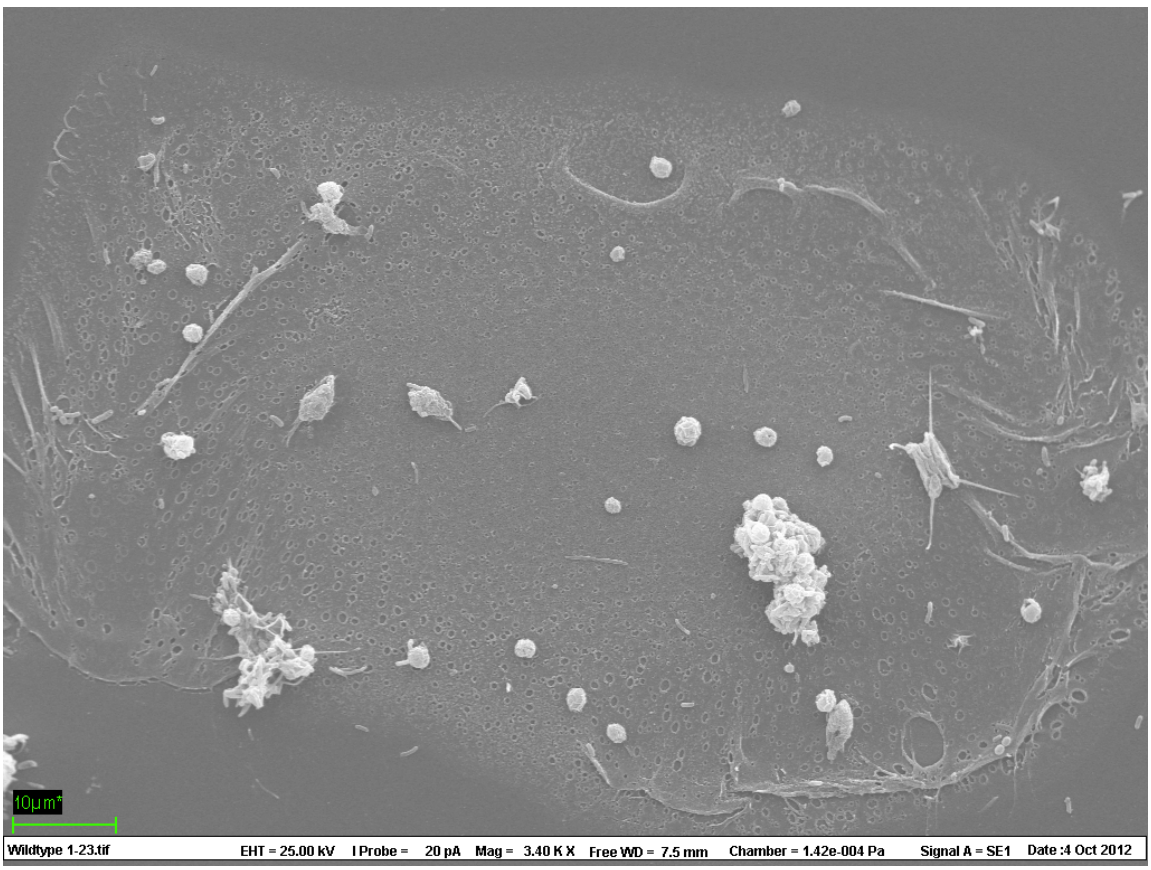


Figure 24 *Wild type sp. Lake Årungen* inoculum 060712. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

