The effect of geographical orientation on the efficiency of microalgae production.

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Abstract

This report describes the work performed during an internship at a project to clean drain-water in the Mexican greenhouse industry using microalgae. For this project a pilot plant facility was created, all systems were connected and trouble shooting was performed on the three LGem MK2-750 tubular photo-bioreactors. All systems were found to be functional and the first experiments performed in the systems are described here. This report describes the initial problems faced when starting the pilot plant facility and the results of the first experiments performed.

For the design of a tubular photo bioreactor pilot plant facility the geographical orientation of the systems is of great importance as this will influence the light input to the systems during the day and will therefore give a change in productivity and efficiency. Here two geographical orientations are compared and tested. One systems placed on the North-South line and one system oriented in the East-West direction. The systems are analysed for biomass productivity, efficiency of cooling water, photosynthetic efficiency and nutrient consumption. From the results it was found that the North-South oriented systems had a higher average biomass production (0.46 vs 0.39 g/l/day) while using less cooling water per unit of biomass produced (1.42 vs 1.54 m^3/kg). The photosynthetic efficiency was found to be higher in the North-South oriented reactor as well. Although all systems are operational the pilot plant facility will require more improvements and additional equipment to facilitate better data production and more efficient biomass production in future experiments.

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1 Introduction

Agropark located in Queretaro, Mexico is a large complex of greenhouses hosting a number of multi-nationals for the production of food crops mainly for the North-American market, using the Mexican sun. This large-scale production of food crops consumes a total of 4.0M m^3 water annually (Aqua-Terra Nova, 2015). Currently all of the water used in the Agropark is pumped from an aquifer that contains groundwater and is filled naturally with rainwater from the mountains. Due to the high usage of water in the Agropark the groundwater level in the aquifer drops by 0.7m annually as was investigated by Aqua-Terra Nova in a survey on the use of water in Agropark. Part of the created drain-water is currently disposed of without any treatment into cannels that are present at the Agropark. This water contaminates the surrounding surface water and eventually possibly the ground water. This, combined with the already present water scarcity in Mexico has become a point of concern for the Agropark as the current water management will cause an increase in further water scarcity. Working towards future plans of a more sustainable Agropark and the planned increase with the build of Agropark phase 2, the Agropark is currently looking for methods to secure their water reserves. Reusing and recycling of the current water and therefore reducing the uptake of water from the aquifer are possible options.

Algae have been shown to be capable of growing well on horticultural waste water and have shown to be able to reduce the amount of nutrients and other contaminations in the water by significant amounts (Hultberg, 2013), (Dong, 2014), (Posadas, 2014) (Dalrymple, 2013). Therefore the growth of algae is considered a possible method to be part of the plan to reduce the use of the water. Earlier experiments on the drain-water of Finka, one of the Agropark companies, have shown that the alga species *Chlorella vulgaris* is able to grow well on this drain-water. This was proven at lab scale under highly controlled conditions using the Algaemist photo bioreactor. Upscaling this experimental phase was done using an outdoors hanging bag systems. Growth of the algae species on the drain-water was proven in these systems on a slightly larger and less controlled scale. In this internship project the growth of algae on the drain water of Finka will be studied on pilot plant scale as a method to clean the water for full-scale algae production. The pilot scale reactors are placed in different geographical orientation to study the effect of this change on the systems.

This project will focus on the growth of algae in three pilot plant reactors that will be placed at the Agropark, Queretaro. Three LGem MK2-750 pilot plant vertical tubular photo-bioreactors are used in this study. Of these reactors two reactors are placed in the North-South direction and one is placed directed East-West. The geographical is an important choice of any vertical tubular photo-bioreactor system as the design of such reactors is typically a fence like structure, long and narrow. It is hypothesized that the choice of orientation will influence the productivity. The goal here is to study the effect of the geographical orientation on the efficiency of the reactors and compare the total biomass production of the systems versus the needed energy inputs in the form of cooling while maximizing the available sun-light energy for the reactors and comparing photosynthetic efficiencies.

The internship project will include the start-up of the newly placed pilot plant PBRs and trouble shoot all necessary systems for algae production. Building and troubleshooting the PBRs and making sure all systems, such as cooling and pH control, function as needed is the first challenge. After this phase the effect of the geographical orientation on the productivity and water usage will be studied. The parameter that is different between the systems will only be the geographical orientation, all other parameters such as pH, temperature and culture density will be set at pre-determined values to be able to completely compare the effect of the geographical orientation on the systems.

Key words: algae, geographical orientation, biomass productivity, photosynthetic efficiency, cooling efficiency, water usage, pilot plant scale, outdoor cultivation.

Goal of project

Starting, trouble-shooting and operating pilot-plant scale vertical tubular photo-bioreactors in the Mexican climate and; studying the effect of geographical orientation on the efficiency of the production of biomass while making use of the naturally available sunlight in Mexico in these systems.

2 Material and methods

Algae are cultivated using multiple systems of different scales. The smaller scale systems are necessary to create the correct volume at a high cell density for inoculation of the pilot plant photo-bioreactors (PBRs). The reactors are used for the actual experiments to reach the described goal of the project. The smaller scale systems are shortly described under chapter 2.1. The pilot plant PBRs and the required measurements to obtain the required data for the calculations are described under 2.2 and 2.3 respectively. The experimental design is described in detail under 2.4 whereas chapter 2.5 describes the calculations that are performed to analyze the data to be able to compare productivity

2.1 Upscaling

The upscaling of the biomass production for the inoculation is performed in two systems. A system with 1.0liter bottles is used and with the biomass produced in this system the second step is inoculated. The second step of upscaling consists of hanging plastic bags that are placed outdoors, this system is called the hanging bag system. Both systems are described shortly in the materials and methods followed by a more detailed description of the pilot plant photo bioreactors.

2.1.1 Bottle system

From a small culture that is grown in an Erlenmeyer flask in the lab larger volumes are inoculated. These larger volumes are grown in Photo-bioreactors that consist of a 1.0Liter glass laboratory bottle (Simax) with a diameter of 10cm. Aeration of the flasks is performed using aquarium equipment such as small air-pumps, valves, hoses and a bubbling stone.

Figure 1: Bottle system when only using the first light source.

The bottle PBRs are placed inside in the laboratory where the temperature is more or less constant at about 22°C. Artificial light is provided using 2 different light sources. The light is controlled by a timer set up to provide the algae with a 12/12 day/night cycle simulation. This is done to grow algae in conditions similar to the conditions applied in the reactors.
The first light used is a Sanelec LED-light model1362, 2100lumen and is used in for cultivating algae with low culture densities, this light is shown in Figure 1. When the culture reaches higher densities the second light sources is also connected to provide more light to the culture, this secondary light sources can be seen in Figure 2 and consists of a Neolux Model D15W/865 fluorescent light bulb producing 53lumen/W. The addition of the second light source will prevent light limitation in the bottles for the algae cultures when they reach higher densities. The cultures are grown on 0.1% Bayfolan®-medium to provide a clean, stable and sterile medium. This medium ensures a controlled and easy to maintain growth in the bottle system.

Figure 2: Bottle system using both light sources for

2.1.2 Hanging bag system

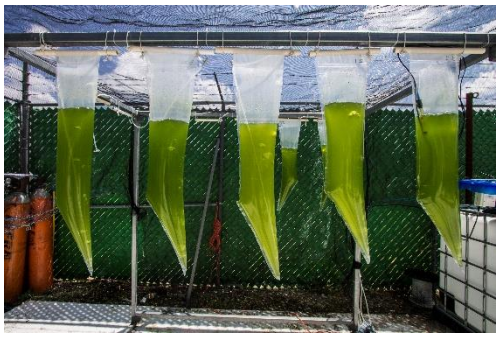

Figure 3: Hanging bag system, outdoors.

The PBR system created for the production of inoculation volumes for the reactors consists of hanging plastic bags with a diameter of the bags of approximately 14cm. Bags have a culture depth of about 60-75cm depending on the used diameter. The bag systems are always inoculated to contain a total of 5.0L of algae culture. Due to inconsistent manufacturing of the plastic, bag diameters fluctuates. Figure 3 shows the bag system placed outdoors and under shadow mesh on the hanging system. The different sizes and shapes of the bags can clearly be observed in this picture. The bags are placed outside in the sun reducing the need for artificial lights for this system. No pH or temperature control is applied to the system. Bags are placed under shadow-mesh reducing the total flux of light received by the bags with 50% in order to prevent overheating of the systems during the day. The system is provided with aeration using aquarium air hoses and valves and an industrial air pump. The complete system of the plastic bag and the part of the air hose into the plastic bag is fully constructed of disposable materials to reduce contamination risks and need for cleaning. Bags are typically inoculated using 0.5L of inoculum, produced using the bottle system. The total volume of a single bag is brought to 5.0L using the medium of choice. Concentration of a newly started bag is always kept at about 3-5e10^6 cells/ml. The standard medium used for algae growth in the bags is the drain-water obtained for the green-house companies at Agropark as this is also used in the reactors. Bags normally grow of 8-10days before being used to inoculate more bags or a reactor. During growth in the bags a culture is monitored on growth and purity by cell-counting and visual inspection using a Euromex Bioblue microscope.

2.2 Pilot plant photo-bioreactors

Pilot plant scale microalgae cultivation is performed in three photo-bioreactors that were purchased at LGem. The three MK2-750 systems contain a fully automated temperature and pH control regulated by the control panel and sensor that are part of the reactors.

The reactors are installed at Agropark, Queretaro, Mexico. Substrate for algae growth is provided by the green-house companies located at the Agropark.

CO_2 is provided to the systems using 25kg cylinders of CO_2 purchased at Infra, Queretaro.

Cooling of the systems is done using different types of systems. Three different systems are created to prevent the reactors from overheating during the day. These three systems are: Shadow mesh above the reactors, a dripping system and a sprinkler system.

The shadow mesh is built to prevent high light input to the culture especially at low culture densities after inoculation and to prevent heating of the systems. Shadow mesh at 50% solar radiation reduction is used in this system. Shadow mesh is placed 50cm above the reactors, the total size of these pieces of shadow mesh are 11.0x1.65m. These are placed above the reactor to protect the reactor from overheating during the moments of the day that the sun is directly above the reactors.

