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Comparison of growth of *Tetraselmis* in a tubular photobioreactor (Biocoil) and a raceway pond

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Abstract

Microalgae cultivation systems can be divided broadly into open ponds and closed photobioreactors. This study investigated the growth and biomass productivity of the halophilic green alga *Tetraselmis* sp. MUR-233, grown outdoors in paddle wheel-driven open raceway ponds and in a tubular closed photobioreactor (Biocoil) at a salinity of 7 % NaCl (*w*/*v*) between mid-March and June 2010 (austral autumn/winter). Volumetric productivity in the Biocoil averaged 67 mg ash-free dry weight (AFDW) L^{-1} day^{-1} when the culture was grown without CO_2 addition. This productivity was 86 % greater, although less stable, than that achieved in the open raceway pond (36 mg L^{-1} day^{-1}) grown at the same time in the autumn period. The *Tetraselmis* culture in the open raceway pond could be maintained in semi-continuous culture for the whole experimental period of 3 months without an additional CO_2 supply, whereas in the Biocoil, under the same conditions, reliable semi-continuous culture was only achievable for a period of 38 days. However, stable semi-continuous culture was achieved in the Biocoil by the addition of CO_2 at a controlled pH of ~7.5. With CO_2 addition, the

volumetric biomass productivity in the Biocoil was 85 mg AFDW L^{-1} day^{-1} which was 5.5 times higher than the productivity achieved in the open raceway pond (15 mg AFDW L^{-1} day^{-1}) with CO_2 addition and 8 times higher compared to the productivity in the open raceway pond without CO_2 addition (11 mg AFDW L^{-1} day^{-1}), when cultures were grown in winter. The illuminated area productivities highlight an alternative story and showed that the open raceway pond had a three times higher productivity (3,000 mg AFDW m^{-2} day^{-1}) compared to the Biocoil (850 mg AFDW m^{-2} day^{-1}). Although significant differences were found between treatments and cultivation systems, the overall average lipid content for *Tetraselmis* sp. MUR-233 was 50 % in exponential phase during semi-continuous cultivation.

Keywords: Chlorophyta; Outdoor cultivation; Raceway pond; Closed photobioreactor; Productivity

Introduction

It is clear that microalgal cultivation has positioned itself as one of the promising biotechnologies of the last decades (Pulz and Gross 2004). Some current and potential future applications of new algae-derived products include the production of fine chemicals and bioactive compounds, dietary supplements for human and animal nutrition, aquaculture feeds and wastewater treatment (Borowitzka 2013; Richmond and Hu 2013). The potential of oleaginous algae which produce high amounts of triacylglycerides is also being extensively studied for the production of algae-derived biofuel (Benemann 1997; Chisti 2007; Fon Sing et al. 2013).

The polyphyletic diversity of microalgae makes it inevitable that there exists a wide diversity in possible cultivation systems and culture processes. While open ponds are the most promising cultivation systems for large-scale commercial production, especially in geographical locations such as Australia (Fon Sing et al. 2013), closed photobioreactors may play a role in scaling up the biomass and algal production in other climatic conditions (Bosma et al. 2007; Zitelli et al. 2013).

The major operational differences between open and closed culture systems, each with their own advantages and disadvantages, are the number of factors that can be regulated and influenced to optimise and stimulate growth (Apt and Behrens 1999; Grobbelaar 2009). Algae cultivation in large open raceway pond systems, in use since the 1950s, is low cost but offers limited control over growth conditions, evaporation of water and invasion of non-desired species (Brennan and Owende 2010; Borowitzka 1999). Closed photobioreactors, on the other hand, are characterised by the better regulation and control of many of the important biotic and abiotic limiting growth factors. The ability to grow microalgae at very high biomass concentrations in closed photobioreactors holds promise for the future of algal biotechnology. However, closed photobioreactors also have several disadvantages such as cooling requirement which increases operational costs and greater O2 build-up which reduces productivity (Grobbelaar 2009).

Most microalgal biotechnological research has been carried out using Chlorophyta (Pulz and Gross 2004). The halophilic flagellate microalga *Tetraselmis* sp. MUR-233 (Chlorodendrophyceae) used in this study is a flagellated unicellular alga, 10 to 15 μm in diameter (Norris et al. 1980; Hori et al. 1982). The genus *Tetraselmis* is known to withstand a wide range of salinities and temperatures (Kirst 1979; Fabregas et al. 1984; Fon Sing 2010) and is widely used in aquaculture and mariculture feeds. *Tetraselmis* MUR-233 is also a potential source of lipids for biofuel production.

