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Production of *Rhodomonas* sp. at pilot scale under sunlight conditions

P.C. Oostlander[a,b,*,1], C. Latsos[b,1], J. van Houcke[b], R.H. Wijffels[a,c], M.J. Barbosa[a]

[a] Bioprocess Engineering, Wageningen University, P.O. Box 16, 6700 AA Wageningen, the Netherlands
[b] Research group Aquaculture, HZ University of Applied Sciences, Edisonweg 4, 4382 NW Vlissingen, the Netherlands
[c] Faculty of Biosciences and Aquaculture, Nord University, N-8049, Bodø, Norway

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ABSTRACT

Rhodomonas sp., is an important microalga for aquaculture feed applications and gained increased research interest over the past few years. While efforts to optimise cultivation of the strain have been studied in detail under laboratory conditions, *Rhodomonas* sp. has never been grown in photobioreactors at large scale under outdoor light conditions. To study the industrial potential of this strain, we cultivated *Rhodomonas* sp. in three identical tubular photobioreactors with 200 l working volume each, located in a greenhouse using sunlight conditions only. Growth experiments were performed from February with winter light conditions (< 10 mol m^{-2} d^{-1}) up to high light conditions of summer (> 50 mol m^{-2} d^{-1}) in July, representing all sunlight conditions in the Netherlands. All nutrients were supplied in surplus and temperature and pH were maintained at optimum values for growth of *Rhodomonas sp.*, based on lab data. The total light per reactor was calculated using a ray-tracing analysis to allow calculations based on the light reaching each individual reactor. *Rhodomonas* sp. grew under all tested light conditions. Biomass yield on light decreased with increasing light conditions from 0.43 ± 0.21 g mol^{-1} to 0.18 ± 0.04 g mol^{-1} at 0–10 mol_{ph} m^{-2} d^{-1} to 30–40 mol_{ph} m^{-2} d^{-1}. Biomass productivities increased with increasing light from 0.09 ± 0.04 g l^{-1} d^{-1} to 0.19 ± 0.06 g l^{-1} d^{-1}, for 0–10 and 30–40 mol_{ph} m^{-2} d^{-1}. We obtained a 2–5 fold increase in biomass productivity compared to previous reports on *Rhodomonas* sp. cultivation using artificial light at large scale. Our results show that *Rhodomonas* sp. can be grown at pilot scale using sunlight conditions and further improvements can be reached in the future.

1. Introduction

In aquaculture, specifically for hatcheries or copepod production, the use of the microalga *Rhodomonas* sp. is of great importance [1–4]. The production of *Rhodomonas* sp. however has been poorly described in literature and the strain has been known for its difficulty to grow in mass-culture [1,5]. *Rhodomonas* sp. cultures are reported as unstable with sudden culture death [1]. Recent studies at lab scale showed large improvements in respect to growth rates and stability of *Rhodomonas sp.* [6–10]. We recently published advancements in continuous cultivation of the strains in lab-scale photobioreactors [11]. As a next step we would like to demonstrate production at larger scale. The increased interest in large scale production of this important strain for aquaculture is evident from recent studies [8,9,12]. Vu et al. showed continuous *Rhodomonas* sp. cultivation in a bubble-column type reactor at a size of 84 l working volume and Thoisen et al. in a 200 l tubular reactor [9,12]. In both aforementioned studies artificial light conditions were used for consistent growth conditions. In these two studies, the biomass productivity from one single reactor and was in the range 0.02–0.13 g l^{-1} d^{-1}. The growth of *Rhodomonas* sp. using only sunlight conditions has never been shown before and the behaviour of this strain in tubular reactors has not been described with enough detail to allow scale-up to industrial processes. Microalgae production outdoors is subjected to fluctuating light conditions throughout the year and day, therefore being not as stable and robust as indoor production under continuous and constant illumination. To study the industrial potential of outdoor cultivation of *Rhodomonas* sp. we cultivated this microalga in three tubular reactors. The reactors are located in a greenhouse

Abbreviations: C_x, biomass concentration (g l^{-1}); D, daily dilution rate (d^{-1}); PFD_{total}, total photon flux density ($mol_{photons}$ m_{ground}^{-2} d^{-1}); $PFD_{reactor}$, photon flux density reaching reactor tubes m_{ground}^{-2} ($mol_{photons\text{-}on\text{-}tubes}$ m_{ground}^{-2} d^{-1}); R_{xA}, areal biomass productivity (g m_{ground}^{-2} d^{-1}); R_{xVol}, volumetric biomass productivity (g l^{-1} d^{-1}); $V_{harvest}$, harvested volume (l d^{-1}); V_r, reactor volume (l); $Y_{x,ph}$, biomass yield on light (g_x mol_{ph}^{-1})

* Corresponding author at: Bioprocess Engineering Group, AlgaePARC, Wageningen University & Research, PO Box 16, 6700 AA Wageningen, the Netherlands.
E-mail address: Pieter.oostlander@wur.nl (P.C. Oostlander).

