Biobased Chemistry and Technology

Thesis Biobased Chemistry and Technology

Validation of Algae Productivity Models for Outdoor Conditions

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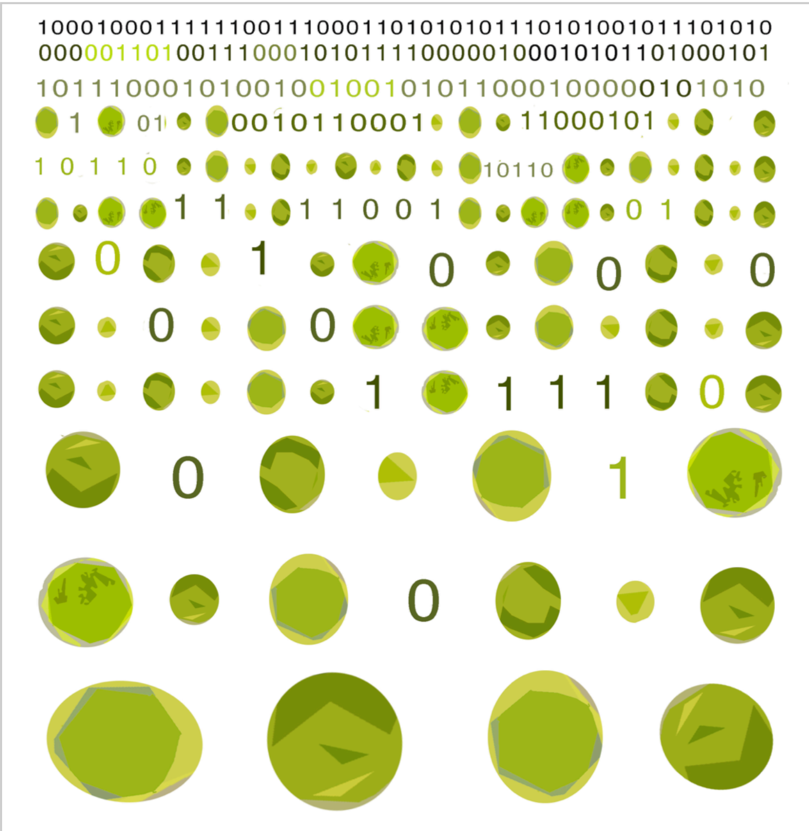

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Abstract

The ability of accurately predicting microalgae productivity at commercial scale under outdoor conditions is crucial to evaluate the potential of algae as renewable feedstock for food, feed, chemicals and biofuel. However, most of the published assessments used algal productivity projections based on laboratory data or on models that have not been fully validated under relevant conditions. The uncertainty attributed with the use of such productivity estimates is a significant concern. Overestimating full-scale productivity will significantly bias the estimated cost-efficiency and environmental performance. It is therefore crucial to validate algae productivity models for outdoor conditions before application in life cycle, techno-economical and scalability assessments.

The aim of this study was to independently validate the algae biomass productivity modelling framework developed by Slegers et al. [1]–[3]. The scenario models incorporate a time-resolved simulation of microalgae growth on solar irradiation, culture temperature, species-specific characteristics and photobioreactor geometry. The accuracy of the productivity models was assessed against data collected from two pilot-scale algae production systems: (1) raceway pond, (2) horizontal tubular photobioreactor (PBR) at AlgaePARC.

The models were found to accurately predict productivity under outdoor conditions in the Netherlands. An overall accuracy of +3.23 % over 45 days of cultivation in the raceway pond and -3.55 % over 121 days of cultivation for the horizontal tubular PBR was obtained. A global uncertainty/sensitivity analysis showed that the uncertainty of the model output was in a range of ±14.65 % for the raceway pond and ±11.27 % for the horizontal tubular PBR. The main attributors for this model uncertainty were indicated for each system. With the range of productivity prediction and the associated model uncertainty the fitness of the model for economic and environmental assessments was investigated. The model predictions were found to be in the same range to reported outdoor productivities and could be categorized in the lower range of used productivity assumptions in previous assessments. With the attributed model uncertainty it was shown that uncertainties in biofuel cost are reduced by more than half when compared to results found in literature.

The validation of this modelling approach is an important step for refining feasibility assessments of algae biotechnology.

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Greek Symbols

α	Functional cross section of the photosynthetic apparatus	[g_C mol^{-1}_{ph} m^2 $g^{-1}_{chl\ a}$]
β_T	Temperature curve modulating factor	[--]
θ_a	Chlorophyll a: carbon ratio in the cell	[$g_{chl\ a}$ g^{-1}_C]
μ_{growth}	Specific growth rate	[s^{-1}]
μ_{max}	Maximum growth rate	[s^{-1}]
λ	Longitude	[degree]
Φ	Latitude	[degree]
ω_{direct}	Interior light angle of direct light	[degree]
$\omega_{diffuse}$	Interior light angle of diffuse light	[degree]

Latin Symbols

C_x	Biomass Concentration (DW)	[g L^{-1}]
f_T	Temperature dependent factor	[--]
I_{total}	Total light intensity	[µmol m^{-2} s^{-1}]
I_{direct}	Direct light intensity	[µmol m^{-2} s^{-1}]
$I_{diffuse}$	Diffuse light intensity	[µmol m^{-2} s^{-1}]
K_{abs}	Spectrally averaged absorption coefficient	[m kg^{-2}]
P_m^c	Maximum carbon specific rate of photosynthesis	[s^{-1}]
P_{cumul}	Cumulative productivity over the production period	[g m^{-2}]
P_{exp}	Measured algae productivity	[g m^{-2} d^{-1}]
P_{pred}	Predicted algae productivity	[g m^{-2} d^{-1}]
R_{ground}	Reflection of diffuse light on ground surface	[--]
R_{direct}	Reflection of direct light on reactor surface	[--]
$R_{diffuse}$	Reflection of diffuse light on reactor surface	[--]
r_{max}	Maintenance associated respiration rate	[s^{-1}]
T_{let}	Lethal temperature	[°C]
T_{opt}	Optimal growth temperature	[°C]
$T_{culture}$	Culture temperature	[°C]
Turb	Turbidity	[NTU]
$Y_{x/ph}$	Yield of biomass on photons	[g mol^{-1}]
z	Reactor depth	[m]

Acknowledgment

One of the reasons why I came to Wageningen, was my great interest to learn more about the BioBased Economy and its future potential. During the course "Algae Biotechnology" I got more and more fascinated by algae as a renewable feedstock for biobased products. Therefore I decided to learn more on this topic. Since I always apprehended the power of modelling I wanted to deepen my knowledge into this field of research as well. This resulted in this thesis I was working on for the last eight months. But I couldn't have done it without the help of some people I want to mention at this point.

First of all I want to thank Ellen for all her guidance during my thesis. I was studying almost all her work and I am really impressed what she achieved so far. She is an inspiration to me and I learnt a lot of her structured and precise way of working. It was a pleasure to work with her together. Second I would like to mention Jeroen, whose help during the process of my thesis, I very much appreciate. He gave always very clear and direct feedback on my work and helped me to be more concise and to improve my writing, meeting and presentation skills. Even though I did not have the chance to meet Rouke often during my thesis, his input on my work was very valuable and helped me to tackle several problems.

From my amazing colleges I want to thank first Alex, who supported me scientifically very much during this work. I will miss the long discussions we had accompanied with tea. Francesca and Iris made my days at BCT very joyful. They always had the ability to cheer me up when I was stuck in a problem. A person who could always help out, especially in regards to issues in Matlab, was Tim, better known as 'Magic' Tim. He is a brilliant mind and I would like to thank him for his helpful tips and motivations. In addition I want to thank Johnny Petrakis 'Marketing', for his help with statistical analyses. One day we will conquer the universe!

Of course I also would like to thank my girlfriend Katharina for all her unconditional support especially in the last weeks of my thesis. In addition my family that helped me however they could, even though 1000km were in between us.

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1 Introduction

At the moment our society is heavily based on fossil fuel, which is nowadays widely accepted as unsustainable due to depleting resources and its involvement in the accumulation of greenhouse gases in the atmosphere [4]. Facing a future shortage of petrochemicals, the biobased economy aims at the replacement of fossil feedstocks by renewable feedstocks to obtain biobased products [5].

During the past years microalgae got increased attention as a renewable feedstock (third-generation) for food, feed, chemicals, and biofuel [6]. Microalgae are characterized by high areal yields and photosynthetic efficiency, the possibility to be produced on arid land, generally a year-round cultivation and a low water footprint [7][8]. An interesting feature of several microalgae species is the ability to accumulate various commodities like carbohydrates, lipids, proteins and pigments in high amounts. Currently, microalgae are already used for several applications like feed, bio-fertilizer, and as ingredient in several cosmetics and health foods for human consumption, due to their high content in polyunsaturated fatty acids and anti-oxidants [9].

Various reactor designs for algae cultivation are currently available. The reactor layouts are ranging from the classical raceway pond (RP) to tubular PhotoBioReactors (PBR) and flat panel PBR, to more sophisticated systems like the biofilm PBR and the foam PBR [10] [11] (figure 1-1). Shape and dimension of the used bioreactor strongly determine the achievable algae productivity on sunlight. A very important factor is the light path of the bioreactor. At systems with a short light path, it is possible that not all the light is absorbed by the algae. This leads to less optimal light usage and lower photosynthetic efficiencies. When systems have a very long light path, algae mostly respire and the biomass productivity will decrease when light is lacking too sustain the biomass. The optimal design therefore searches for the best combination of reactor dimensions in relation to the local light conditions and growth properties of the algae species.

Figure 1-1. Four different algae production systems operated at the research facility AlgaePARC at WUR. From left to right: (1) Raceway Pond; (2) Horizontal tubular PBR; (3) Vertical stacked tubular PBR; (4) and Flat panel PBR [12].

Although the number of studies on outdoor production of algae in experimental, pilot or commercial scale is increasing, there is still a lack of large scale facilities to cover the future demand of algae biomass [2]. Various life cycle (LCA), techno-economical, and resource assessment studies have evaluated the potential of commercial scale algae production for biofuel and biochemicals based on predictions of high areal yields [13]–[19]. Most of the published assessments used projections of algal productivity based on growth models extrapolated from laboratory-scale data or on non-validated models that have not been fully validated under outdoor conditions [20]. The uncertainty associated with the use of such productivity estimates is a significant concern because overestimating full-scale productivity would significantly bias the estimated cost-efficiency (i.e. overestimation of projected revenues) and environmental performance [21].

The life cycle, techno-economic, and resource assessments in literature are strongly influenced by wide range of assumptions regarding yearly areal productivity and composition of the algae mass, resulting in a large spread of end results [19] [21] [27] (table 1-1).

Study	Type of Study	Cultivation System	Location	Productivity [g m^{-2} day^{-1}]
[16]	LCA	PBR/open pond	UK	27.4
[19]	Microalgae Potential	PBR/open pond	NS	11.0 – 22.0
[18]	Microalgae Potential	PBR immersed in water basin	USA	4.4 – 14.8
[14]	LCA	Open Pond	Australia	30.1
[22]	Cost-Analysis	Open Pond	New Mexico	20.0 – 30.1
[23]	Techno-Economical Assessment	PBR/open pond	Japan	30.0 – 40.0
[24]	Cost-Analysis	Open Pond	NS	19.3 – 24.8
[25]	Techno-Economical Assessment	Open Pond	Guinea-Bissau; Spain; Sweden	4.7 – 14.8

Table 1-1. Areal productivity [g m^{-2} day^{-1}] found in different life cycle assessments, techno-economical assessments, cost analysis and literature focused on the potential of microalgae. Abbreviations: UK – United Kingdom; NS – not specified; USA – United States of America;

For example Wijffels et al. [19] used in their study an algae productivity in PBRs between 11.0 – 22.0 g m^{-2} day^{-1}, while Wang et al. [24] used a biomass productivity of 30.0 – 40.0 g m^{-2} day^{-1} in open ponds as a starting point, resulting in different outcomes. Other studies have based the evaluation of the geographical productivity potential on a conversion of solar irradiance to biomass using the photosynthetic efficiency [18]. In the study of Jonker et al. [25] a micro-algae growth model is introduced, which accounts for sunlight intensity, temperature and mixing. Further light conversion efficiency, photo-inhibition and dark respiration are taken into account. However, monthly irradiance is used as a model input, not representing the daily variations in light conditions. In addition, the model predicts algae growth by using a photosynthetic efficiency of 9%, which was never achieved during outdoor cultivation [26], and fails to incorporate cultivation architecture.

In order to predict algae productivity a large variety of empirical and mechanistic models for divers production systems have been developed [15] [17] [25] [30]–[36]. Since outdoor algae productivity is affected by many factors, such as weather conditions, characteristics of algae species, reactor design and operating conditions, models are very divers in the assumption that have been made. In the paper of Béchet et al. [33] 40 different algae growth modelling approaches are reviewed and compared to each other. All of the addressed models describe the relationship between algae and light intensity and make use of the Photo Irradiance (PI) curve. The PI curve gives the influence of light on the rate of photosynthesis (figure 1-2). Béchet et al. categorized the different models in three groups depending on how they address light intensity over the algae culture (incident, average or local), photo acclimation, photo saturation and photo inhibition [34]. Type I model are characterized by expressing the rate of photosynthesis of well-mixed cultures as a function of the average light intensity (I_{av}) over the culture, called "light integration" [35]. In this case the assumption is made that every cell experiences the same light conditions and hence has the same rate of photosynthesis. At the same time photo acclimation, photo saturation and photo inhibition is neglected.

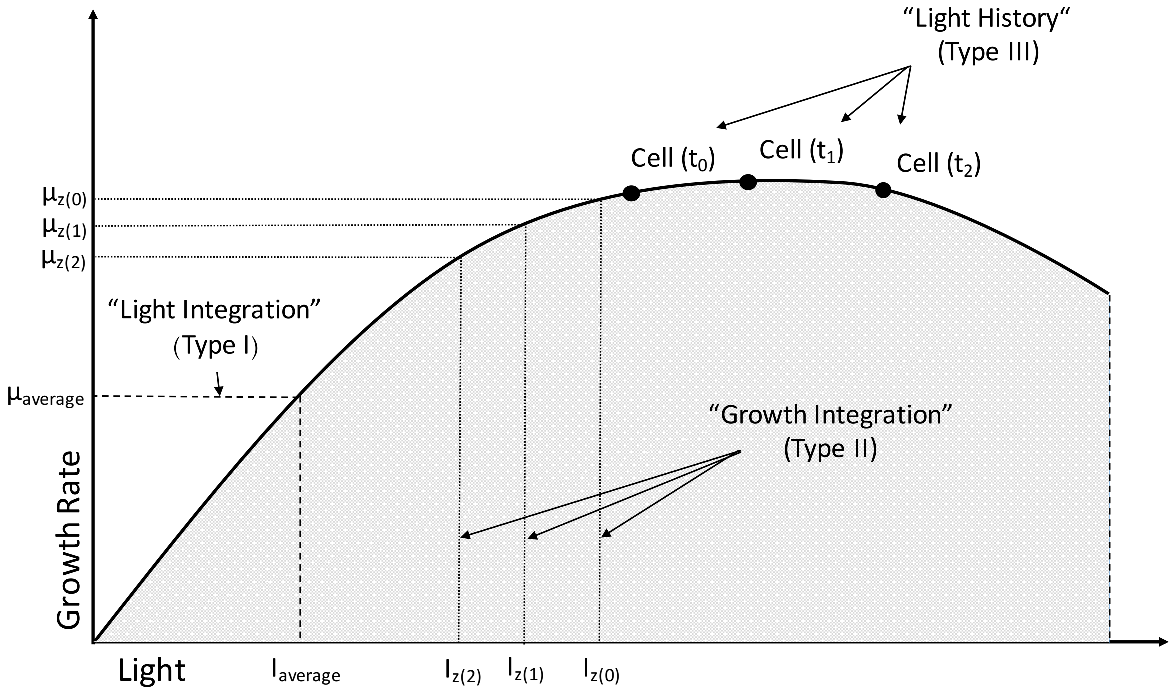


Figure 1-2. PI-Curve illustrating the three different model types. Type I: "Light Integration" uses the average light conditions over the cultivation system; Type II: "Growth Integration" uses a light gradient along the light path to calculate the local growth rate at reactor depth (z); and Type III: "Light History" focuses on single cells and the light fluctuations they experience at the time (t).

Type II model are taking the light gradient, which occurs within the broth, into account, called "growth integration". First the light distribution in the reactor is calculated, usually using Lambert-Beer law, and then the biological model expresses the local rate of photosynthesis as function of the local light intensity. Finally, the local rates of photosynthesis are summed up to obtain the global rate of photosynthesis. Type III model are the most complex approach to calculate algae productivity. In this approach the rate of photosynthesis of an individual cell is considered as a function of its "light history". A way to calculate the flow field in the reactor and the trajectory of the algae cell is the Computational Fluid Dynamics (CFD) [36] [37] [38]. The complexity of the model increases from type I to type III, which increases the capability of the model to describe outdoor production. However with increasing complexity more parameters are necessary for the calculation. This implements the increasing effort to gather the parameters and the risk of including noise of the measurements [34] [33].

Several studies validated algae productivity models with experimental data. However, the majority of the studies used data obtained from lab-scale experiments under continuous light conditions; often using artificial light sources [32]. For this reason the application of those models to field conditions is limited [20]. Only limited studies focused on validation of the model with outdoor productivity data.

Bosma et al. [35] predicted the volumetric algae production in a pilot-scale bubble column reactor under outdoor conditions using a regression model for the biomass growth equation. All light angles are used to derive the light path for Lambert-Beer law to predict the light gradient in the culture volume. However the regression-based equation makes it difficult to apply the model to light and temperature conditions outside the employed range of conditions. Pruvost et al. [39] developed a model to study different harvesting regimes in ponds and flat panels and included also scattering by algae cells. In addition, measured weather conditions are used and a two-dimensional light path is applied to Lambert-Beers law. However the effect of temperature on algal growth is neglected. Since temperature fluctuations can significantly influence productivity the application of this model to outdoor conditions is limited [40][27]. Quinn et al. [8] found a good fit for lipid productivity predictions compared to a commercial scale production system of Solix©. However, this model is limited to the specific production system and was only validated within a narrow temperature range of 19 – 26 °C. Since, culture temperature can vary up to 15 °C per day [41] the application of this model is limited as well. Recently, Béchet et al. [20] demonstrated a validation of an algae productivity model previously developed [41] using pilot-scale outdoor productivity data. The modelled productivity was compared with experimental pilot-scale data and a good fit was found. However, the short-term experiments for model parameterization introduce a considerable model uncertainty. Even though the models above described were validated against outdoor productivity data, these only consider one situation and one algae species and are not designed to predict productivity in various reactor designs. Therefore, future life cycle, techno-economic, and scalability assessments need models that consider the integration of geographically and temporally resolved biological growth modelling, which can be adapted to specific algae species and reactor types, in order to increase accuracy.

Slegers et al. [9] [23] [24] addressed this issue and developed a modelling framework, capable of predicting algae productivity for different algae species, locations, reactor designs and weather conditions. This framework was used in various scenario studies on reactor design, operational concepts and environmental conditions, and the performance of algae production, cultivation supply logistics and processing of biomass. Although the annual productivity predictions of the models are in the same range as annual productivity data found in literature, a further evaluation of the model framework is necessary [14][42].

In this study the productivity models that were developed by Slegers et al. [1]–[3] were validated with outdoor productivity data obtained at AlgaePARC. Validation with experimental productivity data is crucial to determine the model accuracy under outdoor conditions. In addition a successful validation will verify the use of these productivity models in future life cycle, techno-economic, and scalability assessments.

During this work several research questions are addressed:

- Which production parameter or variable is the most influential on algae productivity in the raceway pond and horizontal tubular PBR at AlgaePARC?
- How do the two productivity models of Slegers et al. [1]–[3] behave in respect to the different model inputs and their attributed uncertainties?
- How does the model fit improve to the obtained dataset at AlgaePARC when providing model inputs in different intervals?
- What is the accuracy of the model prediction and are there explanations for deviations between measured and predicted productivity?

2 Validation Approach

The validation approach taken in this thesis is presented in figure 2-1. Techniques used in other validation approaches found in literature (appendix E: table E-2) are taken into account and are applied if they are considered as suitable. The following steps (1. – 6.) are performed sequentially during the validation procedure of both productivity models and are categorized in four different groups: (1) data analysis (1. – 3. step); (2) model analysis (4. step); (3) comparison measured/predicted (5. step); (4) model evaluation (6. step).

1. Data Cleaning

For both systems a dataset obtained at AlgaePARC in 2014 is used as model input to compare the measured productivity with predicted productivity. Therefore, it is crucial to investigate if the dataset exhibits gaps and to detect possible measurement errors, for the successful validation the productivity models. Data gaps are filled according to the approach of Slegers et al. [2] where gaps smaller than 10 measurements are filled with the average of the two neighbouring measurements. Otherwise the missing dataset is replaced by data of a neighbouring day at the same daytime. Larger gaps than a day are excluded of the validation procedure.

2. Comparison of Measured/Modelled Productivity

Subsequently, the predicted productivity of the models, using location, reactor and algae specific inputs, is compared to the measured productivity. A statistical comparison is made based on relative deviation (%) between the value of measured and predicted productivity and visually analysed by using a parity plot. This plot is used in statistical validation [43] and was already used in the validation approach of Bosma et al. [35]. Possible explanations for deviations are identified with detailed weather information obtained from the weather station Veenkampen in Wageningen, and recorded operational problems during operation at AlgaePARC. Days that exhibit a large relative deviation (>±100%) and/or show an operational problem are excluded from model validation.

3. Statistical Evaluation

After the first comparison between measured and predicted productivities was made, a principal component analysis (PCA) and a bivariate correlation analysis (BCA) is performed to explore the cleaned data set and to understand the influence of production conditions on measured algae productivity. These methods are commonly used to analyse and identify patterns in large and complex data-sets [44].

4. Global Uncertainty/Sensitivity Analysis

A global uncertainty and sensitivity analysis is performed to investigate the influence of biological and physical parameters and variables on the model prediction. In the study of Quinn et al. [8] and Béchet et al. [20] the importance of a uncertainty/sensitivity analysis was stressed. Quinn et al. used a local analysis, neglecting interactions between the model inputs, and assumed a fixed uncertainty range for the model inputs of ±20 %. Béchet et al. utilized a global sensitivity analysis that considers interactions among model inputs. The uncertainty attributed with the parameterization of the model inputs was used in this sensitivity analysis. Since a global uncertainty/sensitivity analysis proofed to be superior, this analysis is taken in this thesis. The analysis used in this thesis is based on Monte Carlo Sampling demonstrated by Saltelli et al. [45]. The uncertainty range used is obtained from the error attributed with experimental determination of model input. For model inputs where no experimental data is available uncertainty ranges used in other sensitivity analyses found in literature are used.

(*5. Modelling Scenarios*)

After the determination of the influence of the model inputs and their associated variation, several model scenarios are carried out and compared on accuracy of prediction. The predictions of the different scenarios are compared visually based on cumulative productivity, as proposed by Béchet et al. [20]. Further, the outcome is analysed statistically in respect to relative deviation to the measured daily and cumulative productivities of a production period. Parallel to this the influence of model inputs on the relative deviation is investigated. This method was used by Bosma et al. [35] during validation of a model predicting volumetric algae productivity. In addition, the influence of the used time interval on the accuracy of the model prediction is evaluated.

(*6. Evaluation of Model Performance*)

Finally, the model scenarios that predicted productivity the most accurately for each type of photobioreactor are chosen and are further evaluated. The model performance is assessed including the model uncertainty obtained from the global uncertainty analysis. The model uncertainty of the raceway pond and the horizontal tubular system are plotted as upper and lower bound 95% confidence interval in the graphs displaying cumulative productivity. With this visual approach we can identify if the prediction inaccuracies are the result of the uncertainty in model inputs or due to model assumptions. In addition the fitness of the model for economic and environmental assessments is addressed, by comparing the prediction to results of different modelling studies and reported outdoor productivities.—At the end, possible improvements for future work are suggested.

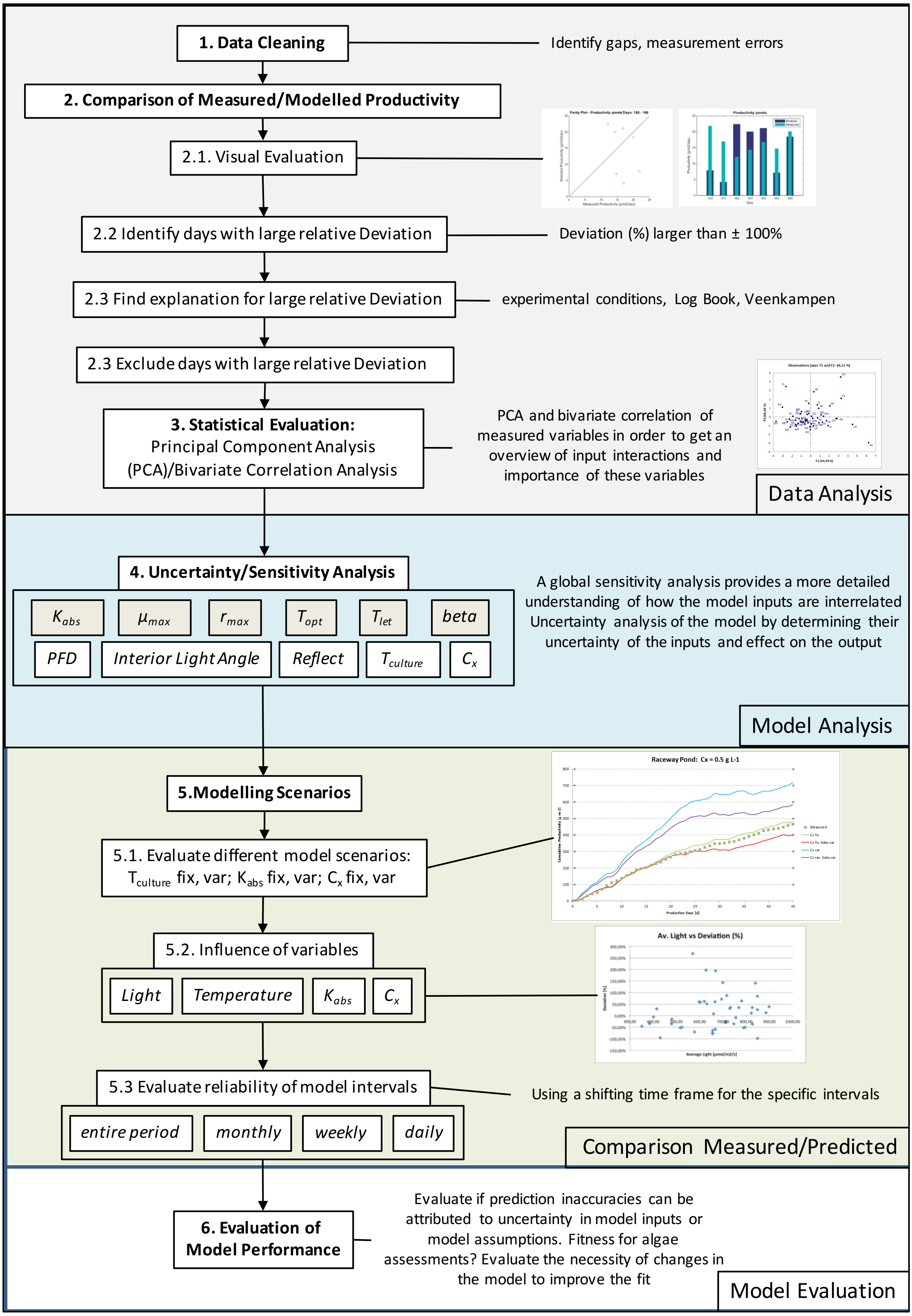


Figure 2-1. This is the step-by-step validation approach used in this thesis, which is categorized in four major parts: (1) Data Analysis, (2) Model Analysis, (3) Comparison Measured/Predicted, and (4) Model Evaluation. Abbreviations: K_{abs} – spectrally averaged absorption coefficient; μ_{max} – maximum specific growth rate; r_{max} – maintenance associated respiration rate; T_{opt} – optimum growth temperature; T_{let} – lethal culture temperature; beta – modulation temperature curve factor; PFD – photon flux density [µmol m^{-2} s^{-1}]; Reflect – surface and ground reflection; $T_{culture}$ – culture temperature; C_x – biomass concentration;

3 Material & Methods

In this part of the thesis the materials and methods, used to validate the algae productivity models of Slegers et al. [1]–[3] are presented. First, the pilot-scale outdoor production systems used at AlgaePARC are described. Followed by the introduction of the model framework of Slegers et al. [1]–[3]. Subsequently, the materials and methods used during the data analysis and model analysis are described. Finally, the comparison between measured and predicted productivity is discussed.

3.1 Production Systems

The productivity data from the following two algae production systems at AlgaePARC are used for model validation in this thesis: (1) open raceway pond and (2) horizontal tubular PBR. These design are pilot-scale photobioreactors, which occupy about the same ground area (25 m^2), installed at the same location (longitude: 5.66°; latitude: 51.99°) and are exposed to the same climatological conditions. The systems are controlled via a supervisory control and data acquisition (SCADA) system for automatic operation, online data collection and generation of alarms in cases of malfunctions. The algae production systems were operated in turbidostat, the biomass concentration is kept constant. When the biomass concentration exceeds the turbidostat set point a harvesting is started and new water and nutrients are added. Culture temperatures are controlled between a low and high set point (20 °C/30 °C), except for the raceway pond where cooling occurs via natural cooling by evaporation of water.

Figure 3-1. The two algae production systems operated at the research facility AlgaePARC at WUR. From left to right: (1) Raceway Pond; (2) Horizontal tubular PBR;

3.1.1 Raceway Pond

The dimensions of the raceway pond are 3 × 9 m (W× L) and is designed with central pillars at the side, which are connected with a plate to create a loop/raceway flow. The system is operated with a liquid level of 0.20 m resulting in a total volume of 4.7 m^3. During the operation period in 2014 the raceway pond is run in turbidostat with a biomass concentration of 0.5 g L^{-1}. During rainfall, liquid culture is automatically harvested to keep the liquid level at 0.20 m. Mixing of the algae broth is realized through a paddle wheel at 0.25 m s^{-1}. Temperature is measured and controlled by means of active heating via two heat exchangers. Since the lower range (20 °C) for the raceway pond cannot be kept due to limited heating capacity, the operation period is limited to late spring to early autumn. Further specifications can be found from table 3-1.