Here, we describe the outdoor semi-continuous growth of a halophilic *Tetraselmis* sp. in open and closed cultivation systems. The main aim of this study was to investigate the biomass productivity between these systems under the climatic conditions of Perth, Western Australia.

Materials and Methods

Tetraselmis sp. strain MUR-233, a strain isolated from a local salt lake, obtained from the Murdoch University Microalgae Culture Collection was maintained in F/2 medium (Guillard and Ryther 1962) with NaCl added to increase the salinity to 7 % (*w*/*v*). Studies carried out by Fon Sing (2010) indicated that 7 % NaCl was the optimum salinity for growth rate, photosynthesis and biomass

productivity for MUR-233. The outdoor experiments were conducted between mid-March and June 2010 (austral autumn and winter) at the Algae R&D Centre of Murdoch University, Perth, Western Australia. Daily 10-min interval measurements of the average irradiance (W m^{-2}) were obtained from the Murdoch University Weather Station (http://wwwmet.murdoch.edu.au).

The 1-m2 paddle wheel-driven open raceway ponds were operated at 20-cm depth with a mixing speed of 0.22 m s−1 (Moheimani and Borowitzka 2006). One paddle wheel-driven raceway pond was operated without the addition of CO2, while the other was operated with the addition of CO2 at a controlled pH of ~7.5. The helical tubular photobioreactor, based on the Biocoil design of Robinson et al. (1988), consisted of 60-m food grade clear polyvinyl tubing (25 mm I.D., 30 mm O.D.), wrapped around a 1-m high, 0.7-m diameter, steel mesh frame (Moheimani et al. 2011). The Biocoil had a total volume of 40 L and an illuminated surface area of 4 m2 (using the equation for a hollow cylinder). The open raceway ponds and Biocoil tubular photobioreactor were inoculated with a Tetraselmis MUR-233 culture that had been acclimatised to outdoor conditions in paddle wheel-driven open raceway ponds in semi-continuous culture for 10 months (Fon Sing 2010).

The effect of CO_2 on the growth and biomass productivity of *Tetraselmis* sp. was studied using a pH-stat system according to the principles of Hayes (1978). The pH, in both culture systems, was maintained at 7.5. Water losses due to evaporation were compensated by adding the required volume of fresh water prior to harvesting. Samples for cell counts, biomass concentration (AFDW in mg L^{-1}) and lipid determination were taken daily at 10 a.m. The semi-continuous cultures were partially harvested three times a week (Monday, Wednesday and Friday at 10 a.m.), and the medium removed was replaced with a fresh medium. Cell counts were carried out using a Neubauer haemocytometer. The specific growth rate was calculated according to Guillard (1973). Culture productivities (mg AFDW L^{-1} day^{-1}) were measured three times a week in triplicate based on the biomass concentration based on the interval between harvests. Ash-free dry weight was determined according to Moheimani et al. (2013), and total lipid content was measured using the method of Bligh and Dyer (1959) as adapted by Mercz (1994) and described in Moheimani et al. (2013).

Flow rates in the cultivation systems were measured with the tracer method by introducing 1 M HCl or 1 M NaOH (Molina et al. 2001; Moheimani et al. 2011). The hydrodynamics in the open raceway pond and Biocoil are described in Table 1. The characteristic of the turbulence velocity was used to estimate the radial velocity, which represents the light/dark frequency of the cells (t_c) (Janssen et al. 2003). No superficial gas hold-up measurements were carried out to calculate Reynolds numbers in the riser and downcomer. The average dark/light residence time of a single cell in the riser and downcomer was 20 % of the total time inside the Biocoil. Raceway pond and Biocoil temperatures were monitored continuously using an underwater temperature logger (Tiny Tag TG-3110). The maximum temperature in the Biocoil was maintained at about 25 °C with an automated evaporative cooling system. The systems were not heated.

Results

Initially, both cultivation systems were operated in batch mode to determine the maximum cell densities in stationary phase and to identify appropriate harvesting cell densities for the semi-continuous culture mode. Remove paasive voice

The open raceway pond and Biocoil were inoculated at an initial cell density of 25×10^4 cells mL^{-1}. At the end of the batch period, maximum cell concentrations were 97×10^4 cells mL^{-1} in the raceway pond and 216×10^4 cells mL^{-1} in the Biocoil (Fig. 1, pond and Biocoil in batch mode). The average specific growth rates were 0.64 ± 0.04 day^{-1} in the raceway pond and 1.06 ± 0.07 day^{-1} in the Biocoil. After stationary phases were reached in batch mode, both cultivation systems were operated in semi-continuous mode for the different growth and cultivation experiments.