[1] Both authors contributed equally.

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Fig. 1. Picture of three tubular photobioreactor operated with *Rhodomonas* sp. as seen from the North-East.

under sunlight conditions. Multiple experimental runs were done over the course of half a year representing all sunlight conditions (from low light < 10 mol m^{-2} d^{-1} up to summer conditions of > 50 mol m^{-2} d^{-1}) found during a full year of outdoor cultivation.

2. Materials and methods

2.1. Experimental setup and reactor operation

Three tubular photobioreactors (LGem MK1–200, LGem, The Netherlands) with a working volume of 200 l, each, located in a greenhouse in Vlissingen, the Netherlands were used for *Rhodomonas* sp. cultivation (Fig. 1). *Rhodomonas* sp. was supplied by the Dutch aquaculture industry, as a commercially used strain. The strain was characterized by 18S sequencing and confirmed as *Rhodomonas sp.* (data not shown). Sunlight was the only light source during all experiments. Recirculation of the culture in the reactor was provided by air only with an approximately 60 l air volume in the tubular section of a reactor. The air was recirculated over the reactor with a fresh flow of approximately 4 l min^{-1} (filter sterilized at 2.0 and 0.2 μm). The pH was measured online and maintained at 7.5 ± 0.3 by on-demand CO_2 addition in the gas phase of the reactor. Temperature of the cultures was maintained at 21.0 ± 2.0 °C by circulation of hot or cold water over a heat exchanger located in the reactor tank with the cold and hot water provided by a heatpump (30RQ 017 CHE, Carrier, The Netherlands). Offline daily measurements (OD_{750}, cell-count, $V_{harvests}$) and online measurements (temperature, pH, light intensity) were performed to monitor the culture in all experimental conditions. These measurements are used to calculate the biomass productivity and biomass yield on light. Online measurements (temperature, pH, light intensity) were logged in 10-min intervals using the reactor control systems (APEX Fusion, Neptune Systems, USA). The light intensity in the greenhouse was measured by a PAR-sensor located inside the greenhouse on top of the middle reactor.

Reactors were operated in chemostat mode, i,e. with a constant daily dilution rate during the full length of the experiment. A dilution rate of 6.5 ± 1.0 l hr^{-1} was applied during the initial 20-days in of the experiment (experiment day 10–30) and 5.5 ± 1.0 l hr^{-1} during all other days. A dilution time of 10 h day^{-1} was applied for the duration of the experiment with the exception of experimental days 59–66 when a dilution time of 12 h day^{-1} was used corresponding to dilution rates of 0.30 ± 0.025 d^{-1} for the full duration of the experiment. The cultivation medium consisted of saline groundwater from a 30 m deep groundwater-well located at the reactor location. The groundwater was aerated to oxidize soluble iron to insoluble precipitates and further removed by a sand filter. Peristatic pumps were used for the mixing of nutrient stocks with the ground water. Addition of nutrient stocks from the L1-culture medium [13] were added in 10 ml l^{-1} or 20 ml l^{-1} to maintain a nutrient-rich condition during reactor operation. Water mixed with nutrients was filter sterilized (0.5 μm and 0.2 μm – Supa-Pleat, AmazonFilters, UK) before use in the reactors.

The three reactors were operated over a 167-day period in multiple reactor runs from February till July. A selection of data points from all available data was made to create the final data set used for data analysis. This selection only contains data points of operational days without nutrient limitations or temperatures outside the selected range. A batch phase of a newly started reactor was excluded from the final dataset. A final dataset of 143 data points over a total of 67 operational days was assembled for final data analysis of *Rhodomonas* sp. cultivation. A detailed timeline of reactor operation and selected data points is found in the supplementary files.

