ABSTRACT: Volumetric productivity of *Monodus subterraneus* cultivated in an outdoor pilot-plant bubble column was predicted with a mathematical model. Two border cases to model the photobioreactor were chosen. Firstly, a model with no light integration in which it is assumed that microalgae can adapt immediately to local light conditions. Secondly, full light integration implicating that microalgae can convert all absorbed light with a photosynthetic yield based on average light intensity. Because temperature and light conditions in our photobioreactor changed during the day, photosynthetic yields at any combination of temperature and light intensity were needed. These were determined in repeated-batch lab-scale experiments with an experimental design. The model was evaluated in an outdoor bubble column at different natural light conditions and different temperatures. Volumetric productivities in the bubble column were predicted and compared with experimental volumetric productivities. The light integration model over-estimated productivity, while the model in which we assumed no light integration under-estimated productivity. Light integration occurred partly (47%) during the period investigated. The average observed biomass yield on light was 0.60 g mol⁻¹. The model of partly light integration predicted an average biomass yield on light of 0.57 g mol⁻¹ and predicted that productivity could have been increased by 19% if culture temperature would have been maintained at 24°C.

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Introduction

In closed photobioreactors, high volumetric productivities are desired in order to reduce photobioreactor size (Janssen, 2002). To study productivity of a bubble column in the Dutch climate, a pilot-plant bubble column was constructed and operated continuously at different dilution rates outdoors. In a photobioreactor, high light intensities occur at the reactor wall and because of absorption of light by microalgae, light intensities will decrease with increasing radial depth (Fig. 1A). Individual cells grown in dense cultures experience a fluctuating light environment. They are exposed to light/dark cycles with high light intensities close to the reactor surface and by travelling through a light gradient, finally darkness in the interior of the photobioreactor.

Our aim was to develop a mathematical model that predicted daily volumetric productivities of this outdoor pilot-plant bubble column in the Dutch climate. Such a model can be used to optimise volumetric productivity, to estimate the effect of temperature control and to predict productivities in other climates. To model the photobioreactor two border cases were chosen, that is, full light integration and no light integration (Terry, 1986). The non-integrated approach assumed that microalgae that move along the light gradient adapt instantaneously their growth rate to the new conditions (Grima et al., 1996). This means that they experience local light intensities and because of that, photosynthetic yields are calculated with local light intensities. At the reactor wall, where local light intensities are highest (Fig. 1A), photoinhibition or photosaturation occurs and photosynthetic yields are low (Fig. 1B). When algae are located more inside the photobioreactor,
photosynthetic yields increase until the maximum yield is obtained in the dark interior where local light intensities are lowest.

In the second approach, it is assumed that the reactor is fully light integrated, meaning that algae are adapted to the average light intensity in the photobioreactor and photosynthetic yield is calculated with this average light intensity (Fig. 1). Light integration takes place when light/dark cycles approach the turnover time of the photosynthetic unit, the time it takes to convert one molecule of carbon dioxide (Richmond et al., 2003). This phenomenon was called the flashing light effect and was observed at light/dark cycles smaller than several ms and a light/dark ratio of about 1:10 (Janssen, 2002; Kok, 1956; Qiang et al., 1998). In this approach, overall microalgal productivity is high because photosaturation and photoinhibition are prevented (Grima et al., 1997; Richmond et al., 2003; Terry, 1986).

We chose as model organism the freshwater algae species Monodus subterraneus, which can produce eicosapentaenoic acid at a 4% w/w concentration (Cohen, 1994, 1999). Eicosapentaenoic acid has therapeutic potential in the treatment of cardiovascular problems, a variety of cancers and inflammatory diseases (Simopoulos, 2004). Monodus subterraneus is generally grown between temperatures of 23 and 32°C (Cohen, 1994, 1999; Lu et al., 2002; Qiang et al., 1997; Richmond et al., 2003; Vonshak et al., 2001). The optimal temperature to grow this species was not known. In addition, it was not known how light and temperature affected photosynthetic yield. To be able to model the outdoor photobioreactor, a central composite design was used to determine photosynthetic yields at different combinations of temperature and light intensities in lab-scale bubble columns. The response area was fitted by a second-order polynomial function and this function was used to predict photosynthetic yields at any combination of temperature and light intensity.

Finally, predicted volumetric productivities via both approaches were compared with measured volumetric productivities and the amount of light integration in our photobioreactor was determined. We used the model to predict the increase in photosynthetic yield if culture temperature in our photobioreactor would have been maintained at 24°C.

Materials and Methods

First, the outdoor pilot-plant bubble column to be modelled is presented. After that, both modelling approaches are addressed. Then, the methods to determine the effects of temperature and light intensity on photosynthetic yield in lab-scale experiments are given. Finally, the method to calculate measured volumetric productivity in the outdoor bubble column is given.