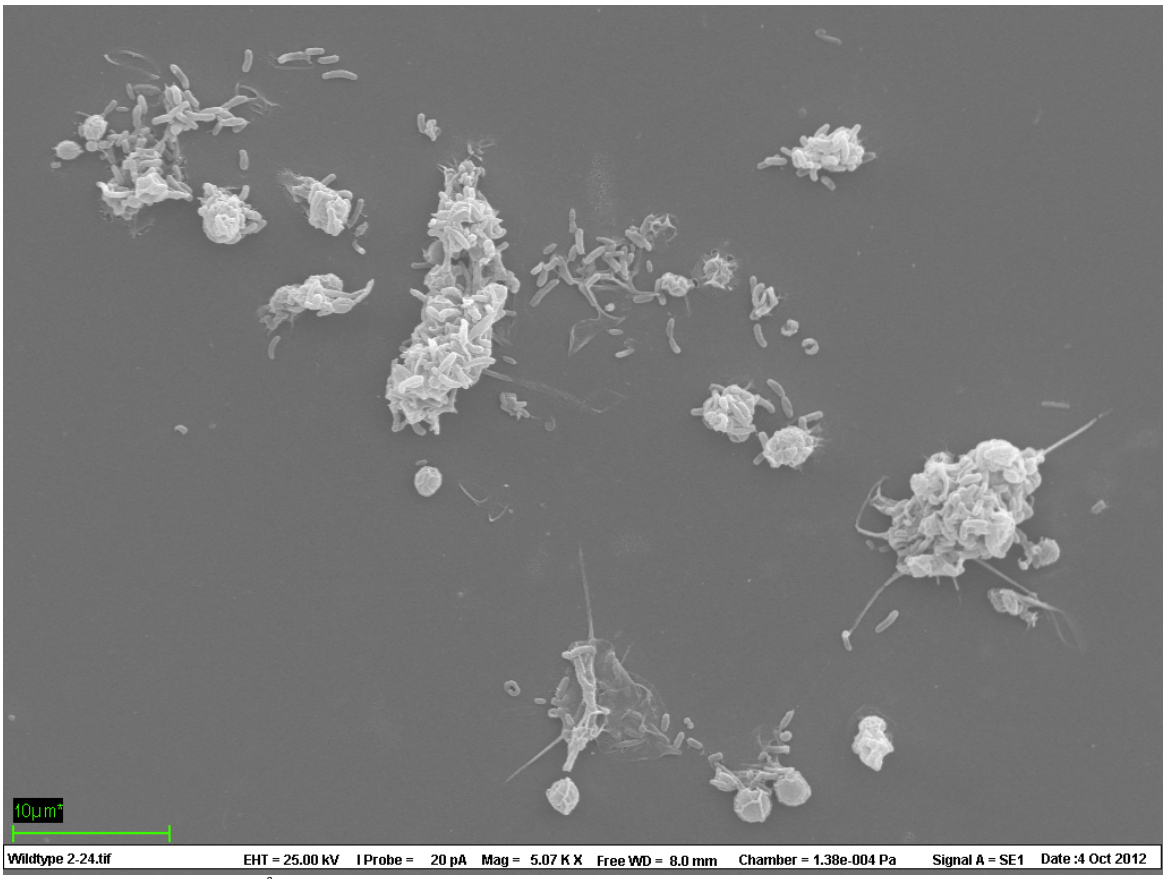


Figure 25 *Wild type sp. Lake Årungen* inoculum 060712. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