2.2. Measurements

2.2.1. Daily measurements

The biomass concentration (C_x) and harvest volume ($V_{harvest}$) were determined daily during the morning. The biomass concentration was determined by optical density (OD_{750}) and cell count measurements in duplicate. OD_{750} was determined by UV-VIS Spectroscopy at 750 nm, (DR5000, Hach, USA). Cell-count was done using a Coulter Counter equipped with a 100 μm aperture tube (MultiSizer 3 Coulter Counter, Beckman Coulter, USA) and samples diluted 100× using Isotone II diluent. Total harvest volume was determined daily by measuring the water level (in cm) in the cylindrical harvest vessels of known diameter. Weekly measurements of nitrogen and phosphate content of the culture medium were performed to ensure non-limiting conditions of nutrients for the full duration of the experiment. Nitrogen and phosphate concentrations were determined by UV-VIS Spectrophotometer using Hach test kits for nitrate (NO_3^- - method number 10049, Hach, USA), ammonia (NH_3 - Nessler Method, Hach, USA) and orthophosphate (PO_4^{3-} - method number 8048, Hach, USA). The nutrient concentration (10 ml l^{-1} or 20 ml l^{-1} of L1-culture medium stocks) was adjusted accordingly (data not shown). The biomass concentration (g l^{-1}) was calculated based on the cell-count (cells ml^{-1}) and an assumed average cell mass (120 pg $cell^{-1}$), based on literature and previous results under laboratory conditions [11,14–16].

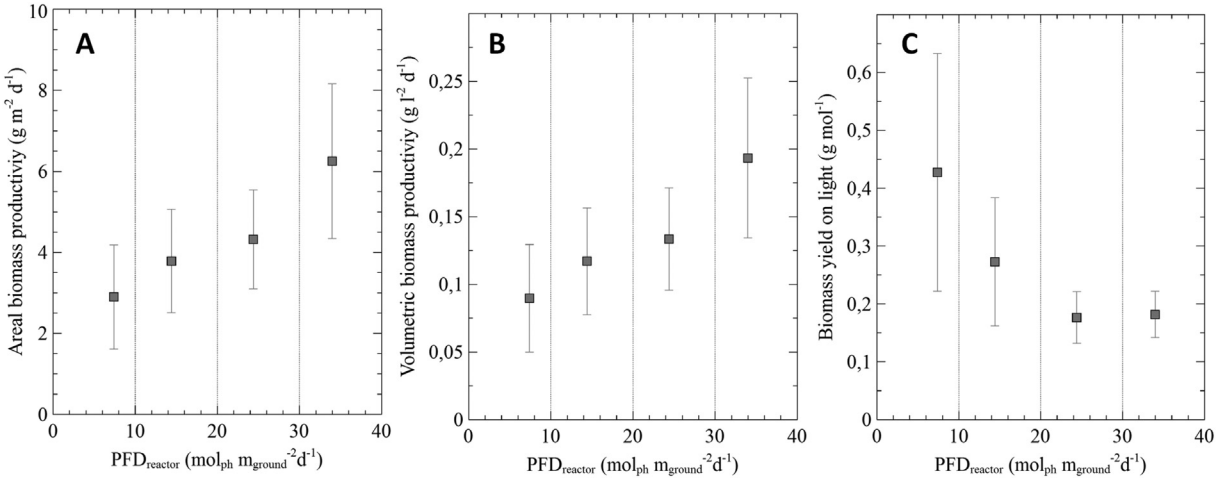

Fig. 2. Areal biomass production rate (panel A), Volumetric production rate (panel B) and biomass yield on light (panel C) averages over the total available light on the tube surface area per unit ground area (Photon Flux Density - $PFD_{reactor}$). Data points show average per interval of 10 for $PFD_{reactor}$ with error bars representing the standard deviation (0-10, 10-20, 20-30 and 30-40 with n = 39, 60, 33 and 6). Data of three independent reactors was combined to create the full dataset.