When the open raceway pond and Biocoil were operated as a semi-continuous culture, they were regularly harvested to initial cell densities of 40×10^4 cells mL^{-1} in the raceway pond and 80×10^4 cells mL^{-1} in the Biocoil (Fig. 1, pond and Biocoil; treatment I). The average volumetric productivity was 36 ± 2 mg AFDW L^{-1} day^{-1} in the open raceway pond and 67 ± 5 mg AFDW L^{-1} day^{-1} in the Biocoil. The average illuminated areal productivity was

7,200 ± 400 mg AFDW m^{-2} day^{-1} in the open raceway pond and 670 ± 50 mg AFDW m^{-2} day^{-1} in the Biocoil. A decline in the ash-free dry weight per cell in each of the cultivation system was observed, possibly as a result of the diminishing solar radiation over this 10-day period (Fig. 1, top panel). Specific growth rates in the open raceway pond (0.11 ± 0.02 day^{-1}) and Biocoil (0.10 ± 0.02 day^{-1}) were similar, resulting in higher biomass productivity in the Biocoil because of the higher standing stock and higher harvesting cell density (Table 2).

A thin biofilm development in the Biocoil resulted in reduced light levels and a poor and declined growth rate of *Tetraselmis.* The importance of the operating cell densities, the targeted cell densities during harvest, in semi-continuous mode were therefore lowered in a short-term experiment in order to compensate for light attenuation in the cultivation systems. The raceway pond and Biocoil harvesting cell densities were reduced from 40×10^4 to 30×10^4cells mL^{-1} and from 80×10^4 to 40×10^4 cells mL^{-1}, respectively (Fig. 1, pond and Biocoil; treatment II). Increased irradiance, due to the lower operating cell densities, enhanced the specific growth rate from 0.11 ± 0.02 to 0.35 ± 0.03 day^{-1} in the open raceway pond and from 0.10 ± 0.02 to 0.33 ± 0.03 day^{-1} in the Biocoil. The AFDW per cell was significantly lower in both cultivation systems when growth rates were higher (Mann–Whitney test, $p < 0.001$).

The productivity in the Biocoil, during semi-continuous mode without additional CO_2, declined with time (Fig. 1, bottom panel, Biocoil) due to the formation of a thick biofilm on the walls of the tube (Fig. 2). After 38 days in semi-continuous mode, the cell density in the Biocoil decreased to 20×10^4 cells mL^{-1} due to extensive biofilm formation. Therefore, $CO_2$was added using a pH-stat system to maintain the pH at 7.5. The biofilm build-up on the inside of the helical tubing started slowly to detach due to increased photosynthetic activity during the CO_2 treatment, forming small bubbles in the biofilm and leading to detachment of the biofilm. Cell density increased from 20×10^4 to 160×10^4 cells mL^{-1} in 7 days, probably due to this detachment of the biofilm. Fifty percent of the Biocoil volume was harvested on May 21. Failure of the air compressor and CO_2 supply, during the controlled CO_2 treatment, on May 22 resulted in an interruption of growth. CO_2 supply was restored on May 25, and the culture returned to exponential growth (Fig. 1, bottom

panel, Biocoil). Twenty percent of the Biocoil volume was harvested on May 28. Both harvests removed the build-up biomass from the biofilm. Biofilm formation did not occur during the rest of the experimental period while CO_2 was being supplied. These results indicated that the *Tetraselmis* culture was carbon limited and unstable without an additional CO_2 supply.