A dripping systems that provides cooling by applying cold water to the tubes is placed to function as the initial cooling system. This type of system typically uses high amounts of water for cooling. The dripping system consist of a perforated white-tube placed on top of the highest tube of the reactors. Tube diameter 20mm, perforation holes 0.5-1.0mm with a distance between dripping locations at 15cm. The water used for the cooling is water directly from the aquifer of Agropark. Cooling water has a typical temperature of 22-30degrees varying per day and time. Pressure on the cooling system is provided by the pressure on the

water line and cooling is controlled by a solenoid valve operated by the IKS aquastar control panel of the PBRs
The sprinkler system consists of Naandanjain SuperFogger X2 blue sprinkler heads to create water droplets of 55microns. The sprinkler system provides cooling by the evaporation of the spayed water. Sprinklers are placed 3.0 meters apart, 30cm above the reactors. This system is build based on recommendations of LGem, the supplier of the PBRs. Water for the sprinkler system is processed by a water softener and the water line is provided with at least 4bars of pressure using a Hygrophor. Due to problems with suppliers and obtaining all parts for this cooling system the system was not ready to be tested within the time period of the research in this internship.

The three reactors are placed in different geographical orientations in an effort to study the effect of these changes to the reactors operation parameters and differences in growth efficiency. The placement of the reactors can be seen in Figure 4.
Reactor number 1 is placed in an East-West orientation with the tank and control panel placed on the East side of the reactor. Reactors number 2 and 3 are placed in a North-South orientation with a lateral distance of 3.0meters between the reactors. The tank units of these PBRs are located on the north side of the reactor and the control panel is place west of the tank unit. Spacing between reactor number one and number 2-3 is 1.5meters. The floor under the reactors is painted in a bright white color to maximize light reflection to the lower tubes of the reactor as this is indicated to be beneficial to algae production in tubular PBR systems (Slegers P. M., 2012). Reactor #1 is referred to as the East-West oriented reactor as the length of the reactor is on the East-West line. Whereas reactor #2 and #3 are placed on the North-South line. Reactor #3 is therefore referred to as North-South. These orientation are chosen based on suggestions of R. Bosma and on the work of Slegers. The work of Slegers concludes that for single vertical plate PBRs a North-South orientation yields a higher biomass productivity whereas on longer altitudes the East-West orientation would be more productive (Slegers P. M., 2011).
The area between the reactors 2 and 3 is 3.5 meters; this reduces the possible canyonning effect on the reactors and increases productivity (Slegers P. M., 2012). The PBRs are placed in such a way that all reactors are completely independent and do not receive any shadow of the other PBRs or nearby objects. Due to limitations in the space available of the provided area for the reactors, the PBRs are not fully orientated in the described North-South and East-West directions as described above. The reactors deviate 40-degrees from their described orientations. Figure 4 shows a schematic lay-out of the reactors on the floor with a compass rose indicating the actual north.
The reactors are placed exactly level on the floor to function properly. To do so the N-S oriented reactors are placed on long screws on the South end of the reactors as the floor was not leveled accordingly. The medium used for growing the algae in the reactors is the green-house drain water in all experimental phases unless stated otherwise in the results section. The pH and temperature of the reactors are controlled by the systems, the pH and temperature set-points used differ per experiment and are mentioned per experiment. Read outs of reactor conditions, changing settings and monitoring of reactor conditions is performed using IKS AquasSoft software. Data is logged for each reactor every 5 minutes. All data is processed using MS-excel.

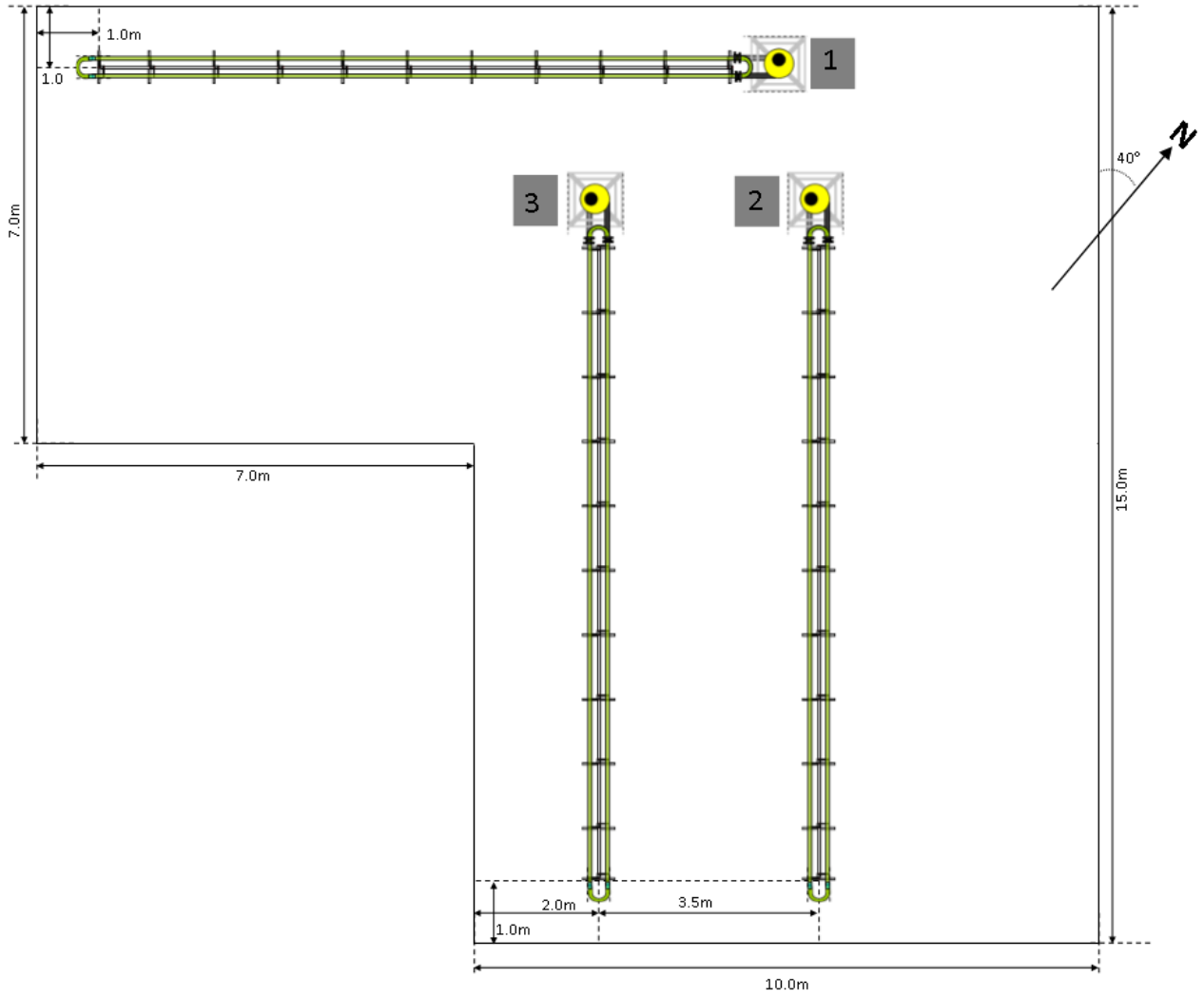

Figure 4: Representation of the pilot plant facility in Agropark, Queretaro, Mexico. Geographical orientation of the reactors is referred to as North-South (NS) for system #2 and #3 whereas reactors one is oriented East-West (EW).

2.3 Measurements

For all the performed experiments multiple parameters of the reactor are chosen as set-points and fixed parameters, such as cooling system, use of shadow flow rate setting for air circulation and CO_2 and all pump settings. Other parameters of the algae growth will be monitored using measurements to determine these parameters during the experiments. All these parameters can be categorized in: Biomass determination, Nutrient concentrations and reactor parameters.

2.3.1 Biomass determination

To determine the biomass concentration in the reactor multiple methods are used during this study. The three chosen methods for biomass determination are: optical density, dry-weight and cell-count.
Due time limitations no calibration curve could be created for the optical density to dry-weight correlation. The results of dry-weight and optical density are compared to each other to detect changes in the culture and to asses if optical density is a good method for the biomass determination in further

experiments. Literature has indicated that it is difficult to create a reliable calibration curve for algae species due to morphological changes as a result of cultivation conditions (Griffiths M. J., 2011).
Optical density is measured using a ThermoSpectronic Genesys 20 spectrophotometer. Measurements are performed 750nm as these wavelengths were found to be the best to determine the biomass concentration by (Griffiths M. J., 2011) (Griffiths M. J., 2014). Measurements are always kept within the 0.3-0.8 range of OD_{750} detection of the used spectrophotometer to ensure accurate measurements, to do so the sample is diluted accordingly.
To determine the dry-weight 5.0-30.0ml of a sample is filtered using filter Whatman™ GF/A microfiber filter and an Evar EV-40 vacuum pump. The volume of sample applied to the filter is adjusted according to the optical density measurement. Sample volumes are decreased with higher optical densities. The sample volume is always maintained at a volume that prevents complete blockage of the filters but allows for a significant different in weight of the filers. Filters are dried using a Felisa FE-292 oven on 80°C for 24hours before and after the sample is applied. Weight of the samples is determined using a Pioneer™ Ohaus PA224 balance which is accurate at 4 decimals.
Cell-counting is performed manually using a Euromex Bioblue series microscope and a Marienfeld Neubauer-improved haemacytometer counting chamber; at least 300 cells are counted for each sample to achieve an accurate number for the cell-count.
Each of the determinations is performed in triplo for each of the sampling times.