3.1.2 Horizontal Tubular PBR

The tubes used in the horizontal tubular PBR have an inner and outside diameter of 0.046 m and 0.05 m, respectively. A distance of 0.05 m between the tubes is used; implicating that only 50% of the ground area is covered in the horizontal system. This system consists of 3 loops, each of 80 m long [46]. Algae cultivation is performed in three different turbidostat regimes. A low biomass concentration of 0.75 g L^{-1}, a medium biomass concentration of 1.50 g L^{-1} and a high biomass concentration of 2.50 g L^{-1} are used. High dissolved oxygen (DO) could be growth inhibiting for algae [47]. Therefore oxygen produced by algae is removed continuously in a vertical column by the injection of ambient air. To prevent biofilm formation, elastomers (3-5 mm) are circulating with the culture broth to clean the system. Further specifications can be found from table 3-1.

Specification	Raceway pond	Horizontal tubular PBR
Light path [m]	0.20	0.046
Tubular length [m]	--	240 (3x80)
Volume [m^3]	4.73	0.56
Illuminated volume (%)	100	73
Ground area occupied (m^2)	25.4	27.0[a]
Illuminated surface A/V ratio [m^2 m^{-3}]	5	63.7
Biomass concentration [g L^{-1}]	0.5	0.75; 1.5; 2.5[b]

[a] including half of the ground area occupied by the dummies at northern and southern sides of the reactor
[b] the system was operated at three different biomass concentrations

Table 3-1. Specifications of the pilot-scale photobioreactors at AlgaePARC [46]

3.1.3 Production Organism

In both pilot-scale production systems the algae species *Nannochloropsis sp.* is cultivated during experiments at AlgaePARC. This unicellular seawater alga is categorized as an oleaginous species due to high accumulations of lipids found in the cell and therefore gains high interest as renewable resource for biodiesel production [6] [7] [50].

More detailed information on the pilot-scale algae production facility at AlgaePARC can be found in the study of Bosma et al. [46] and in appendix A.

3.1.4 Online Measurements

During operation of the production systems several online measurements are performed. A summary of the measurements used during this thesis can be found in table 3-2. Measurements for light and temperature are used as model inputs. The other measurements are used during data cleaning and comparison of measured and predicted productivity to assess the operational conditions. A detailed list of all the online measurements performed during cultivation and the manufacture of the sensors used can be found in table A-1 in appendix A

Measurements	*Unit*	*Interval*
Pyranometer	Direct and diffuse light	10 s
Temperature	°C	60 s
Turbidity	NTU	60 s
Water flow	$m^3 h^{-1}$	60 s
Water level	m	60 s
Carbon dioxide	$L kg^{-1}$	60 s
Recirculation flow[a]	$m^3 h^{-1}$	60 s
Airflow[a]	$m^3 h^{-1}$	60 s

[a] only tubular system

Table 3-2. Online measurements conducted during algae cultivation in the raceway pond and the horizontal tubular PBR at Algae PARC [46]

3.1.5 Offline Measurements

Besides the online measurements, offline measurements (table 3-3) are performed to determine the biomass concentration of the algae in the production system. Optical density measurements 750 nm were performed on a daily basis (samples taken between 09:00-10:00 am) to measure biomass concentration. In addition dry weight measurements are done three times a week (09:00-10:00 am) and the correlation between OD_{750} and dry weight is determined, which are used to calculate the turbidostat set point.

Measurements	*Unit*	*Interval*
Biomass Concentration (OD750)	--	daily
Biomass Concentration (DW)	$g L^{-1}$	3x per week
Absorption Coefficient	$m^2 kg^{-1}$	3x per week

Table 3-3. Offline measurements performed at AlgaePARC during algae cultivation in the raceway pond and the horizontal tubular PBR

3.2 Algae Productivity Models

In this study the mathematical models of Slegers et al. [1]–[3] are used to predict outdoor productivity of algae for two pilot-scale production systems at AlgaePARC: (1) raceway pond, and (2) horizontal tubular PBR. The productivity model uses location specific light angles, day lengths and reported direct and diffuse light intensities, reactor variables like geometry and wall material, ground material and algae characteristics. Further, detailed bio-physics-based models are applied to determine the light input on the reactor surface and the light gradient in the bioreactor. The local light intensity is then used to calculate the specific growth rate at a point along the light path.

The specific growth rate is calculated according to a modified version of the model developed by Geider et al. [51]. This growth model connects the photosynthetic activity of the algae cell to the local light intensity and irradiance dependent chlorophyll a: carbon ratio (equation 3-1). Since the chlorophyll a: carbon ratio in the cell θ_a ($g_{chl\ a}\ g^{-1}_{C}$) and the functional cross section of the photosynthetic apparatus α ($g_C\ (mol^{-1}_{ph})\ m^2\ g^{-1}_{chl\ a}$) are difficult to determine experimentally, the model was adapted to the needs at AlgaePARC; instead of the chlorophyll a: carbon ratio and the photosynthetic apparatus, the yield of biomass on photons ($Y_{x/ph}$) and the absorption coefficient (K_{abs}) were used in the productivity models [13] [14] (equation 3-2). The modified growth model used in this thesis is displayed in equation 3-3:

$$\mu_{growth}(z,t) = P_m^c\left(1 - exp\left(\frac{-\alpha\ I_{PFD}(z,t)\ \theta_a(z,t)}{P_m^c}\right)\right) - r_{max} \qquad \text{Equation 3-1}$$

$$\propto \theta_a(z,t) = Y_{x/ph}(t)\frac{K_{abs}(t)}{1000} \qquad \text{Equation 3-2}$$

$$\mu_{growth}(z,t) = P_m^c\left(1 - exp\left(\frac{-I_{PFD}(z,t)\ Y_{x/ph}\frac{K_{abs}}{1000}}{P_m^c(t)}\right)\right) - r_{max} \qquad \text{Equation 3-3}$$

Both the yield on biomass on photon ($Y_{x/ph}$) and the absorption coefficient (K_{abs}) can be determined experimentally for a specific algae strain. However, both parameters in the model were assumed to be constant, and thus neglect cellular acclimation of the algae to specific light conditions.

The maximum carbon specific rate of photosynthesis depends on the maximum specific growth rate μ_{max} (s^{-1}) and the maintenance coefficient as given by:

$$P_m^c(t) = \mu_{max} f_T(t) + r_{max} \qquad \text{Equation 3-4}$$

3.2.1 Modelling the influence of temperature on algae growth

Since algal growth is not only influenced by light, temperature can strongly influence algal growth. As temperatures in outdoor cultivations vary significantly it is important that the growth is modelled as a function of light and temperature. The model first established by Geider et al. [53] is fully light dependant and lacks the influence of temperature [52]. In order to include the effect of temperature in the model a temperature dependent factor (f_T) is introduced, which was first proposed by Blanchard et al. [54]. This approach was chosen by Slegers et al. [2] and avoids complex equations, which deal with nonlinear relations between light and temperature dependent growth.

$$f_T(t) = \left(\frac{T_{let} - T_{culture}(t)}{T_{let} - T_{opt}}\right)^{\beta_T} exp\left(-\beta_T\left(\frac{T_{let} - T_{culture}(t)}{T_{let} - T_{opt}}\right) - 1\right) \qquad \text{Equation 3-5}$$

where T_{let} (°C) is the lethal temperature, T_{opt} (°C) the optimal temperature, $T_{culture}$ (°C) the culture temperature and β_T (-) the curve modulating constant.

Further information on the productivity model can by found in the publications of Slegers et al. [1]–[3].

3.2.2 Modelling light attenuation

In order to calculate the light the algae receives at a certain culture depth the light path has to be determined. The law of Lambert-Beer is used to calculate the light gradient in the culture broth:

$$I_{PFD}(z,t) = I_{PFD,in}(t)e^{-(\varepsilon + K_{abs}C_x)z} \qquad \text{Equation 3-6}$$

where $I_{PFD}$is the local light intensity in [µmol m^{-2} s^{-1}] at a certain depth (z [m]), $I_{PFD,in}$ is the light intensity in [µmol m^{-2} s^{-1}] at the reactor surface, ε is the extinction coefficient of the culture broth, which is taken as 1 for seawater, K_{abs} is the spectrally averaged absorption coefficient [m^{-2} kg^{-1}]and C_x is the biomass concentration in the bioreactor [g L^{-1}].

3.2.3 Model Input

To validate the algae productivity models established by Slegers et al. it is necessary to provide the experimentally determined model parameters (e.g. absorption coefficient K_{abs}, dimensionless parameter for temperature fit β) and measured model variables (e.g. light and temperature) from the operating period of 2014. The specific biological model parameters for *Nannochloropsis* sp. were determined by previous studies carried out by De Vree [46] and Van Dam [34] at AlgaePARC. The absorption coefficient was obtained by taking the average of the measured values over the production period of the two different production systems, biomass concentration and run. As biomass concentration, the turbidostat set point or the average biomass concentration over a production run is taken. Location specific parameters and reactor specific parameters are known. In the case of biomass concentration and absorption coefficient the input is taken constant or variable depending on the model scenario used (find explanation later in the text). A summary of all model parameters used as model input can be found in table 3-4 and table 3-5.

Symbol	Description	Unit	Value
Algae Specific Parameters			
μ_{max}	Maximal specific growth rate	[d^{-1}]	0.81
r_{max}	Maintenance associated respiration rate	[d^{-1}]	0.084
$Y_{x/ph}$	Theoretical maximum yield	[g mol^{-1}]	1.175
β	Dimensionless parameter fit to moderate μ(T) curve	[--]	3.646
T_{opt}	Optimal growth temperature	[°C]	25
T_{let}	Lethal temperature	[°C]	38
Location Specific Parameters			
λ	Lambda = longitude East is positive West is negative	[degree]	5.66
Φ	Phi = latitude North is positive South is negative	[degree]	51.99
timezone	Time zone in which the location is in	[--]	UTC+1
Reactor Specific Parameters			
Raceway Pond			
w	Width of the raceway pond	[m]	3.0
L	Length of the raceway pond	[m]	9.0
d	Depth of the raceway pond	[m]	0.2
Horizontal tubular PBR			
d	Light path	[m]	0.046
l	Length of the tube	[m]	80
γ	Orientation of the reactor	[--]	N-S

Table 3-4. Model parameters used for validating the algae productivity models of Slegers et al. [1]–[3]

System	K_{abs}	C_x
	[m^2 kg^{-1}]	[g L^{-1}]
Raceway Pond		
Run 1	146.96	0.44
Horizontal tubular PBR		
Cx = 0.75 g L^{-1}		
Run 1	160.66	0.71
Run 2	121.81	0.95
Cx = 1.50 g L^{-1}		
Run 1	150.60	1.67
Run 2	175.70	1.54
Cx = 2.50 g L^{-1}		
Run 1	210.66	2.71
Run 2	213.34	1.87
Run 3	250.53	2.33

Table 3-5. Averaged absorption coefficient (K_{abs}), averaged biomass concentration (C_x) and average culture temperature ($T_{average}$) recorded during cultivation and used as model input for the different runs of the production systems at AlgaePARC.

The model variables for the different production systems, such as light, culture temperature, biomass concentration and absorption coefficient, are measured and provided for the year 2014 (table 3-6). Biomass concentration and absorption coefficient are used as constant and varying input, therefore they appear both as parameters and variables. The light data is obtained by a BF5 Sunshine Sensor by Delta-T Device Ltd. This sensor has three output channels: (1) Total (global) solar irradiation, (2) Diffuse irradiation and (3) Sunshine status; all measured on a horizontal plane. Since direct and diffused light is required as model input the direct light has to be calculated. Direct light is obtained by subtracting the diffuse irradiation from the total irradiation:

$$I_{direct} = I_{total} - I_{diffuse} \quad \text{Equation 3-7}$$

The radiation output of the sensor is set to PAR spectrum in photon flux density [μmol m^{-2} s^{-1}].

Symbol	Description	Unit	Value
I_{direct}	Direct light intensity	PAR, [μmol m^{-2} s^{-1}]	--
$I_{indirect}$	Diffuse light intensity	PAR, [μmol m^{-2} s^{-1}]	--
$T_{culture}$	Culture Temperature	[°C]	--
C_x	Biomass Concentration	[g L^{-1}]	--
K_{abs}	Spectrally averaged absorption coefficient	[m^2 kg^{-1}]	--

Table 3-6. Model variables, which are used to validate the algae production models by Slegers et al. [1]–[3]

3.2.4 Model Interval

Since the measurements of light and temperature are in different time intervals, 10s and 60s respectively (table 3-2), the minimal interval of the model input and therefore also the model output is set to 10 min. The measurements of light and temperature within the 10 min time frame are averaged over the period. As an example the averaging procedure for the culture temperature of the production system is presented:

$$T_{culture}(t) = \frac{1}{n}\sum_{i=1}^{n} T_{i,culture}(t) \quad \text{Equation 3-8}$$

where $T_{culture}$ is the averaged culture temperature [°C 10min^{-1}], $T_{i,culture}$ is the original measured culture temperature [°C min^{-1}], and n is the number of measurements during the model interval (10 min).

3.2.5 Model Output

The model predicts according to the model inputs algae productivity every 10 min. Since these precise predictions are not necessary and measured algae productivities are only available per day, the output of the model is chosen to be daily productivity predictions [g m^{-2} day^{-1}]. According to the requirement of the analysis on the prediction, the daily productivity predictions are summed up for 2 days, 3 days, weekly, monthly or the entire modelling period.

3.3 Data Analysis

3.3.1 Data Cleaning

For both systems the data set obtained at AlgaePARC in 2014 was used as model input and as well as experimental data set to compare the modelled algae biomass productivity with the biomass productivity. Therefore it is crucial to investigate if the data set exhibits gaps and to detect possible measurement errors, in order to successfully validate the algae productivity models.

Gaps and possible mismatches in the data are found by using developed function in Excel that detects gaps in a time series (appendix B). Gaps in the time series are filled in Excel in three ways: (1) larger gaps than 10 measurements are filled with data from a day that exhibited similar weather conditions. These days are selected by using weather information from Veenkampen and should be within the same month or season, preferably the day before or after the day that contains the data gap. (2) Smaller gaps (< 1 hour) are filled with the average of the data neighbouring data points. (3) Gaps larger than 24 hours are excluded from the validation procedure and values are set to 0. A detailed list of all gaps found in the obtained data from the three pilot-scale systems can be found in the appendix B (equation B-1).

As a first step of finding measurement errors daily productivity measured and predicted are compared to each other on the basis of relative deviation. Relative deviations bigger than 100% are investigated carefully on the operational and weather conditions. The measurements on light, temperature, biomass concentration, pH, and salinity, gave valuable insights into operation conditions. At AlgaePARC a logbook is kept where all operational problems, which occur, and actions, which are taken, during production are documented. In addition exact weather conditions for the production period from De Veenkampen, a weather station west of Wageningen, the Netherlands, were obtained. With the help of these sources the deviations between measured and predicted productivity can be explained and can be categorized into three different groups: (1) operational conditions (including weather), (2) operational problems, and (3) model related deviations.

Generally after inoculation the production system was run in a start-up phase where no consistent turbidity control and harvesting regime was applied. Daily productivity values in this phase of the production period were not taken for further analysis, since culture conditions were not stable and not representative.

3.3.2 Principal Component Analysis (PCA) and Bivariate Correlation Analysis (BCA)

Since the pilot scale system at AlgaePARC delivers a large variety of measured variables it is necessary to explore the obtained data. A step further to investigate the data set from the raceway pond and horizontal tubular PBR in more detail, is to perform a principal component analysis (PCA).

PCA is a way of identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences. Since patterns in multivariate data can be hard to find, PCA is a powerful tool for analysing data. The main objective of performing a PCA is to gain insight into the interactions between the different measured variables during cultivation. With this method measured variables during the algae production will be analysed.

In addition to the PCA a bivariate correlation analysis (BCA) between the measured variables and the calculated areal productivity was performed. BCA is the analysis of two variables to explore the association between them. The main aim of the BCA is to get more information on the effect of operational conditions on productivity.

The PCA and the BCA are applied to a series of measured variables: (1) Light (average, total), (2) temperature (average, minimum, maximum), (3) biomass concentration (measured), (4) absorption coefficient (measured), (5) productivity (measured), and (6) pH (measured). The list of measured variables can be found in table 3-7. Before these variables can be used for PCA their distribution had to be verified for normality using a Kolmogorov-Smirnov test. Variables, which are not significant for normality according to the Kolmogorov-Smirnov test, have to be excluded from further analysis.

Both analysis, the PCA and BCA, are performed in IBM SPSS Statistics® (version 22) using a build-in function of the software. A detailed description of the procedure and the settings used in SPSS are provided in appendix B.

Symbol	Description	Unit
P_{exp}	Measured algae productivity	[$g\ m^{-2}\ d^{-1}$]
$I_{average}$	Average light input	[$\mu mol\ m^{-2}\ d^{-1}$]
I_{total}	Total light input	[$\mu mol\ m^{-2}\ d^{-1}$]
$T_{average}$	Average culture temperature	[$°C\ d^{-1}$]
T_{min}	Minimum culture temperature	[$°C\ d^{-1}$]
T_{max}	Maximum culture temperature	[$°C\ d^{-1}$]
$Turb_{average}$	Average turbidity	[$NTU\ d^{-1}$]
$Turb_{min}$	Minimum turbidity	[$NTU\ d^{-1}$]
$Turb_{max}$	Maximum turbidity	[$NTU\ d^{-1}$]
C_x	Experimentally determined dry weight	[$g\ L^{-1}$]
K_{abs}	Spectrally average absorption coefficient	[$m\ kg^{-2}$]
pH	Power of hydrogen	[--]

Table 3-7. Measured variables, which are used for the Principal Component Analysis (PCA)

3.4 Model Analysis

3.4.1 Global Uncertainty/Sensitivity Analysis

Most of the model inputs are well known universal constants, but others are specific to the cultivation conditions of the experiments described in this study and are experimentally determined in situ. Experimental error on these specific parameters and variables may cause inaccuracy on model predictions. In order to get a better understanding on the behaviour of the model in respect to the different model inputs and their attributed uncertainty a global uncertainty and sensitivity analysis was performed. In contrast to the local uncertainty/sensitivity analysis, which uses one at a time variations around the operation point, the global analysis includes interaction between parameters inside a search space [55]. The sensitivity analysis is based on a Monte Carlo sampling which means that each parameter varies randomly between the given minimum and maximum (table 3-8). The ranges were either calculated from the standard deviation of the available experimental data or based on data found in literature [8], [12], [20]. The uncertainty ranges were used as inputs to determine the Saltelli-Sobol coefficients based on Saltelli [45]. The total Sobol coefficients quantitatively indicate the influence of each parameter on the variance of the predicted productivities and are a measure of the importance of each parameter on the model prediction, which is algae productivity. The analysis is performed using the modelling framework of Yao [55] developed in MatLab.

Symbol	Description	Unit	Value	Range
Biological Parameters/Variables				
K_{abs}	Spectrally averaged absorption coefficient	[m kg^{-2}]	-- [d]	± 12 % [a]
μ_{max}	Maximal specific growth rate	[d^{-1}]	0.81	± 8 % [a]
r_{max}	Maintenance associated respiration rate	[d^{-1}]	0.084	± 5 % [a]
β	Dimensionless parameter fit to moderate μ(T) curve	[--]	3.646	± 10% [b]
T_{opt}	Optimal growth temperature	[°C]	25	± 4 % [c]
T_{let}	Lethal temperature	[°C]	38	± 2.5 % [c]
C_x	Biomass concentration	[g L^{-1}]	-- [d]	± 8.5 % [a]
Physical Parameters/Variables				
I_{direct}	Direct light input at reactor surface	[μmol m^{-2} s^{-1}]	--	± 10 % [b]
$I_{diffuse}$	Diffuse light input at reactor surface	[μmol m^{-2} s^{-1}]	--	± 10 % [b]
R_{direct}	Reflection of direct light on reactor surface	[--]	--	± 10 % [b]
$R_{diffuse}$	Reflection of diffuse light on reactor surface	[--]	--	± 10% [b]
R_{ground}	Reflection of diffuse light on ground surface	[--]	--	± 10% [b]
ω_{direct}	Interior light angle of direct light	[degree]	--	± 10 % [b]
$\omega_{diffuse}$	Interior light angle of diffuse light	[degree]	--	± 10 % [b]
T	Culture temperature	[°C]	--	± 4 % [c]

[a] uncertainty range from experiments; [b] range after Slegers et al. [12]; [c] range after Béchet et al. [20]; [d] value dependent on production system used (table 3-5)

Table 3-8. Model parameters/variables chosen to use in global uncertainty and sensitivity analysis and the range in they vary for Monte Carlo Simulation

Parameters and variables chosen for the sensitivity analysis are divided into two categories: (1) biological inputs and (2) physical inputs. A summary of all used inputs can be found in table 3-8. The biological inputs for *Nannochloropsis sp.* were previously determined by experiments performed at AlgaePARC or obtained from literature [46] [34]. Therefore, it is crucial to investigate the importance of accuracy of the acquisition of those inputs for future application of the model to different algae species. The physical inputs chosen are related to the calculation of the light path and the culture temperature during production. The investigation on variables influencing the light path ranges from the primary light input at the reactor surface, to the reflection of light at the reactor surface, to the interior light angle in the culture broth.

First, the model inputs are investigated separately to observe interaction in the biological and physical part of the model individually. Later both categories of model inputs are investigated together to observe combinatory effects and to evaluate the importance of biological inputs over physical inputs.

More information on the global uncertainty/sensitivity analysis can be found in appendix C.

3.5 Comparison between measured and predicted productivity

3.5.1 Parity Plot

In order to evaluate the fit of the model, the parity plot is used as a visual method. In this graph the observed (y) daily productivity is directly plotted versus the predicted (ŷ) productivity, with the line y = ŷ marked to indicate the position of the 'perfect fit'. Within visual techniques, observed vs. predicted plots are shown to have superior diagnostic capabilities compared to the more widely-used time-series plots [43]. Bosma et al. used the parity plot for validating a model for volumetric algae productivity [35].

3.5.2 Cumulative Productivity

Next to daily areal/volumetric productivities, productivities are cumulated to evaluate the performance of the model over time. For this method the data obtained after the data-cleaning step are taken as a consecutive data set and the model productivity is summed up production day after production day. In this way the overall performance of the model can be investigated and trends are better visible. However, the model performance on a daily basis is not so apparent, since daily deviations between measured and predicted are hard to detect.

The cumulative productivity is calculated as following:

$$P_{cumul}(t) = \sum_{n=1}^{N} P_{day}\,(t) \qquad \text{Equation 3-9}$$

where P_{cumul} is the cumulative productivity over the production period [g m^{-2}], P_{day} is the daily productivity [g m^{-2} day^{-1}] and N is the number of production days [day].

3.5.3 Overall Accuracy

The overall accuracy (Δ) of the model was calculated from the mean absolute percentage error defined as follows [43]:

$$\Delta = \frac{\sum\left|P_{cumul,pred} - P_{cumul,exp}\right|}{\sum\left|P_{cumul,exp}\right|} \qquad \text{Equation 3-10}$$

where $P_{cumul,pre}$ and $P_{cumul,exp}$ (g m^{-2}) are the cumulative predicted and measured productivities in the outdoor reactors over the period of cultivation. The root-mean-square error (RMSE, in g m^{-2} d^{-1}) was also used to quantify the error on the daily productivity and was defined as follows [43]:

$$RMSE = \sqrt{\frac{1}{N}\sum P_{cumul,pred} - P_{cumul,exp}} \qquad \text{Equation 3-11}$$

where N is the total number of days and $P_{cumul,pre}$ and $P_{cumul,exp}$ are the predicted and experimental cumulative productivities (g m^{-2} d^{-1}).

3.5.4 Influence of Model Input on Overall Accuracy

Originally the two productivity models incorporate the steady state conditions of cultivation. For the raceway pond, biomass concentration is kept constant and culture temperature is modelled using local climatological data. For the horizontal tubular system both biomass concentration and temperature were assumed to be constant over the production period. For both systems the absorption coefficient in the growth model adapted for AlgaePARC (equation 3) was taken constant, neglecting photo acclimation [1]. From the online measurements on temperature and the offline biomass concentration measurements show that values for temperature, biomass concentration and absorption coefficient vary significantly over time. Temperature is a very dynamic variable, which can vary up to 10 °C per day. Biomass concentration and absorption coefficient are deviating in a more narrow range, but exhibit daily variations depending on irradiance and temperature. In order to evaluate the improvement of accuracy of the model on a daily basis, certain model input are changed from constant to varying inputs. The recorded data at AlgaePARC on biomass concentration, temperature and absorption coefficient are taken as new inputs for the model. Temperature measurement every 10 minutes are takes as input, while biomass concentration and absorption coefficient are taken as daily inputs. Since biomass concentration measurements are only performed 3 times per week and always in the morning of a production day, missing values for the simulations were filled with the average value over the whole production period. The model performance on each of these runs is evaluated separately with various scenario settings. Predictions with fixed and varying biomass concentration, fixed and varying culture temperature and fixed and varying absorption coefficient and every combination between them are considered.

The temperature regime at the raceway pond is not changed, since assuming constant culture temperature was not reasonable due to large temperature fluctuations over the production run. To evaluate the performance of the model of the horizontal tubular system several different model scenarios were done. Different model input options for biomass concentration, culture temperature and absorption coefficient are considered. As biomass concentration input three different values are used: (1) the biomass concentration of the turbidostat set-point, (2) the averaged biomass concentration over the production period and (3) the biomass concentration values obtained from the DW-measurements. In respect to culture temperature the averaged temperature over the production period and the temperature measured online while production was used as a model input. Further the averaged absorption coefficient over the production period and the measured absorption coefficient of *Nannochloropsis* sp. was used for evaluation. An overview of all the modelling scenarios, which are conducted, can be found in table 3-9.

The results of these scenarios are compared visually based on cumulative productivity and statistically in respect to relative deviation to the measured daily and overall cumulative productivities of a production period.

Production System	Scenario	C_x	$T_{culture}$	K_{abs}
Raceway Pond (RP)				
	1	fixed	measured	fixed
	2	fixed	measured	measured
	3	measured	measured	fixed
	4	measured	measured	measured
Horizontal Tubular PBR (HT)				
	1	fixed	fixed	fixed
	2	fixed	measured	fixed
	3	measured	fixed	fixed
	4	measured	measured	fixed
	5	fixed	fixed	measured
	6	fixed	measured	measured
	7	measured	fixed	measured
	8	measured	measured	Measured

Table 3-9. Modelling scenarios used for the raceway pond and the horizontal tubular PBR in order to evaluate the model fit with changing model inputs. Abbreviations: C_x – biomass concentration; $T_{culture}$ – culture temperature; and K_{abs} – spectrally averaged absorption coefficient; fixed – model input is taken constant over the production period; measured – the measured value obtained during production is taken as model input with an interval: C_x (daily), $T_{culture}$ (10 min), K_{abs} (daily);

3.5.5 Influence of measured variables on relative deviation

Since light, temperature and biomass concentration are variable, it is interesting to investigated how the model prediction perform in comparison to the measured productivity at the pilot-scale algae production systems. The productivity models will eventually over- or underestimate measured daily productivities. In addition the correlation of the relative deviation between measured and predicted productivity on these variables is investigated. In this way the model behaviour is explored and it is researched if this behaviour can be address to a specific part of the model, which is receiving light, temperature and biomass concentration as input. In order to evaluate the correlation, the relative deviation is plotted against one of the variables (biomass concentration, temperature, light) as demonstrated by Bosma et al. [35].

4 Results and Discussion

In this part of the report the results of this thesis are presented and discussed. First the data analysis is presented consisting out of a data cleaning step to find gaps and measurement errors, followed by a principle component analysis (PCA) and a bivariate correlation analysis in order to explore the data set obtained at AlgaePARC. Subsequently, the model predictions are compared to the measured productivity of the pilot scale production systems and explanations for deviation are searched. A global uncertainty and sensitivity analysis is performed to investigate the impact and importance of biological and physical input parameters on the model output. With this knowledge several model scenarios are carried out and compared to each other in terms of accuracy of their prediction. Parallel to this the influence of model inputs on the relative deviation between measured and predicted daily productivity is investigated. Finally, the model that predicted the most accurate algae productivity in the given situations is chosen and discussed in detail.

1.1 Selecting Data for Model Validation

The data set of the raceway pond contained 37 gaps, and the data set of the horizontal tubular system had 43 gaps. Filling procedures were applied according to the description in materials/methods, where smaller gaps (<1 hour) were filled with the value before and after the event, and bigger gaps were replaced with data from a day exhibiting similar weather and culture conditions. Larger gaps than 24 hours were left out of the validation procedure, which led to a total amount of 62 days and 148 days for the raceway pond and the horizontal tubular PBR, respectively.

As a first step to identify measurement errors measured productivity and predicted productivity were compared to each other on the basis of relative deviation (%). With the knowledge on weather conditions, operational conditions and operational problems the deviations could be categorized in three different groups: (1) operational conditions (including weather), (2) operational problems, and (3) model related deviations (figure 4-1).

Operational conditions and operational problems strongly influenced the daily measured productivity outcome. During production the turbidity signal could show unstable values, influencing harvest of algae broth. In the raceway pond, high light intensities, culture temperatures and rain had the biggest influence on biomass fluctuations. High light intensities and culture temperature led to evaporation and thereby concentration of the algae broth, while rain led to a dilution of the culture broth. Dilution and evaporation of the algae broth also resulted in fluctuation in salinity, nitrate and other nutrients, affecting algae growth. Since the productivity models assume optimum growth conditions the predicted productivity could be significantly different from the measured one.