In order to be able to compare the effect of CO_2 on growth and biomass productivity between the two cultivation systems, an additional open raceway pond was inoculated and grown with CO_2 addition using the pH-stat system for a period of 2 months (Fig. 1, pond + CO_2). The supply of CO_2 resulted in a stable culture with significantly higher specific growth rates and volumetric productivities in both May and June, compared to the raceway pond without a supply of CO_2 (Mann–Whitney test, $p < 0.001$), thereby indicating the positive effect of CO_2addition. The pH-regulated supply of CO_2 in the Biocoil, however, resulted in higher volumetric productivities compared to the open raceway ponds both with and without CO_2addition. The pH-regulated CO_2 supply in the Biocoil resulted in a 5.5 times higher volumetric productivity (85 ± 11 mg L^{-1} day^{-1}) compared to the open raceway pond with a CO_2 supply (15 ± 1 mg L^{-1} day^{-1}) and an 8 times higher productivity compared to the raceway pond without a supply of CO_2 (11 ± 1 mg L^{-1} day^{-1}), during the austral winter period (Fig. 1, pond, pond + $CO_2$and Biocoil; treatment IV). On the other hand, the illuminated area productivities highlight an alternative story. The average illuminated area productivity was 3,000 mg m^{-2} day^{-1} in the open raceway pond, and this was ~3 times higher than the 850 mg m^{-2} day^{-1} in the Biocoil. The pH-controlled supply of CO_2 in the open raceway pond resulted in significantly higher per cell AFDW compared to the Biocoil (Mann–Whitney test, $p < 0.001$). Data on growth rates, biomass and volumetric productivity are summarised in Table 3 to compare the effect of CO_2treatment between the open and closed cultivation systems.

Lipids

The average lipid content of *Tetraselmis* sp. cells when both cultures reached stationary phase in batch mode was 36.4 ± 4.6 % of AFDW, when grown in the open raceway pond, and 36.9 ± 4.6 % of

AFDW in the Biocoil. No significant differences were found between these lipid values (Mann–Whitney test, *t* test, $p > 0.5$). After batch mode, the open raceway pond and Biocoil were operated in semi-continuous mode with operating cell densities of 40×10^4 cells mL^{-1} in the raceway pond and 80×10^4 cells mL^{-1} in the Biocoil (Fig. 1, treatment I). The cells in the open raceway pond had an average lipid content of 42 ± 4.9 % of AFDW, and this was significantly lower than the lipid content of 55 ± 5.8 % in the Biocoil (Mann–Whitney test, $p < 0.001$). Higher total lipid contents of the algal cells in the raceway pond (*t* test, $p = 0.002$), but not for the Biocoil (*t* test, $p > 0.05$), were measured when the operating cell densities were reduced from 40×10^4 to 30×10^4 cells mL^{-1} and from 80×10^4 to 40×10^4 cells mL^{-1} in the raceway pond and Biocoil, respectively (Fig. 1, pond and Biocoil; treatment II). The significantly higher lipid content per cell in the raceway pond showed statistically higher lipid productivities (*t* test, $p = 0.002$) when operating cell densities were lowered during harvest. No significant differences were found in lipid productivities when operating cell densities were lowered in the Biocoil (*t* test, $p > 0.05$) (Table 2).

The average lipid content of the *Tetraselmis* cells, when cultures were operated with a CO_2supply, was significantly higher in the open raceway pond compared to the cells in the Biocoil (*t* test, $p = 0.009$, treatment IV). The average lipid content was 46.5 ± 2.8 % of AFDW when grown in the open raceway pond and 33.1 ± 1.9 % of AFDW in the Biocoil. The higher volumetric productivity in the Biocoil, however, resulted in a 4-fold higher lipid productivity compared to the open raceway pond (Table 3). Although significant differences in lipid content were found between the treatments, no significant relation was found between the cell lipid content of *Tetraselmis* MUR-233 and solar radiation (non-linear regression, $p = 0.884$) during the cultivation period in autumn and winter. Averaging all the lipid data showed that the lipid content remained constant at about 50 %, for both the open pond and the Biocoil during this cultivation period.

Light and temperature

Success in microalgae cultivation is determined by the physical, chemical and biological factors that limit algal growth or increase the net algal productivity. Light and temperature are the most critical limiting parameters for photosynthetic organisms including algae.

Volumetric productivity and total solar radiation were positively correlated in the open raceway pond, both without and with CO_2 treatment ($r = 0.63$, $p = 0.0015$). Volumetric productivity in the open raceway pond without CO_2 decreased significantly from 36 ± 2 mg L^{-1} day^{-1} in April to 12 ± 1 mg L^{-1} day^{-1} in June due to declining solar radiation. Unlike the raceway pond cultures, the volumetric productivity in the Biocoil showed no linear relationship with solar radiation suggesting that the cultures were not light limited and that volumetric productivity in the Biocoil is affected by more than one physical parameter rather than solar radiation alone.