2.3.2 Nutrient concentrations

To monitor the amount of nutrients present in the culture medium during growth and to assess the water-cleaning capacities of the algae, nutrient concentration are measured. These measurements are performed as often as the biomass determination for Nitrates. Phosphates are measured once a day as these measurements are very laborious. The measured nutrients parameters are: the concentration of phosphates, the concentration of nitrates and the conductivity of the medium.
Nitrates are measured using a Horiba Ltd. TWINNO_3^- B-34X. Measurements are performed measuring ppm with a non-diluted sample. The concentration of phosphates is determined with a HANNA HI713 with a dilution of 1:100 or 1:50 of the sample.
Before determining the nitrate and phosphate concentrations the samples are centrifuged for 10 minutes at 3600rm using a Hailmton Bell co., inc. Montvale Angle centrifuge; model 1550 to remove biomass from the sample which influences nutrient measurements. During the experiments the centrifuge was replaced with Minisart syringe filters 33mm, 0.20µm pore size to filter the biomass from the sample.
The conductivity of each sample is determined using an IKS conductivity module connected to the IKS aquastar control panel of the reactors.
All these measurements are compared and verified with a sample of water that was send to Relab den Haan horticultural consultancy, the Netherlands for full analysis.

2.3.3 Reactor parameters

The control of the pH in the reactors is fully automated with the use of the control panel and CO_2 supply. A set-point for the pH is selected using the IKS aquastar or the AquasSoft software and the system will automatically maintain the pH at the set-point. CO_2 is supplied using a 25kg CO_2 tank obtained from Infra Mexico. The temperature is also controlled using the control panel and the software. Cooling is controlled by a solenoid valve which is operated by the control panel. Cooling water usage is set to an equal flow for both reactors and the total water consumption is measured using Gardena water meters (model 9188). Based on the initial experiments and literature the set-point for T and pH for the experiments are chosen. The flow of CO_2 and circulation air can be set with flow meters on the reactors control box. The values for these flows are determined during the initial experiments.

2.4 Experimental design

The experimental part of the internship contains two main parts of experiments on the PBRs. The first stage of experiments consists of trouble shooting experiments and start-up experiments. These experiments are performed to understand the full-functioning of the PBRs in the Mexican climate and to adjust the available cooling methods and to make sure all other operational parameters are set for the comparative experiment. The second stage of experiments, which is the comparative stage of experiments, involves two pilot plant systems at different geographical orientations. These experiments are performed to be able to conclude on the goal of this thesis: Determine the effect of the geographical orientation on these systems in the Mexican climate. The materials and methods for analysis of samples are equal for both the experimental phases and are described in chapter 2.3.

2.4.1 Start-up and troubleshooting experiments

These experiments, also referred to as initial experiments are performed in a somewhat unstructured manner. The experiment is a large combination of small tests of operational systems of the reactors and changes to the created systems. The experiments are highly dependent on the available equipment and money for the project. Also these experiments rely highly on the help of companies and employees of the Agropark for constructing installations.

Changes and improvements to the systems are made continuously during the start-up period of the reactors. The goal of these initial experiments is to determine growth settings for the reactors during the comparative test and to find out if all systems are functional to run the reactors in a reliable manner to produce data in the comparative experiments. The experiments and results of the start-up and troubleshooting experiments are structured in 4 categories: Cooling, daily operation, cleaning and operational parameters.

The results of these initial runs of the reactor are crucial for the understanding of the functioning of all systems and will help to set all parameters for the comparative experiments. Also these experiments act as a control for all available equipment and protocols, equipment and protocols are adapted according to results of the troubleshooting phase to be ready for the comparative experiments.

2.4.1.1 Cooling

When purchasing the LGem PBRs they are delivered without a cooling system in place. Due to the high amount of heat and sun-light in Mexico an adequate cooling mechanism needed to be created. The goal is to create a system that uses minimal water for cooling. To compare cooling strategies three methods for cooling are envisioned to be created on the LGem reactors in Mexico:

- Shadow mesh 50% above the reactors to prevent heating of the system.
- Dripping cooling water system to cool the tubes with cold water.
- High pressure sprinkler system using evaporation energy to cool the reactors.

Due to a lack of time and the lack of availability of all three cooling systems no structured experiment was performed to compare the three systems. Smaller test and troubleshooting experiments are performed on the systems using shadow mesh and the drip cooling system.

2.4.1.2 Daily operation

The troubleshooting experiments that were done to specify daily operation parameters are small tests and experiments that have not been documented under materials and methods. Testing of all filling, emptying and running protocols lead to a combination conclusions and changes of protocols as shortly described in the results section.

2.4.1.3 Cleaning

During the initial runs of algae in the systems contaminations were introduced. All three systems were inoculated with this algae mixed culture and run for several days. Afterwards all reactors are cleaned and cleaning protocol equipment and procedures are performed. From these test cleaning moments changes were made to the protocols.

2.4.1.4 Operational parameters

The operational parameters such as air flow, CO_2-flow, temperature set point and computer monitoring of the systems is performed in the initial runs. These parameters are changed when problems occurred and when needed changes were made to the controlling systems and or protocols.

2.4.2 Comparative experiment

The performed experiment focusses on the productivity for the different geographical orientations. In this experiment two reactors are compared while keeping both reactors on identical growth parameters such as temperature and pH set-points, growth-medium, harvesting methods and times as well as reactor parameters such as CO_2-flow rate, circulation flow and pump settings. For this experiment reactor #1 and #3 are used, see Figure 4. Reactor #1 is referred to as the East-West oriented reactor as the length of the reactor is on the East-West line. Whereas reactor #2 and #3 are placed on the North-South line. Reactor #3 is therefore referred to as North-South.
After confirming all reactors are running stable and the cooling and pH controls function properly the comparative experiment is started.

The reactors are started with the same culture and run for 6days days in batch mode. The biomass production and other reactor parameters are monitored for the complete duration of the growth. The productivity and the cooling are compared between the two systems. The nutrient concentration is kept to a non-limiting concentration by the addition of Bayfolan medium to the reactor. Nitrogen is considered limiting at levels below the accurately detectable rang of the equipment used which is 63mg/L NO_3^-. These concentrations of nitrate are suggested to be sufficient to be considered nitrogen replete conditions according Griffiths et al. (Griffiths M. J., 2014). Reactors are sampled at least three time a day, samples are taken in triplo from each reactor with 3-5minute intervals between the samples to make sure a sample is take of multiple different parts of the reactor.

For every sample is the OD_{750}, dry-weight and, where possible, cell-count are determinate to monitor the growth of biomass. Also the conductivity and the concentrations of nitrate and phosphate are measured as described before.
During the experiment set values are chosen for the pH value and temperature set-points for the culture. All conditions for the two reactors will be kept exactly similar and solar radiation and outside temperature are monitored during the experiment. The chosen parameters for this experiment are concluded from results of the initial experiments that will were performed to test all the systems on the reactors and gain practical knowledge about operating the systems. These values and values for T and pH are obtained from literature and compared with the earlier experiments to choose the set-point values. The chosen values for the set-points during the experiment are T=30°C and pH=6.8.
From literature it is concluded that *C. vulgaris* is typically cultivated at temperatures somewhat lower than the 30°C set-point used here. pH was found to be mostly used at 6.8 (Razzak, 2013), (Přibyl, 2012), (Qin, 2014).
30 °C was used in these experiments as it was found in the initial experiments that it is not feasible to cool the reactors lower than 30°C efficiently with the currently available cooling systems (data not shown).

Also, previous experiments indicated that *C. vulgaris* grows well under these conditions for the pH and T combination. These values for T and pH were also used during the lab experiments that are part of the project and indicated the results for scaling up the project. Combining this data with the data from the previous experiment the settings are selected for the reactor. The settings can be found Table 1.

Table 1: Reactor settings and parameters used in the comparative experiment

Parameter or setting	Value	Unit
pH-set-point	6.8	-
Temperature-set-point	30	°C
Shadow mesh (50%)	24	h/day
CO_2-flow	2-4[1]	l/min
Circulation air-flow	6	l/min
Liquid pump	On	-

[1]changed during experiments, always kept similar in both PBRs.

2.5 Calculations

Multiple calculations are made to be able to conclude on the results of the experimental phase.
Firstly there are the calculations performed before the experimental phase that will serve as a reference. These calculations will include theoretical biomass productivity maximums for the used nutrients, available light and reactor parameters. These are described in chapter 2.5.1.
Calculations on the obtained data are performed to give the productivity numbers in a way that they are comparable with literature and the other calculations. Calculations for the experimental results and the units of all variables are described in chapter 2.5.2.

2.5.1 Predictive calculations

Predictive calculations are performed on the PBRs and the light conditions to be able to compare the predicted outcome of the experiments with the achieved results and values found in literature.

Calculations on the photosynthetic efficiency are performed using an excel sheet obtain from Wageningen University. These calculation include the data of the actual irradiance of the sun during the experiment duration of the experiment. The area of the reactors used for the photosynthetic efficiency calculations is 16.5m^2, this is the advised width of floor space for the reactor (1.5m) by the length of the tubular system; as advised by LGem. The constant of ''irradiance not missing PBR'' was set at one. This value is chosen based on observed shadows and estimations of the amount of light hitting the reactors. The reactors are placed in such a way that they will not create shadow for each other. Therefore the reactors create a much larger shadow on the first and last hours of the day. This means they effectively obtain more sunlight than the floor area described. Around noon the sun is directly above the reactor, creating a shadow much smaller than the described floor area. It is estimated that these effects are of about equal influence on this parameter, setting it a 1.0.
The excel sheet was adjusted using the actual solar irradiation data that was obtain from United Farms/Finka, Agropark Queretaro for the days of the experiment. Adjustments were made to make the sheet represent daily production in g/l/reactor rather than tonnes/h/year.
The company that provided the solar data is the one with sensor located closest to the reactor location (200m) and monitors all climate parameters continuously using a Hortimax MultiMa XP logging system.

The expected biomass production is calculated for theoretical photosynthetic efficiencies of 1-5% for period of the experiment. These calculations are compared with the observed results for biomass production in the systems.

With the data of the nutrient concentrations of the used medium and the biomass composition of the used species of algae predictive calculations are made for the total biomass production using the drain-water. This will also identify possible limiting nutrients in the medium and indicated suggestions for medium adjustment.