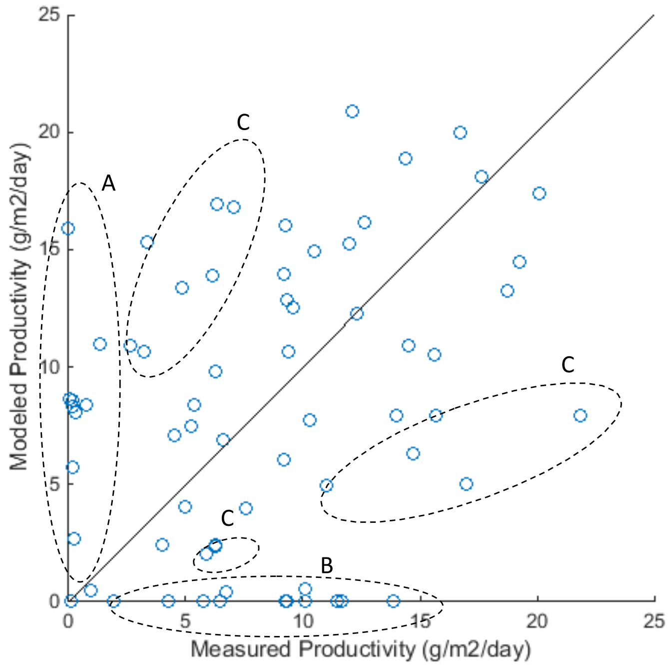


Figure 4-1. Parity plot of the measured against the predicted daily productivity of the raceway pond in between day 150 and 227. Marked comparison of measured and predicted productivity (encircled by dashed line) are excluded from further analysis. A – measurement errors due to operational problems; B – model related errors; C – errors due to operational conditions.

In the horizontal tubular system fluctuations in turbidity are mainly addressed to biofilm formation in the tubing of the system. In addition biomass concentration varied significantly at days with high light intensities and temperatures close to the lethal temperature of *Nannochloropsis* sp. As a counter measure for biomass these fluctuations the turbidity set point was adapted to the current situation to sustain the set biomass concentration in the culture broth. However, at several points during production the horizontal tubular system had to be stopped and cleaned properly to restart cultivation.

Even though the turbidity sensor was cleaned daily for the raceway pond and weekly for the horizontal tubular PBR, biofilm formation was an issue during measurements. Formation of biofilm at the sensor led to higher measured turbidity values and could lead to an earlier harvesting procedure, which distorted the measured daily productivity by underestimating the value of the day where biofilm formation occurred. On the other hand daily productivities could be overestimated when the control software (SCADA) did not initiate harvesting, even though the turbidity exceeds the turbidity set point. In other cases the volume that had to be harvested, exceeded the volume that could be harvested in the maximum harvesting time set in the control system. This resulted in a harvesting time alarm. A common action that had been taken at AlgaePARC was to reset the SCADA software in order to restart harvesting. Such an event could bias the daily productivity as well, since a delay in harvesting could have occurred.

Further, a reason for large deviations between measured and predicted biomass productivity was the behaviour of the model at unfavourable growth conditions. At these days, the solar input was not enough to sustain the culture, as respiration was larger than growth and therefore for some of these days the model calculated a negative daily biomass production. Similar trends were seen in the scenario study of Slegers et al. [1]–[3] on the raceway pond, tubular PBRs and vertical flat panel PBRs for France and the Netherlands [3,4]. However compared to the measured daily productivity of the same days in most of the cases a slightly positive productivity was recorded. There are two explanations: Firstly, that biology of algae could handle harsher conditions in terms of low light in comparison to the assumption made in the model. Since light adaptation was not included in the model and a constant absorption coefficient is used, algae are assumed to be light limited at higher light intensities. Secondly, the effect of temperature on algae growth could be responsible for this deviation. Beta (β_T) is a value, which is fitted to experimental data obtained under continuous light and temperature (24 hours). However the culture temperature can increase or decrease within an hour significantly. Therefore the adaptation dynamics of biology to constantly changing temperature is not included in the temperature model. This simplification of biology can as well create a significant difference between the model prediction and the observed productivity.

Days that exhibited the conditions or problems described above and resulted in a relative deviation larger than 100 % were excluded from validation. Since this resulted in very fractured data set, the production days during validation were treated as if they would be consecutive. An extensive list of all the days, which were excluded and the reason for doing so can be found in the appendix B. An overview of the amount of available days for further data analysis and validation of the productivity models of each system and run is given in table 4-1.

System	Days Available	Days Excluded	Days Used
Raceway Pond			
C_x = 0.5 g L^{-1}	71	26	45
Horizontal Tubular PBR			
C_x = 0.75 g L^{-1}	30	6	24
C_x = 1.50 g L^{-1}	90	29	61
C_x = 2.50 g L^{-1}	43	14	29
Total	163	49	114

Table 4-1. Overview of the available data of the different production systems and the number of used days for validation

Important to note is that the data selection procedure used during validation might have an influence on the outcome of the study. The criteria used were chosen to the best of our knowledge, but could differ from researcher to researcher and may bias the result of every further analysis performed. Therefore it is crucial to be critical about the steps taken during this thesis.

4.1 Investigation of Trends and Patterns in the Data Set obtained at AlgaePARC

After selection of the dataset, a PCA was performed to investigate on trends and patterns in the data. In addition a BCA was conducted to examine the correlation of productivity to system variables. Since the results obtained from both analyses are similar and support one another they are presented together for each production systems. The analyses generally showed commonly known influences of cultivation conditions on algae growth. However the analyses also revealed operation conditions, which limited productivity during the cultivation period. A graphical representation of the PCA and BCA can be found in appendix B.

4.1.1 Raceway Pond

The PCA of the raceway pond resulted in two principal components with a total variance explained of 74.6 %. The first principal component is associated with "light", since both average light and total light input, the absorption coefficient and productivity are attributed with the first column of the component matrix. The second principal component is associated with "temperature", since both average and minimal temperature, average turbidity and measured biomass concentration are in the second column of the component matrix. From the PCA and the correlation factors of the BCA it is shown that the measured productivity exhibit a positively correlation with light and temperature. Further, the maximal temperature is positively correlated with light and productivity, which is due to the reason that productivity increased on days with higher culture temperatures and culture temperature was increased at higher light intensities.

The PCA and BCA show that biomass concentration and productivity is positively correlated with temperature. Therefore an increase in temperature generally led to an increase in biomass concentration and productivity during cultivation. This relation can be explained due to the reason that the average culture temperature over the production period was lower than the optimal growth temperature of *Nannochloropsis* sp. Therefore days with higher average, minimal or maximal temperatures are associated with higher productivities. Biomass concentration however showed a negative relation to productivity, leading to the conclusion that the system exhibited a too high biomass concentration during production. Since the raceway pond has the longest light path (0.20 m) the effect of biomass concentration on productivity was strong. The optimum biomass concentration for *Nannochloropsis sp*. in the pilot scale raceway pond at AlgaePARC was found to be at 0.15 g L^{-1} using the algae productivity model by Slegers et al. [3] (Appendix D: figure D–2). Below the optimal biomass concentrations excess sunlight energy could not be employed for growth, while self-shading of algae as a consequence of too high biomass concentrations resulted in enhanced cellular respiration [3]. The negative correlation of biomass and productivity indicates that at a biomass concentration of 0.5 g L^{-1} light was could not reach the lower layers of the culture and algae growth was thus limited by the amount of light.

4.1.2 Horizontal tubular PBR

The PCA and BCA of the horizontal tubular system were performed on the data sets for different turbidostat operations (0.75 g L^{-1} (low), 1.50 g L^{-1} (mid), 2.50 g L^{-1} (high)). In every case the first principal component is dominated by light and temperature, while the second principal component is associated with productivity and biomass concentration. Light and temperature exhibit the strongest positive correlation in all production runs (appendix B), since culture temperature was increasing with the light input significantly during operation. This effect is addressed to the smaller culture volume and the stronger influence of light energy on temperature compared to the raceway pond.

However, the PCA and the BCA resulted in divers observations in regard to the interactions between the measured variables at different turbidostat operations. The production at 0.75 g L^{-1} and 1.5 g L^{-1} exhibited similar behaviour, since similar weather conditions are recorded. Whereas, the production at 2.5 g L^{-1} showed different correlations in between the measured variables. The production run was during mid-summer and resulted in a higher average culture temperature and higher average light intensities compared to the other two production scenarios.

Measured productivity at low and mid biomass concentration shows a positively correlation with light, temperature and biomass concentration. Generally with decreasing absorption coefficient the productivity increased and they were similar negatively correlated as observed at the raceway pond. However at the highest biomass concentration the productivity appeared negatively correlated with light and temperature. As discussed above light had a strong influence on the culture temperature of the system. At several occasions the cooling system of the horizontal tubular system was not able to keep the upper temperature boundary (30 °C). This led to temperatures close to the lethal temperature of *Nannochloropsis* sp. Generally the culture temperature was higher than the optimal growth temperature leading to higher productivities at lower average temperature.

Since the light path of the horizontal tubular system is shorter than in the raceway pond, a change in biomass concentration did not exhibit a strong effect on productivity as compared to the raceway pond. At the low biomass concentration the productivity increased with biomass concentrations, while at the middle biomass concentration a change in biomass concentration did not affect productivity significantly. At the highest biomass concentration the adverse effect was observed, since higher productivities were generally associated with lower biomass concentration. This led to the conclusion that the optimal biomass concentration for the horizontal tubular system must be located in the near surroundings of 1.5 g L^{-1}. With the aid of the productivity model [1] the optimal biomass concentration for the horizontal tubular system was found to be at 1.25 g L^{-1} (appendix D: figure D–3).

4.2 Model Sensitivity to Uncertainties in Model Inputs

The results of the global uncertainty/sensitivity analysis for both systems are shown in figure 4-2. The model inputs are categorized into biological related (green) and physical related (blue) inputs. The bars represent the calculated Total Sobol-Coefficients, which are indicating the impact of the uncertainty of a parameter on the uncertainty of the model output. From the analysis the model inaccuracy, caused by the uncertainty attributed to the input, is quantified. Further, the reduction of the model inaccuracy by addressing uncertainties in the model inputs is evaluated.

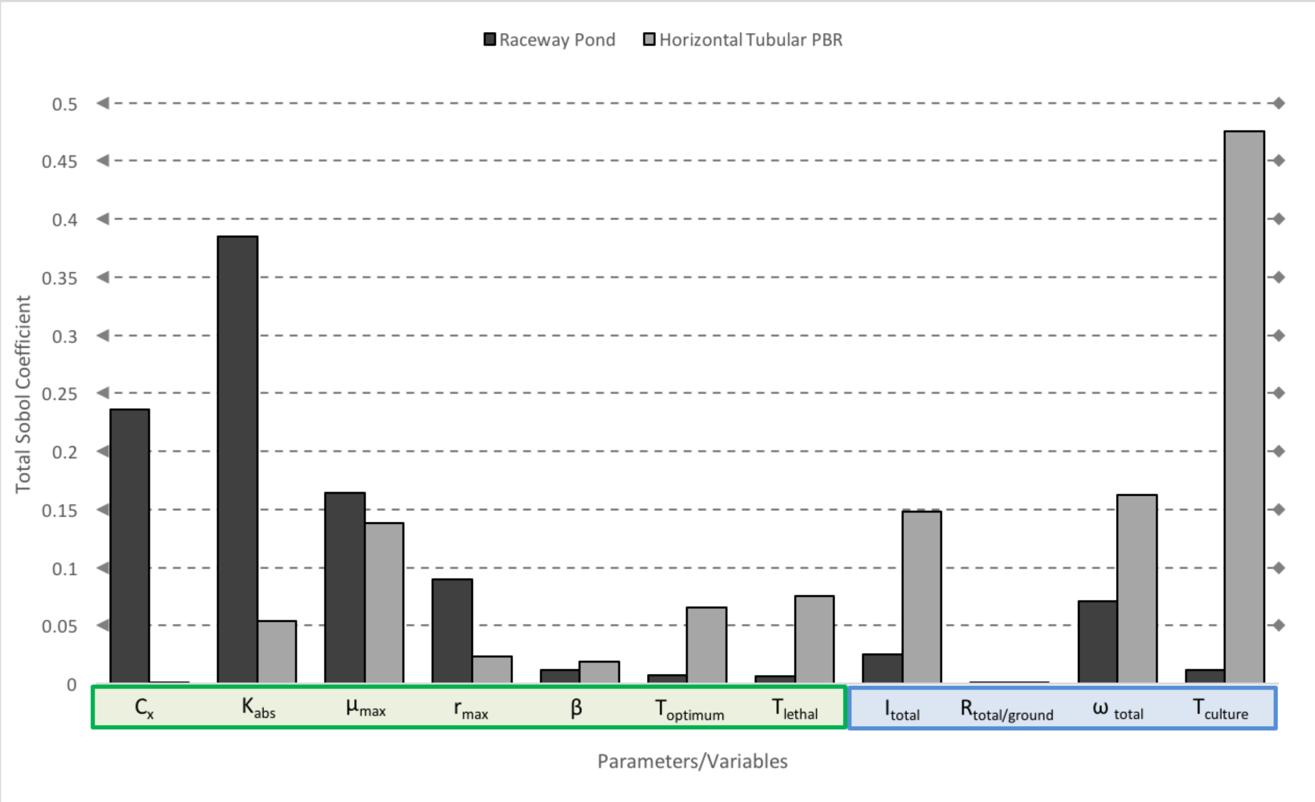


Figure 4-2. Total Sobol Coefficients of the global sensitivity analysis. Parameters/variables in the green box are associated with biology and parameters/variables in the blue box are associated with physics. Abbreviations: C_x – biomass concentration; K_{abs} – spectrally averaged absorption coefficient; μ_{max} – maximum specific growth rate; r_{max} – respiration rate; β_T – temperature curve modulating factor; $T_{optimum}$ – optimum growth temperature; T_{lethal} – lethal temperature; I_{total} – total light input; R_{total} – reflection of total light on water surface; R_{ground} – ground reflection of total light; ω_{total} – interior light angle of total light; $T_{culture}$ – culture temperature;

4.2.1 Raceway Pond

The model inputs contributing the most to the output of the productivity model of the raceway pond are: the spectrum-averaged light absorption coefficient (K_{abs}), biomass concentration (C_x), maximum specific growth rate (μ_{max}), respiration rate (r_{max}) and the interior angle of total light (ω_{total}). These biological parameters are very influential since the algae productivity in the raceway pond is highly determined by the growth characteristics of the algae. The absorption coefficient influences the available light in the calculation of the light gradient and the usage of photons along the light path. In addition the biomass concentration plays a major role in the calculation of the light attenuation (equation 3-6) along the light path and therefore strongly influences the algae productivity. Therefore a lower value for K_{abs} and C_x results in a further penetration of light in the culture and will therefore result in higher growth/productivity. Further the total light input (I_{total}), the interior light angle of total light, which determine the length of light path and therefore the light intensity the algae receives, show the highest influence on the model output from the physical model inputs investigated. Further, the temperature model, including beta (β_T), optimum growth temperature ($T_{optimum}$), lethal temperature (T_{let}), exhibits a considerable influence on the model output. However, compared to light related model inputs the temperature related model inputs are less influential. The reflection of light on the water surface of the raceway pond exhibit the lowest Sobol-coefficients and thereby the lowest contribution to the model output. Generally, the biological parameters are explaining the majority of influence on the model output of the raceway pond. The physical aspects are most related to light transfer than to temperature. However, the physical aspects are still of secondary importance for the model output, since light has a stronger effect on algae biology than temperature [56].

From the global uncertainty/sensitivity analysis it resulted that the uncertainty of the model inputs led to an overall model accuracy of ±14.62 %. Further the analysis showed that by addressing the uncertainty in the measurements of the absorption coefficient, maximal specific growth rate and respiration rate had the potential to increase the accuracy of predicted biomass productivity with 83%. However this model uncertainty is expected to decrease significantly, when light conditions are not limited for algae growth. By either reducing the biomass concentration or operating the system under the same regime at a location with elevated irradiances, would notably reduce the impact of the absorption coefficient and biomass concentration on the model output.

4.2.2 Horizontal tubular PBR

The analysis of the horizontal tubular system shows that the model output is the most influenced by physical inputs like culture temperature, total light input and interior light angle. The biological inputs have generally less impact on the output and are dominated by the maximum specific growth rate, the optimum growth temperature and the lethal temperature. The strong influence of temperature on the model outcome is explained by the on average higher culture temperatures compared to the raceway pond and the higher sensitivity of the productivity model to these elevated temperatures (appendix D: figure D-1). This is also shown by the increased importance of the biological parameters (β_T, $T_{optimum}$, T_{lethal}) associated with the temperature calculation, which were of minor importance in the raceway pond. Light related inputs such as the total light input and the total interior light angle of the total light input proof to be the second most influential factors in the productivity calculation. These model input determine the light path and the amount of light the algae receives during cultivation and therefore exhibit this strong relationship. However the reflection of diffuse light at the ground surface (R_{ground}) did not affect the model output strongly, which is comparable to the reflection of total light at the water surface of the raceway pond.

The maximum specific growth rate showed a similar influence in both production systems. However, the absorption coefficient and the respiration rate had a comparably small impact on the model output in comparison to the raceway pond. This difference is explained due to the geometry of the two reactor systems. Since, the horizontal tubular PBR has a smaller light path, the formation of dark zones in the culture broth is reduced. Thereby the importance of the absorption coefficient and the influence of dark respiration are diminished. The same applies for the biomass concentration, which strongly determine the light distribution and the light that the algae receive. The short light path makes the effect of the biomass concentration on the algae productivity negligible compared to the other parameters and variables investigated.

The global uncertainty/sensitivity analysis shows that the effect of the uncertainty attributed with the model inputs led to an overall model accuracy of ±11.27 %. Further, from the analysis it follows that addressing the uncertainty in the measurement of the culture temperature, the total light intensity and interior light angle, the growth rate and respiration rate has the potential to reduce the variance in predicted biomass productivity with 89%. Since temperature variations can be of higher order at locations with increased irradiances, the model uncertainty can be elevated as well. Due to the high sensitivity of the productivity model to temperature fluctuations, it is important to further evaluate the temperature model before modelling scenarios are performed for southern locations, e.g. Italy, Spain, Algeria.

The global sensitivity analysis gives valuable insights into the differences between the two algae productivity models. While the model output for the raceway pond is highly influenced by the accuracy of biological parameters/variables such (K_{abs}, C_x, μ_{max}, r_{max}), the model output of the horizontal tubular system is highly dependent on the physical input temperature that influences the temperature model. This information provides useful knowledge what model inputs have to be determined precisely and what part of the model should be investigated in order to improve the model fit. For further analysis we focussed on the effect of the absorption coefficient and biomass concentration for the raceway pond and temperature for the horizontal tubular PBR,

4.3 Effect of used model input interval on overall accuracy

For every production run different operational regimes were applied and every run experienced different climatological conditions. The total light input, average temperature, average biomass concentration and absorption coefficient in the two systems during the various runs can be found in appendix A (table A-2). Originally the two productivity models were designed to incorporate the steady state conditions of cultivation. In the case of the raceway pond, biomass concentration was kept constant and culture temperature was either kept constant or modelled using local climatological data. In the case of the horizontal tubular system both biomass concentration and culture temperature were assumed to be constant over the production period. In the used productivity models the absorption coefficient was assumed constant for both systems neglecting photo acclimation. From the online measurements on temperature and the biomass concentration measurements in the morning of a production day showed that values for temperature, biomass concentration and absorption coefficient vary significantly over time (appendix A).

The global sensitivity analysis showed that absorption coefficient and biomass concentration had a large impact on prediction of algae productivity for the raceway pond, and temperature in the prediction for the horizontal tubular system. Therefore, it was investigated if by using measurements from the production period as model inputs the accuracy of the model on a daily basis could be improved. The model inputs such as biomass concentration, absorption coefficient and culture temperature (only horizontal tubular system) were addressed. The model performance for each production run was evaluated using various scenario settings (table 3-9). Predictions with fixed and varying biomass concentration, fixed and varying culture temperature and fixed and varying absorption coefficient and every combination between them were evaluated. The results of these scenarios were compared in respect to relative deviation to the measured daily and cumulative productivities of a production period.

4.3.1 Raceway Pond

The scenario evaluation of the raceway pond is illustrated in figure 4-3. Four different scenarios are displayed; two for fixed and varying biomass concentration and two for fixed and varying absorption coefficient. From the graph we see that the biomass concentration had the biggest influence on the model prediction. This was already indicated by the global uncertainty/sensitivity analysis. In the first scenario both biomass concentration (set at 0.50 g L^{-1}) and absorption coefficient (146.96 m^2 kg^{-1}) were used as model input. An accurate estimation of algae productivity was made with an overall relative deviation of 3.23 %. When the measured biomass concentration was used, the model overestimated the productivity by more that 50 %. Biomass concentrations were determined on samples taken in the morning; due to night biomass loss this value can deviate from the set point for harvesting. The model predicts higher growth at lower biomass concentration, since the dark zone is smaller, resulting in an overestimation of the productivity. The optimal biomass concentration, 0.15 g L^{-1}, for the raceway pond was calculated by the productivity model [3]. Consequently, using biomass concentrations lower than 0.5 g L^{-1} will result in higher algae productivity predictions.

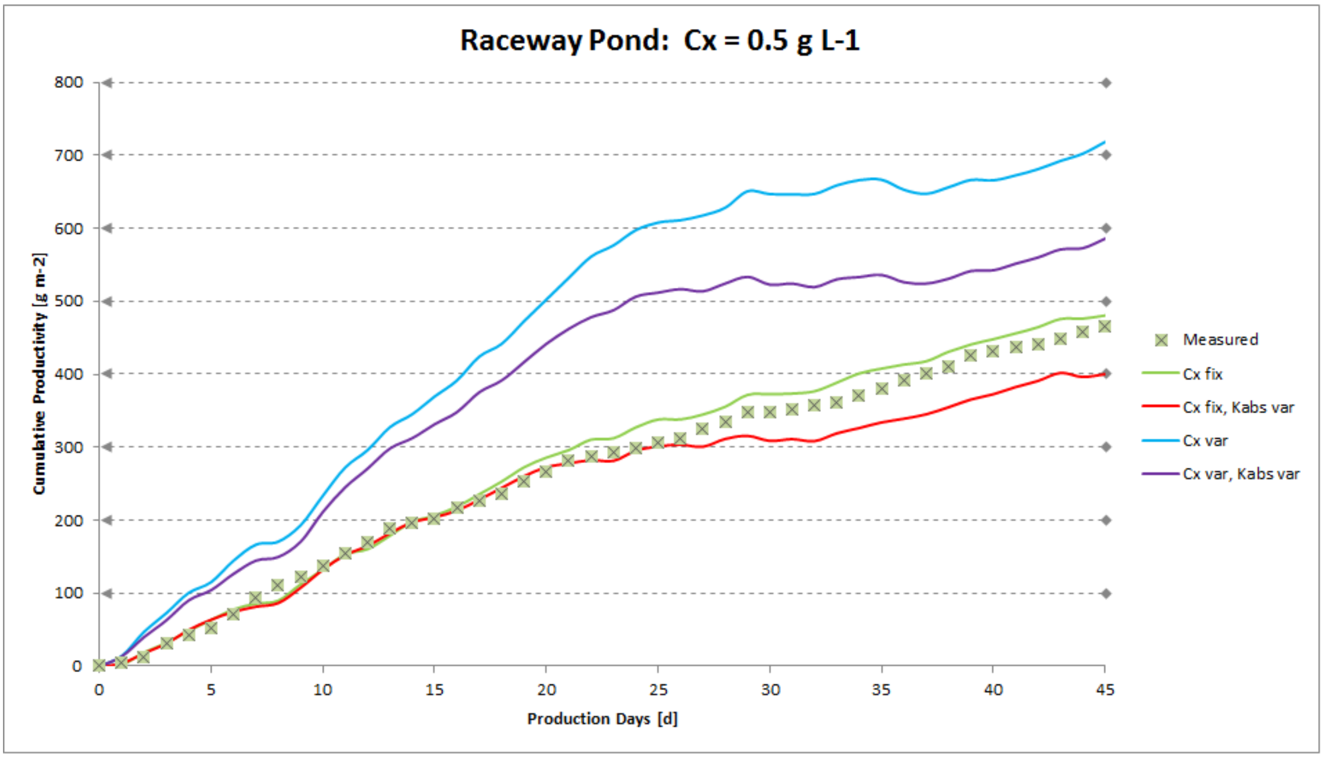


Figure 4-3. Different scenarios for the raceway pond expressed in cumulative production [g m^{-2}]. Abbreviations: Cx fix – fixed value for biomass concentration, Cx var – measured biomass concentration, K abs – fixed value for absorption coefficient, K abs var – measured absorption coefficient.

When implementing the measured absorption coefficient as model input in both scenarios with fixed and varying biomass concentration the algae productivity prediction was lower than using the fixed value. In the majority of the modelled production period the values of the measured absorption coefficient were close to the mean value of 146.96 m^2 kg^{-1}. However, in between production day 25 and 33 and after day 43 the measured absorption coefficient values were often higher than the mean. This could be explained due to climatological conditions. Within this period light intensities were higher than at other days. Therefore the algae was adapting to the new environmental conditions by changing their pigment content, resulting in a higher value for the absorption coefficient.

The highest accuracy of the model was found when using the turbidostat set point for biomass concentration and the averaged absorption coefficient over the production period as input. Implementing measurements for those two model inputs resulted in larger deviation from the measured productivity. The sensitivity analysis showed that both inputs have a very large influence on the model prediction, making the accuracy of the prediction low. However for fully evaluating the implementation of varying biomass concentration and absorption coefficient more data on both variables has to be obtained.

4.3.2 Horizontal tubular PBR

In figure 4-4 the results of the scenario evaluation are depicted for different turbidostat operations and runs. Four of the initially eight scenarios are shown, since differences between some chosen scenarios were not significant. Two of the scenarios are with fixed and varying biomass concentration and two with fixed and varying temperature. For HT: 1.5 g L^{-1} Run 2, HT: 2.50 g L^{-1} Run 3 only limited data points were available and operational issues (biofilm formation in the tubing) biased the reported productivities. Therefore these two production runs are left out of the validation procedure. The graphs for cumulative productivity can be found in the appendix D (figure D-4, figure D-6).

The scenarios using different absorption coefficient are not displayed, since using the measured values did not have a large impact on the predicted productivity. Only limited data points were available and the sensitivity of the model to the absorption coefficient is rather low, as indicated by the performed sensitivity analysis. In most of the cases using fixed or varying biomass concentration as input, no significant difference between the modelling outcomes are observed. In almost every case the introduction of the measured biomass concentration as model input led to an underestimation of the predicted algae productivity. Measured biomass concentrations were generally lower at concentrations of 0.75 and 1.50 g L^{-1} and higher at the concentration of 2.50 g L^{-1} compared to the set point. The optimal biomass concentration in respect to maximum productivity for *Nannochloropsis* sp. in the horizontal tubular PBR was found at 1.25 g L^{-1}. In figure D-3 (Appendix D) the behaviour of the model at different biomass concentrations is illustrated. At biomass concentrations lower than 0.75 g L^{-1} and higher than 2.50 g L^{-1} the model underestimated algae productivity. At 1.50 g L^{-1}, measured biomass concentrations were lower than the set point; therefore the model overestimated algae productivity.

A much bigger difference in productivity prediction was observed by selecting either a fixed culture temperature (averaged over the production period) or the measured culture temperature as model input (10 min). Generally the use of a fixed temperature input resulted in the best model fit. However, the use of the measured culture temperature led in the most cases to an underestimation of productivity. This is explained due to the high sensitivity of the productivity model to changes in temperature. Generally the average temperature of each production run was very close to the optimum growth temperature of 25 °C. However, the temperature within a day could vary significantly at different biomass concentrations and runs. Therefore, it was very important to consider this in the analysis on the effect of temperature on the model output. As shown by Van Dam [34] the curve of the temperature factor f_T for *Nannochloropsis sp.* is declining faster at temperatures higher than the optimal temperature (appendix D, figure D-1).

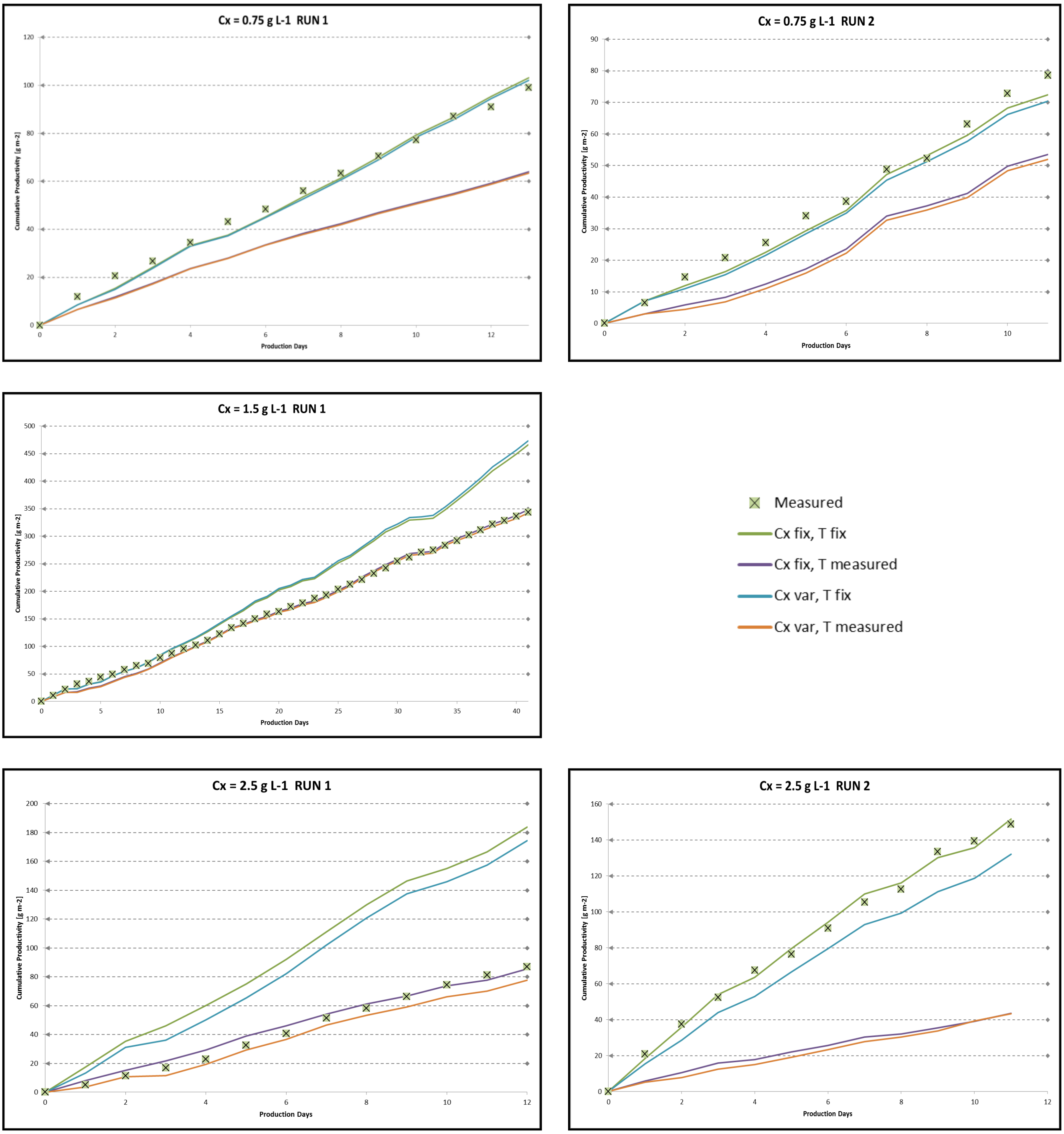


Figure 4-4. Different modelling scenarios for the horizontal tubular system at different biomass concentrations (0.75 g L^{-1}, 1.50 g L^{-1}, 2.50 g L^{-1}) and runs represented in cumulative productivity [g m^{-2}]. Abbreviations: Measured – measured areal productivity, for modelling scenarios: Cx fix – biomass concentration is fixed, Cx var –measured biomass concentration is used, T fix – culture temperature is fixed, T measured – measured culture temperature.