Culture temperatures of outdoor cultures are influenced by air temperature and weather conditions. During these experiments, the average air temperatures decreased from 21.7 °C in March to 11.9 °C in June. The open raceway pond and Biocoil culture temperature profiles over a 24-h period on a warm and cold day recorded on the 30th of March and the 14th of May 2010 are shown in Fig. 3.The culture temperature profiles show that the Biocoil had a greater diurnal temperature range. The temperature inside the Biocoil increased quickly in the morning due to the greater surface-to-volume ratio and the shorter light path but cooled quickly after sunset. The Biocoil reached the temperature of 20 ± 5 °C in approximately 3 h on colder days, whereas the open raceway pond remained well below this temperature during colder days. On a cold day, minimum raceway pond temperatures increased from 5 to 14.8 °C in approximately 7 h, in comparison to a warm day where the maximum raceway pond temperature increased from 13.9 to 25.1 °C. On a cold day, the Biocoil temperatures increased from 1 to 23.7 °C in approximately 7 h, whereas the temperature on a warm day increased from 12.2 to 28.9 °C. Better temperature regulation along with increased irradiance in the Biocoil resulted in higher volumetric productivities (Tables 2 and 3).

In this study, *Tetraselmis* sp. MUR-233 was tested for its lipid production potential in an outdoor open raceway pond and a helical tubular photobioreactor (Biocoil) under low average irradiances and temperatures in austral autumn and winter. The volumetric productivity in the Biocoil with added CO_2 was 8 times higher compared to the open raceway pond without a CO_2 addition and 5.5 times higher compared to the open raceway pond which received a CO_2treatment. CO_2 supplemented also showed an increased volumetric productivity. Although significant differences were found in lipid contents between treatments and cultivation systems, the overall average lipid content for *Tetraselmis* MUR-233 was 50 % of AFDW in the exponential phase during semi-continuous cultivation. It should be highlighted that long-term seasonal lipid data are preferable to detect changes in lipid content.

Critical aspects in microalgae cultivation system designs are the supply, distribution and utilisation of light (Richmond et al. 2003). The average irradiance to which outdoor cultures are exposed in different cultivation systems will depend on the geometry of the design, diurnal irradiance, variable cloudiness, light reflection due to water waves, solar elevation angle from sunrise to sunset, increased light scattering and attenuation in turbulent flows and dilution rate of the culture (Fernández et al. 2000; Cervený et al. 2009). The advantage of the coiled structure of the Biocoil is that it is self-supporting and allows the operation of relatively long tubes on a small surface area (Tredici and Zittelli 1998). The design of the tubular photobioreactor improved light conditions significantly, as the surface-to-volume ratio was ten times higher compared to the open raceway pond. The effect of enhanced light distribution inside the Biocoil was clearly shown in higher volumetric productivities and growth rates on days with low solar irradiance compared to the open raceway pond. The higher volumetric productivity in the Biocoil compared to the open raceway pond showed that closed systems are less affected by weather conditions during colder winter periods as they warm up more rapidly than the open ponds, reaching more optimal temperatures for *Tetraselmis* photosynthesis (Fon Sing 2010). The shorter light path in the Biocoil tubing can explain the higher standing stock but would also result in the accumulation of high O_2 concentrations which inhibit photosynthesis. High oxygen concentrations are one of the most important factors limiting the productivity in closed

systems. The experiments in this study showed furthermore that a reliable cultivation of *Tetraselmis* sp. MUR-233 in the Biocoil can be achieved in austral autumn and winter when additional CO_2 is supplied using a pH-stat system.

Successful commercial microalgae cultivation requires the growing of algae in continuous or semi-continuous cultures over long periods. Improving cultivation management by seasonal adjusting cell densities, regulating pond depths and enhancing kinetics and dynamics of the algal cells could be further steps to achieve higher biomass productivities (Borowitzka and Moheimani 2013). Our data showed that lower post-harvest cell densities resulted in higher specific growth rates and higher lipid productivities, especially in the raceway ponds where the algae cells are likely to be light limited. A high lipid productivity for cost-effective cultivation of microalgae for biofuels is essential (Griffiths and Harrison 2009), and *Tetraselmis* MUR-233, unlike many other microalgae species, has a higher cell lipid content with increasing growth rate, thus increasing the lipid productivity. These data are valuable in understanding and improving the main challenge in microalgal oil production: "obtaining and cultivating a robust algal strain with high average annual lipid productivity per unit area at a low cost" (Fon Sing et al. 2013).