2.5.2 Equations for calculations on experimental data

Calculations on the obtained data are performed to be able to compare the achieved experimental results with numbers found in literature and theoretical production based on the predictive calculations. The equations used for the calculations are described here. For all calculations performed that give a total water consumption or biomass production per day the measurements of 09:00 were used of both days. Therefore including a total 24hour period in each data point, eliminating problems with overlapping time intervals and incorrect cool water allocations.

Equation 1.1 gives the efficiency of the use of cooling on the reactor by dividing the used amount of cooling water consumed (in m^3) during a given time interval (days) by the amount of biomass produced in the reactor (in kg) during the same time interval. This determines the efficiency of cooling of the reactor in m^3 of cooling water that was required to produce one kilogram of biomass. This equation is not true for all chosen time intervals due to continues-batch mode of operation on the reactor. The time interval should be chosen in such a way that the used cooling water and the corresponding harvest are within the same time interval. The equation will be therefore only be used to calculated the cooling water efficiency of the system over larger time intervals. Typically 24hour intervals are used as described above. To compare the different systems the exact time interval will be used for both calculations. This will create an equal discrepancy for both systems.

$$\text{eq. 1.1} \quad \text{Coolingwater efficiency} = P_{CW} = \frac{(V_{cw(t)} - V_{cw(t-1)})}{\left((C_{x(t)} - C_{x(t-1)}) * V_r\right) + (C_{x(harvest)} * V_h)} \qquad \text{Unit: } [\,m^3 \,/\, kg_{biomass}]$$

Equation 1.2 gives the volumetric biomass productivity by dividing the total produced amount of algae over a time interval over the volume of the reactor and the time that was needed to produce this amount of biomass. The total produce amount of biomass is defined as the sum of the difference of total biomass in the reactor on the time intervals and the total biomass harvested if a harvest was performed.

$$\text{eq. 1.2} \quad \text{Volumetric biomass productiviy} = P_{x,vol} = \frac{\left((C_{x(t)} - C_{x(t-1)}) * V_r\right) + \left(C_{x(harvest)} * V_H\right)}{V_r} \Big/ \Delta t \qquad \text{Unit: } [kg/m^3/day]$$

Equation 1.3 describes the areal biomass productivity. This number gives the production of biomass per square meter of used land area for the PBR. This is a number frequently used in literature and will be used to compare achieved experimental results during this study with values found in literature.
This equation is similar to equation 1.2 with the only difference that here the ground area is used instead of the total reactor volume.

$$eq.\,1.3 \quad Areal\ biomass\ productiviy = P_{x,area} = \frac{\left(\left(C_{x(t)} - C_{x(t-1)}\right) * V_r\right) + \left(C_{x(harvest)} * V_H\right)}{A} \Big/ \Delta t \qquad Unit: [kg/m^2/day]$$

The equation used to calculate the amount of volume that has to be harvested from the reactor at each moment of diluting the culture is displayed in equation 1.4.
This equation can be used on any of the measurements that determine biomass before harvesting, for practical reason only OD and cell-count can be used in this equation to determine the volume for harvesting. After determining the dry-weight concentration the calculation is also performed with this data to compare all different measurements.

$$eq.\,1.4 \quad Harvesting\ volume = V_{harvest} = \left(1 - \left(\frac{1}{C_{x(t)}/C_{x(0)}}\right)\right) * V_r \qquad Unit: [m^3]$$

The growth speed of the culture is calculated using equation 1.5. This equation can be used on all measurement for the biomass determination. Comparing the calculated growth speed and dilution factor with between different measurements gives indications on the accuracy and consistency of the different methods used to determine the biomass.

$$eq.\,1.5 \quad Growth\ speed = \mu = \frac{\ln\left(\frac{C_{x(t)}}{C_{x(t-1)}}\right)}{\Delta t} \qquad Unit: [day^{-1}]$$

Table 2: List of abbreviations for calculations; full name symbols and units.

Definition	**Symbol**	**Unit**
Cooling water used	V_{CW}	m^3
Cooling water efficiency	P_{CW}	$m^3_{CW} \cdot kg_{biomass}^{-1}$
Harvested volume	V_H	m^3
Volumetric biomass productivity	$P_{X,vol}$	$kg \cdot m^{-3} \cdot day^{-1}$
Reactor Volume	V_r	m^3
Biomass concentration set-point / time = 0	$C_{x(0)}$	$kg \cdot m^{-3}$
Biomass concentration at time t	$C_{x(t)}$	$kg \cdot m^{-3}$
Time	t or Δt	days
Growth speed	μ	day^{-1}
Dilution rate	D	day^{-1}
Volume to harvest	$V_{harvest}$	m^3
Ground area of PBR	A_g	m^2

3 Results and discussion

The results are divided in the two phases of experiments performed as described under 2.4 in material and methods results of the calculations are included in the discussion of the results of the comparative experiment.

3.1 Start-up and initial experiments

Although these experiments consumed the majority of the time of the internship the results of these experiments are not described in much detail. This is due to the unstructured nature of these experiments. Important findings and conclusions that are used in the comparative experiments will be described shortly, the findings are categorized under the following sub-categories: cooling, daily operation, cleaning, and operational parameters.

3.1.1 Cooling

Due to a lack of money and long delivery times it was not possible to build all three cooling systems that were planned for the reactors. Table 3 shows the three systems and the hypothesized pros and cons of these systems.

Table 3: The three cooling systems that will be built for the reactors and their hypothesized benefits and drawbacks

Cooling type	Hypothesized benefits	Hypothesized drawbacks
Shadow mesh 50%	Less cooling needed	Reduces available sun-light for algae growth
Dripping system	Low start-up costs	High water usage of the cooling due to inefficient cooling. Extensive need of cleaning of reactors due to salt deposits.
Sprinkler system	Highly efficient / low water usage. Proven to be functional on similar reactors before	High costs involved to build system due to required water quality standards for sprinklers

Due to a shortage of money combined with difficulties of obtaining all the required parts needed for the construction of the sprinkler system it was not possible to test this system on the reactor during this internship. The sprinkler system is hypothesized to use a maximum of 65L/hour of runtime based on specifications of the used materials, this systems will therefore use a lot less water than the dripping systems which is set at 300L/hour of use. Also it is hypothesized that the sprinkler system will cool much more efficiently and will therefore be needed less time per day to maintain the temperature in the reactor.

The dripping system was constructed using white plastic tube running on top of the reactor. A small hole for dripping the cooling water was created every 15 cm. The flow was set on 5l/min for the cooling in both reactors. Initial experiments showed that the cooling of the reactors was successful as the reactors were kept at the temperature set-point of 30°C with this cooling system (data not shown). The drip cooling system was found only to be sufficient when also using shadow mesh. Removing shadow mesh from the systems caused the reactors to overheat while the cooling systems were always on, indicating insufficient cooling capacity for the cooling of the reactors with this system when not using shadow mesh. A high amount of water was needed to be able to cool; 500-1000L/day was needed depending on the density of the algae culture present in the systems and the pressure of the water line (which is not controllable). The water used for this cooling system was taken from the aquifer of the Agropark and generally had a

temperature of 25-30°C depending on the time of day. Due to high salt concentrations in this water large amount of salts were deposited on the reactor tubes, therefore requiring cleaning of the tubes on a weekly basis. The pressure on the line varied per day and hour of the day. This caused a sometimes fluctuation flow on the cooling of the reactors. Both reactors were set to a flow of 5L/min at the same moment. Therefore the flow would be similar in both reactors. This will still create inaccuracies for the total used cooling water if the reactors are cooling at different times of the day and these moments would have different pressure on the cooling water. This is something that was not controllable in the situation of the experiment.

The shadow system was created to prevent the reactor from heating up too much. This system was constructed as a temporary measure for preventing the systems to heat up. Part of the goal of the complete project is to maximize sun-light use and therefore minimizing shadow use. The shadow is created using a 50% shadow mesh with a 1.65 x 11.0m piece of 50% shadow mesh and placed 0.50m above the reactors. Experiments with and without the use of this shadow mesh showed the effect of the shadow mesh system; the drip cooling systems was unable to maintain the temperature of the reactor at the set-point without the shadow whereas it did when shadow was used (data not shown). This test was performed with a low culture density. This implicates that when higher density cultures are used the reactor will heat up even more.
Another option for shadow-mesh usage could be to only use the shadow mesh if the reactor is heated up. The systems need to warm up in the morning. This could be faster if no shadow-mesh is applied (not tested) and the system could be provided with shadow when the system measures the temperature reaching a set-point. Such a system is not created on the reactors yet. It is there chosen that during the comparative experiment the shadow mesh is applied for 24hours a day to keep both systems under equal conditions.

3.1.2 Daily operation

Due to the size and volume of the reactors a lot had to be learned about the daily operation of the reactors. During the comparative experiments daily harvesting was planned, due to problems with water supply and the lack of a fast harvesting method while keeping the reactor mixed resulted in a changed plan. Proper mixing of the reactor while adding new medium is a challenge. Some of the important concerns during daily operation include:

- Harvesting has to be performed quickly using a pump. Otherwise the slow flowing culture will form a biofilm in the tubes. The culture being stationary for a longer time causes temperature and pH increase in the systems and the control is turned off.
- Harvesting moments should generally be done in the early morning to prevent pH and T fluctuations in the pipes. Harvesting times however are dependent on the water source.
- Lower CO_2 flows (2-3l/min) will create lower fluctuations around the set-point.
- CO_2 consumption during the night indicates contaminations of the culture.
- CO_2-tank pressure should be carefully monitored, very difficult to observe an empty tank. When tank pressure drops below the 700PSI the tank is empty within a few hours. Depending on the amount of reactors that are operated and on what settings.
- Operating the software can be challenging. This is solved by setting up

3.1.3 Cleaning

All cleaning procedures were tested and sources of contamination were identified. This yielded the following results and operational methods concerning the cleaning of the PBRs:

- Using 3ml/L of Cloralex® (a commercial chlorine-solution) to clean the systems works well. Due to air circulation flow when running the chlorine water in the reactor the chlorine is removed and will allow contaminations to grow.
- A stagnant culture in the tubes quickly causes biofilm formation. This can be caused while harvesting but is mostly caused by power-outages during rain.
- Biofilm cannot be removed with chlorine treatment.
- Biofilm should be fully yellow/dead before cleaning with the sponge to fully remove the biofilm.
- Sponge cleaning only works well on large contaminations when using water flow only.
- Any sticking contamination will not be removed just by chlorine treatment of the system.
- Fully cleaning after a serious contamination takes 2-4days per reactor.
- Cleaning lime-scale deposits on tubes as result of cooling can be done using vinegar. Proper cleaning can only be done when reactor is not being cooled and takes about 4-5hours.