At HT: 0.75 g L^{-1} Run 1, Run 2, and HT: 2.50 g L^{-1} Run 2 the model underestimated algae productivity when introducing the measured temperature values over the day. Regarding the temperature distribution over the whole production period most of the measured values were above the optimal temperature with several values close to the lethal temperature. While using the average temperatures of these runs led to a good model fit, using the varying temperature input led to an underestimation of productivity by the model. Comparing this model behaviour at other turbidostat settings and production runs a different model behaviour was found. In the cases of HT: 1.5 g L^{-1} Run 1, HT: 2.5 g L^{-1} Run 1 an overestimation of algae productivity was obtained when the fixed culture temperature was used as model input. However, introducing the measured culture temperature resulted in a good model fit. During these production runs the temperatures were generally lower and normally distributed around the average temperature over the production. Further, they did not result in extreme temperatures as compared to the other runs.

A possible explanation for this model behaviour is the high sensitivity to culture temperature and the shape of the current temperature curve (equation 3-5), which is modulated by the factor beta (β_T). Regarding the fit of the temperature curve to the experimental data of Sukenik et al. [48], growth rates at lower temperature appear to be overestimated and higher temperatures appear to be underestimated (appendix D, figure D-1). The influence of modelling variables on the deviation between measured and predicted daily productivities support this by showing that generally the model underestimates at high temperatures (appendix D). This could explain the underestimation of the model at HT: 0.75 g L^{-1} Run 1, Run 2, and HT: 2.50 g L^{-1} Run 2, due to the high temperature values in the measurements used as a model input. The usage of the average temperature as model input on the contrary neglected the influence of these high temperatures and therefore resulted in a good fit. The overestimation of productivity at HT: 1.5 g L^{-1} Run 1, HT: 2.5 g L^{-1} Run 1 when using the average temperature as model input can be explained due to the slight overestimation of the temperature model at these temperatures. However, using the measured values as model both over- and underestimation of productivity occurred. This resulted in a good model fit, since the over- and underestimation compensated each other.

In addition the current temperature model neglect the dynamic of adaptation of algae cells to changes of temperature in time. The model assumes an instant effect of temperature on the growth rate. However, biology processes usually take time and follow a certain dynamic. Therefore we assumed that the growth rate of algae is dependent on temperature and time as well, since an exposure to high temperatures for several minutes or several hours would have a different impact on growth. The underestimation by the model at high temperatures could be also a result of this dynamic of biology when using temperature inputs with short time intervals. The algae cells can possibly grow under high temperatures for a certain period of time, whereas the model assumes no biomass productivity.

At locations where greater temperature fluctuations during the day are expected (e.g. Italy, Spain, Algeria), the impact of temperature changes on the model input is assumed to be even larger. Therefore a detailed evaluation of the current temperature model is suggested, to eliminate the model uncertainty attributed to temperature variations.

Even though similar light and temperatures conditions at HT: 2.50 g L^{-1} Run 1, Run 3, and HT: 1.50 g L^{-1} Run 2 were recorded, different results were obtained when implementing varying model inputs such as temperature. In these three runs operational issues occurred; biofilm formation in the tubing caused fluctuating turbidity. The turbidity set point was frequently adapted during the operational period, which could bias the measurements. This had the strongest influence on HT: 1.50 g L^{-1} Run 2 where measurements are in between the modelling scenario outcomes. Therefore it is assumed that the measured productivities are lower due to the operational issues and suggested to be critical about the results of these runs.

A summary of the discussed model scenarios for both systems at different operational conditions and production runs in terms of their model performance can be found in table 4-2. The values written bold represent the model scenario, which resulted in the best model fit. The best prediction for the raceway pond deviates +3.23 % from the actual recorded productivity. The model for the horizontal tubular PBR predicts with an overall accuracy of -3.55 % when taken the average over all turbidostat regimes and production runs. The relative deviation over the different turbidostat regimes in the horizontal tubular PBR resulted in: C_x = 0.75 g L^{-1} with +1.19 %; C_x = 1.50 g L^{-1} with +1.35 %; C_x = 2.50 g L^{-1} with -6.09 %.

System	Scenario	Relative Deviation [%]	Scenario	Relative Deviation [%]
Raceway Pond				
Run 1 (45 days)	**C_x fix, K_{abs} fix**	**3.23**	C_x var, K_{abs} fix	54.30
	C_x fix, K_{abs} measured	-14.02	C_x var, K_{abs} measured	25.85
Horizontal Tubular PBR				
C_x = 0.75 g L^{-1}				
Run 1 (	**C_x fix, $T_{culture}$ fix**	**2.97**	C_x var, $T_{culture}$ fix	4.07
	C_x fix, $T_{culture}$ measured	-35.37	C_x var, $T_{culture}$ measured	-35.98
Run 2	C_x fix, $T_{culture}$ fix	111.57	C_x var, $T_{culture}$ fix	100.49
	C_x fix, $T_{culture}$ measured	**-1.78**	C_x var, $T_{culture}$ measured	-10.80
C_x = 1.50 g L^{-1}				
Run 1	C_x fix, $T_{culture}$ fix	35.50	C_x var, $T_{culture}$ fix	37.66
	C_x fix, $T_{culture}$ measured	**1.35**	C_x var, $T_{culture}$ measured	-2.35
C_x = 2.50 g L^{-1}				
Run 1	**C_x fix, $T_{culture}$ fix**	**-7.95**	C_x var, $T_{culture}$ fix	-10.45
	C_x fix, $T_{culture}$ measured	-32.00	C_x var, $T_{culture}$ measured	-33.88
Run 2	**C_x fix, $T_{culture}$ fix**	**1.86**	C_x var, $T_{culture}$ fix	-11.32
	C_x fix, $T_{culture}$ measured	-70.76	C_x var, $T_{culture}$ measured	-70.87

Table 4-2. Summary of Relative Deviation [%] between predicted and measured productivity for different production systems, turbidostat set points and runs. The scenarios, which resulted in the best model fit, are in bold.

Generally, the change in biomass concentration input at the raceway pond and the temperature input at the horizontal tubular PBR had the strongest influence on productivity prediction. Using the turbidostat set point for biomass concentration and the average absorption coefficient over the production period as model input for the raceway pond resulted in the most accurate prediction. For the horizontal tubular PBR in the most cases using the fixed temperature input lead to the best model fit. Using varying temperature input led to accurate predictions when temperatures are below and close to the optimum growth temperature. In case where culture temperatures are above the optimum temperature and close to the lethal temperature the model tended to underestimate algae productivity significantly, which can be addressed to the current temperature model used.

The model settings resulting in the best model fit were taken for further evaluation on the influence of time interval on accuracy of model prediction. In addition it was investigated if the model inaccuracy can be addressed to the uncertainties in the model inputs or to the assumptions made during establishing the productivity models.

4.4 Influence of model interval on accuracy of prediction

When comparing measured and predicted productivity on daily basis, the relative deviation between the two productivities was observed to be large at certain days. A visual representation of the model performance on predicting daily algae productivities for the raceway pond and horizontal tubular PBR can be found in figure 4-5. Compared to the model of the raceway pond, the model for the horizontal tubular PBR generally predicts more accurate on daily basis. This is explained due to less variation in measured conditions in the horizontal system. Since the tubular system provides a closed environment for algae cultivation, the growth conditions are more controlled compared to the raceway pond leading to less variation in parameters, which are essential for algae growth. As previously discussed, operational conditions varied in the raceway pond more compared to the horizontal tubular system. Salinity, nitrogen and other nutrients fluctuated over the production days in the raceway pond, affecting algae growth. Due to the fact that the model assumes optimal growth conditions in terms of salinity and nutrients, this could lead to larger deviations in daily algae productivity compared to the horizontal tubular system, where conditions are closer to the optimal growing conditions for *Nannochloropsis* sp.

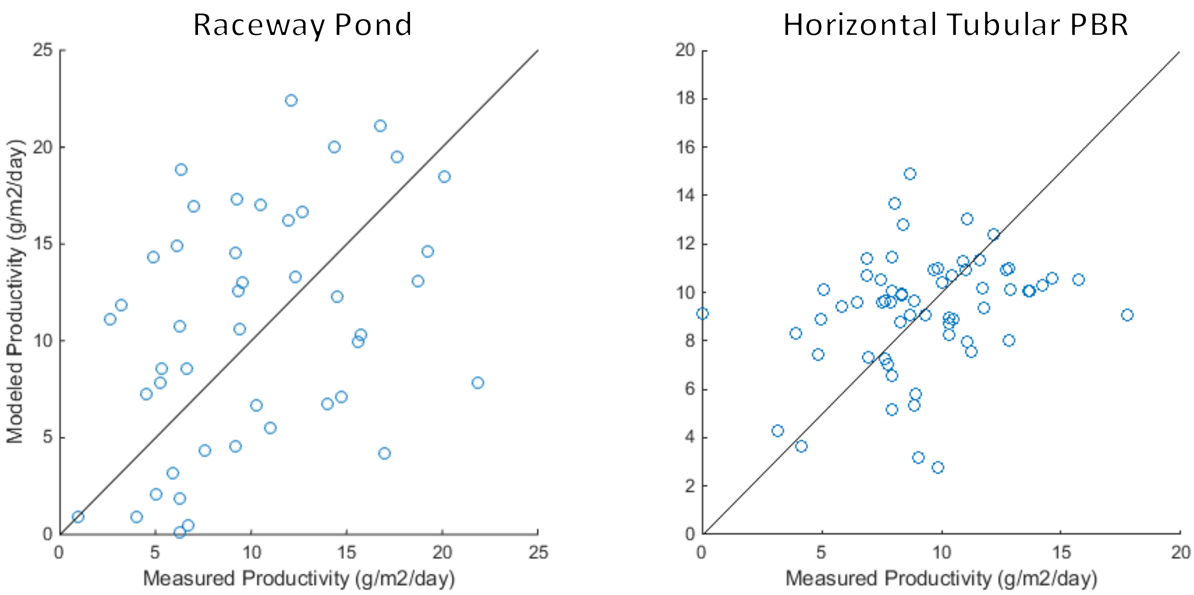


Figure 4-5. Parity plot of measured against modelled areal productivity from (1) Raceway Pond, (2) Horizontal Tubular PBR.

Although the model the prediction deviate significantly from the measurement on a daily basis and prediction appears very scattered, the overall prediction on algae productivity of an entire production period is reasonable accurate. As shown previously, the model for the raceway pond predicted algae productivity with a relative deviation of +3.23 %, and the model for horizontal tubular PBR with an average relative deviation of -3.55 % over all production runs. In order to determine the reliability of model predictions on a timescale, the accuracy of the model was evaluated at different time frames: 1 day; 2 days; 3 days; week; month; entire period. In figure 4-6 the performance of the raceway pond and the horizontal tubular system at different intervals are compared. A distinctive trend can be observed in this graph. At daily productivities the relative deviation ranged in between ±80 %, leading to the conclusion that on this level the predictions cannot be taken as accurate. However, moving to larger time frames the relative deviations gets smaller and smaller with the best result when predicting productivity of the entire production period. Generally for both systems the deviation between measured and predicted productivity levelled out when going to larger time frames.

While the model implements variations in light and temperature immediately, algae cells need time to adapt to new environmental conditions meaning that biology is slower than the models assume. Photo acclimation of the algae cell is a process, which is not happening immediate with changing light conditions. Increasing or decreasing the pigment content for optimal light uptake can take algae cells many hours up to several days [9]. When temperature changes of the culture broth, micro algae are adapting their biomass composition in order to regulate their membrane fluidity, the amount and activity of Rubisco, and starch and sucrose content [57] [58]. This process takes time as well and is not happening instantly [59].

At certain points during production this delay of adaptation of biology was observed. Occasionally, measured daily algae productivities still resulted in a considerable high value the following days after a significant decrease in total light input or temperature from one day to another. A delay in productivity was also observed when the light input or temperature significantly increased from one production day to another. Usually the adaptation of the system could be observed 1 or 2 days after a change in light or temperature input. An example for this effect can be found in appendix D (figure D-9).

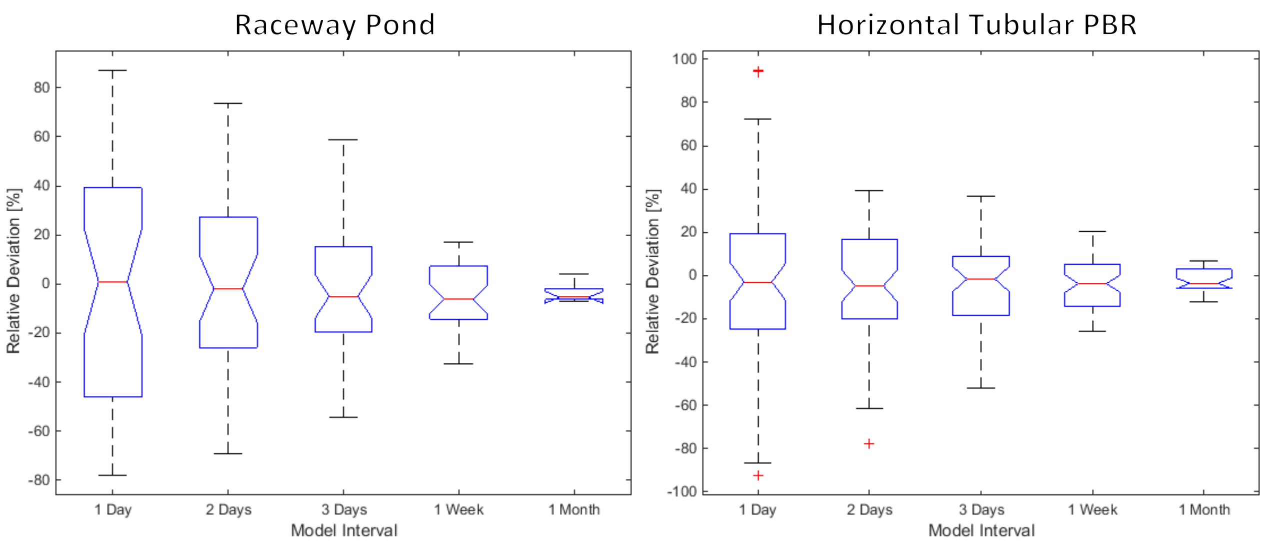


Figure 4-6. Box plot of relative deviations [%] between measured and predicted areal productivity at different model intervals: 1 Day; 2 Days; 3 Days; 1 Week; 1 Month. Left: Raceway Pond. Right: Horizontal Tubular PBR.

4.5 Evaluation of model accuracy considering model uncertainty

The model settings, which resulted in the best model fit in respect to the observed productivities were taken to model productivity for the operational period of the different systems and biomass concentrations. The model uncertainty calculated in the global uncertainty/sensitivity analysis of the raceway pond and the horizontal tubular PBR are plotted as upper and lower bound in the graphs displaying cumulative productivity. With this visual approach it is possible to identify if the prediction inaccuracies are the result of the uncertainty in model inputs or due to model assumptions.

1.1.1 Raceway Pond

For the raceway pond using a fixed biomass concentration and fixed absorption coefficient resulted in the most accurate prediction of productivity. In figure 4-7 measured and predicted cumulative productivities are displayed. The upper and lower boundaries of the 95% confidence interval calculated from the model uncertainty are included. The overall model uncertainty is ±14.62 %. In the first 10 days of production various measurements are outside of the boundaries of prediction, meaning that the model under- or overestimated. However, after the 10th day of production the measurements were found to fall within the confidence interval of the predicted productivities. This indicates that after this period the uncertainties on the model inputs can explain the overall inaccuracies of the productivity predictions. This observation is supported by the results of the analysis of the used model interval on the accuracy of the model. There it was shown that deviations between measured and predicted are considerable large on smaller time frames less than a month.

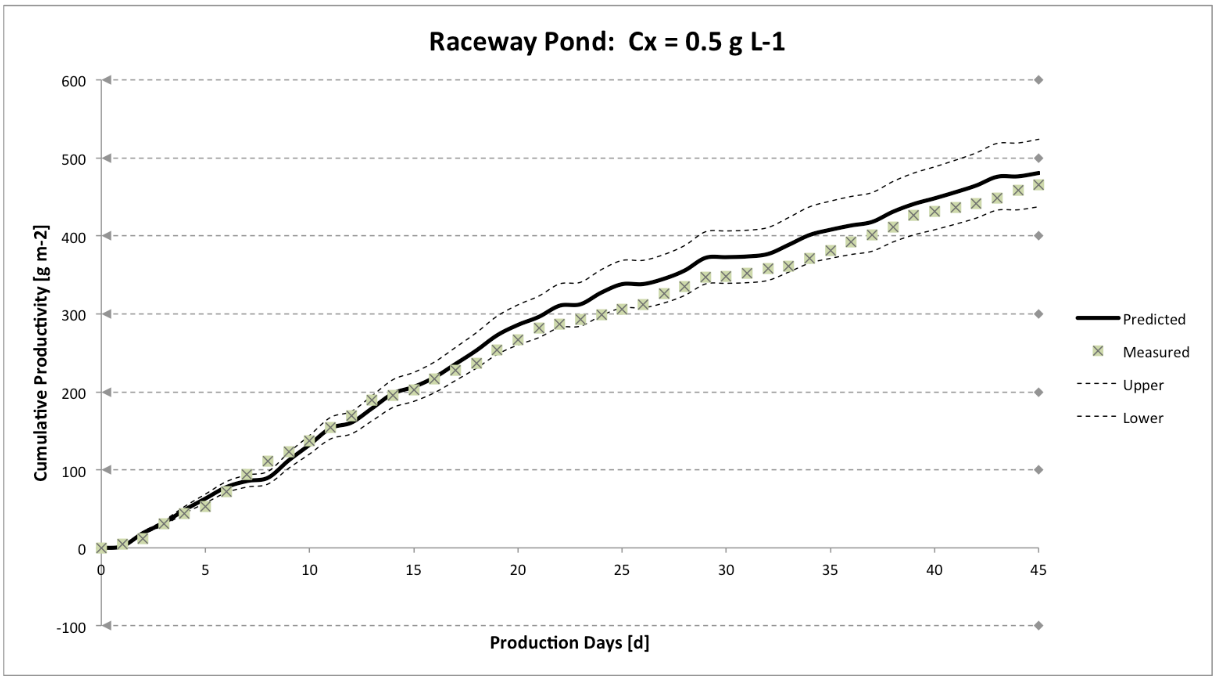


Figure 4-7. Cumulative productivity in the raceway pond between May and August 2014. Comparison of experimental data and model prediction. Grey dashed line represents the upper and lower boundaries of the confidence interval of the model attributed uncertainty (±14.62 %)

4.5.1 Horizontal Tubular PBR

From the modelling scenarios of the horizontal tubular system different model settings resulted in the most accurate prediction of algae productivity. In most of the cases fixed biomass concentration, fixed absorption coefficient and fixed temperature resulted in the smallest deviation between measured and predicted productivity. Fixed values for these model inputs where used at production runs HD: 0.75 g L^{-1} run 1, run 2, HD: 1.5 g L^{-1} Run 2, and HD: 2.5 g L^{-1}. However during HD: 1.5 g L^{-1} Run 2, HD: 2.5 g L^{-1} Run 1 and Run 2 the best results were achieved when introducing the measured culture temperature as model input. This model behaviour was explained due currently used temperature model.

In figure 4-8 the outcome of the different model settings, which fitted the best to the respective production scenario, are illustrated. The upper and lower boundaries of the confidence interval of the model accuracy are included in this graphical representation. The boundaries were calculated from the model uncertainty obtained from the global uncertainty analysis, which was found to be ±11.27 %. In the most cases the measurements in the beginning of a production process were outside of the boundaries of the model prediction. In early phase of production the model over- or underestimated model productivity. However, after approximately a week of production the measurements fell inside the uncertainty range of the model prediction, leading to the conclusion that inaccuracies of the productivity predictions on a larger time frame could be addressed to the uncertainty of the model inputs. For production run HD: 1.5 g L^{-1} Run 2 and HD: 2.5 g L^{-1} Run 3, the measured algae productivity was found to be outside of the model prediction. However those two production runs are short and operational issues occurred which biased the calculated algae productivity. As previously discussed above, biofilm formation led to unreliable productivity measurements, which make the validation of the model with these production runs not feasible not feasible. Therefore they were excluded from the main body of this thesis.

Generally, the fit of the model prediction of Slegers et al. [1] [2] [14] to the measured data obtained at AlgaePARC was very accurate over longer periods of time. However, at smaller time frames the model the deviation between measured and predicted productivity could be rather large. This is explained due to the delayed response of biology to changes in cultivation conditions, while the models assumed instant adaptation and operational issues (early or delayed harvesting). However, daily predictions are not needed when evaluating the potential of algae at commercial scale. Productivities predictions larger than a month are sufficient in order to assess the economical and environmental impact of algae biotechnology. As a last part the productivity models of Slegers et al. [1]–[3] are compared to reported outdoor productivities and the compared other modelling studies used in various assessments.

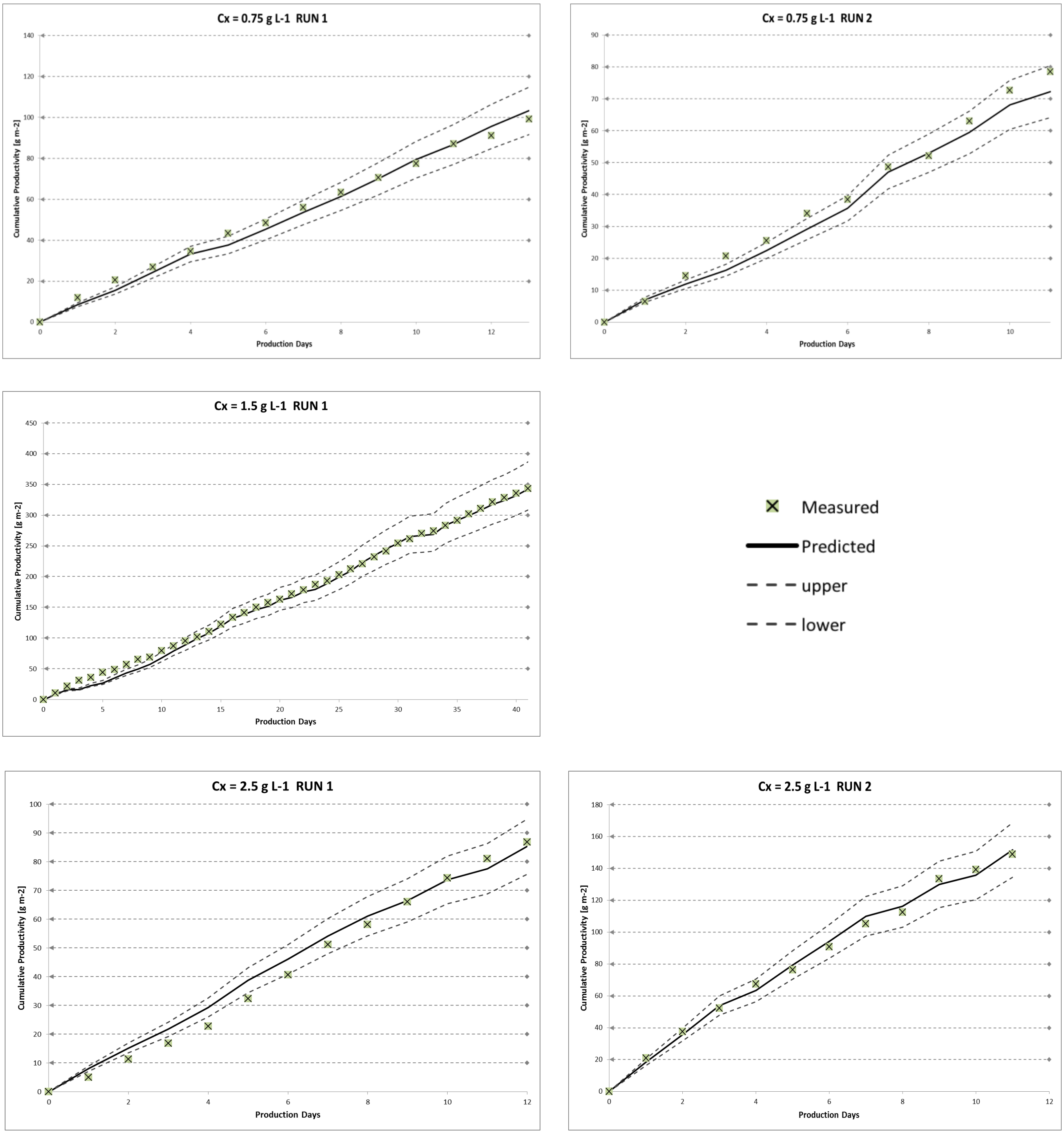


Figure 4-8. Cumulative productivity in the horizontal tubular system at different biomass concentrations (0.75 g L^{-1}, 1.50 g L^{-1}, 2.50 g L^{-1}) and production runs between May and October 2014. Comparison of experimental data and model prediction is made. Grey dashed line represents the upper and lower boundaries of the confidence interval of the model attributed uncertainty (±11.27 %)

4.6 Fitness of the model for economic and environmental assessments

In the Introduction it was stated that a wide range of algae productivity assumptions are used in past assessments. This wide range resulted from the large uncertainty associated with full-scale productivity predictions. The productivity predictions of the models of Slegers et al. [1]–[3] were found to be in the lower third of the productivity assumptions reported in various assessments. An average daily productivity of 10.7±1.0 [g m^{-2} day^{-1}] is predicted for the raceway pond for the Netherlands. When compared to literature the prediction for the raceway pond was found to be in between reported productivities in England (6.8 g m^{-2} day^{-1}) [60] and Italy (12.9 g m^{-2} day^{-1}) [61]. Daily productivities for the horizontal tubular PBR were depended on the biomass concentration used during production with the highest productivity found at a biomass concentration of 1.5 g L^{-1}. The three averaged daily productivities predictions obtained for the three different turbidostat regimes are displayed in table 4-3. The average productivity over all production runs is 9.8±1.1 g m^{-2} day^{-1}. However there is only limited reported productivity data for tubular systems using various reactor set-ups mainly in southern locations such as Spain. Therefore a comparison of productivities for the Netherlands is not feasible.

System	Biomass Concentration	Averaged Daily Productivity
	[g L^{-1}]	[g m^{-2} day^{-1}]
Raceway Pond	0.5	10.7 ± 1.0
Horizontal Tubular PBR	0.75	7.2 ± 0.8
	1.50	11.7 ± 1.3
	2.50	10.4 ± 1.2
		9.8 ± 1.1 (average)

Table 4-3. Averaged daily productivities [µmol m^{-2} day^{-1}] predicted by the productivity models of Slegers et al. [1]–[3] *for the raceway pond and horizontal tubular PBR at different turbidostat regimes.*

In the scenario studies of Slegers et al. [1] [3] algae productivity in the Netherlands, France and Algeria for cultivation in raceway pond and horizontal tubular PBR was assessed (appendix D: table D-1). In this study a relative increase in productivity of 57.9 % for France and 115.1 % for Algeria, in respect to the Netherlands, for the horizontal tubular PBR was reported. For the raceway pond only Algeria (69.9 % productivity increase) was investigated, since climatological data for France were missing. The productivity predictions obtained in this thesis were extrapolated with these relative productivity increases. This resulted in productivity projections for the raceway pond of 18.2±1.7 g m^{-2} day^{-1} for Algeria and for the horizontal tubular PBR in 15.5±2.1 g m^{-2} day^{-1} in France, 21.2±2.8 g m^{-2} day^{-1} in Algeria. The productivity projection of the raceway pond for Algeria is comparable with a productivity of 16.4 g m^{-2} day^{-1} obtained in California, which is on a similar latitude than Algeria [62]. The productivities obtained for the horizontal tubular PBR are comparable to productivities found in literature; for France a modelled productivity between 14.1 – 16.8 g m^{-2} day^{-1} was obtained by Fernández et al. [63]; in Spain productivity of 19.1–19.8 g m^{-2} day^{-1} [29] are reported and for Brazil modelled productivities of 25.0 g m^{-2} day^{-1} [42] are obtained – these productivities are comparable to the model predictions for Algeria. However further validation for these locations and climatological conditions are needed to verify the use of the productivity models under these conditions.

In the study of Rogers et al. [64] the biofuel production costs using a raceway pond are investigated. In a sensitivity analysis performed during this study, the uncertainty attributed with the productivity was assumed to be ±33 %. In comparison the productivity model of Slegers et al. [1]–[3] showed an associated uncertainty of ±14.62 % (raceway pond) and ±11.27 % (horizontal tubular PBR). When translated into the biofuel cost variation the range in which the biofuel cost are varying can be reduced by 55.7 % and 65.8 %. This would significantly reduce the uncertainty in economical assessments and will help to evaluate the potential of algae as a renewable feedstock.