Outdoor Experiments

Pilot-plant Bubble Column

A Plexiglas bubble column with dimensions \((H \times D)\) 2.0 × 0.21 m was constructed on a roof at the Energy research Centre of the Netherlands (ECN) located in Petten, The Netherlands (52°46’N, 4°40’E) (Fig. 2). An overflow tube was placed at a height of 1.85 m giving a culture volume of 64 L. The photobioreactor was operated continuously by diluting the column from sunrise to sunset with dilution rates between 0.03 and 0.38 d⁻¹. The pump (Iwaki metering pump) was turned off during the night. Carbon dioxide enriched air (gas velocity 10 L·min⁻¹) was supplied via mass flow controllers (Brooks Instruments 5850S, 5851S) and sterile filtered with a 0.2 µm filter (Pall, Acro® 50 Vent...
Temperature was continuously measured with a thermocouple and when culture temperature rose above 28°C, a thin water film was sprayed over the reactor wall to cool the algal culture. On top of the reactor, a LI-COR 190-SA 2π quantum sensor measured total horizontal solar radiation within Photosynthetic Active Radiation (PAR) range (400–700 nm). Biomass concentration was measured with a turbidity sensor (Solids Content Sensor CUS41-W, Endress & Hauser) that was calibrated with off-line dry-weight determinations. pH was measured with a Yokogawa electrode (SC21/AGP24) and dissolved oxygen was measured with an AppliSens Dissolved Oxygen sensor (APS101). Measured data was stored using the data acquisition programme WizCon for off-line analysis.

Organism and Cultivation Conditions

*Monodus subterraneus* UTEX 151 was obtained from the University of Texas Culture Collection and cultivated in test tubes containing BG-11 medium (Rippka et al., 1979) containing 1% agar. The cultures were grown in a light climate cabinet at a temperature of 25°C, a light intensity of 50 μmol m⁻²·s⁻¹ and a 16 h/8 h day/night rhythm. After growing, algae were transposed to 250 mL Erlenmeyer flasks containing adjusted BG-11 medium and grown under the same conditions. In the modified medium iron-ammonium-citrate was replaced by iron-chloride and citric acid was omitted to prevent bacterial growth. Medium was pumped through a sterile 0.2 μm filter (Capsule filter, Pall) before entering the reactor.

Mathematical Model

**Short Overview**

Our goal was to predict volumetric daily productivity in the outdoor bubble column from measured total solar radiation on a horizontal surface, temperature and biomass concentration. The general structure of the model is shown in Fig. 3. Our model was programmed in Mathcad 11.0.

Measured total horizontal solar radiation (also called global irradiance) is the sum of incident diffuse radiation...
and direct normal irradiance projected onto the horizontal surface. Direct light is characterised by having a specific direction while diffuse light has not. In our model, measured total horizontal light intensities were first converted to direct and diffuse horizontal light intensities. These direct and diffuse light intensities were converted to direct and diffuse light intensities falling on the surface inside the vertical photobioreactor. Light gradients inside the photobioreactor were calculated yielding local light intensities in the photobioreactor. The no light integrated approach used these local light intensities to determine local photosynthetic yields. Local absorbed light was multiplied by local photosynthetic yields to obtain local productivities; these were summed to get total productivity. The integrated approach started by calculating the average light intensity in the photobioreactor from local light intensities. Then, this average light intensity was used to calculate average photosynthetic yield. Productivity was calculated by multiplying the total amount of absorbed light inside the photobioreactor by this average photosynthetic yield. In both modelling approaches, for each half hour, productivity was calculated. These productivities were summed and divided by reactor volume to get the daily volumetric productivity of the photobioreactor.

**Conversion of Measured Total Horizontal Irradiance in a Diffuse and Direct Component**

First, the theoretical amount of sunlight, which would have been measured if no clouds were present, was calculated (Velds et al., 1992). Total horizontal radiation, consisting of a diffuse and a direct fraction depending on cloudiness, was measured on top of the reactor each half hour. This measured total horizontal radiation was compared with the theoretical amount of sunlight that would fall on the sensor if no clouds had been present. Via equations derived by de Jong for De Bilt (Netherlands), described in Velds et al. (1992), measured total radiation was split into a direct and diffuse component, which made it able to calculate light gradients in our reactor.

**Calculation of Light at the Photobioreactor Walls**

Light could not enter the photobioreactor via the horizontal area at top of the reactor. For that, light falling on the reactor top was converted to the amount of light falling on the vertical surface of the bubble column (Camacho et al., 1999). With a combination of Fresnels Law, Snell’s Law and refractive indices, the amount of light entering the photobioreactor at the walls was calculated (van Heel, 1964). These light intensities at the wall were used to calculate light gradients inside the photobioreactor.