2.2.2. Available light and ray-tracing

The reactors were placed in a north-south facing orientation with a total distance of 132 cm between two reactors and a total length of the tube section of 450 cm. The control panels of all reactors are located on the east side of the tube section. Buildings were present in the immediate facility of the reactors location. The total available light to all reactors (PFD_{total}) was determined by the 10-minute interval measurements by a PAR-sensor located in the greenhouse in the middle of the reactor tube section area. A ray-tracing analysis was performed to determine the total available light for each reactor in the used location as a function of the measured light from the PAR-sensor. Calculations were performed based on a CAD-drawing of the full scenery including building as used in the experiments. From the results of this ray-tracing model a monthly average of light received by each individual reactor ($PFD_{reactor}$) is determined as a function of the total available light in the greenhouse (PFD_{total}) as measured by the PAR-sensor inside the greenhouse. These light correction values are summarized in Table 1 of the supplementary files. With this specific method, only the light reaching the culture is used in the calculations.

2.2.3. Biomass productivity

The biomass productivity is calculated using the biomass concentration at the time of sampling (C_x) and the total volume harvested ($V_{harvest}$) between samples. Actual biomass concentration in the harvest could not be determined due to fast precipitation, cell death and continued cell division in the harvest volume. It is therefore assumed that the biomass concentration in the harvest volume ($C_{x\text{-}harvest}$) is equal to the biomass concentration measured in the reactor ($C_{x\text{-}reactor} = C_{x\text{-}harvest}$). The biomass productivity is determined as both volumetric and areal biomass productivity as given in Eqs. (1) and (2).

$$\textit{Volumetric biomass productivity } R_{xVol}\,(g * l^{-1} * d^{-1}) = \frac{C_{x-harvest} * V_{harvest}}{(V_R/\Delta T)} \quad (1)$$

$$\textit{Areal biomass productivity } R_{xA}\,(g * m^{-2} * d^{-1}) = \frac{C_{x-harvest} * V_{harvest}}{(A/\Delta T)} \quad (2)$$

Where $V_{harvest}$ = The volume of harvest between two measurements (litre), V_r = Culture volume of a reactor (litre), A = ground area for a reactor (m^2) and ΔT the time between two measurements (days).

2.2.4. Biomass yield on light

The biomass yield on light ($Y_{x,ph}$) describes the efficiency of light use by the algae culture. The biomass yield on light is calculated using Eq. (3) where R_x is the biomass productivity (in g $reactor^{-1}$ day^{-1}) and photon flux density is the total available light that reaches the reactor ($PFD_{reactor}$ in $mol_{photons}$ $reactor^{-1}$ day^{-1}).

$$\textit{Biomass yield on light } (g * mol^{-1}) = \frac{R_x}{PFD} \quad (3)$$

3. Results and discussion

Cultivation of *Rhodomonas* sp. under sunlight conditions showed variable results based on the available light. During the experiments the three reactors behaved equally during days of operation, indicating good reproducibility. Continuous cultivation was maintained during multiple reactor runs under all available sunlight conditions. The overall trends showed increased biomass productivities at higher light levels but with decreased biomass yield on light. These trends were all similar to trends observed for outdoor production of other algae species [17]. No unexpected behaviour, such as culture crashes as described in literature, were observed under controlled conditions. With cultivation parameters maintained within the boundaries set (temperature below 25 °C, non-limiting nutrients and pH 7.5 ± 0.3) no biofilm formation or sudden culture dead was observed. Deviating from the set points resulted in the formation of biofilm in the reactors and decreased biomass productivity. Data of reactor with biofilm formation was not included in the final dataset.

All data points were combined in average values per photon flux density (PFD) intervals of 10 $mol_{photons}$ m^{-2} d^{-1} (0–10, 10–20, 20–30 and 30–40 mol m^{-2} d^{-1}) with error bars representing the total standard deviation of all days represented within each PFD interval (Fig. 2). Visualisation of all data points within the selected dataset can be found in the Supplementary files. The results of single data points fluctuate between biomass concentrations of 0.10 and 0.90 g l^{-1}, biomass productivities of 0.03 to 0.30 g l^{-1} d^{-1} and biomass yield on light between 0.07 and 0.88 g mol^{-1}. The light conditions on the reactor surface ($PFD_{reactor}$) fluctuate between 1.7 and 38.6 mol_{ph} m^{-2} d^{-1}.