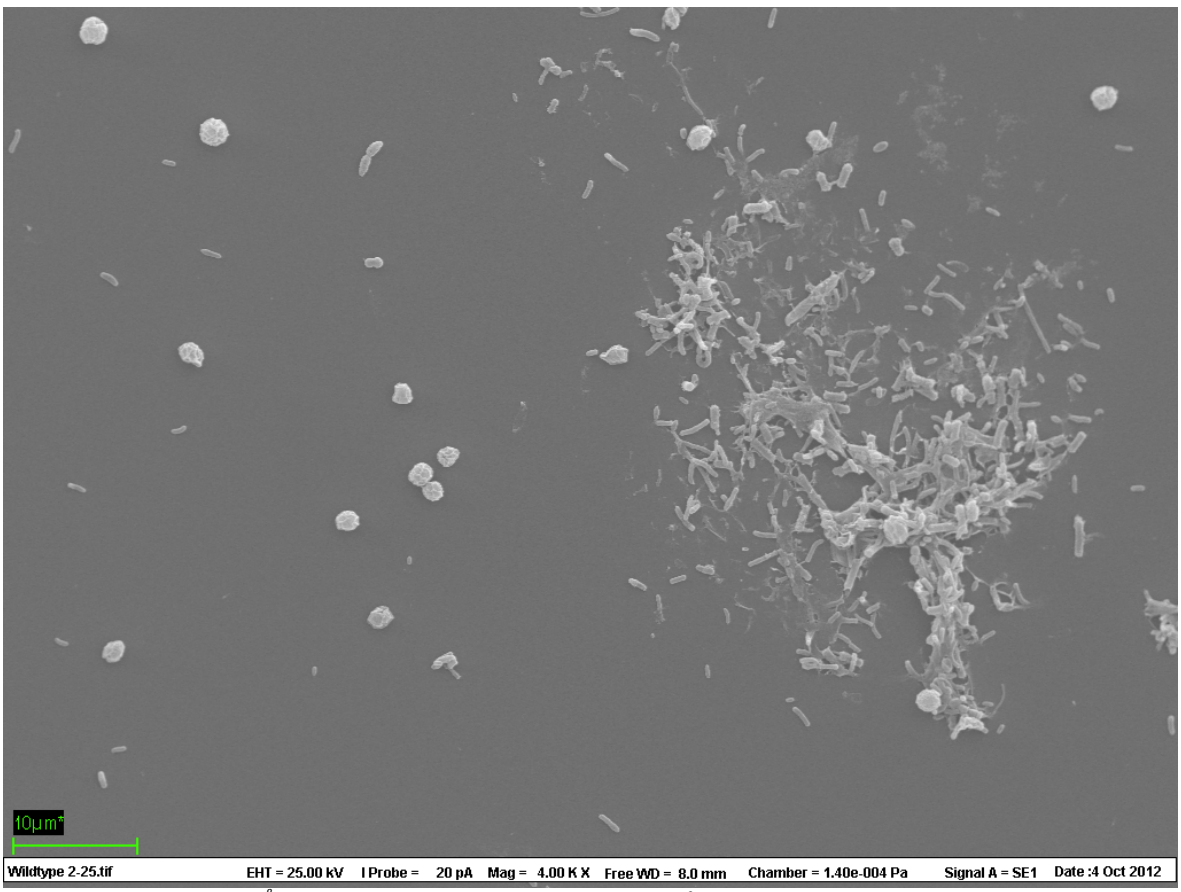


Figure 26 *Wild type sp. Lake Årungen* inoculum 060712. Cryopreserved at 4°C. SEM 041012. Photo Elin Ørmen

References

Paerl, H. W. and Shimp, S. L. (1973), Preparation of filtered plankton and detritus for study with scanning electron microscopy, Limnology and oceanography, **18**: 802-805.

Appendix 1
Critical Point Dryer

Type:
BAL-TEC CPD 030

Producer:
BAL-TEC AG
Neugruet 7
FL-9496 Balzers
Germany
www.bal-tec.com

Application:
Critical point drying is an efficient method of drying delicate samples without damaging its structure through surface tension that occurs when changing from the liquid to the gaseous phase.

Product specification:
- Critical point drying for biological specimens
- Low CO2 consumption
- Automatic cooling- / heating- system

Sputter Coater

Type:
Polaron SC 7640

Producer:
Quorum Technologies Ltd
2 Acorn House, The Broyle
Ringmer, East Sussex, BN8 5NN
UK
www.quorumtech.com

Application:

Product specification:
The SC7640 is an ideal all-round SEM sputter coater. Operation is switchable between automatic and manual. Automatic operation saves operator time and allows inexperienced sputter coater users to prepare samples. A unique "cool" sputtering head with annular target means that fine grain coatings are routinely achieved. The large target diameter (82mm) ensures that coatings have an even thickness.

The SC7640 is supplied with a gold/palladium sputtering target. A routine cycle time for sputter coating SEM samples with a typical conductive coating (5 - 7 nm) of gold or gold / palladium will be less than three minutes.

Scanning Electron Microscope (SEM)

Type:
Zeiss EVO - 50 - EP

Producer:
Carl Zeiss SMT Ltd
511 Coldhams Lane
Cambridge CB1 3JS
UK
www.smt.zeiss.com/nts

Application:

SEM provides a flexible and productive tool for surface imaging at both high and low resolution. Our SEM is a so called Extended Pressure (EP) instrument where the specimen environment can be controlled. This allows us to visualize native "live" specimens (no specimen preparation). Another aspect is that the specimen environment can be controlled so that liquid water can be condensed onto materials.