**Light Gradients**

With the amount of diffuse light at the wall, biomass concentration and absorption coefficient known, a diffuse light gradient inside the photobioreactor was calculated (Evers, 1991). Camacho determined the 3D direct light path \( (p_{\text{direct}}) \) for radial positions using sun altitude and refractive indexes of air and water (Camacho et al., 1999). This equation was adapted to determine local light intensities \( (I_{\text{dir}}) \) for each half hour \( (a) \), certain photobioreactor angle \( (b) \) and changing locations \( (z) \) as shown in Eq. 1, which is further explained in Figure 4.

\[
I_{\text{dir}}(a, b, z) = I_{\text{dir}}(a, b + \Delta b(a, b, z))e^{-\alpha X \frac{\Delta b(a, b, z)}{C_1} X \frac{\Delta b(a, b, z)}{C_2}}
\]  

\( (1) \)

In Eq. 1, \( \alpha \) is the corrected absorption coefficient, \( X \) is biomass concentration and \( I_{\text{dir}} \) is the direct light intensity falling on the wall. Adaptation was needed because local productivities in the no light integrated approach were first summed for each local position in a radial position, while Camacho integrated light intensity over the whole reactor starting with radial position.

A sunlight corrected absorption coefficient of 0.20 m\(^2\) g\(^{-1}\) was used that was measured in lab-scale experiments at 23.5\(^o\) C and 50 \( \mu \)mol m\(^{-2}\) s\(^{-1}\). This absorption coefficient was chosen because a light intensity of 50 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) was about the average light intensity (integrated over the whole reactor) that was experienced by the microalgae during cultivation in our photobioreactor. Cells adapt to this average light intensity because acclimation processes are
much slower than the light/dark cycles in the photobioreactor (Torzillo et al., 2003; Zonneveld, 1998). It was assumed that the absorption coefficient was not affected by temperature differences.

**Photosynthetic Yield**

The approach of no light integration calculated photosynthetic yield at each radial position from local light intensities and measured temperature using the polynomial equation of photosynthetic yield determined in lab-scale experiments. The light integrated approach started by calculating the average light intensity over the cross-section of the bubble column from local light intensities (Eq. 2).

$$I_{av} = \frac{\int 2 \cdot \pi \cdot z \cdot I_{locabs}(a, b, z) dz}{\pi \cdot r^2}$$  \quad (2)

Average light intensity and measured temperature were used in the polynomial equation of photosynthetic yield to calculate average photosynthetic yield.

**Productivity**

In the approach of no light integration, local absorbed light \(I_{locabs}\) was calculated by taking the difference between two local light intensities. To calculate productivity, local absorbed light was multiplied by the average photosynthetic yield \(Y_{av}\) between these two locations and illuminated photobioreactor surface \(A_{light}\). Productivity was summed for locations \(z\), radial directions \(b\) and finally for each half hour \(a\) (Eq. 3).

$$P_{numint} = \sum_a \sum_b \sum_z Y_{av}(T(a), I_{tot}(a, b, z)) \cdot I_{locabs}(a, b, z) \cdot A_{light} \cdot 0.5 \text{ h}$$  \quad (3)

In the approach of light integration, the total amount of absorbed light \(\sum I_{locabs}(a, b, z)\) was multiplied by average photosynthetic yield \(Y(T, I_{av})\) and illuminated photobioreactor surface and summed for each half hour (Eq. 4).

$$P_{int} = \sum_a \left[ Y(T(a), I_{av}(a)) \cdot \sum_b \sum_z I_{locabs}(a, b, z) \cdot A_{light} \cdot 0.5 \text{ h} \right]$$  \quad (4)

**Model Criteria**

Days from 18 July until 26 October 2001 were modelled if the average weighed temperature during the light period was between 17.5 and 29.5°C and biomass concentration at sunrise did not deviate more than 10% from biomass concentration at sunset. Criteria of temperature were established because outside these temperatures photosynthetic yield could not be predicted accurately by the polynomial equation (Eq. 6). Criterion of 10% deviation of biomass concentration was chosen, because our model assumed steady state during the day. With these criteria, 72 of in total 98 days were modelled.