The productivity models of Slegers et al. [1]–[3] were found to accurately predict algae productivity in the Netherlands and showed similar productivity values when compared to other reported outdoor productivities in similar climatological conditions. Extrapolation productivities to locations such as France and Algeria resulted in comparable values compared to reported productivities in literature. In addition it was demonstrated that the model accuracy has the potential to eliminate uncertainties in production costs. Therefore the productivity models are considered as suitable for application in life cycle, techno-economical and scalability assessments in the Netherlands.

5 Conclusion

In this study the productivity models for the open raceway pond and the horizontal tubular PBR developed by Slegers et al. [1]–[3] were validated with productivity data obtained at AlgaePARC with outdoor systems. A principal component analysis (PCA) and bivariate correlation analysis (BCA) showed a strong correlation between light, temperature, biomass concentration, absorption coefficient and measured productivity during algae production in the pilot-scale systems. The strong negative correlation between biomass concentration and absorption coefficient with productivity indicate that during cultivation the raceway pond was light limited. While in the horizontal tubular PBR productivity was highly dependent on the culture temperature.

The model accuracy was assessed using algae species specific, reactor specific parameters and climatological data during the production period as model inputs. The models were found to accurately predict productivity for the raceway pond and the horizontal tubular PBR under outdoor conditions. The model of the raceway pond predicted with an overall accuracy of ±3.23 % over 45 days of cultivation. While the model of the horizontal tubular PBR predicted with an overall accuracy of ±3.55 % over 121 days of cultivation. When compared to other studies the accuracy of the productivity models of Slegers et al. was found to be higher. The best model fit was found when model inputs are taken constant by using the average measured value over the production period.

As experimentally determined parameters and variables were necessary as model input, it was assessed to what degree the uncertainty of the model inputs had an influence on the uncertainty of the model output. By using a global uncertainty/sensitivity analysis, the uncertainty of the model output was found to be ±14.67 % for the raceway pond and ±11.26 % for the horizontal tubular PBR. While the calculation of the light path and the growth model had the largest influence in the productivity model of the raceway pond, the temperature model primarily determined the output of the horizontal tubular PBR. In general the physical part of the model showed a larger impact in the horizontal tubular system compared to the raceway pond where the biological model was the most influential. This can be explained by the stronger link to light and temperature in the productivity model of the horizontal tubular system. With the results of the uncertainty analysis it was shown that differences between measured and predicted productivity were primarily caused by experimental errors, rather than by model assumptions.

With the range of productivity prediction and the obtained model uncertainty the fitness of the model for economic and environmental assessments in the Netherlands was investigated. The model was found to predict in the same range as reported outdoor productivities. In addition it was shown that the productivity models have the potential to reduce uncertainties in biofuel cost by half. This relative small model uncertainty and the good representation of outdoor cultivation on longer production periods (>1 month), makes the productivity models suitable for application in life cycle, techno-economical and scalability assessments. In this way, the validated productivity models will help to evaluate the potential of microalgae as a renewable feedstock for food, feed, bio-chemicals and biofuel in the future.

6 Perspectives

The models developed by Slegers et al. [1]–[3] were found to accurately predict algae productivity for the raceway pond and the horizontal tubular PBR in the Netherlands under outdoor conditions. The overall accuracy for the raceway pond is +3.23 % over 45 days of cultivation and for the horizontal tubular PBR -3.55 % over 121 days of cultivation. The best model fit was obtained when the model inputs were taken constantly (using the average measured value over the complete production period). The global uncertainty/sensitivity analysis showed that the uncertainty of the model output was found to be ±14.67 % for the raceway pond and ±11.26 % for the horizontal tubular PBR. This uncertainty analysis indicated that differences between measured and predicted productivity were caused by experimental errors rather than by model assumptions. The sensitivity analysis of the productivity models also revealed that growth model had the largest influence in model of the raceway pond, the temperature model in the horizontal tubular PBR was more important. In the horizontal tubular PBR the physical part of the model showed a larger impact compared to the raceway pond, since the model is stronger linked to light and temperature. Even though the productivity models showed a good overall performance, various suggestions for future work are proposed: a) A different approach for the calculation of the measured algae productivity, b) validation with data-sets obtained in different years, seasons and location, c) validation of the raceway pond at different biomass concentration, d) a detailed evaluation of the temperature model, e) implementing photo-acclimation and nightly biomass loss.

6.1 Extending/Expanding validation

The validation performed in this thesis was based on productivity data obtained from cultivation experiments in 2014. Since the data set used in this thesis could bias the validation, we suggest that the validation should be extended with datasets of several production runs of various years obtained at AlgaePARC (e.g. dataset of AlgaePARC from 2015). In this way, the influence of the year-to-year variation in weather conditions on the model fit can be investigated. This would allow further investigation and improvement of the accuracy of the productivity models in the Netherlands. In addition, the validation of the productivity models should be further extended to different locations and different climatological conditions where productivity data are available (e.g Southern Europe: Italy, France, Spain). This will extend the understanding of how the model incorporates different weather conditions and location specific parameters. Hence, a successful full validation would verify the use of the productivity models in various algae life cycle, techno-economical and scalability assessments at different location and climatological conditions.

In addition it is suggested to further investigate the influence of the used interval for the model input, especially in respect to light and temperature. In this thesis only the intervals of 10 min and averaged values over the production period are taken as temperature input and only a 10 min interval for light. An evaluation of temperature changes every hour, day, week and month (averaged value of the period) to use as input are therefore suggested. The same is recommended for light as well. This will give further insight into the model behaviour in respect to different intervals used for the model inputs.

6.2 Measured algae productivity

In this thesis the calculation of measured algae productivity was based on the volume harvested from the production system and the average of the dry weight concentration measurement in the morning of a production day (appendix A: equation A-1). This approach could bias the actual algae productivity, since the volume harvested is influenced by rain and evaporation in the raceway pond. Furthermore, the biomass concentration in the morning is not representative for the biomass concentration at which harvesting starts. Therefore, for future research it is suggested to calculate algae productivity on the basis of the actual biomass concentration used as set point for harvesting, e.g with the turbidity value. This will give a more accurate daily productivity measurement, especially for the raceway pond where harvesting occurred also due to liquid level increase by rain.

6.3 Influence of biomass concentration in raceway pond

As shown in Results & Discussion the biomass concentration used during cultivation of the raceway pond led to light limitation during production. Since biomass concentration had a significant impact on the model output it is important to validate the productivity model of the raceway pond against productivity data obtained at different biomass concentrations. Therefore it is suggested that similar to the horizontal tubular PBR the validation should be performed for a low, medium and high biomass concentration (e.g low: 0.15 g L^{-1}, middle: 0.25 g L^{-1}, high: 0.5 g L^{-1}). This will give valuable insight into the model behaviour at different biomass concentration and the influence of the modelling of light attenuation (equation 3-6).

6.4 Temperature model

The temperature model used in the productivity models of Slegers et al. [1]–[3] is dependent on β, the dimensionless parameter fit to moderate the μ(T) curve. This parameter was determined previously by Van Dam [34] by fitting the temperature dependent factor (f_T) (equation 3-5) to growth rates found at four different temperatures (18°C, 25°C – optimal growth temperature, 32°C, 38°C – lethal temperature) and continuous light (24 hours) by Sukenik et al. [48]. A literature comparison performed by Van Dam showed that available data is limited and that during the determination of growth rates at different temperature various biomass concentrations, light intensities and reactor design are used. This significantly influenced the results of the conducted experiment and therefore Van Dam suggested to obtain more reliable data for the determination of β. When looking at the current fit of the curve obtained by Van Dam to the experimental data of Sukenik et al. [48], it can be noticed that between the optimal growth temperature and the lethal temperature the curve declines faster than the experimental value indicated (appendix D: figure D-1). At a temperature of 32 °C the predicted growth rate is almost half of the measured value of Sukenik et al. [48]. At the same time the fit of the curve on lower temperatures than the optimal growth temperature is higher than the experimental value at 18°C leading to a slight overestimation at these temperatures. This observation is coinciding with the model behaviour at the horizontal tubular PBR where the model overestimated at lower temperatures (<25°C) and underestimates at higher temperatures (>25°C). Even though four values are enough for fitting the Arrhenius equation (equation 3-5) the current fit indicates that the fitting procedure of Van Dam [34] could be imperfect. Therefore future work has to focus on correct determination of β for *Nannochloropsis* sp. eventually with experiments determine growth rates at different temperatures conducted at AlgaePARC.

Further, the current temperature model neglects the dynamics of biology to temperature changes. Therefore research should be focus on understanding and mathematically describing the adaption of algae cells to temperature over time. Experiments under changing light and temperature conditions at different time steps (e.g 10 min, 30 min, 60 min, 120 min, 180 min) will give insight into the dynamics of algae biology.

Improving the temperature model would increase the accuracy of productivity prediction especially of the horizontal tubular PBR, since the model output is highly determined by the culture temperature. In addition the model accuracy at location with elevated irradiances and culture temperatures (e.g. France, Algeria) will depend strongly on the temperature range in which the model predicts accurately. A successful determination of the temperature model will significantly increase the model fit in a broader temperature range and will increase the predictions on smaller time scale for both systems.

6.5 Implementing photo-acclimation and nightly biomass loss

As showed in Results & Discussion the model fit is decreasing on timeframes less than a month. It is deviating the most at daily productivity predictions. This deviation could be reduced by addressing several facts: The model tends to over- and underestimates at certain cultivation conditions. In the horizontal tubular PBR it was found that at high light intensities the model overestimates, while at low light intensities the model underestimates. The behaviour at the different light conditions can be explained due the lacking photo-acclimation of the model. The absorption coefficient, which is used as a model input is averaged over the production period and therefore at days with high average light intensities the model overestimates and at low average light intensities the model underestimates, because the adapted growth model (equation 3-3) does not adapt to the current light conditions. However in areas with large variations in light conditions like the Netherlands the incorporating of varying absorption coefficients during the day and year may be appropriate [12]. The implementation of photo-acclimation would increase the model fit on smaller intervals significantly, since absorption coefficient can vary daily. The model of Slegers et al. [1]–[3] used originally the growth relation developed by Geider et al. [51] including light acclimation. Therefore a further adaptation is proposed to the productivity models used in this thesis to incorporate varying absorption coefficients. This will increase the model fit on smaller time intervals significantly.

Another issue that could be addressed when improving the model performance at smaller time interval is the calculation of nightly biomass loss. In a comparison of the average nightly biomass losses measured at AlgaePARC and predicted by the model a significant different trend could be observed (appendix D). The modelled biomass loss showed to be increasing with biomass concentration. This was explained due to the fact that the rate of night time respiration is modelled using first-order kinetics in regard to cell concentration. Further the night time respiration is only associated with the maintenance coefficient and therefore constant over time. However, the opposite trend was observed for the measured biomass loss recorded at AlgaePARC. The calculated biomass loss was decreasing with increasing biomass concentration. Similar results were obtained by Michels et al. [65] for *Tetraselmis suecica*. In that study the highest nightly biomass loss rate was recorded at the optimal biomass concentration of this organism in the production system used during research. Michels et al. explained this trend due to higher maintenance costs due to the increasing growth rate. The night metabolism is determined by numerous environmental factors including prior light intensity history, nutrient status, temperature and the species itself [66]. In the study of Vítová et al. [67] it was shown that the respiration duration of the *C. reinhardtii* cell cycle is affected by both prior experienced light intensity and temperature. The study of Le Borgne et al. [68] stresses that although biomass concentration influences the biomass loss during night, the effect is negligible compared with that of temperature. Therefore, it is suggested to investigate into the modelling of nightly biomass loss and couple it to temperature and specific growth rate. In order to validate the incorporation of biomass loss correctly two biomass concentration measurements could be performed per day; one at sunrise and one at sunset. In this way the algae productivity during the day and the biomass loss during night can be validated independently, as showed previously by Béchet et al. [20]

With even further refinement and validation of the algae productivity model of Slegers et al. [1]–[3] more accurate algae biomass predictions can be made. This will extend the application range in various algae assessments at different locations and will even further leverage the reliability of the model in the scientific and economic community.

Appendix A

In this part of the appendix more detailed information on the research facility AlgaePARC at WUR is provided. Schematic drawings of the raceway pond (figure A-1) and horizontal tubular PBR (figure A-2) are displayed. Further, measurements taken (table A-1), the light and temperature distribution (figure A-3 and figure A-4) and the general operational conditions (table A-2) during the production runs are given. Information that is not shown can be found in the paper of Bosma et al. [46] on the design and construction of this facility.

A–1 Production Systems at AlgaePARC

A–1.1 Raceway Pond

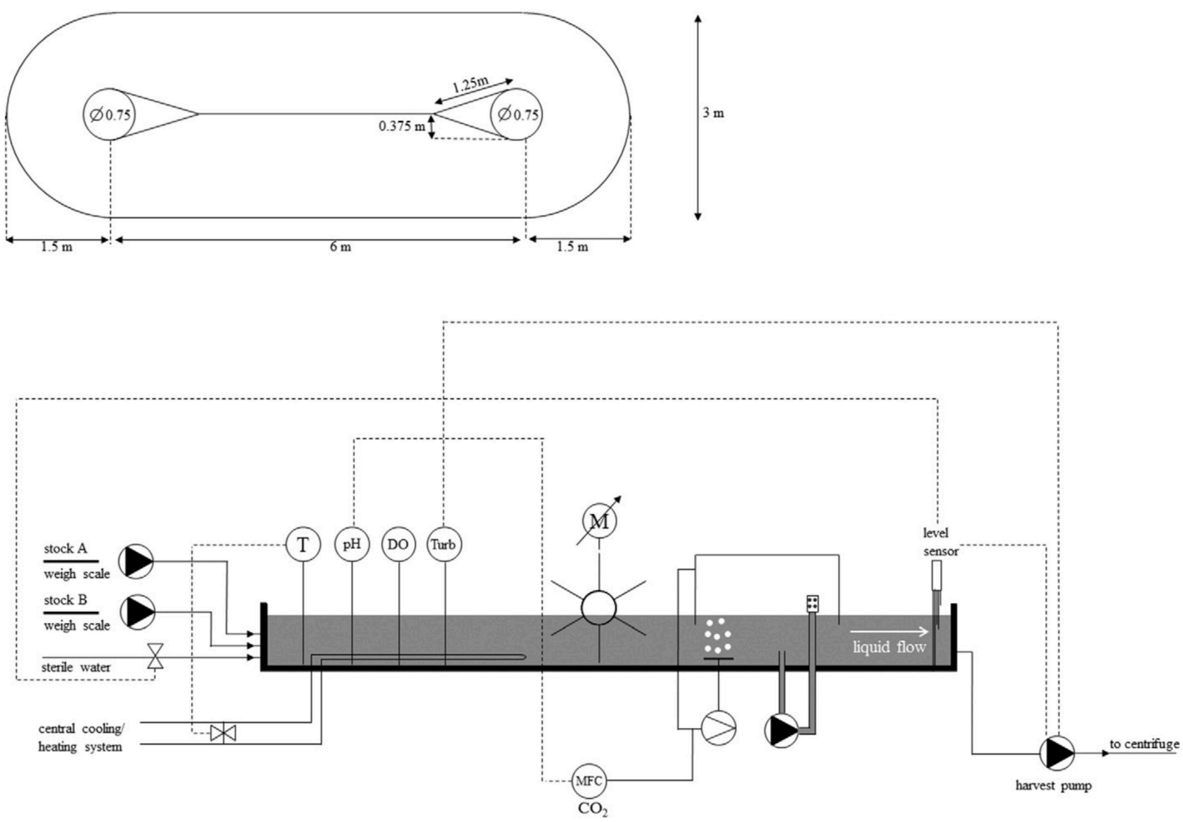


Figure A-1. Schematic drawing of the raceway pond. Dashed lines show control strategies. Abbreviations: DO, dissolved oxygen; MFC,mass flow controller; T, temperature; turb, turbidity [46].

A–1.2 Horizontal Tubular PBR

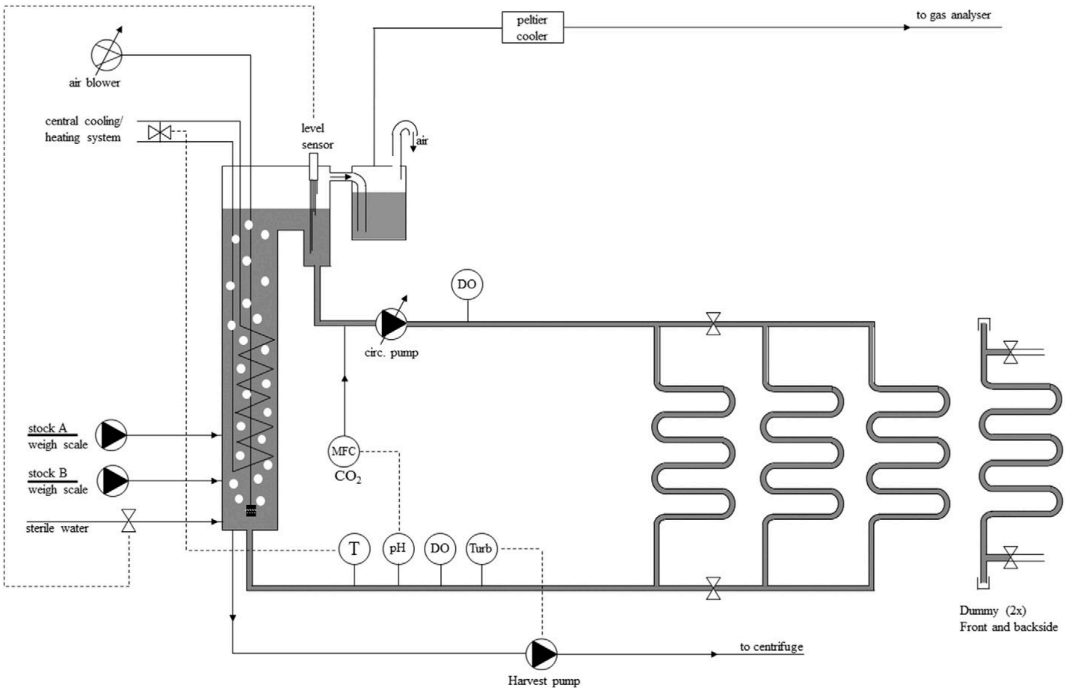


Figure A-2. Schematic drawing of the large horizontal tubular system (HD). Dashed lines show control strategies. Abbreviations: DO, dissolved oxygen; MFC, mass flow controller; T, temperature; turb, turbidity [46].

A–1.3 Measurements performed at AlgaePARC

In table A-1 all the measurements conducted during production at AlgaePARC are listed. In addition the used Sensor is indicated.

Measurements/Equipment	Sensor	Information
Light measurement (PAR)	CaTec Li-Cor LI-190SA	PAR, µmol m^{-2} s^{-1}
Pyranometer	Delta-T devices Sunshine sensor BF5	Direct and indirect light
Gas analysis	Servomex 4100	0–100% O_2 0–2.5% CO_2
Temperature	Endress + Hauser TSM487-AFE	Easytemp
Nutrient addition	Sartorius Midrics MAPP	DC/FE
Water flow	Kobold MIK-5NA-20-A-E34R	MIK
Sample gas cooler	Buhler technologies PKE511	Peltier cooler
Water level	Endress & Hauser FTW31-B2A5CA0A	5 pins
pH/temperature	Elscolab InPro3250/120/PT100	Stratos Pro
Dissolved oxygen	Mettler Toledo InPro6800/12/220	M300
Turbidity	Optek AS16-05	Control 4000
Carbon dioxide	Bronkhorst F201CV	EL-FLOW
Recirculation flow[a]	Endress + Hauser 50 W40-UA0A1AA0AAAA	
Airflow[a]	Kobold DOG-1101L-F25N-S-D	DOG

[a] only tubular system

Table A-0-1. Specification of online measurements and equipment for (1) Raceway Pond, (2) horizontal tubular PBR and (3) vertical stacked tubular PBR [46]

A–1.4 Measured Productivity Data

With the results of the biomass measurements (offline dry weight measurements), the volume added per day and the occupied ground area, the areal productivities can be determined. Daily productivities [g m^{-2} day^{-1}] at AlgaePARC are calculated by:

$$P_{exp} = \frac{X_{average} * V_{added}}{A_{ground}} \qquad \text{Equation A-1}$$

where $X_{average}$ [g L^{-1}]is the averaged dry weight over the production period, V_{added} [L day^{-1}] is the volume of water added per day to the system and A_{ground} [m^2] is the ground area covered.

A–1.5 Light and temperature conditions at pilot-scale production systems at AlgaePARC

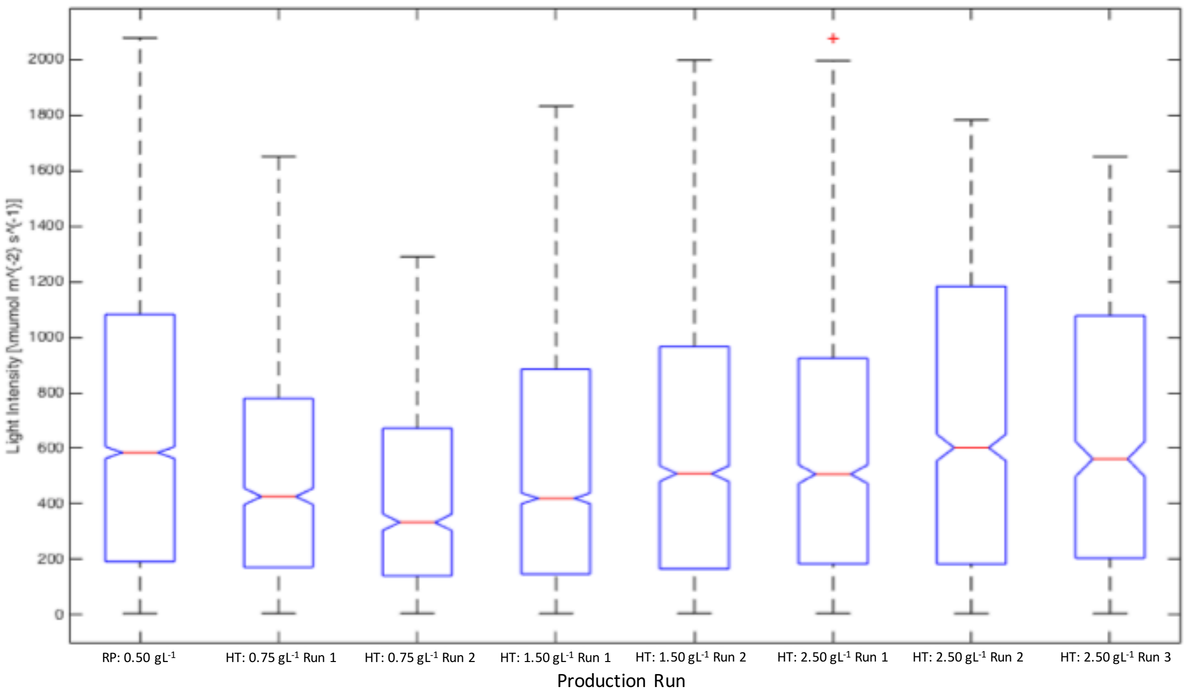


Figure A-3. Boxplot of light Intensity [µmol m-2 s-1] distribution found in the different runs of the raceway pond and horizontal tubular system at AlgaePARC in 2014.

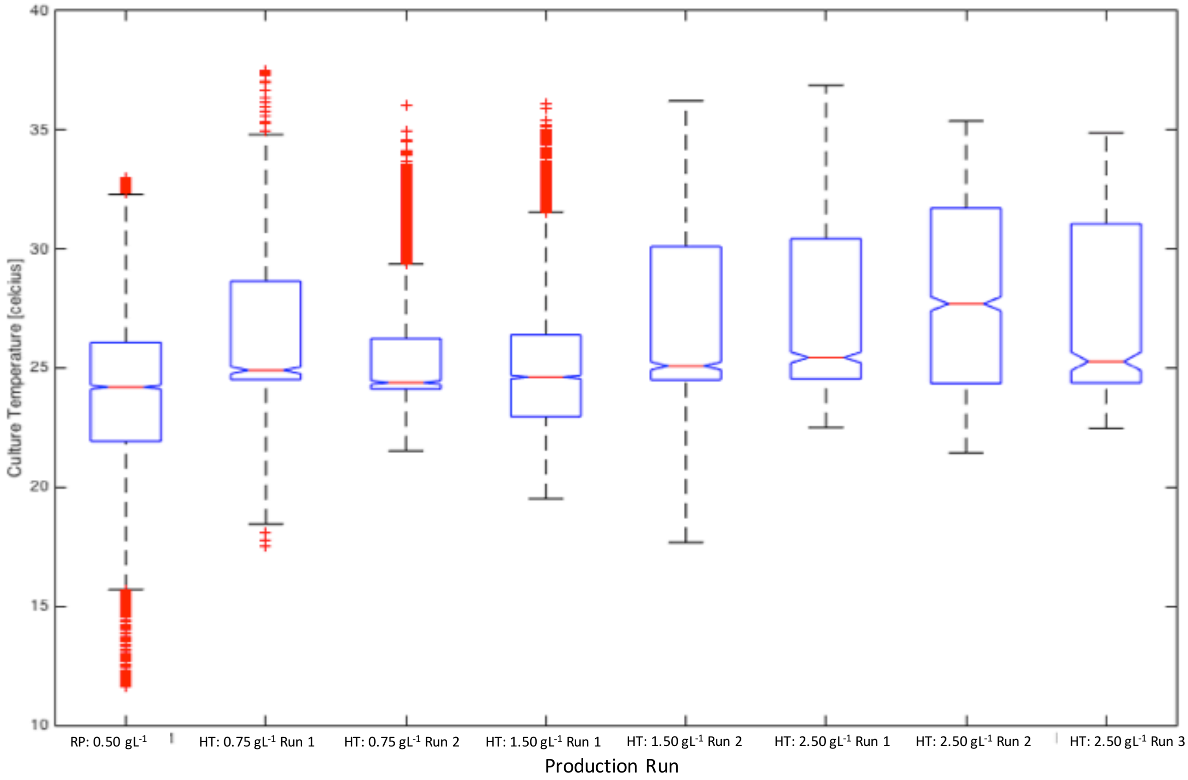


Figure A-4. *Boxplot of temperature [°C] distribution found in the different runs of the raceway pond and horizontal tubular system at AlgaePARC in 2014.*

System	Period	P_{exp}	$C_{x, average}$	$K_{abs, min}$	$K_{abs, average}$	K_{abs}, max	I_{total}	T_{min}	$T_{average}$	T_{max}
		[g m^{-2} day^{-1}]	[g L^{-1}]	[m^2 kg^{-1}]	[m^2 kg^{-1}]	[m^2 kg^{-1}]	[mol m^{-2} day^{-1}]	[°C day^{-1}]	[°C day^{-1}]	[°C day^{-1}]
Raceway Pond										
Run 1	30/05/2014 – 15/08/2014	10.4	0.44	126.5	146.96	189.4	38.26	20.7 (11.5)	24.0	28.01 (33.06)
Horizontal Tubular PBR										
C_x = 0.75 g L^{-1}										
Run 1	24/08/2014 – 08/09/2014	7.6	0.71	118.1	160.66	180.3	26.64	20.7 (18.9)	25.3	30.8 (32.5)
Run 2	18/08/2014 – 30/09/2014	7.1	0.95	105.1	121.81	130.8	18.95	20.9 (21.7)	24.4	28.5 (33.0)
C_x = 1.50 g L^{-1}										
Run 1	02/04/2014 – 22/05/2014	8.4	1.67	112.7	150.60	187.0	30.03	19.6 (16.9)	23.6	28.6 (34.0)
Run 2	29/05/2014 – 21/06/2014	11.6	1.54	154.1	175.70	197.2	38.00	20.4 (17.8)	25.4	31.0 (33.2)
C_x = 2.50 g L^{-1}										
Run 1	25/06/2014 – 07/07/2014	7.2	2.71	192.7	210.66	231.5	37.37	20.8 (19.8)	25.9	32.0 (34.0)
Run 2	17/07/2014 – 29/07/2014	13.5	1.87	165.0	213.34	242.7	37.13	20.2 (17.1)	27.7	31.1 (33.7)
Run 3	30/07/2014 – 06/08/2014	5.5	2.33	246.8	250.53	253.5	40.80	21.2 (20.0)	26.3	31.5 (32.0)

Table A-0-2. Summary of the operational conditions during cultivation in the pilot-scale systems at AlgaePARC. Displayed are the production period; areal productivity; biomass concentrations; minimum, average and maximum absorption coefficient; total light; and minimum, average and maximum temperature of different runs performed.

Appendix B

B–1 Data Analysis

B–1.1 Finding Gaps

Gaps in the dataset were found with a function created in Excel. The function detects greater gaps than the specification in a time series. In the example a gap greater than 10 min (1/1400 of a day) between cell A2 and A1 is searched:

```
=if(A2-A1>1/1400; "Gap"; "")
```
Equation B-1

B–1.2 Data selection

An extensive list of all the days excluded from the validation procedure can be found in the file:

"Productivity Data Evaluation – HT.xlsx"

B–1.3 Exploratory Data Analysis

Before any statistical analysis a dataset has to be explored, otherwise inferences cannot be made. The data exploration was performed using SPSS Statistics (v22).

From the menu in SPSS the following option is selected:

Analyze → Descriptives → Explore

Important are the plots on normality with the statistical test and the Kolmogorow-Smirnow test. The Kolmogorow-Smirnow should be not significant for normality. If the variables are not normally distributed they are not suitable for principal component analysis and bivariate correlation analysis.

B–1.4 Principal Component Analysis (PCA)

PCA can be generalized as correspondence analysis (CA) in order to handle qualitative variables and as multiple factor analysis (MFA) in order to handle heterogeneous sets of variables. Another advantage of PCA is that once patterns have been found they can be compressed, ie. by reducing the number of dimensions, without much loss of information [69]. Mathematically, PCA depends upon the eigen-decomposition of positive semi-definite matrices and upon the singular value decomposition (SVD) of rectangular matrices [44]. The PCA is performed using SPSS Statistics (v22). The settings within the program used for this analysis are as following:

From the menu in SPSS the following option is selected:

- *Analyze → Dimension Reduction → Factor Analysis*

The following options are chosen during conducting the analysis:

- *Descriptives: univariate, initial solution, coefficients, significance levels, KMO*
- *Extraction: unrotated factor solution, screen plot*
- *Options: sorted, supress small coefficients: 0.4*

The solution of the PCA is checked on the number of components. After that it is decided on the number of components that should be used for the further analysis.