3.1.4 Operational parameters

Operational parameters include the measured data and the parameters that can be set on the control panel of the PBR.

- Operating the PBR on air pumps only gives a total volume of about 560L, when operated with all pumps on the culture volume is about 660L.
- When changing pump settings during e.g. harvesting always check if vortex breaker does not block the water flow afterwards.
- Filling a reactor has to be done by pump due to time limitations.
- When inoculating the reactor a sufficient dense culture should be used to minimize contaminations.

3.2 Comparative experiment

Not all measured data is explained and discussed in this report. This report focusses on the effect of the geographical orientation on the productivity of biomass. Only results important for this conclusion will be mentioned in this report. To be able to make conclusions on biomass productivity the results are presented in the graphs found in the Appendix.

The comparative experiment was run for 6days in two reactors. Reactor #1 in the East-West orientation and reactor #3 in the North-South orientation, see Figure 4. Both reactors were started on a similar, but not completely equal, OD_{750} and the growth is monitored closely in the 5.5 days after inoculation. Calculations are performed for time intervals take for 24 hours (09-:00-09:00).

3.2.1 Biomass productivity and cooling efficiency

For the data of the complete experiment the average biomass productivity and cooling water efficiency was calculated using equation 1.1 and 1.2. The results of these calculations are shown below in Table 4. As these values described the average over the complete experiment the values are much lower than the once found in literature for maximum productivities reached. In the calculation also the nights are included reducing the average productivity numbers. Literature describes a productivity for *C. vulgaris* of 0.1-0.5 kg/m^3/day when growing on waste water (Chiu, 2015). Griffiths described a productivity of 0.325g/l/day on a medium with similar nitrate concentrations with a growth up to 1.7-1.9g.l total biomass. The highest reported productivity of *C. vulgaris* found in literature is 2.1g/l/day at a total biomass of 21g/l (Fu, 2012), but these values are created under highly optimized conditions. This indicates that the productivity reached in this experiment falls nicely within the range of reported numbers from literature, although these are averages. For productivity between two data point at which the highest growth was observed values as high as 1.7 g/l/day are obtained (data not shown). These growth rates are higher than

usually found in literature for outside operated reactors. It is suggested that this steep increase in growth is created by the contaminations present in the culture.

Table 4: Volumetric productivity and cooling efficiency for both system over the complete operating period of the batch experiment.

	Volumetric productivity	**Volumetric productivity**	**Cooling efficiency**
	kg/m³/day	**kg/m³/h**	**m³/kg**
#1 (E-W)	0.39	1.64E-02	1.54
#3 (N-S)	0.46	1.93E-02	1.42
difference	18%		-7%

From the calculations of the productivity of the biomass in km/m^3/day for the two systems it is found that the North-South oriented system shows an 18% higher volumetric productivity than the East-West oriented system when comparing the complete period of 5.5days. The absolute biomass concentration that was produced was almost 14% higher in the NS-oriented reactor (data not shown).
The results of the cooling water efficiency show that the NS-oriented reactor required less cooling for the production of a kg of biomass than to the EW-oriented system.
The NS-oriented system used an average of 7% less m^3 cooling water / $kg_{biomass}$ produced. This indicates that the orientation in the North-South direction heats up less than the East-West oriented system under the same conditions. This system therefore maintain the temperature in the reactor with more efficiently. Figure 2 in the Appendix shows the temperature of both reactors and the ambient temperature, the vertical lines indicate midnight. These graphs show that the reactors behave differently during days. Both systems always heat up faster than the ambient temperature and require cooling when the set-point is reached. From the results it is also seen that the cooling was not always sufficient to keep the systems under the set-point. Occasionally the temperature exceed the set-point value slightly. This could also have been caused by a temporary drop in cooling water pressure. This is however ruled out for most situations where the temperature exceeded the set-point as such a change would influence the temperature of both systems equally. The EW-oriented systems generally heats up earlier on the day. This behaviour is not as hypothesized for the EW-oriented systems as the orientation would suggest a low amount of light hitting the reactors surface in the morning. This change of hypothesized behaviour is due to the deviation from the intended reactor orientations and is discussed in more detail under 3.2.3. One clear observation however is that the NS-orienting system needed cooling on the 4th day whereas the EW-oriented system didn't. That day the sun was only shining at the end of the afternoon (indicated with the radiation data in Figure 3 of the appendix), heating up the NS-systems but not the EW-systems. This is most likely due to the fence that is placed north of the EW-system.

From the absolute cooling water consumption per reactor it can be found that the systems behave differently per day (see Appendix Figure1). Also it is seen that the more efficient cooling of the NS system used a larger total amount of cooling water. This is compensated by production of a higher biomass concentration over the same time period as is indicated by the dots in the same graph. The error bars on the dots become larger during the 5day experiment as the biomass increased. The increase in biomass required smaller sample volumes to be applied on the filters to prevent the filter to be blocked, therefore increasing the error.

The solar data, the temperature and cooling data all show similar patterns for each day. Comparing all his data together with the results strengthens the conclusion and helps to explain the differences between the two systems.

Another reason for the differences is the obstruction of the solar radiation to the systems on day 1 and 2 of the experiment. During these days equipment used for the official opening of the pilot plant facility was casting a shadow on the systems preventing accurate data of the effect of the orientation of the systems on the productivity and cooling water efficiency.

3.2.2 Nutrients

During the experiment the nitrate concentration in the medium was monitored, the results of these measurements can be found in Figure 5of the Appendix.
Based on calculation performed on the biomass-composition of *C. vulagris* (Janssen M., 2011) and the composition of the drain-water obtain from the company supplying the water a total biomass concentration of about 3.1-3.8 g/l could be achieved (calculations can be found in Table 1 of the Appendix) The data for this calculation contains about 1050mg/l of nitrates and was the most recent data of the greenhouse drain water available. A change of plants species used in the greenhouses between the last detailed water analysis and the experiment changed the composition of the water significantly; reducing the nitrate concentration to 420mg/l. Literature indicates that this would support a growth of about 4.9 days to a total biomass concentration of about 1.7-1.9g/l (Griffiths M. J., 2014).
When the total supported biomass concentration is calculated based on 420mg/L it is found that it should be possible to grow about 1.2-1.5g/l. The results of the biomass growth show that on the day that nutrients were added to prevent nitrate limitation a growth of about 1.1-1.3g/l was achieved which falls nicely within the calculated range.
Due to limitations in time and the unavailability of the drain-water at the moment of the experiments the reactors were not diluted when the nitrogen concentration in the systems was almost depleted. The reactor was operated in a fed-batch mode and nutrients were added in the form of Bayfolan®-medium. This is a medium that mostly contains NH_4^+ as a nitrogen source. Ammonia is preferred as a nitrogen source by the algae culture in the reactor as is indicated by the stable concentration of measured nitrates in the medium. Medium addition was used to maintain stable levels of measured nitrates in the systems. The change from a nitrate to ammonia rich medium could have benefitted different species of algae in the reactors changing the composition of the culture.

3.2.3 Photosynthetic efficiency

Calculations on the theoretical expected biomass production based on the irradiance were performed with the sunlight data that was measured during the experiments by the HortiMax systems at Finka. The results of these calculation can be found in Appendix Figure 4.
The graph shows the theoretical biomass production per day based on 1 up to 5% of photosynthetic efficiency in bars. The total sunlight input per reactor is calculated with the solar irradiation data and the floor area of the reactor. From the graphs it can been seen that the achieved results for photosynthetic efficiency (PE) are higher for the North-South oriented reactor #3 for all days of the experiment. This is a logical result of the fact that this reactor produce a higher concentration of biomass as for the calculations both systems occupy the same ground area. In reality the difference between the two reactors is most likely different than depicted in the calculations here as the value for ‘’radiation not missing bioreactor’’ in the calculation is dependent on PBR-design and also orientation. This factor was set on 1 as described before. Besides the orientation difference of the systems this factor should also be corrected for the use of the shadow mesh. As the shadow mesh on the reactors is used using equal methods for both reactors this will not create a difference between the two orientations. It could be argued that setting this factor at 1 does not represent reality completely. If more extensive conclusions would be made on PE in the future it would be good to revise this value for a value that more accurately describes the conditions per reactor.

The PE of the reactors falls between 1-3% for all days, which is within the range of expected values based on literature. Literature describes values for the PE with a theoretical maximum at 9 % on the full light spectrum (Janssen, 2003).
An interesting observation in these results is the fact that when the total biomass production lowers due to less available light (during days 4 and 5) the PE increases. This shows that under lower light conditions growth still continues and is in fact more efficient than for higher light conditions, although producing less biomass. Literature also describes that high light intensities create the highest productivity and at biomass concentrations minimizing dark zones (Kliphuis, 2010). Other research describes the highest PE at higher biomass concentrations (Janssen, 2003).
The increase of PE under low light conditions might be amplified by the use of shadow mesh on the reactors for the full length of the experiment. The shadow mesh reduces the amount of direct irradiance to the reactor limiting the total irradiance input, but without correcting for this in the calculations. During the cloudy days the majority of the light that reaches the systems will be diffuse light, which is less affected by the used of shadow mesh.
is not possible to compare the results between the days of the experiments as there are too many factors that influence light availability and growth, of which the impact is mostly unknown. The sunlight was different for each day in intensity and in the hours this intensity occurred, objects casted shadows on the reactors, the biomass constantly changed and the influence of the possible changes of the mixed culture cannot be corrected for.