**Determination of Photosynthetic Yield in Lab-Scale Experiments**

**Experimental Design**

A central composite design was chosen to determine photosynthetic yield as a function of temperature and light intensity. Both parameters were varied at five levels. The centre of the experimental domain was measured four times to estimate repeatability of experimental measurements. Table 1 shows parameters and their minimal and maximal tested levels. The program Design-Expert version 6 was used to construct, analyse and optimise the design. Photosynthetic yield was fitted with the following polynomial equation:

$$Y = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 + b_3 \cdot X_1^2 + b_4 \cdot X_2^2 + b_5 \cdot X_1 \cdot X_2$$  \quad (6)

**Table 1.** Parameter levels in central composite design.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Coded value</th>
<th>Level (-\sqrt{2})</th>
<th>Level (-1)</th>
<th>Level 0</th>
<th>Level (+1)</th>
<th>Level (\sqrt{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light intensity</td>
<td>(\mu \text{mol-m}^{-2-} \text{s}^{-1})</td>
<td>(X_1)</td>
<td>50</td>
<td>270</td>
<td>800</td>
<td>1,330</td>
<td>1,550</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>(X_2)</td>
<td>17.5</td>
<td>19.3</td>
<td>23.5</td>
<td>27.8</td>
<td>29.5</td>
</tr>
</tbody>
</table>
In this equation, $Y$ is the predicted response and $X_i$ variables are coded values of the parameters. The $b$ values correspond to estimated polynomial coefficients: $b_0$ is the intercept term, $b_1$ and $b_2$ represent the main effect for each variable, and $b_3$ and $b_4$ describe square effects and $b_5$ describes the interaction effect between temperature and light. Statistical significant coefficients were estimated by the method of backward stepwise elimination ($P \leq 0.05$). To establish model hierarchy, the linear term of a parameter was introduced when an interaction or a square effect of that parameter was significant.

Organism and Cultivation Conditions

In lab-scale experiments, the same strain was used as outdoors. Medium was not sterile filtered, but medium compounds except iron and phosphate were mixed from stock solutions and heat sterilised at 121°C. Separate from this solution, phosphate solution was heat sterilised and iron solution was sterilised by filtration (0.2 μm). Phosphate and iron solution were added aseptically before inoculation.

Experimental Set-up

*Mona* dulciflum was grown in repeated-batch mode in small bubble columns (450 mL) with an internal diameter of four cm. Ten Sylvania CF-LE 55W dimmable fluorescent lamps provided light. A 16h/8h day/night rhythm was applied in all experiments. Air was sparged through each reactor with a flow of 2 L·min$^{-1}$ by using flow controllers (Brooks, GT1357). Reactors were kept at the desired temperature ± 0.1°C by water baths. In all experiments, pH was maintained at 8.0 ± 0.4 by adding pure carbon dioxide via a pump. Before measuring growth rates, conditions were slowly changed to new ones and then cultures were allowed to adapt for 2 weeks to these new conditions.

Optical Density

The optical densities at 530 nm (OD$_{530}$) and 680 nm (OD$_{680}$) were measured as absorbance on a spectrophotometer (Spectronic 20, Genesys) against medium as blank. Samples reaching an absorbance above 0.9 were diluted with medium.

Specific Growth Rate

Specific growth rates ($\mu$) were calculated by linear regression of the natural logarithm of OD$_{530}$ versus time between OD$_{530}$ values of 0.05 and 1. Between these values, microalgae grew exponentially with a constant growth rate showing that no light limitation occurred. For the lowest light intensity (50 μmol m$^{-2}$·s$^{-1}$), specific growth rate was determined between OD$_{530}$ values of 0.05 and 0.5, because at higher biomass concentrations linear growth was observed. At least five OD$_{530}$ measurements were done per specific growth rate calculation. When the first run had a significantly lower growth rate then succeeding runs, the first run was disregarded because the organism was still not adapted.

Photosynthetic Yield

At the end of a batch run, biomass was used to determine the specific absorption coefficient (Janssen et al., 2000). Instead of protein dry weight, biomass dry weight was used to determine the specific absorption coefficient on dry weight basis (Dubinsky et al., 1986). In the repeated-batch runs, relative spectral distribution of the Sylvania lamps was used for yield calculations; this distribution was determined from 400 to 750 nm with a 0.5 nm interval with a SR9910 spectroradiometer (Macam, SR9910). The relative spectral distribution of sunlight (Wozniak et al., 2003) was used to determine photosynthetic yields of our microalgae on sunlight. These yields were used to model volumetric productivity of our outdoor pilot-plant bubble column.

Dry Weight Determination

A membrane filter (Schleicher & Schuell, NC45) was dried at 80°C for at least 12 h. It was placed in a desiccator to cool to room temperature. It was weighed and 10 mL of the same solution as used for the specific absorption coefficient (OD$_{530}$ of 1) was filtrated under vacuum for 5 min. Then again, the filter was dried at 80°C for at least 12 h, allowed to cool to room temperature in a desiccator and weighed.

Photon Flux Density

Photo flux density (PFD) was measured in the PAR-range (400–700 nm) with a LI-COR 190- SA 2π sensor at both sides of reactor and averaged.