High turbulence moves cells through variations in the quantity and quality of light energy. It has been shown that increased turbulence results in enhanced exchange rates of nutrients and metabolites between the cells and their growth medium and is important for the achievement of high productivities (Grobbelaar 2009, 2012). It is to be noted that high liquid velocities and high turbulence and the method to achieve these can also damage algal cells due to shear stress. Not only mechanical pumps will damage the cells but shear stresses caused by eddies in the medium and air bubbles can also not be neglected (Janssen et al. 2003; Eriksen 2008; Leupold et al 2013). Shear-specific sensitivity stress will need to be tested for each microalgae species considered for out or indoor cultivation. The flow pattern in the open raceway pond showed a turbulent flow with Reynolds numbers above 3,000 in the first 3 m and a laminar flow pattern with Reynolds numbers below 2,000 in the last section of the pond. The importance of turbulent flow can be seen when a 2-m^2 open raceway pond with Reynolds numbers above 5,000 achieved maximum cell densities of

200×10^4 cells mL^{-1} in semi-continuous mode, whereas cell densities in the 1-m^2 open raceway ponds with low Reynolds numbers remained at 100×10^4 cells mL^{-1} (Isdepsky et al. 2010). The Biocoil, with a Reynolds number above 5,000 in the helical tubing photostage, reached a maximum cell density of 220×10^4 cells mL^{-1}. Increased flow velocities and Reynolds numbers in the 2-m^2 open raceway pond system reduced the gap in biomass production and maximum cell density between the open and closed (Biocoil) cultivation systems. Large open raceway ponds, however, usually never achieve these high Reynolds numbers. Furthermore, our data support the view that the advantage of closed tubular photobioreactors lies in the combination of higher Reynolds numbers, higher mass transfer rates, shorter light paths and better temperature control compared to the open raceway pond (Ugwu et al. 2005; Grobbelaar 2009). The average illuminated area productivity between the open raceway pond and the Biocoil shows a different story. The average illuminated area productivity was 3,000 mg m^{-2} day^{-1} in the open raceway pond and three times higher compared to the 850 mg m^{-2} day^{-1} in the Biocoil, when both cultures were operated with a pH-regulated CO_2 supply (treatment IV). Furthermore, the energy input for constructing, operating and maintaining multiple photobioreactor units and associated equipment during upscaling could easily result in a negative energy balance (van Beilen 2010). Scalability of open raceway cultivation systems is less complicated, although capital costs for construction remain a primary economic variable (Darzins et al. 2010). Taking these factors into consideration, the final type of cultivation system will be a trade-off between costs and the rate and reliability of the produced algae biomass.

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Table 1. Description of the hydrodynamics in the paddle wheel-driven open raceway pond and the Biocoil

Hydrodynamics	Location	Flow rate (m s^{-1})	Reynolds number	L/D frequency of cells (s)
Open pond	1 m from paddle wheel	0.22	3,250	1.6
	2 m from paddle wheel	0.25	2,190	1.4
	3 m from paddle wheel	0.21	1,730	1.8
Biocoil	Helical tubing	0.25	5,628	0.33
	Riser	0.15	–	–
	Downcomer	0.04	–	–

L/D light/dark

Table 2. Comparison of the effect of cell density on the growth rate, biomass concentration, volumetric productivity and lipid productivity between the open raceway pond and the Biocoil operated as semi-continuous cultures.

No CO_2	**Open pond**		**Biocoil**	
Post-harvesting cell density (cells mL^{-1})	40×10^4	30×10^4	80×10^4	40×10^4
Specific growth rate (day^{-1})	0.11 ± 0.03^a	0.35 ± 0.03^b	0.10 ± 0.02^a	0.33 ± 0.03^b
AFDW per cell (pg $cell^{-1}$)	385 ± 13^a	323 ± 9^b	425 ± 27^a	320 ± 4^b
Biomass concentration (mg AFDW L^{-1})	200 ± 7^a	160 ± 4^b	430 ± 40^a	220 ± 7^b
Volumetric productivity (mg AFDW L^{-1} day^{-1})	36 ± 2^a	39 ± 1^b	67 ± 5^a	56 ± 2^b
Illuminated area productivity (mg AFDW $m^{-2}day^{-1}$)	$7{,}200 \pm 400^a$	$7{,}800 \pm 200^b$	670 ± 50^a	560 ± 20^b
Volumetric lipid productivity (mg AFDW $L^{-1}day^{-1}$)	15 ± 3^a	25 ± 2^b	37 ± 4^a	43 ± 2^b
Illuminated area lipid productivity (mg AFDW m^{-2} day^{-1})	$3{,}000 \pm 600^a$	$5{,}000 \pm 400^b$		