The following options are chosen for the PCA analysis with reduced number of components:

- *Number of fixed factors: usually 2 or 3 depending on the result of the initial PCA*
- *Rotation: loading plots*
- *Rotated solution of factor analysis is obtained either by using Varimax or Oblimin*

Following criteria have to be checked in the outcome of the analysis conducted in SPSS. When they are not applying the analysis is not significant:

KMO/Barlet < 0.05
Communalities > 0.6
FVE > 0.6
Screen plot of variance explained should be in an elbow shape
Component matrix:
    Classify the variable to the component that has the higher score (loading)
Correlation Matrix:
    Coefficients between 0.3 – 0.9

B–1.4.1 Raceway Pond

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.867	48.672	48.672	4.867	48.672	48.672	4.012	40.121	40.121
2	2.588	25.884	74.556	2.588	25.884	74.556	3.443	34.434	74.556
3	1.608	16.083	90.638						
4	.633	6.332	96.970						
5	.217	2.175	99.145						
6	.060	.605	99.750						
7	.013	.125	99.875						
8	.008	.084	99.959						
9	.004	.036	99.995						
10	.001	.005	100.000						

Extraction Method: Principal Component Analysis.

Table B-0-1. Total variance explained of the PCA of the raceway pond

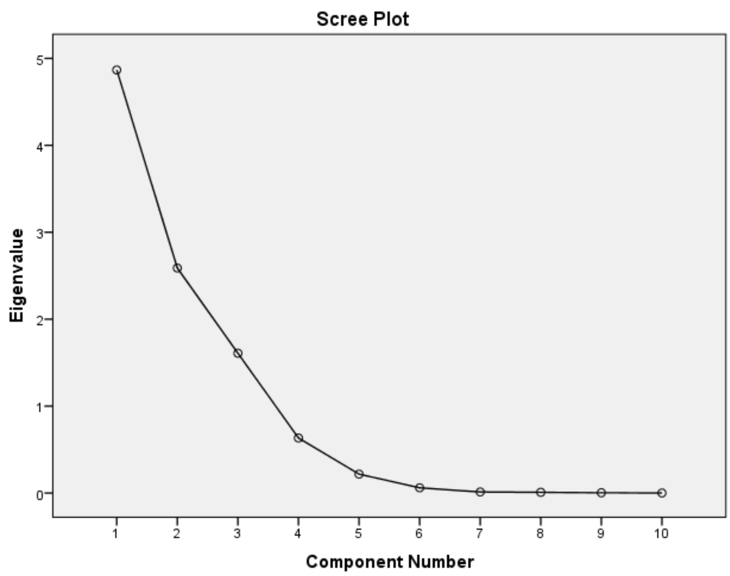


Figure B-1. Plot of the total variance explained of the PCA of the raceway pond

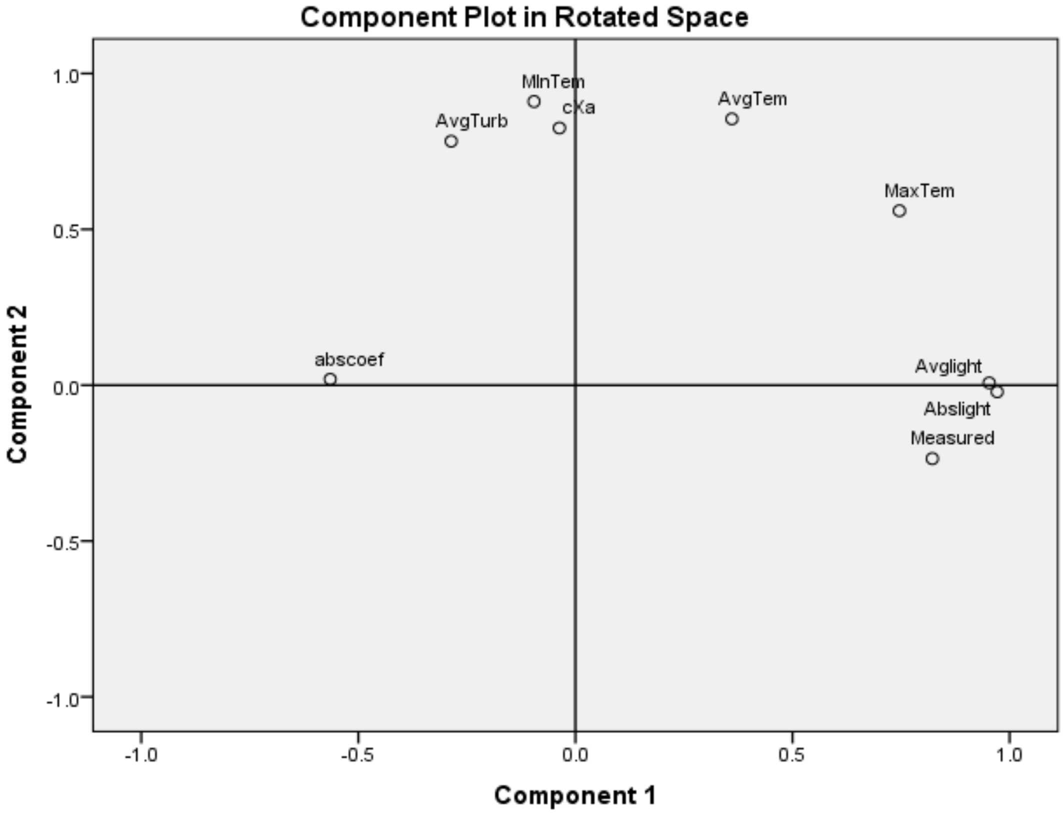


Figure B-2. Rotated component plot of the PCA result performed on measured variables of the raceway pond. Abbreviations: Abslight – total light input [mol m^{-2} day^{-1}]; Avglight – average light input [µmol m^{-2} s^{-1}]; Measured – measured areal productivity [g L^{-1} day^{-1}]; T_{max} - maximum culture temperature [°C day^{-1}]; abscoef – spectrally averaged absorption coefficient [kg m^{-2}]; T_{min} - minimum culture temperature [°C day^{-1}]; $T_{average}$ – average culture temperature [°C day^{-1}]; cXa – measured biomass concentration [g L^{-1}]; AvgTurb – average culture turbidity [NTU];

Rotated Component Matrix[a]

	Component	
	1	2
Abslight	.971	
Avglight	.953	
Measured	.822	
MaxTem	.746	.560
abscoef	-.565	
MInTem		.910
AvgTem		.854
cXa		.825
AvgTurb		.783

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 3 iterations.

Figure B-3. Component matrix obtained from the PCA on the raceway pond. This matrix contains of two principal components. The values represent how large the contribution of a variable is to the component. In the case of MaxTem the the higher value indicate that this variable is attributed with the first component. Abbreviations: Abslight – total light input [mol m^{-2} day^{-1}]; Avglight – average light input [µmol m^{-2} s^{-1}]; Measured – measured areal productivity [g L^{-1} day^{-1}]; T_{max} - maximum culture temperature [°C day^{-1}]; abscoef – spectrally averaged absorption coefficient [kg m^{-2}]; T_{min} - minimum culture temperature [°C day^{-1}]; $T_{average}$ – average culture temperature [°C day^{-1}]; cXa – measured biomass concentration [g L^{-1}]; AvgTurb – average culture turbidity [NTU

B–1.3.2 Horizontal Tubular PBR

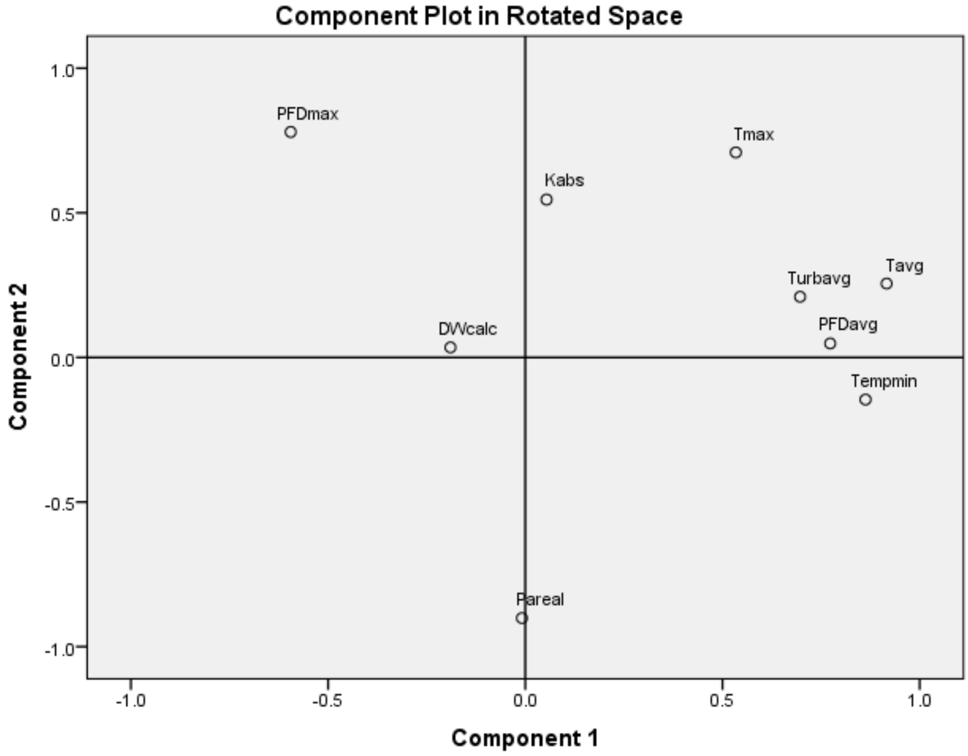


Figure B-4. *Rotated component plot of the PCA result performed on measured variables of the horizontal tubular PBR Cx=0.75 g L-1. Abbreviations: PFDmax – max light input [mol m^{-2} day^{-1}]; PFDavg – average light input [µmol m^{-2} s^{-1}]; Pareal – measured areal productivity [g L^{-1} day^{-1}]; Tmax - maximum culture temperature [°C day^{-1}]; Kabs – spectrally averaged absorption coefficient [kg m^{-2}]; Tempmin - minimum culture temperature [°C day^{-1}]; Tavg – average culture temperature [°C day^{-1}]; DWcalc – measured biomass concentration [g L^{-1}]; Turbavg – average culture turbidity [NTU];*

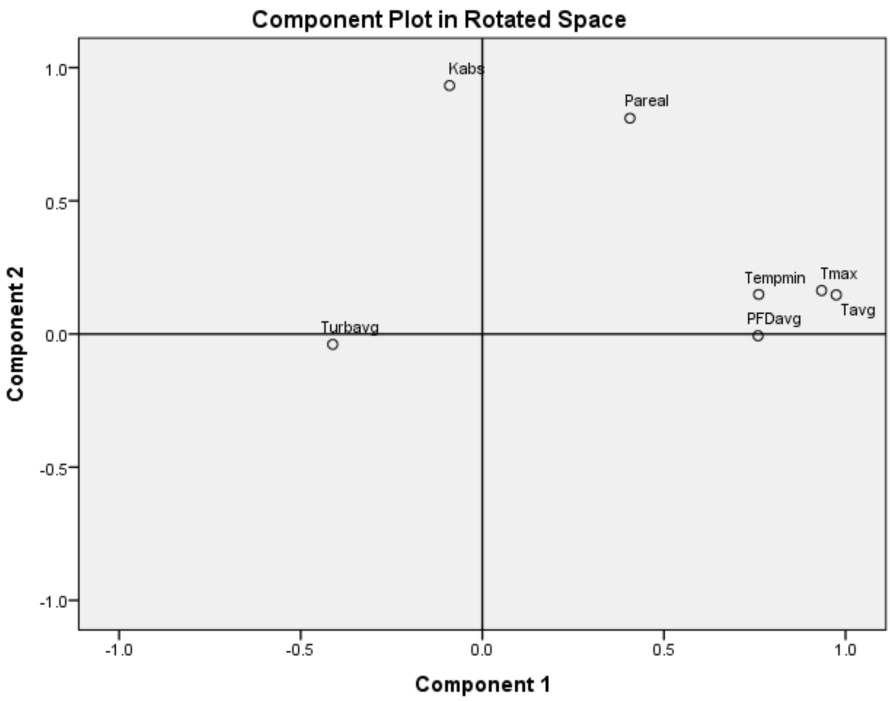


Figure B-5. *Rotated component plot of the PCA result performed on measured variables of the horizontal tubular PBR Cx=1.50 g L-1. Abbreviations: PFDmax – max light input [mol m^{-2} day^{-1}]; PFDavg – average light input [µmol m^{-2} s^{-1}]; Pareal – measured areal productivity [g L^{-1} day^{-1}]; Tmax - maximum culture temperature [°C day^{-1}]; Kabs – spectrally averaged absorption coefficient [kg m^{-2}]; Tempmin - minimum culture temperature [°C day^{-1}]; Tavg – average culture temperature [°C day^{-1}]; DWcalc – measured biomass concentration [g L^{-1}]; Turbavg – average culture turbidity [NTU];*

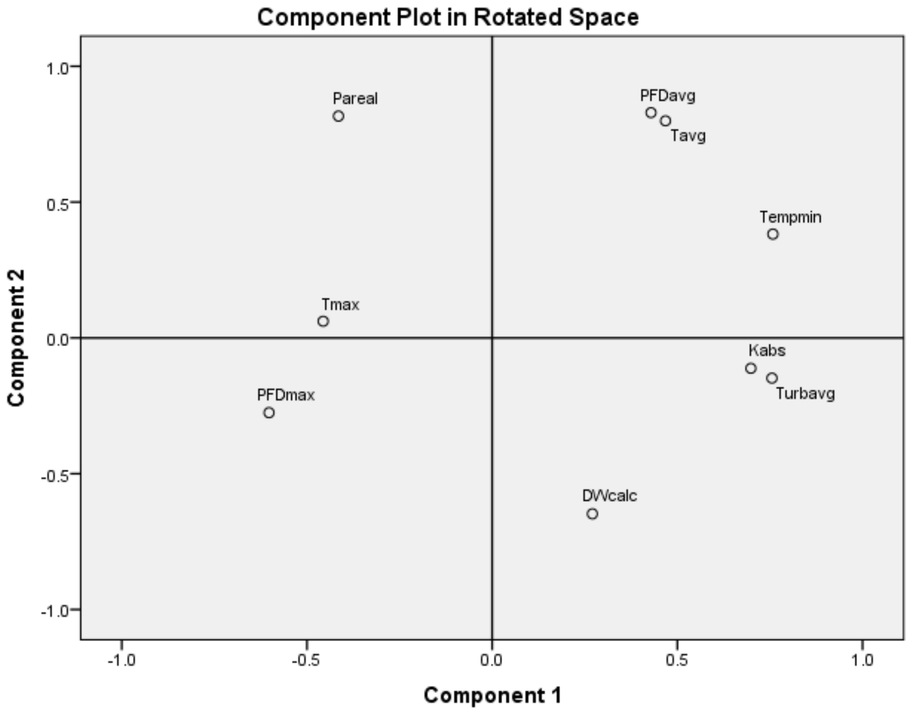


Figure B-6. Rotated component plot of the PCA result performed on measured variables of the horizontal tubular PBR Cx=2.50 g L-1. Abbreviations: PFDmax – max light input [mol m^{-2} day^{-1}]; PFDavg – average light input [µmol m^{-2} s^{-1}]; Pareal – measured areal productivity [g L^{-1} day^{-1}]; Tmax - maximum culture temperature [°C day^{-1}]; Kabs – spectrally averaged absorption coefficient [kg m^{-2}]; Tempmin - minimum culture temperature [°C day^{-1}]; Tavg – average culture temperature [°C day^{-1}]; DWcalc – measured biomass concentration [g L^{-1}]; Turbavg – average culture turbidity [NTU];

B–1.4 Bivariate Correlation Analysis (BCA)

The BCA is performed on the measured variables during the cultivation at AlgaePARC. The analysis is conducted in SPSS Statistics (v22).

From the menu in SPSS the following option is selected:

Correlate → Bivariate

The correlation factors of the analysed variables on measured productivity are displayed in figure B-7.

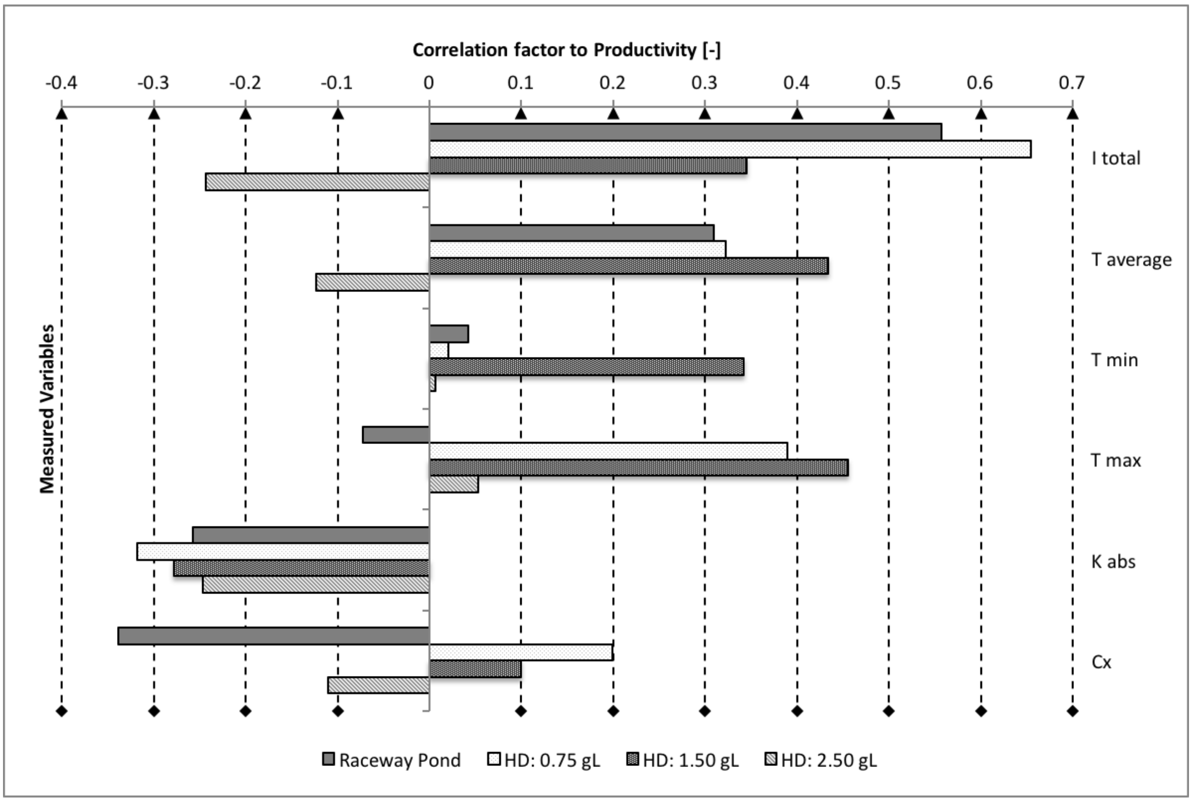


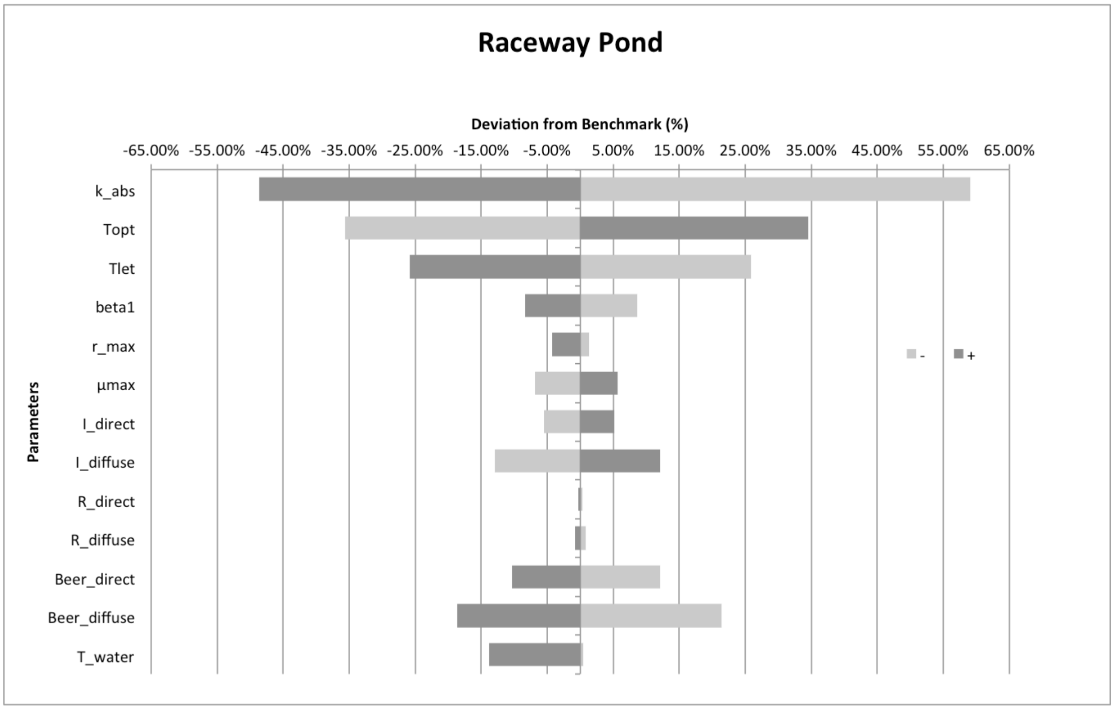
Figure B-7. The correlation factor of measured variables from the raceway pond and the different turbidostat operations of the horizontal tubular system in respect to the measured productivity. Abbreviations: I_{total} - total light input [mol m^{-2} day^{-1}]; $T_{average}$ - average culture temperature [°C day^{-1}]; T_{min} - minimum culture temperature [°C day^{-1}]; T_{max} - maximum culture temperature [°C day^{-1}]; K abs – spectrally averaged absorption coefficient [kg m^{-2}]; C_x – biomass concentration [g L^{-1}].

Appendix C

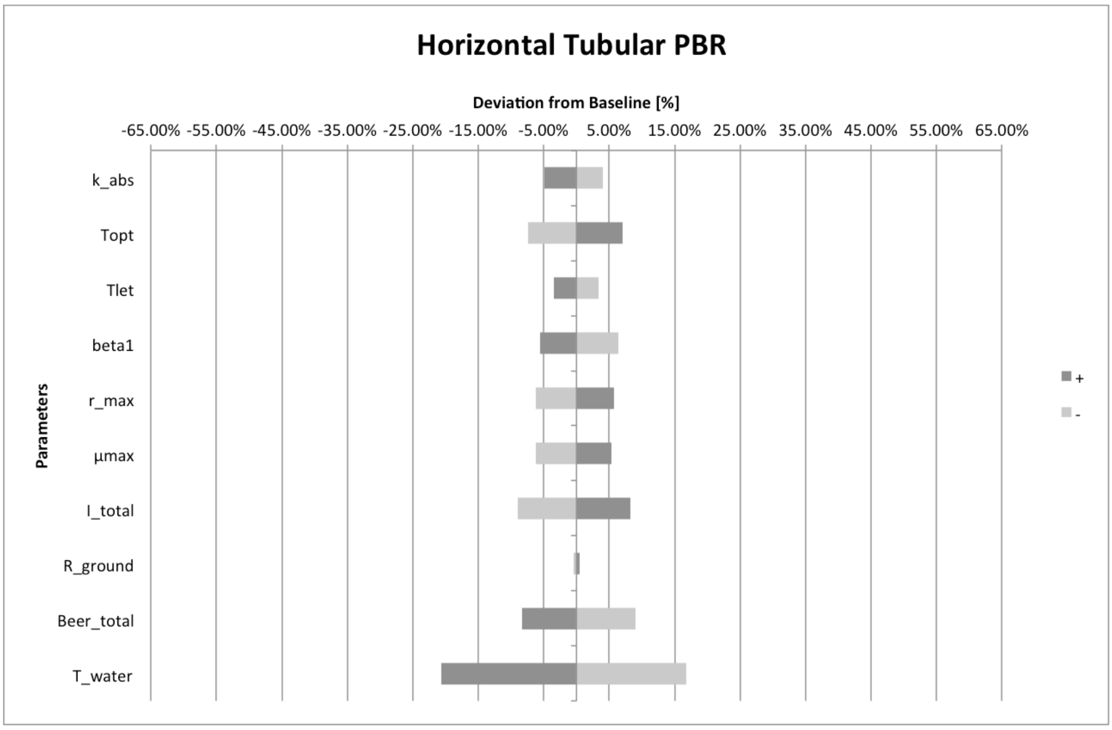
C–1 Local Uncertainty/Sensitivity Analysis

Prior to the global uncertainty/sensitivity analysis a local uncertainty/sensitivity analysis was performed. The drawbacks of such a way of computing sensitivity are that it depends on the linearity of the model, and no interactions of inputs are studied [55]. For this analysis the same uncertainty ranges for the model inputs were chosen as for the global uncertaint/sensitivity analysis (table 3-8). The result of this local uncertainty/sensitivity analysis showed similar importance of the model inputs. In the case of the raceway pond the absorption coefficient, diffused light input and the interior light angle of diffused light showed similar importance. However the optimum and lethal temperature of *Nannochloropsis sp.* weighted more than the maximum specific growth rate and the respiration rate. In the case of the horizontal tubular system a much similar result compared to the global sensitivity analysis was obtained. The culture temperature had the largest effect on the model output, however when looking at beta, optimum and lethal temperature, they were almost equally important as the maximum specific growth rate and respiration rate. On the contrary in the global analysis the difference between these parameters was much more distinct. The tornado charts obtained from the local uncertainty/sensitivity analysis can be found in figure C-1 and figure C-2.

Slegers et al. performed an global uncertainty and sensitivity for the productivity model used for single standing flat panels [2]. Since at this point experimental data were not available, the ranges in which the parameters vary were based on current best knowledge. The absorption coefficient was varied in a range of 50 %, functional cross section of the photosynthetic apparatus by 40 %, maximum specific growth rate and maximum respiration rate by 20 % each. The results obtained in this analysis corresponded with the result of the raceway pond obtained in this study. The most essential parameters found were the spectrum-averaged light absorption coefficient, maximum specific growth rate and functional cross section of the photosynthetic apparatus.



<u>Figure C-1.</u> Tornado Plot of the local sensitivity analysis performed on the productivity model of the raceway pond. Abbreviations: k_abs – spectrally averaged absorption coefficient; Topt – optimum growth temperature; Tlet – lethal temperature; beta1 – temperature curve modulating factor; r_max – maintenance associated respiration rate; µmax – maximum growth rate; I_direct – direct light input; I_diffuse – diffuse light input; R_direct – reflection of direct light; R_diffuse – reflection of diffuse light; Beer_direct – interior light angle of direct light; Beer_diffuse – interior light angle of diffuse light; T_water – culture temperature.



<u>Figure C-2.</u> Tornado Plot of the local sensitivity analysis performed on the productivity model of horizontal tubular PBR. Abbreviations: k_abs – spectrally averaged absorption coefficient; Topt – optimum growth temperature; Tlet – lethal temperature; beta1 – temperature curve modulating factor; r_max – maintenance associated respiration rate; µmax – maximum growth rate; I_direct – direct light input; I_diffuse – diffuse light input; R_direct – reflection of direct light; R_diffuse – reflection of diffuse light; Beer_direct – interior light angle of direct light; Beer_diffuse – interior light angle of diffuse light; T_water – culture temperature.

C–2 Global Uncertainty/Sensitivity Analysis

In order to perform the global uncertainty/sensitivity analysis error factors (e) are introduced to the productivity model (table C-1). These factors cause a variation in the specified uncertainty range of the biological or physical model input that should be investigated. The concept of Monte-Carlo Samplings are used for causing the input fluctuations [55].

Model Input	Implementation of error factor
(1) Absorption coefficient	$K_{abs} = K_{abs} * e(1)$
(2) Specific growth rate	$\mu_{max} = \mu_{max} * e(2)$
(3) Maintenance associated respiration rate	$\beta_T = \beta_T * e(3)$
(4) Temperature curve modulating factor	$r_{max} = r_{max} * e(4)$
(5) Optimum growth temperature	$T_{optimum} = T_{optimum} * e(5)$
(6) Lethal temperature	$T_{lethal} = T_{lethal} * e(6)$
(7) Total Light Input	$I_{total} = (I_{direct} + I_{diffuse}) * e(7)$
(8) Ground reflectivity	$rho = 0.2 * e(8)$
(9) Interior light angle	$I_{local} = I_{total} * e^{-K_{abs} * C_x * z * e(9)}$
(10) Culture temperature	$T = T * e(10)$
(11) Biomass Concentration	$C_x = C_x * e(11)$

Table C-1. Implementation of error factors in the productivity models of Slegers et al. [1]–[3]

C–2.1 Biological model inputs

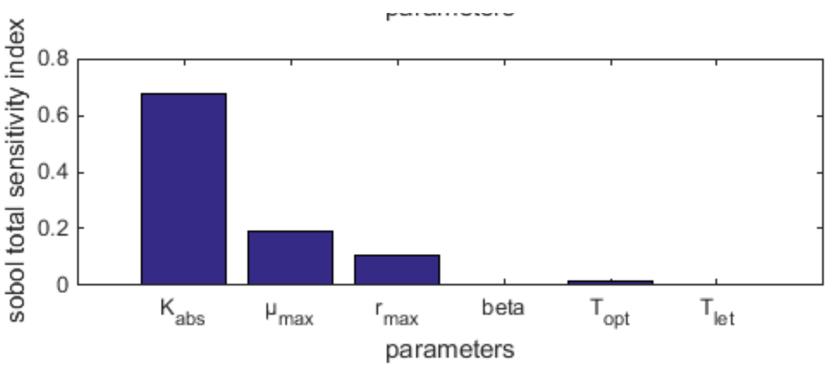


Figure C-3. Total Sobol-Coefficients of the biological parameters/variables investigated in the productivity model of the raceway pond.