3.1. Biomass concentration

For chemostat conditions (fixed dilution rate) the biomass concentration is a result of the total available light. Under fixed light conditions a steady-state could be achieved, with higher biomass concentrations at higher light intensities. Under outdoor cultivation the biomass concentration is constantly adapting to the available light. The biomass concentration is expected to increase with increased light

conditions. The average biomass concentration over the full length of the experiment increased with increasing light availability for 0.30 ± 0.15 g l^{-1} in February with an average a $PFD_{reactor}$ of 8.34 mol_{ph} m^{-2} d^{-1} up to 0.55 ± 0.20 g l^{-1} in June/July with an average $PFD_{reactor}$ of 20.9 mol_{ph} m^{-2} d^{-1}. In literature it is described that higher biomass concentrations could be achieved for other algae species grown in very similar conditions [17]. *Nannochloropsis* sp. is described in vertical tubular reactors, operated in chemostat mode under comparable light conditions (The Netherlands, July and August) and dilution rates (0.27 d^{-1}) obtaining an average biomass concentrations of 2.1 g l^{-1}. *Rhodomonas* sp. showed biomass concentration of to 0.55 ± 0.20 g l^{-1} under similar light conditions and a dilution rate of 0.30 d^{-1}. Although the work with *Nannochloropsis* sp. is the most comparable work available in literature, many differences exist between literature and our work with *Rhodomonas sp.*. Equal outdoor light conditions could be assumed, but in our work with *Rhodomonas* sp. the reactors were located in a greenhouse. An average loss of light of 14.6% is calculated with the ray-tracing analysis for the summer months (June–August) by placing the photobioreactors in a greenhouse. The tube diameter in this work was 6.0 cm compared to the 4.6 cm of the reactors described for *Nannochloropsis sp.*. Besides these differences the difference in strain could also result in very different biomass yield on light and biomass concentrations. The lower biomass concentrations found for *Rhodomonas* sp. does seem to indicate lower biomass yield on light for this strain under chemostat conditions utilizing sunlight, compared to *Nannochloropsis sp.*.

Rhodomonas sp. did show higher biomass concentrations of 1.2–1.5 g l^{-1} in lab experiments using the same nutrient, temperature and pH settings with a dilution rate up to 1.02 d^{-1} [18]. These lab experiments were performed with the light applied at a constant level (600 μmol m^{-2} s^{-1}) for 24 h per day resulting in a total PFD of 52 mol m^{-2} d^{-1}. From these lab results it can be concluded that *Rhodomonas* sp. is able to reach higher biomass concentrations under favourable light conditions. We hypothesize that sunlight conditions with high peak light intensities (> 1500 μmol m^{-2} s^{-1}) had a negative effect on the growth rate of *Rhodomonas* sp. during moments of high incident light intensities. The high incident light intensity combined with the relatively low biomass concentrations (< 0.5 g l^{-1}) during peak light hours of the day may have resulted in photo-inhibition. A lowered photosynthetic efficiency at high light intensities for outdoor production has been described in detail for *Chlorella* sp. species [19]. High light conditions (> 1000 μmol m^{-2} s^{-1}) during midday showed a decrease in photosynthetic activity, specifically under low biomass concentrations photo inhibition is more evident [19]. Photo-inhibition could have halted or decreased growth during hours of high light intensity in our experiments, keeping the biomass concentrations relatively low under the applied dilution conditions.