Biomass Yield

With specific growth rate, absorption coefficient and light intensity known, photosynthetic yield was calculated (Eq. 7) representing the ratio of biomass production over energy consumption, including maintenance requirements (Janssen, 2002).

$$Y_{x,E} = \frac{\mu}{I \cdot \alpha \cdot 10^{-6} \cdot 3600 \cdot 16 \cdot (g \cdot mol^{-1})} \quad (7)$$

This equation should be used for optically thin cultures. Here, specific growth rate was determined from several measurements in a repeated-batch run and it was assumed that the amount of light absorbed was constant. However, a light gradient occurred at the end of the batch phase, which could lead to an under estimation of photosynthetic yield.
To validate predicted volumetric productivities, measured productivities were calculated by Eq (8) taking into account biomass loss via the effluent and biomass accumulation.

\[
P_{\text{measured}} = D \cdot \frac{t \cdot (X_{\text{sunset}} + X_{\text{sunrise}})}{2} + V - \frac{t \cdot (X_{\text{sunset}} - X_{\text{sunrise}})}{2} \text{ (8)}
\]

### Results and Discussion

#### Determination of Photosynthetic Yield

To be able to model the outdoor pilot-plant bubble column, photosynthetic yields at any combination of temperature and light intensity had to be known. These were determined in lab-scale experiments with a central composite design and a second-order polynomial function was derived.

#### Lab-Scale Experimental Results

Table II shows the number of repeated batches \( (n) \), measured growth rates \( (\mu) \), absorption coefficients \( (\alpha) \) and calculated photosynthetic efficiencies of experiments \( (Y) \) with 95% confidence intervals. Yield was corrected with the relative spectral distribution of Sylvania lamps that were used to grow the microalga. Mostly, specific growth rate was constant over different runs, implicating that algae adapted to the new conditions. Then, average growth rate over the sequential batches was taken. However, in four cases, growth rates dropped during sequential batches and algae died within four batches. In those cases, algae were not able to adapt to the new conditions and growth rate was set to zero.

#### Statistical Model

Model coefficients were estimated using Design-Expert version 6 and Eq (6). Figure 5 shows that the polynomial fit predicted photosynthetic yield well with an \( R^2 \) of 0.92. The ANOVA (analysis of variance) for the model had four degrees of freedom, \( F \)-value of 20.3 and probability value of 0.0005. Table III shows the ANOVA table for model coefficients that were corrected with the relative spectral distribution of sunlight.

---

**Table II.** Measured experimental values of the central composite design.

<table>
<thead>
<tr>
<th>Run</th>
<th>Temperature (°C)</th>
<th>Light intensity (µmol m(^{-2}) s(^{-1}))</th>
<th>( \mu ) (day(^{-1}))</th>
<th>Conf. interval (day(^{-1}))</th>
<th>( \alpha ) (m(^2) g(^{-1}))</th>
<th>Conf. interval (m(^2) g(^{-1}))</th>
<th>( Y ) (g mol(^{-1}))</th>
<th>Conf. interval (g mol(^{-1}))</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.3</td>
<td>270</td>
<td>0.50</td>
<td>0.01</td>
<td>0.11</td>
<td>0.02</td>
<td>0.29</td>
<td>0.07</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>27.8</td>
<td>270</td>
<td>0.69</td>
<td>0.07</td>
<td>0.11</td>
<td>0.03</td>
<td>0.40</td>
<td>0.14</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>19.3</td>
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<td>0</td>
<td></td>
<td>0.08</td>
<td></td>
<td>0</td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>27.8</td>
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<td>0.15</td>
<td>0.06</td>
<td>0.01</td>
<td>0.09</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>23.5</td>
<td>800</td>
<td>0.56</td>
<td>0.08</td>
<td>0.09</td>
<td>0.02</td>
<td>0.14</td>
<td>0.05</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>23.5</td>
<td>800</td>
<td>0.61</td>
<td>0.05</td>
<td>0.09</td>
<td>0.02</td>
<td>0.15</td>
<td>0.05</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>29.5</td>
<td>800</td>
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<td>0.04</td>
<td></td>
<td>0</td>
<td></td>
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<tr>
<td>8</td>
<td>17.5</td>
<td>800</td>
<td>0.07</td>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>23.5</td>
<td>1,550</td>
<td>0.08</td>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
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<td>0.08</td>
<td>0.20</td>
<td>0.02</td>
<td>0.73</td>
<td>0.21</td>
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<tr>
<td>11</td>
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<td>800</td>
<td>0.62</td>
<td>0.03</td>
<td>0.05</td>
<td>0.03</td>
<td>0.25</td>
<td>0.15</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>23.5</td>
<td>800</td>
<td>0.56</td>
<td>0.09</td>
<td>0.07</td>
<td>0.04</td>
<td>0.18</td>
<td>0.14</td>
<td>4</td>
</tr>
</tbody>
</table>
Influence of Light and Temperature on Photosynthetic Yield