Data are presented as means with standard errors
[a]Mean±SE, $n = 21$
[b]Mean±SE, $n = 15$

Table 3. Comparison of semi-continuous cultures in the open raceway pond and the Biocoil, both with CO_2added using a pH-stat system

$+CO_2$	Open pond	Biocoil	
Post-harvesting cell density (cells mL^{-1})	40×10^4	60×10^4	120×10^4
Specific growth rate (day^{-1})	0.11 ± 0.02^b	0.6 ± 0.06^a	0.31 ± 0.04^b
AFDW per cell (pg $cell^{-1}$)	333 ± 87^b	387 ± 3^a	489 ± 42^b
Biomass concentration (mg AFDW L^{-1})	152 ± 6^b	290 ± 20^a	500 ± 60^b
Volumetric productivity (mg AFDW L^{-1} $day^{-1)}$	15 ± 1^b	63 ± 6^a	85 ± 11^b
Illuminated area productivity (mg AFDW m^{-2} day^{-1})	$3{,}000 \pm 200^b$	630 ± 60^a	850 ± 110^b
Volumetric lipid productivity (mg AFDW L^{-1} day^{-1})	7 ± 1^b	25 ± 2^a	28 ± 4^b
Illuminated area lipid productivity (mg AFDW m^{-2} day^{-1})	$1{,}400 \pm 200^b$	250 ± 10^a	280 ± 40^b

Data are presented as means with standard errors
[a]Mean±SE, $n = 12$
[b]Mean±SE, $n = 24$

Fig. 1. *Top panel*, the total daily solar irradiation (*diamonds*) and maximum (*open triangles*) and minimum air temperatures (*filled triangles*); *other panels* show log-transformed cell densities and pond pH with different treatments, in descending order: *Pond*, raceway pond without additional CO_2; *Pond + CO_2*, raceway pond with CO_2 supplied using a pH-stat system; *Biocoil* operated under different CO_2 conditions. The *first arrow* indicates commencement of CO_2 supply in the Biocoil, and the *second arrow* indicates the supply after failure of the air compressor and reinitialising CO_2supply. Treatments for the cultivation systems: *I*, post-harvesting cell density of 40×10^4 for the open pond and 80×10^4 cells mL^{-1} for the Biocoil; *II*, harvesting cell density of 30×10^4 for the open pond and 40×10^4 cells mL^{-1} for the Biocoil; *III*, harvesting cell density of 60×10^4; *IV*, 120×10^4cells mL^{-1} for the Biocoil.