3.2. Biomass productivity

From the biomass concentration and the dilution rate of the reactors the daily biomass productivity was calculated. The biomass productivity is represented in Fig. 2A (areal) and Fig. 2B (volumetric). An average areal biomass productivity of 3.11–6.25 g m^{-2} d^{-1} is found. The overall trend of increased biomass productivity with increasing total photon flux density is equal to that described for other algae strains [17,20]. Values observed in literature for a vertical tubular reactor under Dutch summer outdoor conditions described 3-4× higher areal biomass productivities of (10.6–24.4 g m^{-1} d^{-1}) with *Nannochloropsis sp.* [17,21,22]. The lower areal biomass productivity for *Rhodomonas* sp. is also represented in relatively low volumetric biomass productivities. The volumetric biomass productivity of *Rhodomonas sp.* (0.10–0.19 g l^{-1} d^{-1}) is lower than values in literature for *Nannochloropsis* sp. at 0.31–0.71 g l^{-1} d^{-1} [17,21,22]. These lower volumetric and areal biomass productivities for *Rhodomonas* sp. are the result of the low biomass concentration obtained in the reactors. The significantly lower biomass productivity suggests lower biomass yield on lights obtained for *Rhodomonas sp.*. Nevertheless, the productivity data we obtained for *Rhodomonas* sp. at larger scale are higher than described in literature. The average volumetric biomass productivity over the full length of the experiment of 0.12 ± 0.05 g l^{-1} d^{-1} is very similar to that described by Vu et al. growing *Rhodomonas salina* in a bubble column reactor under artificial light conditions (981 ± 133 μmol m^{-2} s^{-1}) at a dilution rate of 0.46 d^{-1} and biomass concentration of approximately 0.29 g l^{-1} resulting in a biomass productivity of approximately 0.13 g l^{-1} d^{-1} [12]. Thoisen et al. obtained a volumetric biomass productivity of approximately 0.02–0.08 g l^{-1} d^{-1} for *Rhodomonas* sp. grown in a tubular photobioreactor under artificial light conditions (225 ± 35 μmol m^{-2} s^{-1}) with a dilution rate of approximately 0.3 d^{-1} [9]. Both literature studies are performed under constant artificial light conditions and 24 h per day of light. It has to be noted that the biomass productivities are presented in g l^{-1} d^{-1} but growth only occurs during the light hours of the day, when photosynthesis takes place [23]. For the outdoor production of *Rhodomonas* sp. this is limited to a maximum of 15–16 h of light per day, whereas artificial light experiments described in literature used constant light intensities 24 h per day. The biomass productivity corrected for hours of light received (g l^{-1} $h_{light}{}^{-1}$) shows a more realistic comparison between the different values from literature and the growth under sunlight conditions. The maximum biomass productivity in our study is found for the highest light conditions with an average of 0.19 ± 0.06 g l^{-1} d^{-1} from 6 data points at a PFD of 30–40 mol_{ph} m^{-2} d^{-1}. A maximum average hourly biomass productivity of 12 ± 3 mg l^{-1} $h_{light}{}^{-1}$ was achieved. Literature described 5.4 mg l^{-1} $h_{light}{}^{-1}$ for Vu et al. and 0.8–3.3 mg l^{-1} $h_{light}{}^{-1}$ for Thoisen et al. [8,12]. The volumetric biomass productivity expressed per hour of light shows that the actual biomass productivity obtained under sunlight conditions in our results is 2-5× higher than those reported in literature. This shows the potential of *Rhodomonas* sp. production under sunlight conditions. Biomass productivities (up to 1.4 g l^{-1} d^{-1} or 60 mg l^{-1} $h_{light}{}^{-1}$) at lab scale have recently been reported [11]. It is therefore likely that with *Rhodomonas* sp. a much higher biomass productivity at large scale production could be achieved than presented here.

3.3. Biomass yield on light

The biomass yield on light (Fig. 2C) clearly shows the negative effect of increased total daily photon flux density on the efficiency of light use. This trend correlates to similar trends described in literature for other algae strains at pilot scale production [17,20,22]. The results for *Rhodomonas* sp. represent inefficient light conversion to biomass by the algae strain under the applied experimental conditions with an average biomass yield on light of 0.29 ± 0.16 g mol^{-1} over the full length of the experiment. Studies on *Nannochloropsis* sp. showed a similar trend. A decrease in biomass yield on light from approximately 0.93 g mol^{-1} at low light conditions of 0–15 mol_{ph} m^{-2} d^{-1} to about 0.44 g mol^{-1} at high light conditions of 45–60 mol_{ph} m^{-2} d^{-1} was described [17,20]. *Rhodomonas* sp. in our study shows an average of 0.43 ± 0.21 g mol^{-1} under low light conditions of 0–10 mol_{ph} m^{-2} d^{-1} to 0.18 ± 0.04 g mol^{-1} at high light conditions of 30–40 mol_{ph} m^{-2} d^{-1}. Our results for *Rhodomonas* sp. only use the light reaching the culture, as calculated using the ray-tracing method. The total light in the studies on *Nannochloropsis* sp. describe all light reaching the ground surface of the reactor including light not reaching the culture. Values of the work with *Nannochloropsis* sp. are expected to be slightly higher if a similar ray-trace approach would have been used for the determination of light reaching the culture, further increasing the gap between our results with *Rhodomonas* sp. and data on *Nannochloropsis sp.* [17]. Although the same trend is observed with decreasing biomass yield on light with an increased PFD, the average values are significantly lower for *Rhodomonas* sp. in our results. It was not possible to calculate a biomass yield

on light for *Rhodomonas* sp. in other studies in literature due to limited data on total available light. Improving the biomass yield on light is key to the large scale success of *Rhodomonas* sp. as a production species for aquaculture. It is likely that the low biomass concentrations achieved during the study resulted in inefficient light use during moments of high incident light intensity. Light not reaching microalgae cells leaves the reactor and is not used for photosynthesis, lowering the biomass yield on light. This loss of light should be avoided to obtain more efficient biomass production.