Photosynthetic yields were affected by light and temperature (Fig. 6). Especially light had a large effect on photosynthetic yield, shown by its low probability values (P-value linear effect 0.0001, P-value squared effect 0.011). The optimal photosynthetic yield for *Monodus subterraneus* was predicted at a temperature of 24°C and a low light intensity. Microalgae can adapt to low light intensities by increasing their antenna size or increase the amount of antennas (Gordillo et al., 2001). At these low light intensities, microalgae try to capture all photons available and algae are able to convert this absorbed light into carbohydrates. This gives a maximum photosynthetic yield because every photon is captured and converted into biomass and no photons are wasted in the form of heat. At higher light intensities, algae cannot convert all light because too much light is received. This leads to waste of energy in the form of heat, thus resulting in lower photosynthetic yields. At high light intensities photoinhibition occurred, giving low growth rates. At even higher light intensities, culture death occurred. Photoinhibition occurs when the photosynthetic apparatus is exposed to excessively high irradiances and photooxidation degrades protein D1 in photosystem II (Gordillo et al., 2001; Han et al., 2000). One should realise that diluted cultures were used and so each organism was subjected constantly to high light intensities. At sub-optimal temperatures, light was absorbed by photosystems, but could not be converted to carbohydrates by enzymes due to lower enzyme activities (Kirk, 1994). Photoinhibition occurred and light energy was wasted in the form of heat and photosynthetic yield dropped severely.

Modelling Results

As stated before, our goal was to predict volumetric daily productivities in an outdoor bubble column. To validate the model, predicted productivities of both approaches were compared with measured productivities and the amount of light integration was determined. With the final model, the influence of parameters on productivity was determined. Finally, the final model was used to predict the effect of temperature control on productivity.

Model Validation

Between 18 July and 26 October 2001, 73% of all days were modelled. Cloudy and cloudless days were included; biomass concentrations ranged between 0.4 and 1.4 g L⁻¹, and weighed temperature varied between 17.5 and 29.5°C. To validate the model, predicted productivities were compared with measured volumetric productivities (Fig. 7). Measured productivities (0.03–0.20 g L⁻¹ d⁻¹) were in the same range as reported previously in which *Monodus subterraneus* was grown in a similar bubble column (Lu et al., 2002). The light integration approach over-predicted productivity because in this model no photoinhibition occurred while the no light integrated approach under-predicted productivity because it over-estimated photoinhibition. Grima et al. (1996) described a model, also based on average irradiance, which used an affinity constant and a fitting parameter to determine the amount of photoinhibition and its effect on productivity. With the models described here, from independent lab-scale experiments, productivities can be estimated if the amount of sunlight, geographical location, day in the year and temperature are known. Because both border cases are modelled, insight is obtained about minimal and maximal productivities reachable without the need to measure productivities. The amount of light integration can be determined if volumetric productivities are measured or can be estimated using literature data (Terry, 1986).

Figure 8 shows local productivities at a certain time in the reactor for both models. The light integrated approach assumes that light/dark cycles are sufficiently fast and the flashing light effect occurs. This means that microalgae can convert absorbed light during the time that they travel in the dark interior of the bubble column with a high photo-

Table III. ANOVA table for coefficients of photosynthetic yield.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Parameter</th>
<th>Value</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>b₀</td>
<td>Constant</td>
<td>−1.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b₁</td>
<td>X₁</td>
<td>−9.45 × 10⁻⁴</td>
<td>57.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>b₂</td>
<td>X₂</td>
<td>0.20</td>
<td>0.8</td>
<td>0.39</td>
</tr>
<tr>
<td>b₃</td>
<td>X₁, X₂</td>
<td>3.60 × 10⁻²</td>
<td>12.2</td>
<td>0.011</td>
</tr>
<tr>
<td>b₄</td>
<td>X₁, X₂</td>
<td>−4.07 × 10⁻³</td>
<td>6.4</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Figure 6. Surface plot showing effects of light intensity and temperature on photosynthetic yield.
synthetic yield based on the average light experienced in the light gradient (Fig. 8A). Productivity in the first 5 mm is high, because much light is available and this light is converted with a high photosynthetic yield because photoinhibition is absent (Fig. 8B). The other approach, in which no light integration occurs, assumes that absorbed light is converted into biomass with a photosynthetic yield based on the light intensity experienced at that position. This means that in this approach, photosynthetic yield at the wall ($z = 0.105$ m) is lowest, because at this position light intensity is maximal and photoinhibition occurs (Fig. 6). Because light is absorbed by the microalgae, less light becomes available when moving to the dark interior and photosynthetic yields increase until it is maximal (Fig. 8A). Productivity is severely reduced in this approach because much energy is wasted in the form of heat, because light intensity in the first five mm is excessively high (Fig. 8B).