Detectors for Backscattered Electron Imaging (BEI) and X-ray analysis make it possible to detect and localize differences in element composition. It is ideally suited to study deposits of inorganic material such as CaOx and Silicates.

Product specification:

The EVO-50 EP is a versatile, research grade extended pressure scanning electron microscope benefiting from a large, multi-ported chamber, state-of-the-art objective lens, and an all axes motorised stage. This combination enables high resolution navigation and imaging with minimal beam scattering of larger specimens all at a class leading X-ray analytical working distance. The standard oil-free turbo molecular pump ensures high specimen throughput and contamination free imaging and analysis.

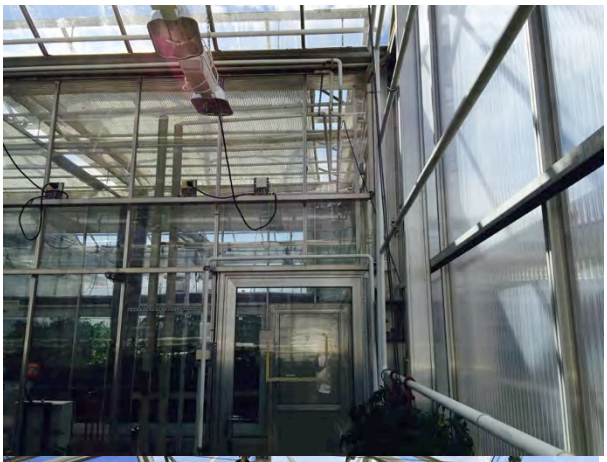
The EVO 50 series provides quality results from a versatile analytical microscope with a large specimen chamber. Whether the specimen requires imaging, at High Vacuum, XVP or EP, the EVO 50 series is able to image to perfection. New scanning modes, higher signal detection and image capture techniques provide the most informative images.

The new XVP operation mode even provides for the introduction of water vapour to prevent dehydration damage in life science and pharmaceutical applications.

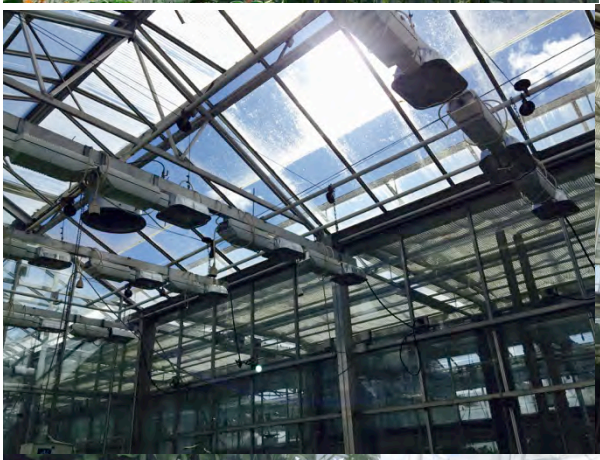
Specification	**Values**
Resolution	3.0 nm at 30 kV (W) 4.5 nm at 30 kV (BSD – EP mode)
Acceleration Voltage	0.2 – 30 kV
Probe current	0.5 pA – 5 µA
Magnification	5 – 1 000 000 x
XVP Pressure range EP Pressure range	1 – 750 Pa with air or water (optional) 1 – 3000 Pa with air or water (optional)
Available detectors	Everhart-Thornley Secondary Electron Detector VPSE (Variable Pressure Secondary Electron) Detector 4Q-BSD (4 Quadrant Backscattered Detector)
Chamber	365 mm (Ø) x 255 mm (h)

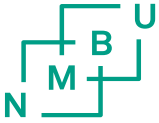
18 Appendix 9. SKP greenhouse room destined for upscaling experiments of semicontinuous microalgae cultivation system TPBR (14 photos – 080515-15:00)

14 different photos taken 090515 at 15:00 showing the different elements of the roof walls and floor of the greenhouse construction impeded the fluctuation and delivery of light irradiation to the algae suspension. This unusual effect affected the light cycle and the photosynthesis performance of microalgae cells growing up in TPBR semicontinuous cultivation system. This effect of disturbance or light dilution was analyzed in detail in the diagrams of the appendix 3 (tables 15-44 and figs. 35-64)







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