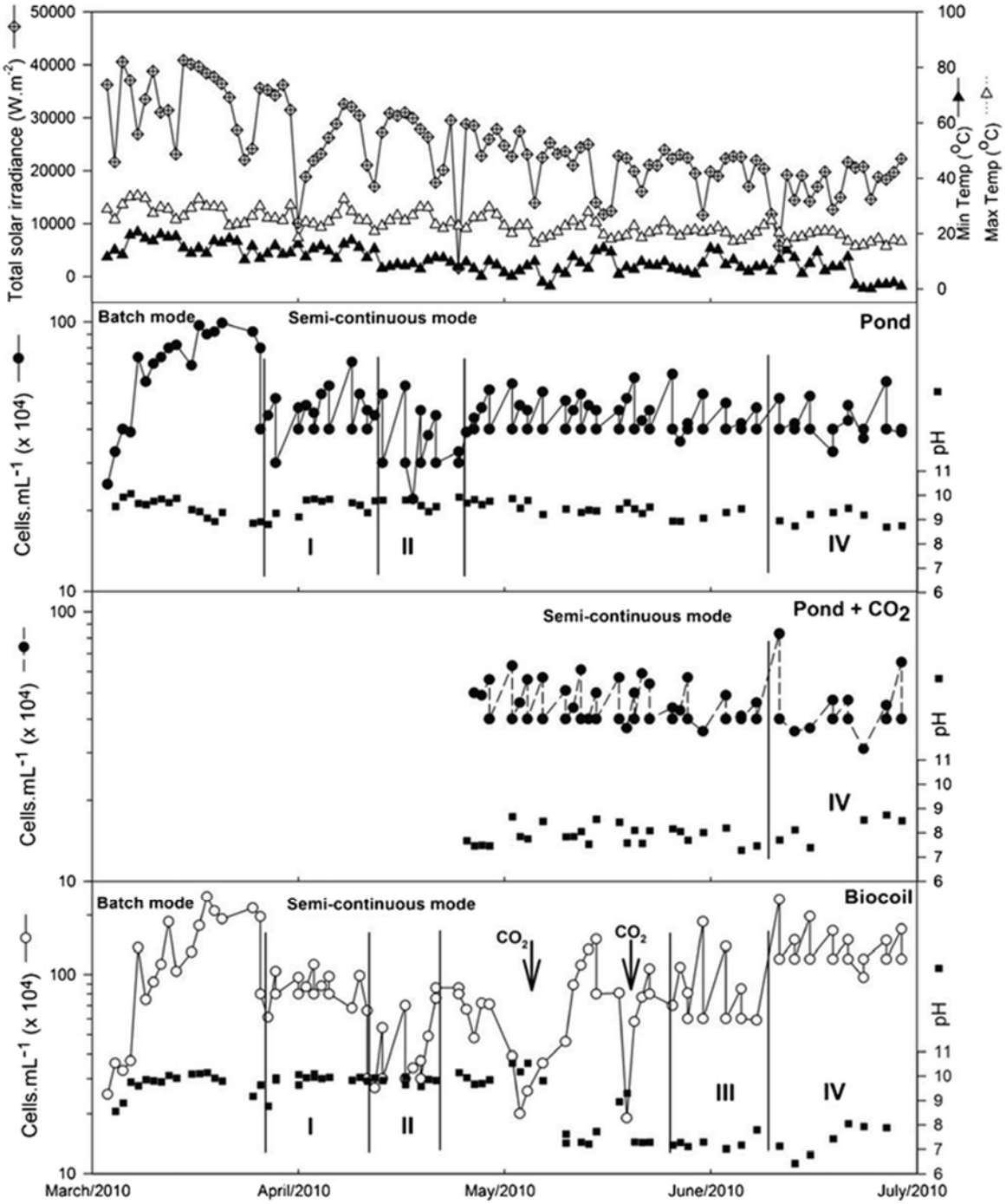

Fig. 2. a Biofilm build-up on the inside of the helical tubing of the Biocoil without CO_2 addition on 2 May 2010. **b** Detachment of biofilm due to increased photosynthetic activity following CO_2 addition on 20 May 2010

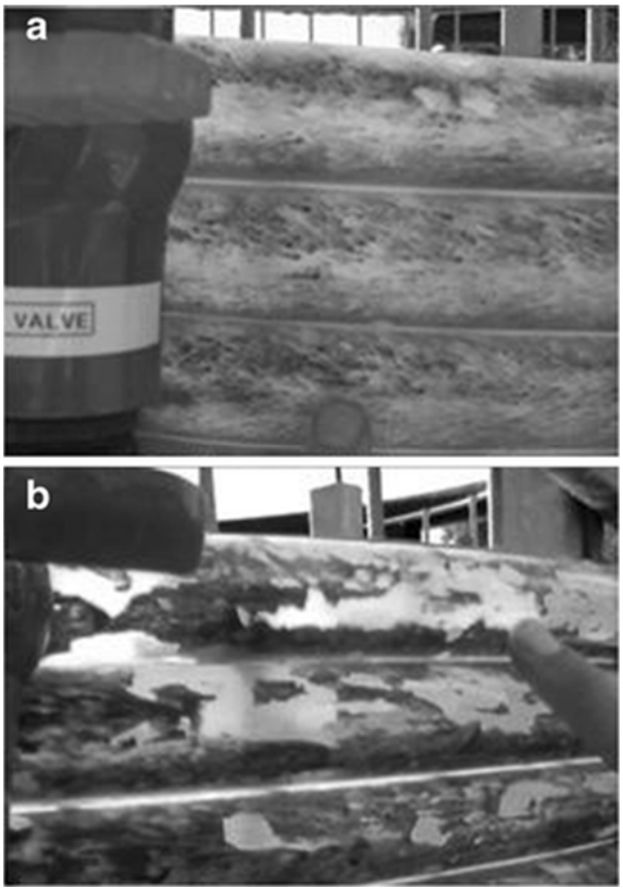

Fig. 3. Diurnal variation in culture temperatures over a cold day (*top*) and a warm day (*bottom*). Open raceway pond (*closed circle*) and Biocoil, PBR (*open circle*)