Measured productivities were between both modelling approaches. It was found that at light/dark cycle times below 100 ms, full light integration occurred and high photosynthetic yields could be obtained (Qiang et al., 1998; Terry, 1986). However, in our reactor, light/dark cycles of an order of seconds were present, implicating that full light integration could not occur. By illuminating diluted cultures with a cycle time of four seconds, about 30% light integration took place (Terry, 1986). We calculated the amount of light integration by minimising the absolute error in volumetric productivity for the period investigated. In our reactor, 47% light integration occurred, which is a bit higher than the value reported by Terry. This was probably because some mixing occurred between the different light zones and consequently, more light integration was obtained. This model of partly light integration predicted measured productivity well if it is taken into account that during each day, temperature and light fluctuated continuously and photosynthetic yield was calculated with a polynomial function obtained by independent lab-scale experiments (Fig. 9).

Figure 7. Parity plot showing measured productivity versus predicted productivity by both modelling approaches. The solid line presents a perfect match.

Figure 8. A: Photosynthetic yield versus position in the photobioreactor for both modelling approaches. B: Productivity versus position in the photobioreactor for both modelling approaches. Model predictions are shown for 22 July 2001 10:00 A.M. for the first 3 cm in our bubble column outdoors in the East position ($b = -90^\circ$).

Figure 9. Parity plot showing measured productivity versus productivity predicted by the model of partly light integration (47%). The solid line presents a perfect match.
**Photosynthetic Yield**

Measured averaged photosynthetic yield for the period investigated was 0.60 g mol\(^{-1}\). As mentioned earlier, the light integrated approach over-predicted productivity as shown by its photosynthetic yield of 0.67 g mol\(^{-1}\). The no light integrated approach under-predicted productivity and predicted a yield of 0.49 g mol\(^{-1}\). With the model of partly light integration, an average photosynthetic yield of 0.57 g mol\(^{-1}\) was obtained.

**Sensitivity Analysis Absorption Coefficient**

Three external parameters influenced productivity: biomass concentration, temperature and light intensity. As mentioned in the model description, absorption coefficient was set (for all days) at 214 m\(^2\) kg\(^{-1}\). This parameter was used to calculate light gradients in our reactor. To evaluate if it was justified to use this absorption coefficient, eight days were selected that had minimal and maximal values for the parameters affecting productivity. For these days, the absorption coefficient value was halved and doubled and the effect on productivity was determined. It was found that the maximum relative error on predicted productivity was 4.2%. Therefore, it was justified to use a constant absorption coefficient of 214 m\(^2\) kg\(^{-1}\).

**Parameters Influence on Volumetric Productivity**

Because biomass concentration, temperature and light intensity changed each day, it was investigated if productivity was correlated to those parameters. As known from other work, under controlled conditions, an optimum of

![Figure 10](image-url)
Biomass concentration was found where productivity was highest (Qiang et al., 1997; Richmond et al., 2003). Here, no biomass optimum was observed, because light and temperature had more impact on algal productivity than biomass concentration. For weighed temperature, measured productivities had a positive relation but varied widely (Fig. 10A). Predicted productivities showed also a positive relationship between weighed temperature and productivity, but we could not distinguish if the relationship was linear or exponential (Fig. 10B). However, this figure shows that by controlling temperature higher productivities can be attained.

Measured productivity showed a direct correlation with light intensity, implicating that our reactor was limited mainly by light (Fig. 10C). This same correlation was also reported in outdoor cultures of other microalgae (Qiang et al., 1998; Tredici et al., 1991; Zhang et al., 1999; Zittelli et al., 1996). Zhang also showed that if temperature was not controlled, like here, that this correlation was less pronounced due to the interaction effect between temperature and light intensity. Our model predicted this linear relationship very well (Fig. 10D) showing that also our model predicted that this photobioreactor was mainly limited by light.

### Controlling Temperature

Temperature control can be used to enhance volumetric productivity by growing the species at its optimal temperature constantly. Here, it was predicted how much effect temperature control has on volumetric productivity using the partly light integrated model (Fig. 11). Productivity could be raised by 19% for all days modelled and an average photosynthetic yield of 0.69 g mol⁻¹ could have been reached. This was close to the photosynthetic maximum of our species (0.72 g mol⁻¹) and consequently a bubble column is a good photobioreactor to grow *Monodus subterraneus* in the Dutch climate. Firstly, this is caused by the vertical arrangement of the bubble column that prevents high light intensities during noon and by that photoinhibition (Camacho et al., 1999). Secondly, light intensities in the Dutch climate are in comparison to southern countries a factor two lower. In countries where more light is available, photoinhibition reduces productivity much more (Lu et al., 2002). In those countries, it is better to use a photobioreactor with a smaller optical path in which more light integration is obtained and photoinhibition can be prevented (Qiang et al., 1998).