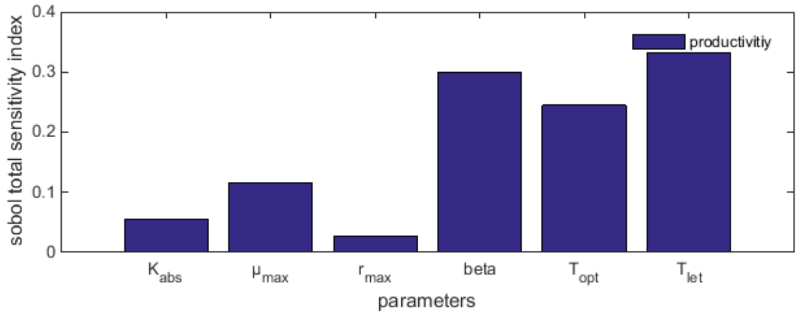


Figure C-4. Total Sobol-Coefficients of the biological parameters/variables investigated in the productivity model of the horizontal tubular PBR.

C–2.2 Physical model inputs

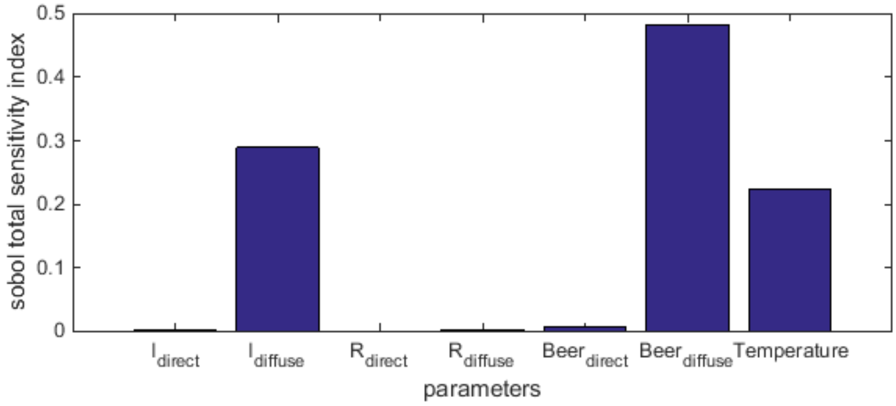


Figure C-5. Total Sobol-Coefficients of the physical parameters/variables investigated in the productivity model of the raceway pond.

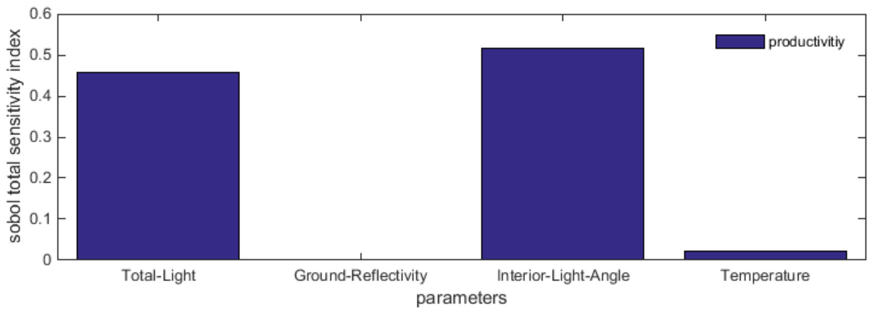


Figure C-6. Total Sobol-Coefficients of the physical parameters/variables investigated in the productivity model of the horizontal tubular PBR.

C–2.3 Combination of biological and physical model inputs

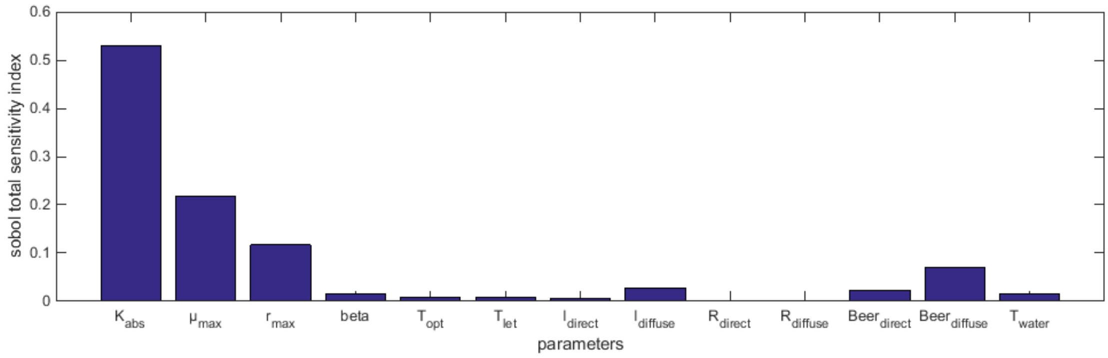


Figure C-7. Total Sobol-Coefficients of the biological and physical parameters/variables investigated in the productivity model of the raceway pond.

For the plot displayed in the main body of the report, the total Sobol-coefficients are combined for the direct and diffuse light in order to compare it to the results of the horizontal tubular PBR.

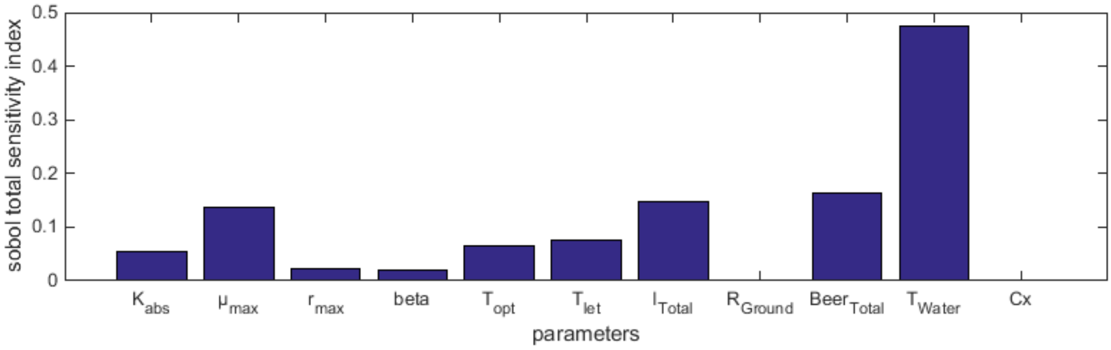


Figure C-8. Total Sobol-Coefficients of the biological and physical parameters/variables investigated in the productivity model of the horizontal tubular PBR.

Appendix D

D–1 Productivity Model

<u>Original version:</u>

The specific growth rate is calculated according to the model developed by Geider et al. [51]. This growth model is connects the photosynthetic activity of the algae cell to the current light intensity and irradiance dependent chlorophyll a: carbon ratio. Photo inhibition is not taken into account [3].

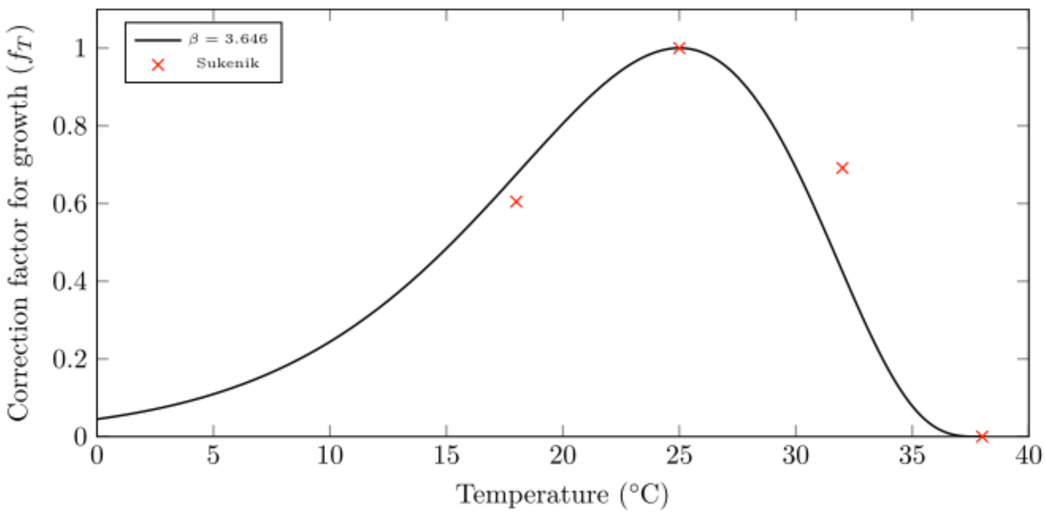
$$\mu_{growth}(z,t) = P_m^c\left(1 - exp\left(\frac{-\alpha\, I_{PFD}(z,t)\,\theta_a(z,t)}{P_m^c}\right)\right) - r_{max}$$ Equation D-1

The specific growth rate depends on the chlorophyll a:carbon ratio in the cell θ_a (g Chl a g^{-1} C) and on the photon flow density I_{PFD} (µmol m^{-2} s^{-1}) experienced by the algae cell at the position in pond depth z (m) and time t. The chlorophyll a: carbon ratio in the algae cell adapts to the according light conditions. The specific growth rate also depends on the maximum carbon specific rate of photosynthesis P_m^c (s^{-1}), the functional cross section of the photosynthetic apparatus α (g C (mol^{-1} photons) m^2 g^{-1} Chl a) and the maintenance metabolic coefficient r_{max}.

The functional cross section α is taken constant. The chlorophyll a: carbon ratio is given by:

$$\theta_a\,(z,t) = \theta_{a,max}\frac{1}{1+\frac{\theta_{max}\,\alpha\, I_{PFD}(z,t)}{2P_m^c}}$$ Equation D-2

D–2 Temperature Model



<u>Figure D-1.</u> Curve of the temperature dependent factor (f_T) used in this thesis. Curve is fitted to data by Sukenik et al. [48] *with a beta of 3.646 by Van Dam* [34].

D–3 Optimal Biomass Concentration

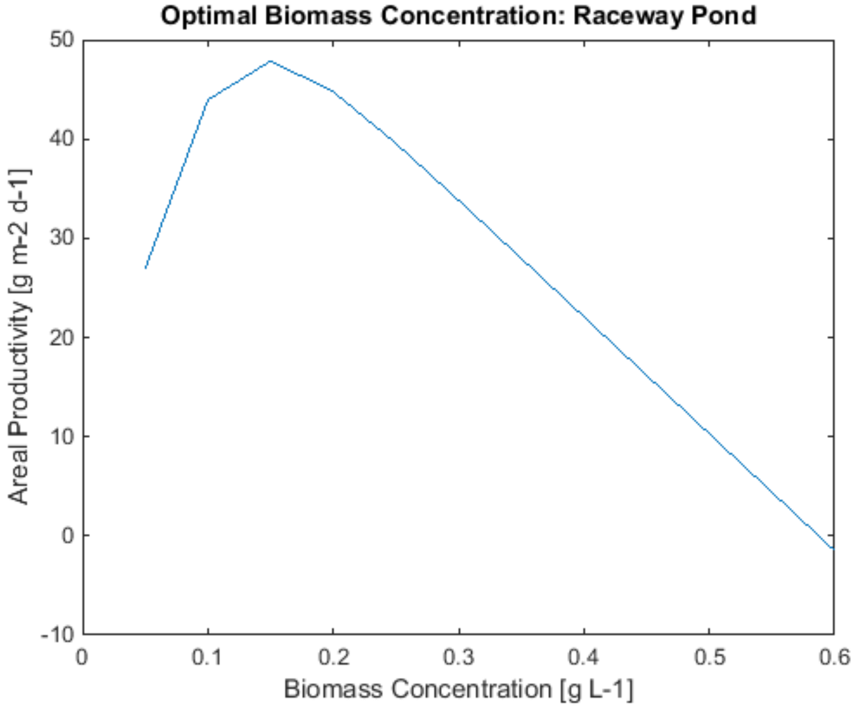


Figure D-2. Influence of biomass concentration on areal productivity [g m^{-2} d^{-1}] in the raceway pond.

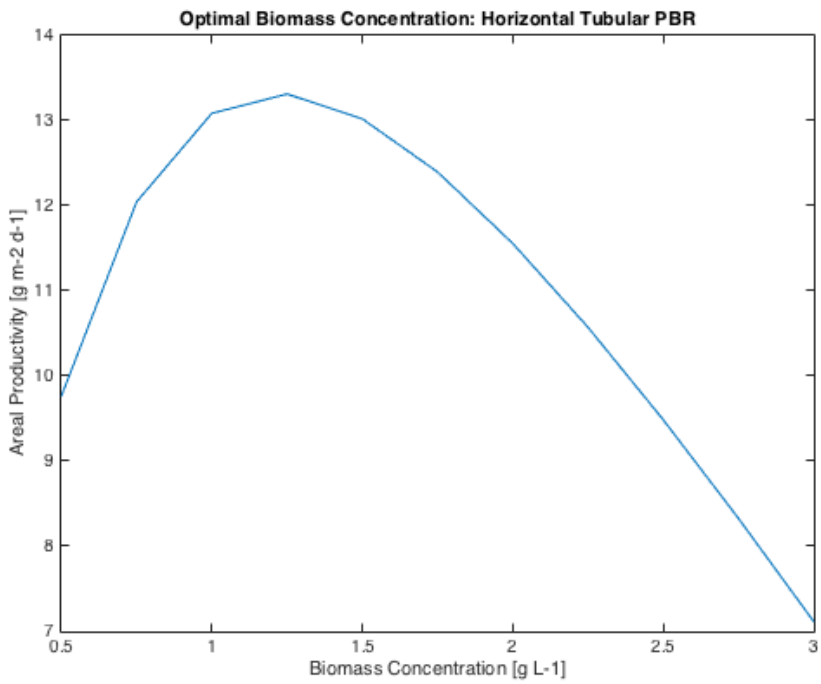


Figure D-3. Influence of biomass concentration on areal productivity [g m^{-2} d^{-1}] in the horizontal tubular PBR.

D–3 Cumulative Productivity

During the production run Horizontal Tubular PBR (HT): 1.5 g L^{-1} Run 2, HT: 2.50 g L^{-1} Run 3 operational issues and limited data points biased the model validation. During both runs biofilm formation led to unstable turbidostat signals. The readjustment of the set point to maintain constant biomass concentration had an significant effect on the harvesting procedure.

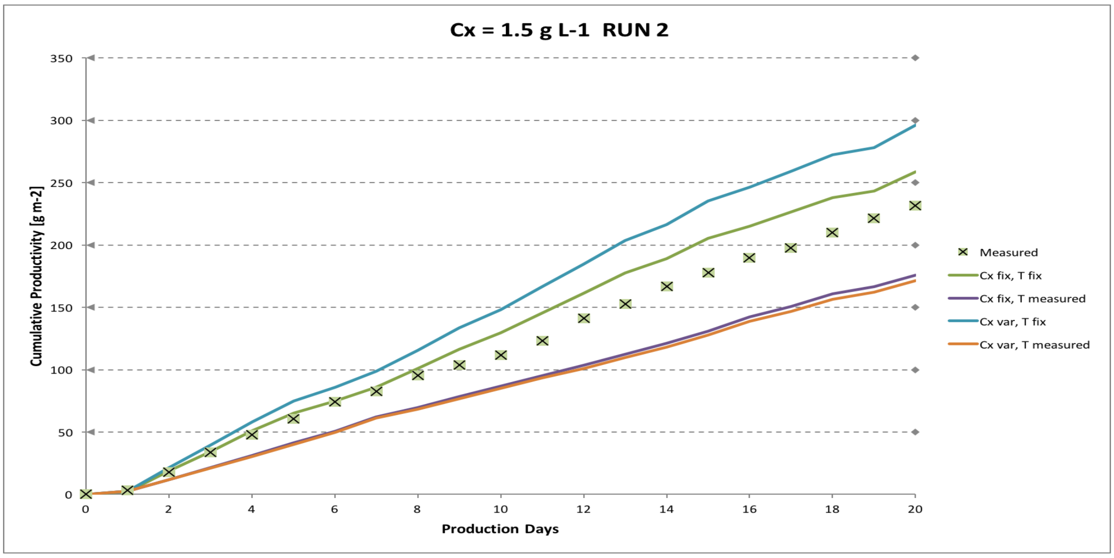


Figure D-4. Different modelling scenarios for the horizontal tubular system at a biomass concentrations of 1.50 g L^{-1} Run 2. Abbreviations: Measured – measured areal productivity, for modelling scenarios: Cx fix – biomass concentration is fixed, Cx var –measured biomass concentration is used, T fix – culture temperature is fixed, T measured – measured culture temperature.

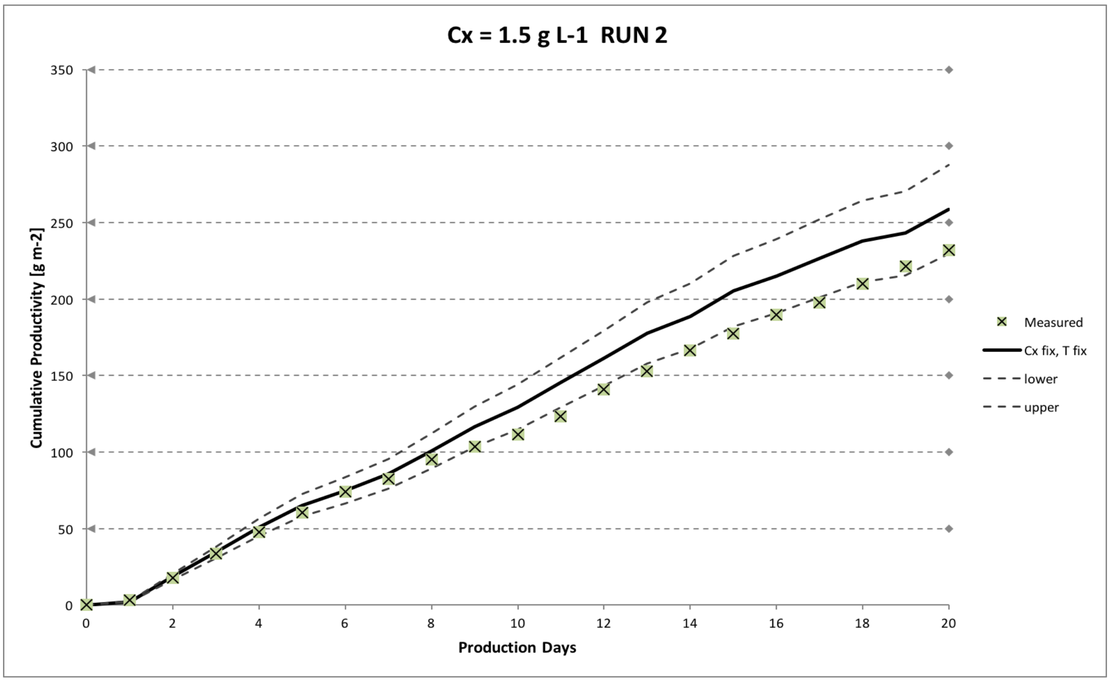


Figure D-5. Cumulative productivity in the horizontal tubular system at a biomass concentrations of 1.50 g L^{-1} Run 2. Comparison of experimental data and model prediction is made. Grey dashed line represents the upper and lower boundaries of the confidence interval of the model attributed uncertainty (±11.27 %)

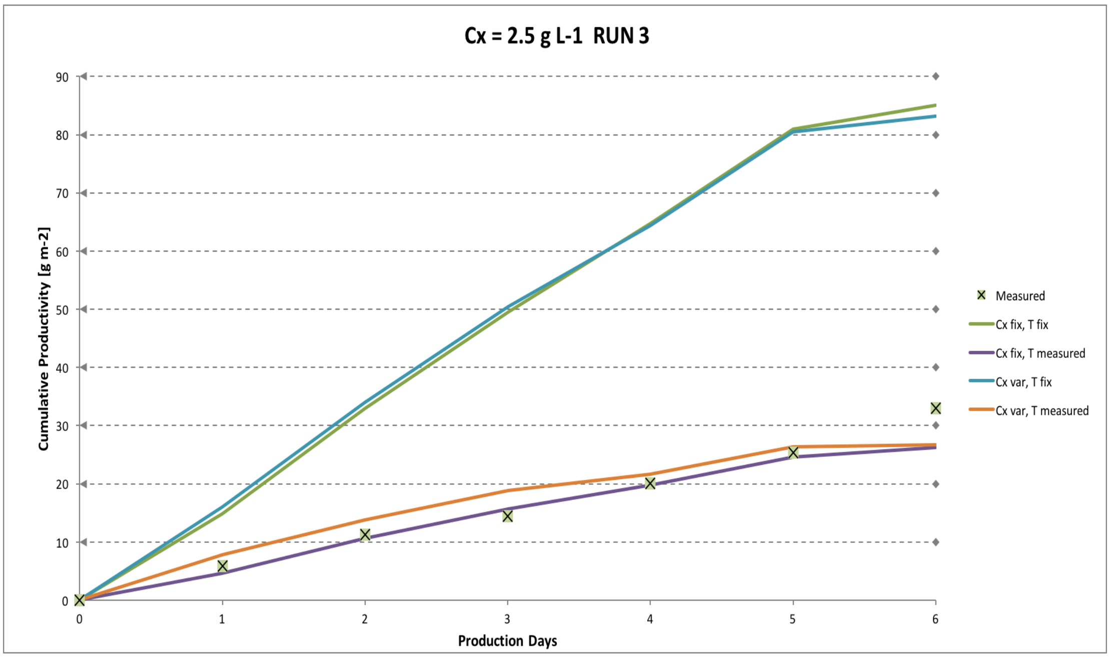


Figure D-6. Different modelling scenarios for the horizontal tubular system at a biomass concentrations of 2.50 g L^{-1} Run 3. Abbreviations: Measured – measured areal productivity, for modelling scenarios: Cx fix – biomass concentration is fixed, Cx var –measured biomass concentration is used, T fix – culture temperature is fixed, T measured – measured culture temperature.

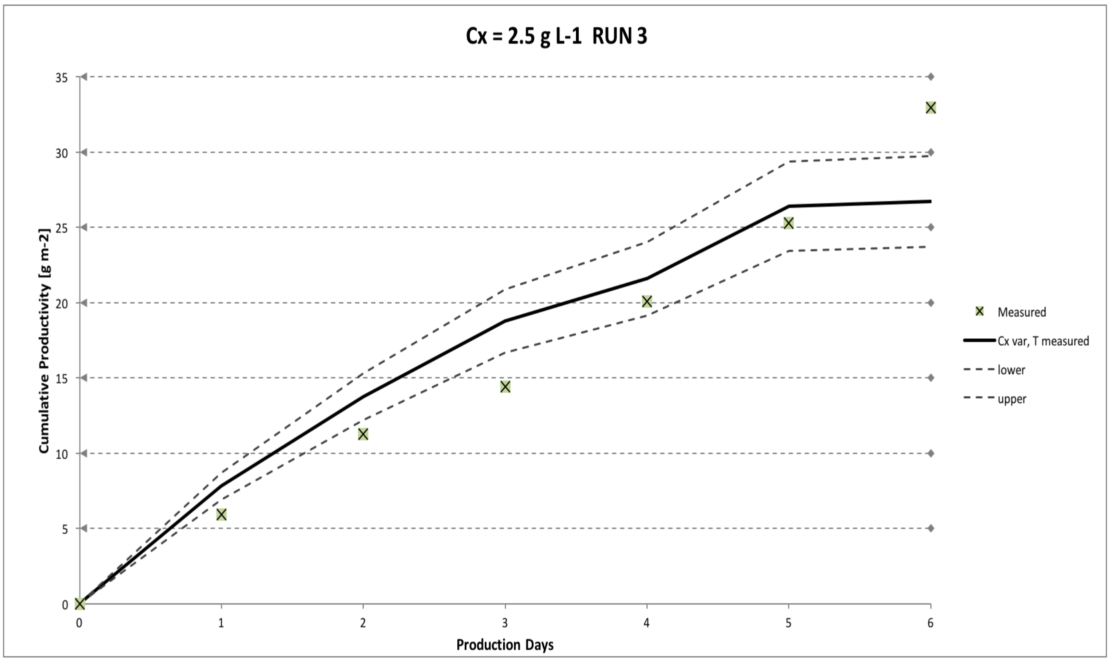


Figure D-7. Cumulative productivity in the horizontal tubular system at a biomass concentrations of 2.50 g L^{-1} Run 3. Comparison of experimental data and model prediction is made. Grey dashed line represents the upper and lower boundaries of the confidence interval of the model attributed uncertainty (±11.27 %)

D–4 Average biomass loss during night

The decrease of the biomass concentration during the night at AlgaePARC is calculated via the measured linear relationship between the turbidity and the biomass concentration obtained at AlgaePARC (equation D-3). The average biomass loss rate due to nightly respiration was determined at each biomass concentration (C_X) used in the different algae production systems. The biomass concentration is converted from NTU into gL^{-1} with the following calculation:

$$C_x = \frac{C_{x,NTU}}{\varphi_{NTU:DW}} \qquad \text{Equation D-3}$$

where C_x is the biomass concentration in the culture [g L^{-1}], $C_{x,NTU}$ is the biomass concnetraion in the culture in [NTU], and $\varphi_{NTU:DW}$ is the ratio between NTU and dry weight.

The average rate of biomass loss during the night was calculated with the following formula used by Michels et al. [65]:

$$Average\ biomass\ loss\ = \frac{\ln{(C_{x,0})} - \ln{(C_{x,t})}}{\Delta t_{night}} \qquad \text{Equation D-4}$$

where $C_{x,0}$ is the biomass concentration at sunset [g L^{-1}], $C_{x,t}$ is the biomass concentration at sunrise [g L^{-1}], and Δt_{night} is the time period of the night from sunset to sunrise [h].

The nightly biomass loss measured at AlgaePARC for the different systems and biomass concentrations is compared to the biomass loss predicted by the productivity models.

D–5 Biomass loss during night

During the night, biomass concentration decreases. The endogenous respiration is the self-evident process responsible for this biomass loss. During respiration compounds like carbohydrates are used to provide the cells with sufficient energy to maintain their metabolic activity [14] [15]. Energy is used at the cost of biomass.

In figure 11 the average nightly biomass losses measured at AlgaePARC and predicted by the model for the raceway pond and horizontal tubular system are compared to each other. Generally the algae productivity models of Slegers et al. [1], [3] result in an overestimation of biomass loss during night, since the biomass concentration was assumed to stay constant during the night. In addition the modelled biomass loss showed to be increasing with biomass concentration. This was explained due to the fact that the rate of night time respiration is modelled using first-order kinetics with regard to cell concentration. An equal biomass loss rate for every biomass concentration is taken, since it is assumed that night time respiration is only associated with the maximum respiration rate maintenance and therefore constant over time. This resulted in higher biomass losses at higher biomass concentrations due to prevailing respiration at night.

However the opposite trend was observed for the measured biomass loss recorded at AlgaePARC. The calculated biomass loss was decreasing with increasing biomass concentration. At a biomass concentration of 0.5 g L^{-1} the biomass loss was recorded to be the highest, having the lowest biomass loss at a biomass concentration of 2.5 g L^{-1}.Similar results were obtained by Michels et al. [65], which studied the effect of biomass concentration and growth rate on the nightly biomass loss rate of *Tetraselmis suecica*. In this study the highest nightly biomass loss rate was recorded at the optimal biomass concentration of this organism in the production system used during research. This nightly biomass loss rate was higher than the ones found for biomass concentrations of 1.5 g L^{-1} and 2.0 g L^{-1}. Michels et al. explained this trend due to higher maintenance costs due to the increasing growth rate.

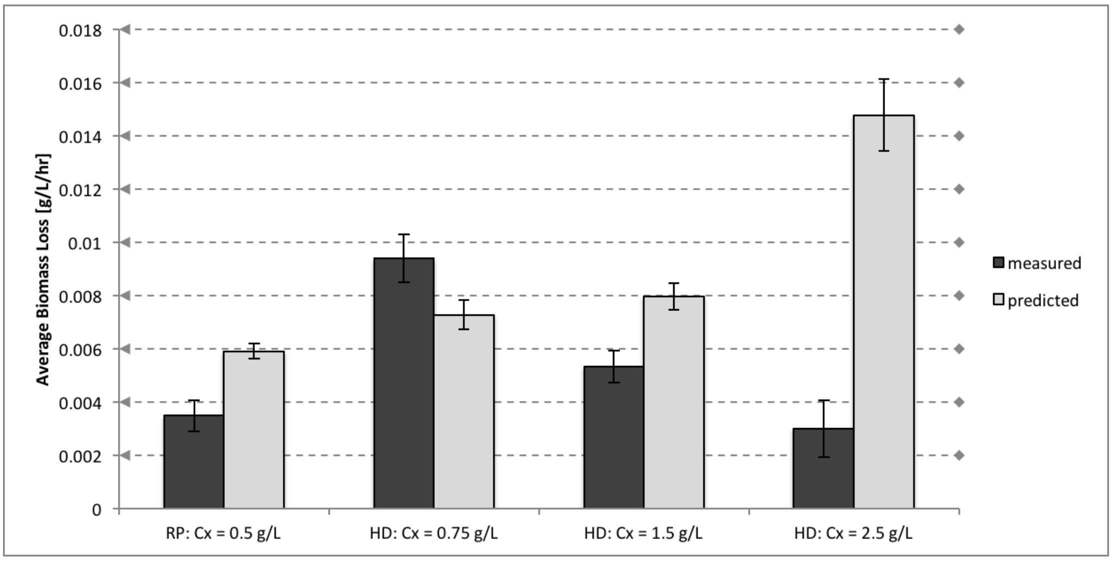


Figure D-8. The measured and predicted average biomass loss of different biomass concentration in the raceway pond (RP) and horizontal tubular system (HD). (Error bars represent the 95% confidence interval)

The night metabolism is also determined by numerous environmental factors including prior light intensity history, nutrient status, temperature and the species itself [66]. In the study of Vítová et al. [67] it was shown that the respiration duration of the *C. reinhardtii* cell cycle is affected by both prior experienced light intensity and temperature. Michels et al. [65] investigated in addition the effect of the growth rate on the nightly biomass loss. Microalgae with a higher growth rate due to higher prior light history were found to store relatively more energy in the formation of carbohydrates, which can then easier be used in the following night for the maintenance of the cells. Therefore, Michels et al. expect a linear correlation between the specific growth rate and the nightly biomass loss rate of the same day. This effect was also studied by Torzillo et al. [72] that found the similar relation ship between biomass composition and biomass loss during night.