4. Improved productivity under sunlight conditions

The results of the biomass concentration, biomass productivity and biomass yield on light of *Rhodomonas* sp. in our study all represent the same overall trend. A lower average biomass concentration resulting in a lower biomass productivity and lower biomass yield on light compared to data in literature for *Nannochloropsis* sp. were found. In lab-scale experiments *Rhodomonas* sp. has shown higher biomass concentrations (over 1.4 g l^{-1}), biomass productivities (up to 60 mg l^{-1} h^{-1}) and biomass yields on light (up to 0.91 g mol^{-1}) than the values found at pilot-scale [11]. Laboratory experiments with *Rhodomonas* sp. showed higher growth rates (> 1.0 d^{-1}) than those obtained at pilot scale utilizing sunlight. For industrial implementation of *Rhodomonas* sp. the gap between what has been achieved in lab studies and what our study shows at large scale should be closed. To achieve this the biomass productivity should be increased under the applied sunlight conditions. In chemostat the biomass productivity is a function of the dilution rate and the biomass concentration. A higher biomass concentration should be maintained under chemostat production to obtain higher biomass production rates. Increasing the dilution rate would reduce the biomass concentration further, not resulting in more efficient biomass production. As discussed in Section 3.1 the biomass concentration is assumed to have remained low as a result of the high incident light intensity combined with a low biomass concentration. It is hypothesized that the high amount of light per cell could have resulted in photo inhibition. Strategies that reduce the total light per cell are therefore expected to result in higher biomass concentration and biomass production rates.

One strategy to decrease the amount of light per cell is an increased biomass concentration. A higher biomass concentration could be achieved by changing the production strategy or the reactor setup. A changed production strategy could include a longer batch phase, increasing the biomass concentration before starting a chemostat or a chemostat operation with a lower dilution rate. If the increased biomass concentration indeed results in higher growth rates with high light conditions it is hypothesized to maintain this higher biomass concentration in the reactor during chemostat operation. Lab scale experiments of *Rhodomonas* sp. under high light conditions with high and low biomass concentration could show the effect of photo inhibition on the cells. Lab scale experiment could facilitate determination of cultivation strategies that could effectively increase the biomass production rate under sunlight conditions. Turbidostat experiments with a low biomass concentration under high incident light intensities could show the photo inhibiting effect. *Rhodomonas* sp. has not been produced at lab scales with light intensities over 600 μmol m^{-2} s^{-1} [11]. Increasing the range of light conditions tested at lab scale could provide valuable information on the biomass yield on light and suitable biomass concentration for these light conditions.

5. Conclusion

Rhodomonas sp. was successfully cultivated at pilot-scale utilizing the sunlight conditions of the Dutch climate from February till July. This is the first reported cultivation of *Rhodomonas* sp. at pilot scale utilizing natural sunlight conditions. Biomass productivities on sunlight conditions are higher than previously published results of *Rhodomonas* sp. at pilot-scale utilizing stable artificial light conditions. Based on literature of *Rhodomonas* sp. production at lab scale and comparison to cultivation of other algae species at pilot-scale outdoors, a large potential for future improvement is still possible. Lab scale studies of *Rhodomonas* sp. with high light levels should determine the further possible improvements of *Rhodomonas* sp. as an industrial production strain for aquaculture using sunlight conditions.

Statement of informed consent

No conflicts, informed consent, or human or animal rights are applicable to this study".

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Author statement

PCO, CL, JvH, RW and MB contributed to the conception and design of the study. Collection of the data was performed by CL. Assembly, analysis and interpretation of the data and drafting the article was done by PCO. JvH RW MB revised the manuscript. All authors approved the final manuscript.

CRediT authorship contribution statement

P.C. Oostlander:Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing.**C. Latsos**:Conceptualization, Investigation, Writing - review & editing.**J. van Houcke**:Conceptualization, Writing - review & editing.**R.H. Wijffels**:Conceptualization, Writing - review & editing.**M.J. Barbosa**:Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2020.101934.

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