Temperature control had only a small effect when the average temperature of the culture during the daylight period was higher than 22°C (Fig. 11). However, at temperatures below 22°C, during spring and autumn, controlling temperature can have a great effect because photosaturation and photoinhibition, due to lower activity of the enzymes in the Calvin-Benson cycle, can be prevented.

In countries with a warmer climate than the Netherlands, for example, Israel, also heating in the morning can be used (Vonshak et al., 2001). They reported, by only heating culture for 2 h in the morning to 28°C, a 60% increase of daily productivity. They found a higher increase in volumetric productivity than we did because light intensities in their case were much higher (up to 2,000 μmol m⁻² s⁻¹) and by heating their culture, photoinhibition was prevented.

### Conclusion

Photobioreactors operated outdoors are mostly limited by sunlight, which is determined by geographic location. The models described in this paper can be used to determine minimal and maximal volumetric productivities at any geographical location from independent lab-scale experiments. Ideally, full light integration is obtained because then productivity is maximal because photoinhibition and photosaturation are prevented. In our photobioreactor, partly light integration (47%) occurred because light/dark cycles were too long to obtain complete light integration. Higher productivities can be reached by reducing optical path to about 0.5–1.0 cm and optimise gas flow rates (Richmond et al. 2003). Then, light/dark cycles start to approach photosynthetic unit turnover time and if growth inhibition is prevented and mass transfer is sufficient, full light integration and by that maximum productivity can be obtained.
Nomenclature

\( a \) counter for time at half hour interval (–)
\( A_{\text{light}} \) illuminated photobioreactor surface (m²)
\( b \) surface azimuth angle (N=180°, E=90°, S=0°, W=90°) (rad)
\( b' \) surface azimuth angle in the case of direct light at different \( z \) (rad)
\( D \) dilution speed (L·day⁻¹)
\( I \) light intensity (µmol·m⁻²·s⁻¹)
\( I_{\text{average}} \) average light intensity in the daylight period (µmol·m⁻²·s⁻¹)
\( I_{\text{ave}0} \) average light intensity in the whole reactor (µmol·m⁻²·s⁻¹)
\( I_{\text{dir}} \) direct irradiance falling at the reactor wall inside the photobioreactor (µmol·m⁻²·s⁻¹)
\( I_{\text{local}} \) light absorbed between \( z \) and \( z=2\Delta z \) inside the reactor (µmol·m⁻²·s⁻¹)
\( I_{\text{dir},2D} \) local direct light intensities (µmol·m⁻²·s⁻¹)
\( I_{\text{ins}} \) total inside irradiance inside the photobioreactor (µmol·m⁻²·s⁻¹)
\( P_{\text{n}} \) direct light path projected over cross-sectional area (2D) (m)
\( P_{\text{dir}} \) direct light path (3D) (m)
\( P_{\text{total}} \) total productivity calculated with the no integrated approach (g)
\( P_{\text{measured}} \) measured productivity (g)
\( r \) reactor radius (m)
\( T \) temperature (°C)
\( T_{\text{weigthed}} \) temperature weighted average in the daylight period (°C)
\( t \) total time that pump was on (sunrise to sunset) (hr)
\( V \) reactor volume (m³)
\( X \) average biomass concentration during a day (kg·m⁻³)
\( X_{\text{nmaximize}} \) biomass concentration at sunrise (kg·m⁻³)
\( X_{\text{nunset}} \) biomass concentration at sunset (kg·m⁻³)
\( Y \) photosynthetic yield (g·mol⁻¹)
\( Y_{\text{ave}} \) average photosynthetic yield (g·mol⁻¹)
\( z \) distance from reactor wall (m)

Greek symbols

\( \alpha \) absorption coefficient (214 m²·kg⁻¹)
\( \Delta b \) angle between \( b \) and \( b' \) (rad)
\( \mu \) specific growth rate (d⁻¹)
\( \Delta \) angle between solar angle and surface azimuth angle (rad)
\( \theta_{G}, \theta_{S} \) angles need to calculate direct light path (rad)
\( \alpha \) solar angle (N=180°, E=90°, S=0°, W=90°) (rad)

This research was financially supported through a grant from the Programme Economy, Ecology and Technology (EET) by the Netherlands Department of Economic Affairs, the Department of Public Housing, Spatial Planning and Environmental Protection, and the Department of Education, Cultural Affairs and Sciences (K99005/398510-1010). We want to thank Wim A. van Spronsen for measuring growth rates and absorption coefficients in the lab-scale bubble columns and Marcel Janssen for his idea to use photosynthetic yield instead of growth rate as a modelling tool.

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