In the study of Le Borgne et al. [68] it is stresses that biomass concentration do have an influence on the biomass loss during night, but the effect is negligible compared with that of temperature. In the modelling study of Collins and Boylen, the specific rate of respiration of *A. variabilis* was shown to increase from 0.2 to 1.0 g carbon/g $_{biomass}$ d^{-1} when temperature varied from 10 °C to 40 °C. As temperature can drop by more than 10 °C at night during outdoor cultivation [41], the impact of temperature on night-time respiration must be considered.

Although the model overestimated biomass loss during night, the net biomass gain could be accurate even if both the rates of growth and respiration were systematically overestimated, thereby cancelling out their respective errors.

D–6 Bar Plot

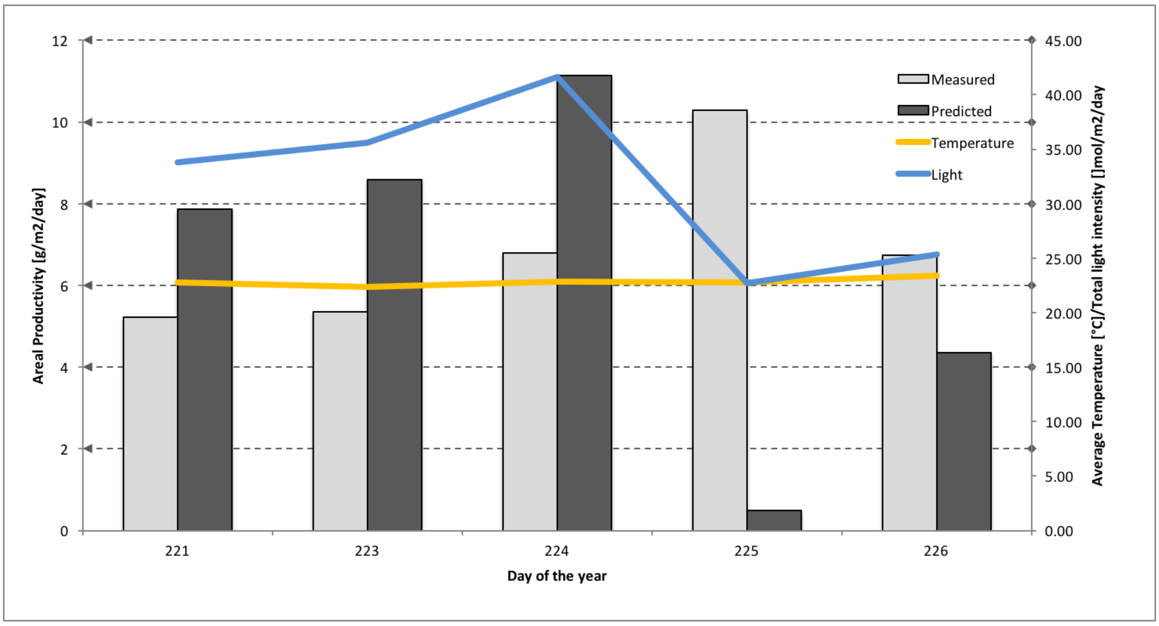


Figure D-9. Bar Plot of the first 16 days of production in the raceway pond at AlgaePARC. In this graph the instant adaption of the model and the delayed reaction of biology to changes in light and temperature can be observed.

In figure D-9 five production days in the raceway pond are depicted. During this period an increase in total light intensity is recorded with a sudden drop at day 225. With increasing light, algae cells are slowly adaption to the new conditions. On the day where the light intensity decreases significantly the highest productivity is recorded meaning that the cells exhibit still a high specific growth rate. Only on the second day of the decrease in irradiance the effect on biology is notable.

D–7 Influence of model variable on relative deviation

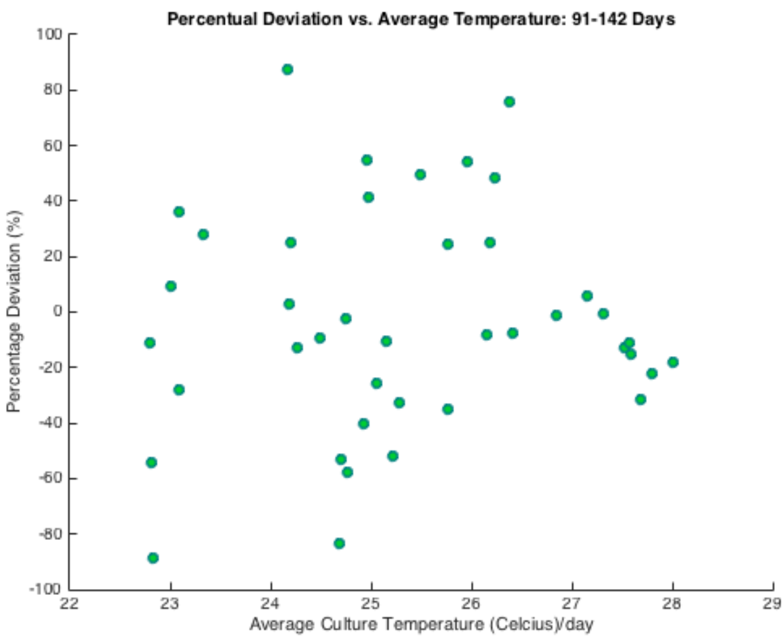


Figure D-10. Influence of average temperature [°C day^{-1}] on relative deviation [%] between measured and predicted productivity.

System /Location	P. tricornum (Kabs=75)	Increase in productivity in respect to the base scenario	T. pseudonana (Kabs=269)	Increase in productivity in respect to the base scenario	Average productivity increase	Extrapolation of Model Prediction	Extrapolation of Model Uncertainty
	[g m^{-2} day^{-1}]		[g m^{-2} day^{-1}]			[g m^{-2} day^{-1}]	[g m^{-2} day^{-1}]
Raceway Pond							
Netherlands (Base Scenario)	11.4		2.2			10.7	1.0
France	--		--				
Algeria	17.5	53.5%	4.1	86.4%	69.9%	18.2	1.7
Horizontal Tubular PBR							
Netherlands (Base Scenario)	12.7		7.4			9.8	1.3
France	19.5	53.5%	12.0	62.2%	57.9%	15.5	2.1
Algeria	26.5	108.7%	16.4	121.6%	115.1%	21.1	2.8

Table D-0-1. Extrapolation of predicted productivity data to other locations such as France and Algeria by using the scenario estimates of Slegers et. al [1], [3].

Appendix E

E–1 Literature research

Study	Cultivation System	Location	Productivity [g m^{-2} day^{-1}]
[73]	Raceway Pond	Australia	24.9
[42]	Raceway Pond	Brazil	10.7
[74]	Raceway Pond	Spain	8.2
[75]	Raceway Pond	Italy	5.5
[61]	Raceway Pond	Italy	12.9
[60]	Raceway Pond	England	6.8
[62]	Raceway Pond	California (USA)	16.4
[12]	Raceway Pond	California (USA)	17.5
[63]	Horizontal Tubular PBR	France	14.1 – 16.8
[29]	Horizontal Tubular PBR	Spain	19.1–19.8
[76]	Horizontal Tubular PBR	Spain	32
[77]	Horizontal Tubular PBR	Spain	21.8 ± 0.3
[42]	Horizontal Tubular PBR	Spain	25.0
[78]	Flat Panel	Czech	23.5 (July) 11.1 (September)
[79]	Flat Panel	Israel	12.8 – 22.5

Table E-0-1. Comparison of area productivities reported in literature.

Study	Modelling Approach	Cultivation System	Validation with	Strength	Weaknesses
[8]	16 model inputs; Includes lipid production;	PBR immersed in water basin	Commercial-scale outdoor productivity data	Considers light and temperature effect on growth; Validation with outdoor productivity data (9 weeks);	Validated in narrow temperature range (19-26 °C); Light calculation not clear; Can not be transferred to other reactor systems; Local uncertainty/sensitivity analysis
[20]	Growth model based on oxygen production rate;	Tubular Airlift	Pilot-scale outdoor productivity data	Considers light and temperature effect on growth; Validation with outdoor productivity data (148 days); Separate validation of day-time production and night-time loss; Global uncertainty/sensitivity analysis;	Short-term indoor experiments for model parameterization introduce large model uncertainty;
[35]	Volumetric Productivity; Regression Model;	Tubular Airlift	Pilot-scale outdoor productivity data	Considers light and temperature effect on growth; Validation with pilot-scale productivity data	Regression Model; Can not be transferred to light and temperature conditions outside the range used to determine the regression model;
[30] [39]	Two dimensional light path; Scattering by algae included;	Rectangular PBR	Pilot-scale outdoor productivity data	Two dimensional light path; Scattering by algae included;	Temperature effect on growth is neglected;
[80] [63]	Hyperbolic growth model; Average light intensity;	Horizontal tubular PBR	Pilot-scale outdoor productivity data	Simple growth model, easy to parameterize;	Temperature effect on growth is neglected; Average light intensity over the reactor is used to calculate growth;
[81]	Two physical and two species-specific biological inputs;	Roux-Flasks/ Raceway Pond	Lab-scale productivity data	Only two physical and two species –specific biological inputs are needed;	Validation with lab-scale experiments under continuous light; Temperature effect not included;
[32]		Raceway Pond	Lab-scale productivity data	Validation with lab-scale productivity data from 15 algae strains at different temperatures;	Not validated with outdoor productivity data; Light attenuation is not included; Experimental set-up not clear;

Table E-0-2. Comparison of modelling approaches found in literature accounting for the cultivation systems, which the model is designed for, the data used for validation and the strength and weaknesses found in the applied procedure. Grey shaded rows are validated against lab-scale experiments under controlled conditions. They are mentioned as example studies for model validation with lab-scale experiments.

Bibliography

[1] P. M. Slegers, P. J. M. van Beveren, R. H. Wijffels, G. van Straten, and A. J. B. van Boxtel, "Scenario analysis of large scale algae production in tubular photobioreactors," *Appl. Energy*, vol. 105, pp. 395–406, May 2013.

[2] P. M. Slegers, R. H. Wijffels, G. van Straten, and A. J. B. van Boxtel, "Design scenarios for flat panel photobioreactors," *Appl. Energy*, vol. 88, no. 10, pp. 3342–3353, Oct. 2011.

[3] P. M. Slegers, M. B. Lösing, R. H. Wijffels, G. van Straten, and A. J. B. van Boxtel, "Scenario evaluation of open pond microalgae production," *Algal Res.*, vol. 2, no. 4, pp. 358–368, Oct. 2013.

[4] P. M. Schenk, S. R. Thomas-Hall, E. Stephens, U. C. Marx, J. H. Mussgnug, C. Posten, O. Kruse, and B. Hankamer, "Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production," *BioEnergy Res.*, vol. 1, no. 1, pp. 20–43, Mar. 2008.

[5] J. W. A. Langeveld, J. Dixon, and J. F. Jaworski, "Development Perspectives Of The Biobased Economy: A Review," *Crop Science*, vol. 50, no. Supplement 1. p. S–142–S–151, 2010.

[6] R. H. Wijffels and M. J. Barbosa, "An outlook on microalgal biofuels.," *Science*, vol. 329, no. 5993, pp. 796–9, Aug. 2010.

[7] N.-H. Norsker, M. J. Barbosa, M. H. Vermuë, and R. H. Wijffels, "Microalgal production--a close look at the economics.," *Biotechnol. Adv.*, vol. 29, no. 1, pp. 24–7, Jan. 2011.

[8] J. Quinn, L. de Winter, and T. Bradley, "Microalgae bulk growth model with application to industrial scale systems.," *Bioresour. Technol.*, vol. 102, no. 8, pp. 5083–92, Apr. 2011.

[9] M. Janssen and P. Lamers, "Microalgae biotechnology," *Trends Biotechnol.*, vol. 5, no. 2, pp. 47–53, 2014.

[10] L. B. Christenson and R. C. Sims, "Rotating algal biofilm reactor and spool harvester for wastewater treatment with biofuels by-products.," *Biotechnol. Bioeng.*, vol. 109, no. 7, pp. 1674–84, Jul. 2012.

[11] W. Blanken, M. Janssen, M. Cuaresma, Z. Libor, T. Bhaiji, and R. H. Wijffels, "Biofilm growth of Chlorella sorokiniana in a rotating biological contactor based photobioreactor.," *Biotechnol. Bioeng.*, vol. 111, no. 12, pp. 2436–45, Dec. 2014.

[12] P. M. Slegers, *Scenario studies for algae production*. 2014.

[13] A. L. Mascarelli, "Algae: fuel of the future?," *Environ. Sci. Technol.*, vol. 43, no. 19, pp. 7160–7161, Oct. 2009.

[14] P. K. Campbell, T. Beer, and D. Batten, "Life cycle assessment of biodiesel production from microalgae in ponds.," *Bioresour. Technol.*, vol. 102, no. 1, pp. 50–6, Jan. 2011.

[15] Y. Chisti, "Biodiesel from microalgae beats bioethanol.," *Trends Biotechnol.*, vol. 26, no. 3, pp. 126–31, Mar. 2008.

[16] P. G. Stephenson, C. M. Moore, M. J. Terry, M. V Zubkov, and T. S. Bibby, "Improving photosynthesis for algal biofuels: toward a green revolution.," *Trends Biotechnol.*, vol. 29, no. 12, pp. 615–23, Dec. 2011.

[17] J. W. Moody, C. M. McGinty, and J. C. Quinn, "Global evaluation of biofuel potential from microalgae.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 111, no. 23, pp. 8691–6, Jun. 2014.

[18] J. C. Quinn, K. Catton, N. Wagner, and T. H. Bradley, "Current Large-Scale US Biofuel Potential from Microalgae Cultivated in Photobioreactors," *BioEnergy Res.*, vol. 5, no. 1, pp. 49–60, Nov. 2011.

[19] R. H. Wijffels, M. J. Barbosa, and M. H. M. Eppink, "Microalgae for the production of bulk chemicals and biofuels," *Biofuels, Bioprod. Biorefining*, vol. 4, no. 3, pp. 287–295, May 2010.

[20] Q. Béchet, A. Shilton, and B. Guieysse, "Full-scale validation of a model of algal productivity.," *Environ. Sci. Technol.*, vol. 48, no. 23, pp. 13826–33, Dec. 2014.

[21] D. A. Walker, "Biofuels, facts, fantasy, and feasibility," *J. Appl. Phycol.*, vol. 21, no. 5, pp. 509–517, Jun. 2009.

[22] L. Lardon, A. Hélias, B. Sialve, J.-P. Steyer, and O. Bernard, "Life-Cycle Assessment of Biodiesel Production from Microalgae," *Environ. Sci. Technol.*, vol. 43, no. 17, pp. 6475–6481, Sep. 2009.

[23] T. Wang, H. Yabar, and Y. Higano, "Perspective assessment of algae-based biofuel production using recycled nutrient sources: the case of Japan.," *Bioresour. Technol.*, vol. 128, pp. 688–96, Jan. 2013.

[24] A. Sun, R. Davis, M. Starbuck, A. Ben-Amotz, R. Pate, and P. T. Pienkos, "Comparative cost analysis of algal oil production for biofuels," *Energy*, vol. 36, no. 8, pp. 5169–5179, Aug. 2011.

[25] J. G. G. Jonker and A. P. C. Faaij, "Techno-economic assessment of micro-algae as feedstock for renewable bio-energy production," *Appl. Energy*, vol. 102, pp. 461–475, 2013.

[26] M. Al-Qasmi, N. Raut, S. Talebi, S. Al-Rajhi, and T. Al-Barwani, "A Review of Effect of Light on Microalgae Growth," *Proc. World Congr. Eng.*, vol. I, pp. 8–10, 2012.

[27] Q. Béchet, P. Chambonnière, A. Shilton, G. Guizard, and B. Guieysse, "Algal productivity modeling: a step toward accurate assessments of full-scale algal cultivation.," *Biotechnol. Bioeng.*, vol. 112, no. 5, pp. 987–96, May 2015.

[28] Q. Béchet, A. Shilton, O. B. Fringer, R. Muñoz, and B. Guieysse, "Mechanistic modeling of broth temperature in outdoor photobioreactors.," *Environ. Sci. Technol.*, vol. 44, no. 6, pp. 2197–2203, Mar. 2010.

[29] F. G. Acién Fernández, J. M. Fernández Sevilla, J. A. Sánchez Pérez, E. Molina Grima, and Y. Chisti, "Airlift-driven external-loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance," *Chem. Eng. Sci.*, vol. 56, no. 8, pp. 2721–2732, Apr. 2001.

[30] J. Pruvost, J. F. Cornet, V. Goetz, and J. Legrand, "Modeling dynamic functioning of rectangular photobioreactors in solar conditions," *AIChE J.*, vol. 57, no. 7, pp. 1947–1960, 2011.

[31] E. Molina Grima, F. Garcia Camacho, J. A. Sanchez Perez, J. M. Fernandez Sevilla, F. G. Acien Fernandez, and A. Contreras Gomez, "A mathematical model of microalgal growth in light-limited chemostat culture," *J. Chem. Technol. Biotechnol.*, vol. 61, no. 2, pp. 167–173, 1994.

[32] O. Bernard and B. Rémond, "Validation of a simple model accounting for light and temperature effect on microalgal growth.," *Bioresour. Technol.*, vol. 123, pp. 520–7, Nov. 2012.

[33] Q. Béchet, A. Shilton, and B. Guieysse, "Modeling the effects of light and temperature on algae growth: state of the art and critical assessment for productivity prediction during outdoor cultivation.," *Biotechnol. Adv.*, vol. 31, no. 8, pp. 1648–63, Dec. 2013.

[34] L. M. Van Dam, "Masters Thesis: Gathering biological parameters to validate productivity models at lab scale and pilot scale," 2015.

[35] R. Bosma, E. van Zessen, J. H. Reith, J. Tramper, and R. H. Wijffels, "Prediction of volumetric productivity of an outdoor photobioreactor.," *Biotechnol. Bioeng.*, vol. 97, no. 5, pp. 1108–20, Aug. 2007.

[36] J. C. Merchuk, F. Garcia-Camacho, and E. Molina-Grima, *Comprehensive Biotechnology*. Elsevier, 2011.

[37] I. Perner-Nochta and C. Posten, "Simulations of light intensity variation in photobioreactors.," *J. Biotechnol.*, vol. 131, no. 3, pp. 276–85, Sep. 2007.

[38] J. Pruvost, L. Pottier, and J. Legrand, "Numerical investigation of hydrodynamic and mixing conditions in a torus photobioreactor," *Chem. Eng. Sci.*, vol. 61, no. 14, pp. 4476–4489, Jul. 2006.

[39] J. Pruvost, J. F. Cornet, V. Goetz, and J. Legrand, "Theoretical investigation of biomass productivities achievable in solar rectangular photobioreactors for the cyanobacterium Arthrospira platensis," *Biotechnol. Prog.*, vol. 28, no. 3, pp. 699–714, 2012.

[40] M. A. Borowitzka, *Wastewater Treatment with Algae*. Berlin, Heidelberg: Springer Berlin Heidelberg, 1998.

[41] Q. Béchet, A. Shilton, J. B. K. Park, R. J. Craggs, and B. Guieysse, "Universal temperature model for shallow algal ponds provides improved accuracy.," *Environ. Sci. Technol.*, vol. 45, no. 8, pp. 3702–9, Apr. 2011.

[42] O. Jorquera, A. Kiperstok, E. A. Sales, M. Embiruçu, and M. L. Ghirardi, "Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors.," *Bioresour. Technol.*, vol. 101, no. 4, pp. 1406–13, Feb. 2010.

[43] D. G. Mayer and D. G. Butler, "Statistical validation," *Ecol. Modell.*, vol. 68, no. 1–2, pp. 21–32, Jul. 1993.

[44] H. Abdi and L. J. Williams, "Principal component analysis," *Wiley Interdisciplinary Reviews: Computational Statistics*, vol. 2, no. 4. pp. 433–459, 2010.

[45] A. Saltelli, "Global sensitivity analysis: An introduction," Jan. 2004.

[46] R. Bosma, J. H. de Vree, P. M. Slegers, M. Janssen, R. H. Wijffels, and M. J. Barbosa, "Design and construction of the microalgal pilot facility AlgaePARC," *Algal Res.*, vol. 6, pp. 160–169, Oct. 2014.

[47] C. Sousa, L. de Winter, M. Janssen, M. H. Vermuë, and R. H. Wijffels, "Growth of the microalgae Neochloris oleoabundans at high partial oxygen pressures and sub-saturating light intensity.," *Bioresour. Technol.*, vol. 104, pp. 565–70, Jan. 2012.

[48] A. Sukenik, "Ecophysiological considerations in the optimization of eicosapentaenoic acid production by Nannochloropsis sp. (Eustigmatophyceae)," *Bioresour. Technol.*, vol. 35, no. 3, pp. 263–269, 1991.

[49] D. Pal, I. Khozin-Goldberg, Z. Cohen, and S. Boussiba, "The effect of light, salinity, and nitrogen availability on lipid production by Nannochloropsis sp.," *Appl. Microbiol. Biotechnol.*, vol. 90, no. 4, pp. 1429–41, May 2011.

[50] Y. Chen, J. Wang, T. Liu, and L. Gao, "Effects of initial population density (IPD) on growth and lipid composition of Nannochloropsis sp.," *J. Appl. Phycol.*, vol. 24, no. 6, pp. 1623–1627, Mar. 2012.

[51] R. J. Geider, H. L. MacIntyre, and T. M. Kana, "Dynamic model of phytoplankton growth and acclimation: Responses of the balanced growth rate and the chlorophyll a:carbon ratio to light, nutrient-limitation and temperature," *Mar. Ecol. Prog. Ser.*, vol. 148, pp. 187–200, 1997.

[52] M. van den Braak, *The effect of temperature on algal growth from both a practical and modelling point of view*, no. June. 2012.

[53] R. J. Geider, "Light and temperature dependence of the carbon to chlorophyll a ratio in microalagae and cyanobacteria: imoplication for physiology and growth of phytoplankton," *New Phytol.*, vol. 106, no. 1, pp. 1–34, 1987.

[54] G. F. Blanchard, J. M. Guarini, P. Richard, P. Gros, and F. Mornet, "Quantifying the short-term temperature effect on light- saturated photosynthesis of intertidal microphytobenthos," *Mar. Ecol. Prog. Ser.*, vol. 134, no. 1–3, pp. 309–313, 1996.

[55] X. Yao, "Sensitivity Analysis for Biorefineries," no. May, 2015.

[56] S. Wahidin, A. Idris, and S. R. M. Shaleh, "The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp.," *Bioresour. Technol.*, vol. 129, pp. 7–11, Feb. 2013.

[57] Y. Li, Y. Gao, X. Xu, Q. Shen, and S. Guo, "Light-saturated photosynthetic rate in high-nitrogen rice (Oryza sativa L.) leaves is related to chloroplastic CO2 concentration.," *J. Exp. Bot.*, vol. 60, no. 8, pp. 2351–60, Jan. 2009.

[58] G. N. Somero, "Adaptation of enzymes to temperature: searching for basic 'strategies'.," *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.*, vol. 139, no. 3, pp. 321–33, Nov. 2004.

[59] A. Converti, A. A. Casazza, E. Y. Ortiz, P. Perego, and M. Del Borghi, "Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production," *Chem. Eng. Process. Process Intensif.*, vol. 48, no. 6, pp. 1146–1151, 2009.

[60] A. D. ANSELL, J. E. G. RAYMONT, K. F. LANDER, E. CROWLEY, and P. SHACKLEY, "Studies on the mass culture of Phaeodactylum. II. The growth of Phaeodactylum and other species in outdoor tanks," *Limnol. Oceanogr.*, vol. 8, no. 2, pp. 184–206, 1963.

[61] P. Bondioli, L. Della Bella, G. Rivolta, G. Chini Zittelli, N. Bassi, L. Rodolfi, D. Casini, M. Prussi, D. Chiaramonti, and M. R. Tredici, "Oil production by the marine microalgae Nannochloropsis sp. F&M-M24 and Tetraselmis suecica F&M-M33.," *Bioresour. Technol.*, vol. 114, pp. 567–72, Jun. 2012.

[62] W. H. Thomas, D. L. R. Seibert, M. Alden, A. Neori, and P. Eldridge, "Yields, photosynthetic efficiencies and proximate composition of dense marine microalgal cultures. I. Introduction and Phaeodactylum tricornutum experiments," *Biomass*, vol. 5, no. 3, pp. 181–209, Jan. 1984.

[63] F. G. A. Fernández, F. G. Camacho, J. A. S. Pérez, J. M. F. Sevilla, and E. M. Grima, "Modeling of biomass productivity in tubular photobioreactors for microalgal cultures: Effects of dilution rate, tube diameter, and solar irradiance," *Biotechnol. Bioeng.*, vol. 58, no. 6, pp. 605–616, 1998.

[64] J. N. Rogers, J. N. Rosenberg, B. J. Guzman, V. H. Oh, L. E. Mimbela, A. Ghassemi, M. J. Betenbaugh, G. A. Oyler, and M. D. Donohue, "A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales," *Algal Res.*, vol. 4, pp. 76–88, Apr. 2014.

[65] M. H. A. Michels, P. M. Slegers, M. H. Vermuë, and R. H. Wijffels, "Effect of biomass concentration on the productivity of Tetraselmis suecica in a pilot-scale tubular photobioreactor using natural sunlight," *Algal Res.*, vol. 4, pp. 12–18, Apr. 2014.

[66] R. L. CUHEL, P. B. ORTNER, and D. R. S. LEAN, "Night synthesis of protein by algae," *Limnol. Oceanogr.*, vol. 29, no. 4, pp. 731–744, 1984.

[67] M. Vítová, K. Bišová, M. Hlavová, S. Kawano, V. Zachleder, and M. Cížková, "Chlamydomonas reinhardtii: duration of its cell cycle and phases at growth rates affected by temperature.," *Planta*, vol. 234, no. 3, pp. 599–608, Sep. 2011.

[68] F. Le Borgne and J. Pruvost, "Investigation and modeling of biomass decay rate in the dark and its potential influence on net productivity of solar photobioreactors for microalga Chlamydomonas reinhardtii and cyanobacterium Arthrospira platensis.," *Bioresour. Technol.*, vol. 138, pp. 271–6, Jun. 2013.

[69] L. I. Smith, "1. A tutorial on Principal Components Analysis Introduction," 2002.

[70] A. J. Martínez-Roldán, H. V. Perales-Vela, R. O. Cañizares-Villanueva, and G. Torzillo, "Physiological response of Nannochloropsis sp. to saline stress in laboratory batch cultures," *J. Appl. Phycol.*, vol. 26, no. 1, pp. 115–121, Jun. 2013.

[71] J. Fábregas, A. Maseda, A. Domínguez, and A. Otero, "The cell composition of Nannochloropsis sp. changes under different irradiances in semicontinuous culture," *World J. Microbiol. Biotechnol.*, vol. 20, no. 1, pp. 31–35, 2004.

[72] G. Torzillo, A. Sacchi, and R. Materassi, "Temperature as an important factor affecting productivity and night biomass loss in Spirulina platensis grown outdoors in tubular photobioreactors," *Bioresour. Technol.*, vol. 38, no. 2–3, pp. 95–100, Jan. 1991.

[73] M. A. Borowitzka, *Marine Bioprocess Engineering, Proceedings of an International Symposium organized under auspices of The Working Party on Applied Biocatalysis of the Eurpean Federation of Biotechnology and The European Society for Marine Biotechnology*, vol. 35. Elsevier, 1999.

[74] C. Jiménez, B. R. Cossío, D. Labella, and F. Xavier Niell, "The Feasibility of industrial production of Spirulina (Arthrospira) in Southern Spain," *Aquaculture*, vol. 217, no. 1–4, pp. 179–190, Mar. 2003.

[75] M. R. Tredici and R. Materassi, "From open ponds to vertical alveolar panels: the Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms," *J. Appl. Phycol.*, vol. 4, no. 3, pp. 221–231, Sep. 1992.

[76] E. Molina, J. Fernández, F. G. Acién, and Y. Chisti, "Tubular photobioreactor design for algal cultures," *J. Biotechnol.*, vol. 92, no. 2, pp. 113–131, Dec. 2001.

[77] Z. Arbib, J. Ruiz, P. Álvarez-Díaz, C. Garrido-Pérez, J. Barragan, and J. A. Perales, "Long term outdoor operation of a tubular airlift pilot photobioreactor and a high rate algal pond as tertiary treatment of urban wastewater," *Ecol. Eng.*, vol. 52, pp. 143–153, Mar. 2013.

[78] J. Doucha and K. Lívanský, "Productivity, CO2/O2 exchange and hydraulics in outdoor open high density microalgal (Chlorella sp.) photobioreactors operated in a Middle and Southern European climate," *J. Appl. Phycol.*, vol. 18, no. 6, pp. 811–826, Nov. 2006.

[79] N. Zou and A. Richmond, "Effect of light-path length in outdoor flat plate reactors on output rate of cell mass and of EPA in Nannochloropsis sp.," *J. Biotechnol.*, vol. 70, no. 1–3, pp. 351–356, Apr. 1999.

[80] F. G. Acién Fernández, F. García Camacho, J. A. Sánchez Pérez, J. M. Fernández Sevilla, and E. Molina Grima, "A model for light distribution and average solar irradiance inside outdoor tubular photobioreactors for the microalgal mass culture," *Biotechnol. Bioeng.*, vol. 55, no. 5, pp. 701–714, 1997.

[81] M. H. Huesemann, J. Van Wagenen, T. Miller, A. Chavis, S. Hobbs, and B. Crowe, "A screening model to predict microalgae biomass growth in photobioreactors and raceway ponds.," *Biotechnol. Bioeng.*, vol. 110, no. 6, pp. 1583–94, Jun. 2013.