During the experiments more factors than the above discussed issues have influenced the collection of accurate data from the reactors making it difficult to conclude on the data depicted in this report. Many factors changed during the time of the experiment or are different than the hypothesized situation when comparing the two orientations.
One of the problems during the experiment was the culture that was used for the batch experiment. During upscaling for the production of the require culture volume for inoculation of the reactors contaminated water was used. Cleaned and sterilized, by filtration and UV treatment, water from the greenhouses is normally used as a medium for growth in these experiments. During upscaling the Hortimax Vitalite system, responsible for cleaning of the water, did not function properly causing contaminated water to end up in the process. Time limitations forced the experiment to continue with the contaminated culture rather than produce a new clean culture.
Changes in the medium during the experiment and the different orientations of the systems might have favoured other combinations of microalgae to thrive in the reactors. Due to the contamination of the systems cell-counting was not possible as a measure for biomass concentration. Only visual inspection of the culture was done on a daily basis. The composition of the culture changed visually during a day, but both cultures remained similar based on visual inspection.
Another change that could influence the results slightly is the sampling time. It was not always possible to exactly measure at the same moment of the day. As calculations and growth determinations can only be performed between sample moments these moments will influence the results slightly. Daily growth data is calculated per 24 hours using the early morning measurements of two days. These measurements fall within the timeframe of 08:30-09:15 with on day far outside this range. Although all produce data given per day with the actual time interval used, it can still influence the results.

Finally it should be noted that the reactors clearly do not follow the intended orientations of NS and EW as can be seen in the schematic representation of the floor plan of the plot plant facility. The reactors deviate about 40° from the orientation. This problem was created when the floor was not made to the

specifications that were given and the reactors placed on the floor according to a floor that was actually placed fully NS and EW oriented. This change in orientation from the intended orientation prevents strong conclusions on the differences of the orientation on any data produced in the reactors as they do not fully represent the described orientation. Observed differences from the expected behaviour (such as the heat up of the EW oriented system in the early morning) are a result of this deviation. The fact that the systems heats up in the morning indicates that the systems receives a lot of light in the early morning which is different from what would be hypothesized based if the system did not deviate from the EW line.

4 Conclusions

Conclusions of the above described results are separated in the results of the start-up phase and the comparative experiment.

4.1 Start-up and initial experiments

The initial experiments provided important information about the operation of the reactors in the comparative experiments. The experiments showed that operating the reactors can be a very laborious process. To reduce the time needed for operation and minimize errors during the operation of the system it is of great importance to fully understand the systems and all available equipment. The initial phase of experiments was crucially for the success of the comparative experiments and to reach the goal of the internship-research.

The experiments showed that the cooling systems functioned as hypothesized and that the dripping cooling is not sufficient in all situations and cannot be used without shadow mesh. Based on the achieved results in the experiments for the cooling it is highly recommended to continue with the production of the sprinkler cooling system as it will high increase efficiency of the cooling and reduce the need of manual cleaning of the PBRs.

Due to limitations of the equipment it was sometimes difficult to completely perform all necessary tasks within time. Guidelines for inoculation, harvesting and operating the systems that were created during the experiments were not always possible as the experiment depends on the help of others.

In short: The pilot plant PBR facility was started successfully, all necessary systems have been installed and are confirmed to work properly. More equipment will be installed in the near future improving the capabilities of the pilot plant facility concerning more accurate data accumulation and more efficient biomass production.

4.2 Comparative

An experiment comparing two geographical orientations was performed during a five-day fed-batch operation of the reactors. Nutrients were non-limiting and pH and temperature are controlled. These systems all worked well and it can be concluded that the LGem PBRs are fully operational and with some additions will be ready for future experiments.

The results of the experiments conclude that the North-South oriented system is more efficient concerning the amount of used cooling water and also has a higher photosynthetic efficiency. The systems produced a higher concentration of biomass over the course of the experiment than the East-West oriented reactor and did this in a more efficient manner. It is therefore concluded that for the location that is used the North-South oriented systems is the best method. It has to be noted that the difference between the systems were not very big and it is important to assess whether these observations show a statistical significant change between the two systems. Another important note is the large amount of changes that have influenced accurate data collection during the experiment. Many factors, including sunlight and shadows to the reactors changed and influence the results.

From the results it is also concluded that the current cooling systems are not sufficient and that at higher biomass concentrations and sunny days these limitations will be more pronounced. Comparing the results to the calculations of biomass production on nutrients and available sunlight show that these models describe the situation correctly and can indeed be used for predictions on total biomass production in the system and to predict the amount of dilution needed.

In short: The experiments performed here show that the North-South oriented system performs better during a short term experiment. Longer term and more accurate data has to confirm these observations.

5 Recommendations

From the results during the experiments of this internship a lot has been learned about the operation and the startup of the pilot plant facility. It has been concluded that the reactors work properly but before proceeding with new experiments in the pilot plant reactors there are some things that could be improved.

First of all it is of great importance to stabilize the inoculum growth procedure. Since the experiments described in this report improvements have been made to the bag system to allow for larger pre-culture volumes and better prevention of contamination. To be able to produce reliable results it is important to create a pure culture to start the reactors.

Secondly the new experiments must be planned very carefully. The operation of the reactors is a very laborious process and does not allow for many other activities during the experiment other than sampling, diluting and analyzing the information to make adequate decision. It is therefore absolutely necessary to fully plan any future experiments meticulously and with great attention to detail. It is advisable to base future experiments on more literature before starting, to better understand the significance of the obtained results during the process.

During the experiments it is important that the chosen sample times and actual sample times are maintained more precise to be able to have more accurate data representation and allow for better comparability of the data. Also it would be advisable to perform statistical relevance analysis on the obtained data to see if the observed results between the NS and EW systems are indeed significant. More predictive calculations should be performed before the experiment is started to assess whether theoretical limitations of the systems and medium are reached or not. It is important to monitor the concentrations of nutrients in the supplied water more often. The nutrients in the water supplied depends on the plant species cultivated in the greenhouses and will be the limiting facotr for the biomass concentration in the reactors during continuous cultivation.

For future experiments it is advised to run for a longer time (multiple months) to obtain accurate data under different weather circumstances and be able to give a reliable average for the production of algae at the pilot plant location. This can be compared with the data of the sunlight intensities on average over the past years and the actual data of the experiment to assess the economic feasibility of a large scale drain-water cleaning facility in Mexico.

Although logistically very difficult it would be advisable to look into options to place the reactors in the exact geographical orientations as intended. The current deviation from these orientations makes it hard to create solid data on the geographical orientation on the efficiency of the reactors. To be able to publish on this topic with these systems, adjusting the position is absolutely necessary. The short experiment performed her already indicated that the 40° deviation influences reactor behavior from what would be expected for the systems. To give solid advice on the geographical orientation for a large scale facility the orientation of the systems has to be accurately described.

More detailed research should be done to the effect of reactor parameters and settings on the effect on the productivity. Optimizing these parameters could significantly improve production of the systems and allow for a more feasible plan for full-scale drain-water treatment. It is advised to perform small scale experiment with the Algaemist on these settings and to do more literature research on these settings for the algal species used in future experiments.

Finally it is of great importance to minimize the dependability of an experiment on other parties. One of these factors is the supply of culturing medium. A sufficient medium supply should be available at the pilot plant facility allowing for dilution in the weekends or at times the forklift and its driver are not available. Other option include structured water delivery, a big medium storage vessel or direct water access at the pilot plant facility. The water quality should be tested before every use as it is of big influence of the experiments whenever the used medium contains contaminations.

6 Other learning outcomes of internship

From working on this internship I have learned and experienced a lot more than what is described in this report. The majority of the work performed at the internship consisted of the building, testing and troubleshooting of all systems required to operate the reactors. Doing this in a different culture with a crew of only two was a very challenging task.

The change to the Mexican culture was of great influence on the work that I was able to perform during the internship. Due to many small factors mostly related to the cultural change I was unable to perform all the experiments as planned in the project proposal as created before the internship started. Another important factor of delay were the challenges that had to overcome to be able to make the reactors fully operational. During the visit to LGem a clear list of what was needed and how to make the reactors function was created. Once in Mexico it turns out that everyday items in Europeans standards simply don't exist here or are really hard to find.

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Appendix

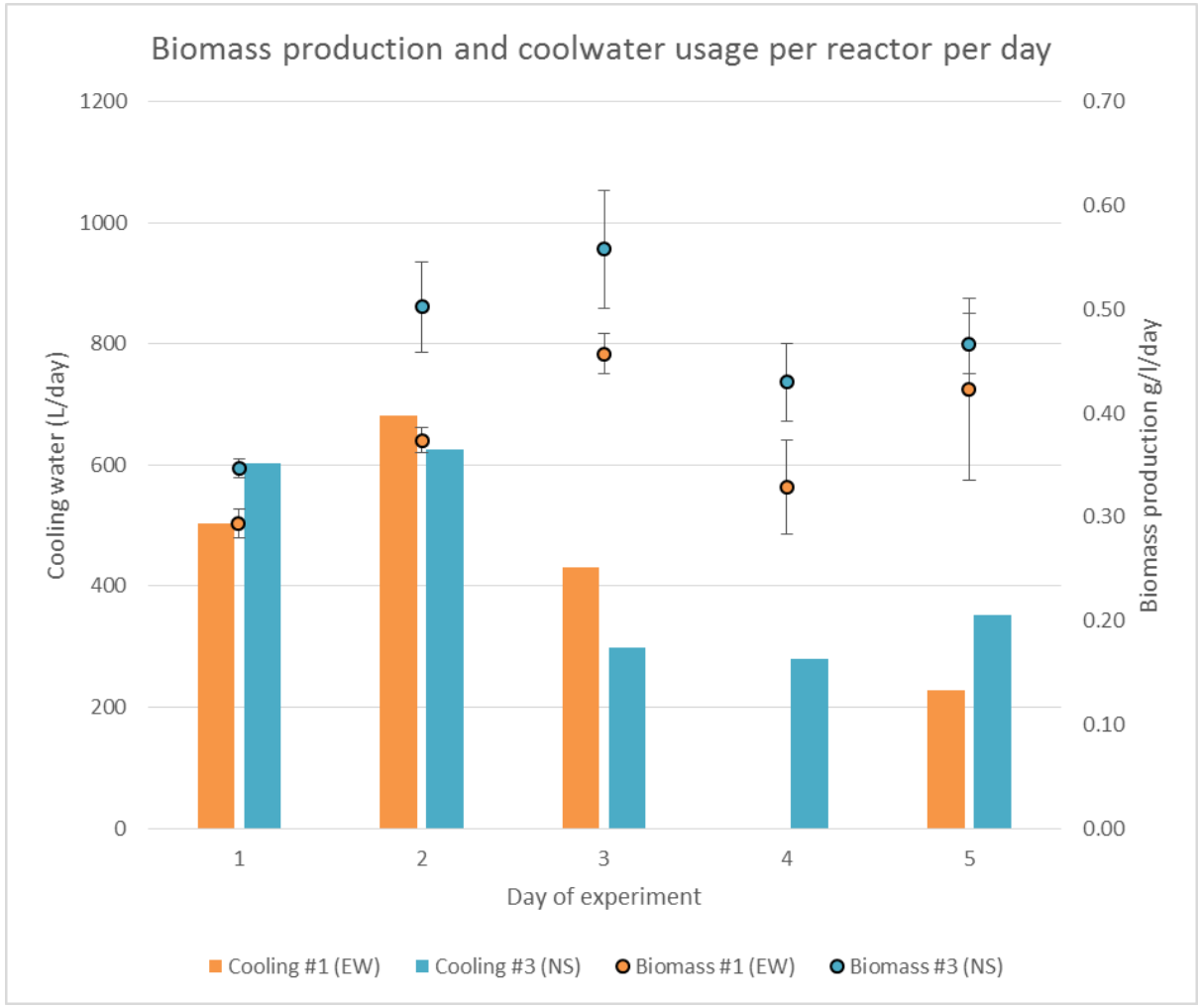

Figure1: Daily biomass production in g/l per reactor (dots) and the total amount of cooling water used during the same time interval (bars). Each data-point is created with data of the 09:00 measurement of two days.

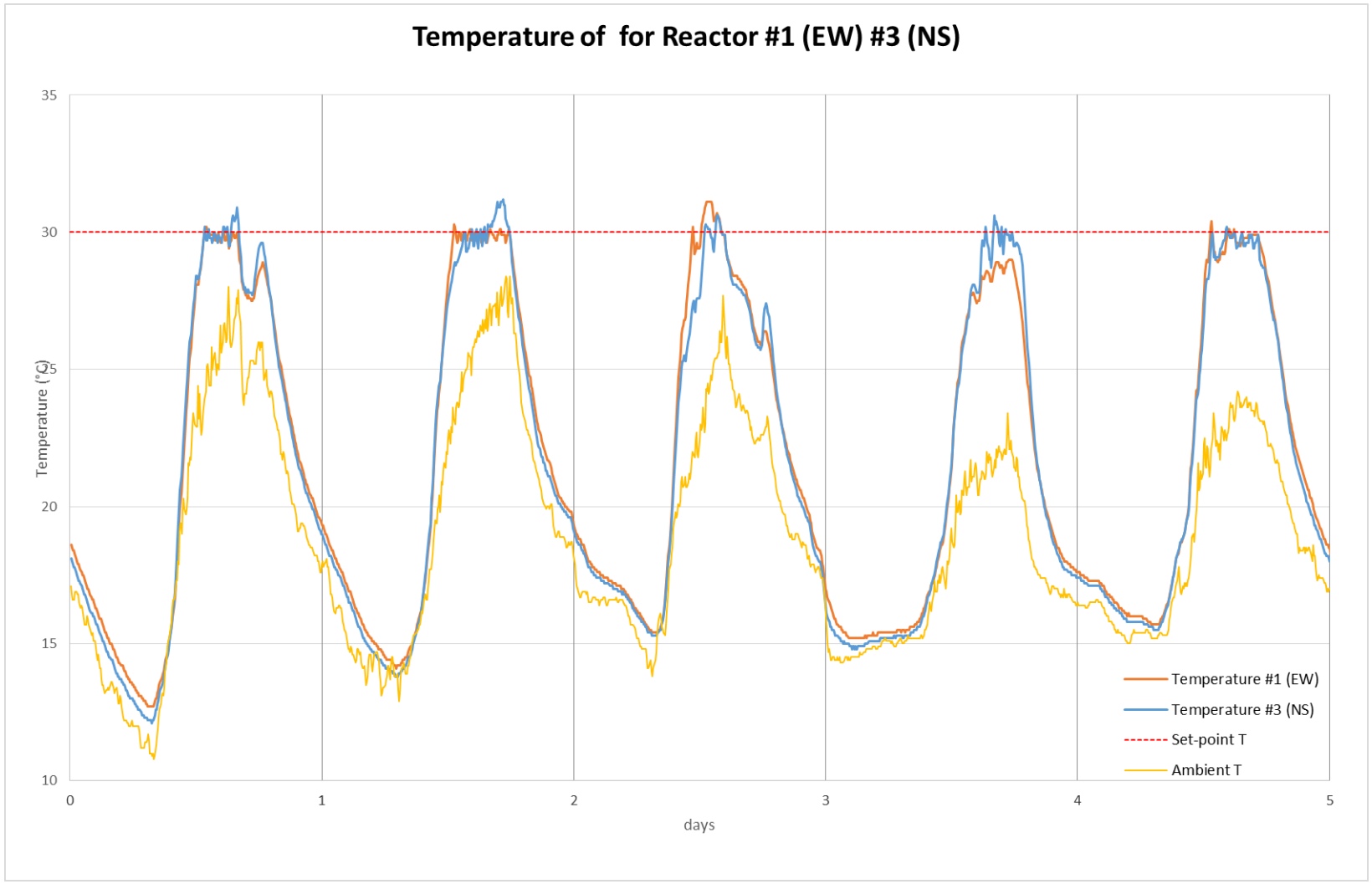

Figure 2: Temperature of the two reactors and the ambient temperature. The ser-point for cooling of the reactors (30°C) is indicated with the red-dotted line. Vertical lines indicate 24:00.

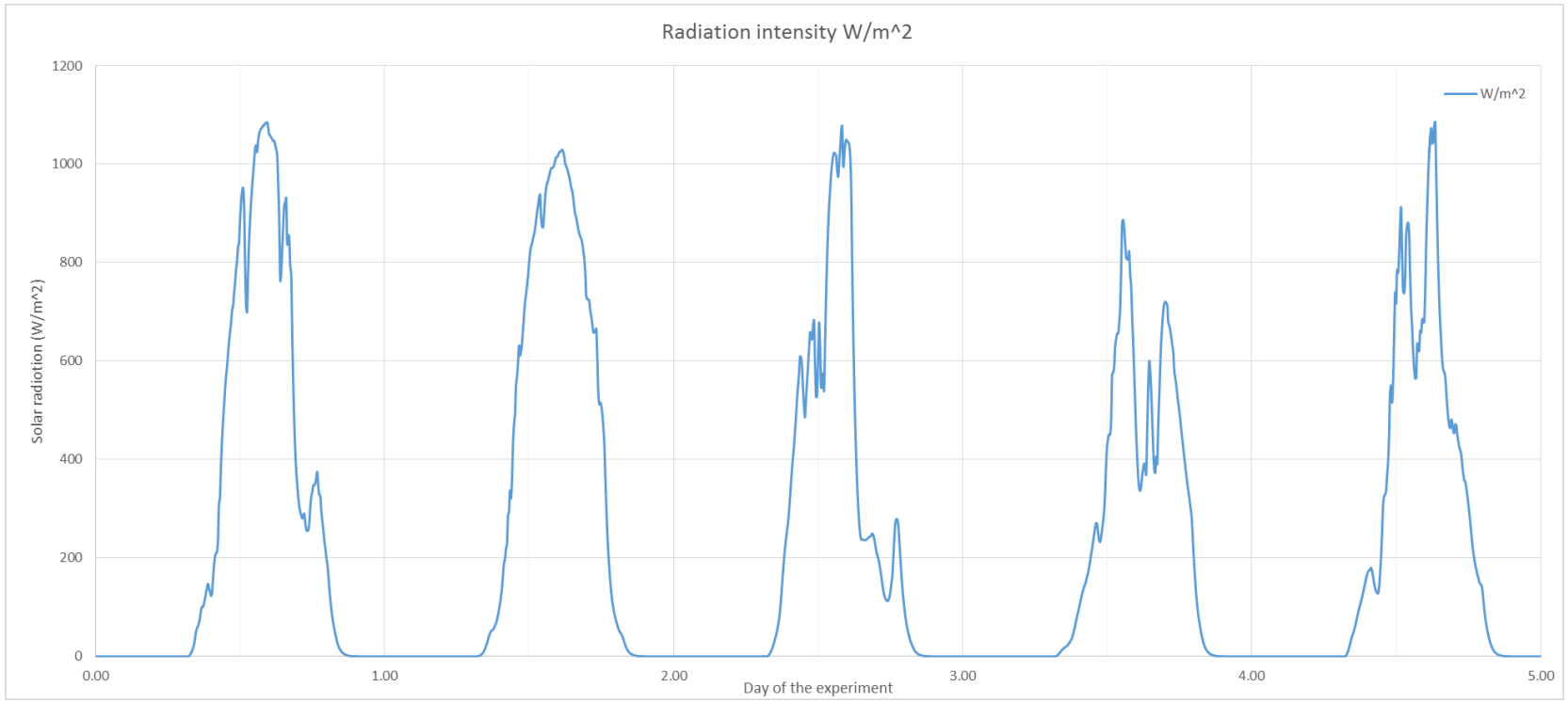

Figure 3: Solar radiation data during the days of the experiment. Vertical lines indicate 24:00 of each day. Data obtained from Finka, Querétaro.

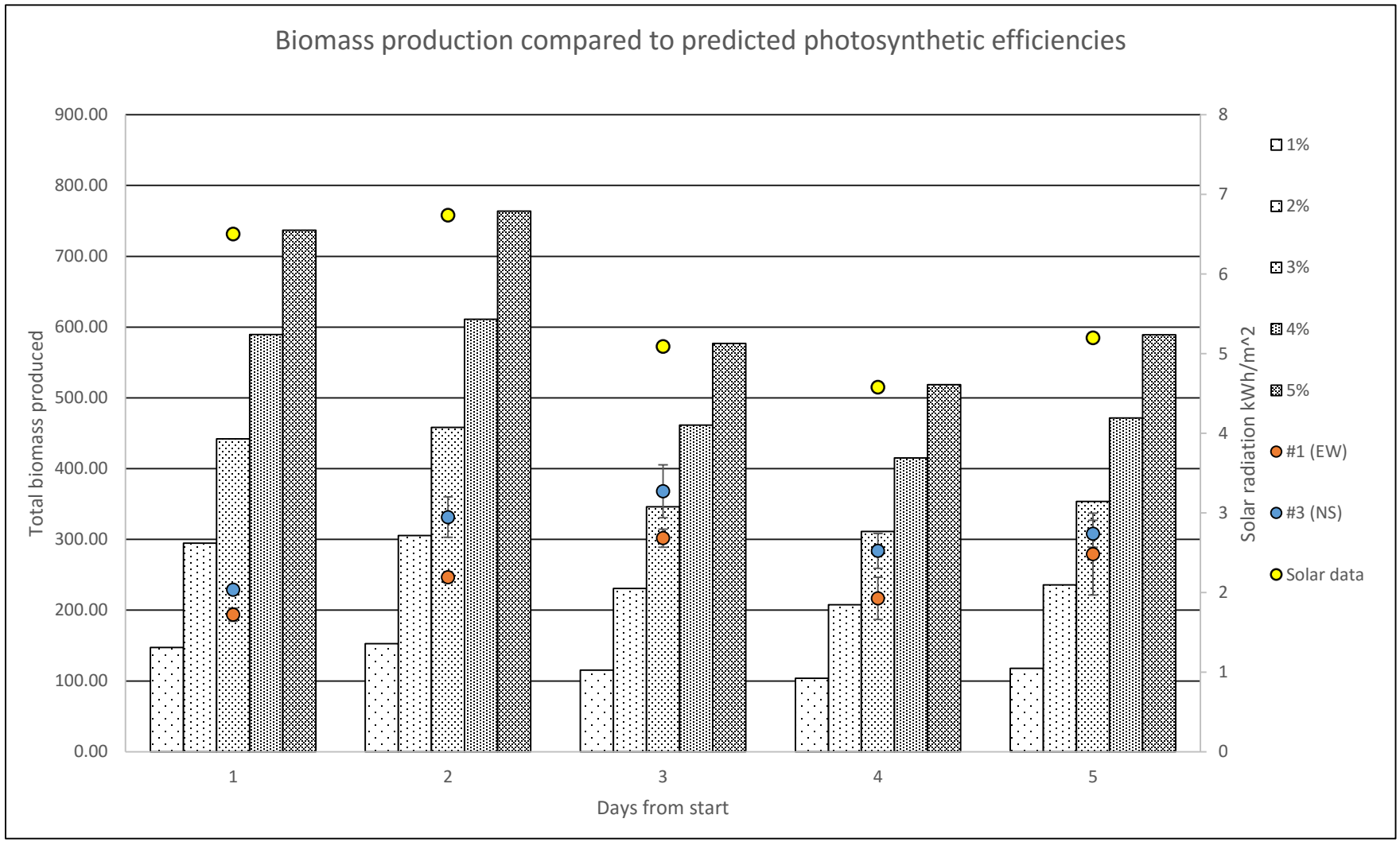

Figure 4: Achieved total biomass accumulation per reactor per day (dots, primary axis) compared to theoretical biomass production based on photosynthetic efficiency (bars, primary axis) calculated with the sunlight data of the experiment. The total solar radiation is represented with yellow dots on the secondary y-axis

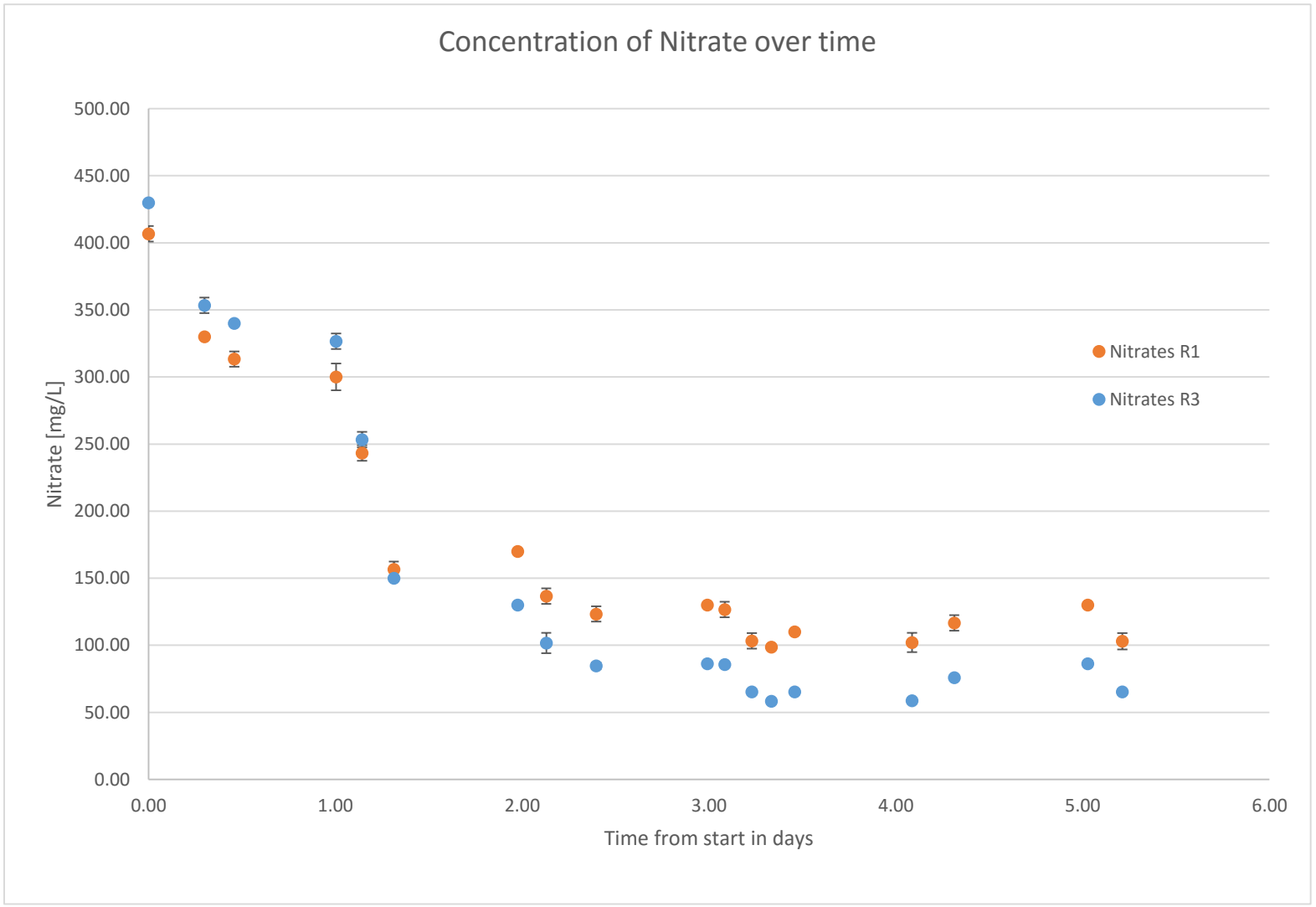

Figure 5: Concentration of nitrates as measured during the course of the experiment. Nitrogen was kept above limiting levels by addition of Bayfolan-medium. This medium contained ammonia as a nitrogen source, creating no change in the nitrate measurement. Medium was added if a decrease of nitrates was observed.

Table 1: Calculation of predicted biomass production with the used drain-water. Minimal and maximal values for biomass composition (g/g) were obtained from the reader of the course of Wageningen University. Used water was sampled and analysed 1 week before the experiments were performed. Below: Actual measured nitrate concentration in the used water during the experiment with the indicated maximum and minimum biomass production in g/l

Composition of Chlorella

Component	Minimal g/g g/g	Maximal g/g g/g	Minimal mol/mol C	Maximal mol/mol C		Finka mg/L	Biomass maximal mg/L	Biomass minimal mg/L
C	0.51	0.73	1	1	C	2.4	4.705882	3.287671
H	0.07	0.1	1.63	1.65	H	0.6	8.64	6.048
O	0.116	0.285	0.169	0.294	O	825.6	7117.241	2896.842
N	0.062	0.077	0.103	0.091	N	239.5	3863.221	3110.645
S	0.0028	0.0039	0.002	0.002	S	153.9	54960	39458.46
P	0.01	0.02	0.008	0.011	P	77.4	7743.25	3871.625
K	0.0085	0.0162	0.0051	0.0068	K	371.4	43698.1	22928.02
Mg	0.0036	0.008	0.0035	0.0054	Mg	116.7	32406.67	14583
Fe	0.0004	0.0055	0.00017	0.00163	Fe	2.0	5026.05	365.5309
Ca	0.00005	0.0008	2.91E-05	0.00033	Ca	288.6	5771232	360702
Zn	0.000006	0.00005	2.14E-06	1.26E-05	Zn	0.2	32690	3922.8
Cu	0.00001	0.00004	3.68E-06	1.04E-05	Cu	0.1	6354.6	1588.65
Mn	0.00002	0.0001	8.51E-06	3.01E-05	Mn	0.1	5768.49	1153.698

mg/L (NO3)	mg/L (N)	Max biomass (g/L)	Min biomass (g/L)
420	94.8	1.53	1.23