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Growth characteristics of *Rhodopseudomonas palustris* cultured outdoors, in an underwater tubular photobioreactor, and investigation on photosynthetic efficiency

Pietro Carlozzi · Benjamin Pushparaj · Alessandro Degl'Innocenti · Antonella Capperucci

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Abstract The underwater tubular photobioreactor is a fully controlled outdoor system to study photosynthetic bacteria. Before growing bacteria cells outdoors, two modified van Niel medium (vN-A, vN-B) were tested under artificial light. During exponential growth, the specific growth rates were 0.0416 and 0.0434 h^{-1}, respectively; vN-B was chosen for outdoor experiments. The growth behavior of *Rhodopseudomonas palustris* was investigated under a natural light–dark cycle (sunrise–sunset, 15L/9D) and a forced light–dark cycle (9:00–19:00, 10L/14D). Relationships between solar radiations, daily growth rates, and biomass output rates were also investigated. After determining the elemental biomass molar composition and its combustion heat, some trends of photosynthetic efficiency (PE) were obtained over daylight. The PE trends were always of the oscillatory type, with the exception of that achieved at low biomass concentration. Under a natural light/dark cycle, the maximum PE (11.2%) was attained at sunset, while under a forced light/dark cycle, the highest PE (8.5%) was achieved in the morning. Three initial biomass concentrations were investigated (0.65, 1.01, and 1.54 g l^{-1}). The stoichiometric equation for bacteria cells indicated that 87.7% of the carbon of acetic acid was converted to biomass and only 12.3% was lost as CO_2.

P. Carlozzi (✉) · B. Pushparaj
Istituto per lo Studio degli Ecosistemi,
Distaccamento di Firenze,
Consiglio Nazionale delle Ricerche,
Polo Scientifico, Via Madonna del Piano n. 10, Sesto F.no,
50019 Florence, Italy
e-mail: p.carlozzi@ise.cnr.it

A. Degl'Innocenti · A. Capperucci
Dipartimento di Chimica Organica,
Università degli Studi di Firenze,
Polo Scientifico, Via della Lastruccia n. 13, Sesto F.no,
50019 Florence, Italy

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Introduction

The purple strains of bacteria are an interesting group belonging to the family of Rhodospirillaceae. In this group, we find *Rhodopseudomonas palustris*, which is known for its ability to produce clean fuel as H_2 (Barbosa et al. 2001), a polymer as poly-β-hydroxybutyrate (PHB) (Carlozzi and Sacchi 2001), a bioactive substance as *cis*-vaccenic acid (antivirus; see Hirotani et al. 1991; Kenyon 1978), and carotenoid type as lycopene (Evans et al. 1990). *Rhodopseudomonas* biomasses are rich in proteins (from 51 to 61% of dry weight) and they can be used very well as feed stock supplements (Vrati 1984).

R. palustris is among the most metabolically versatile phototrophic bacteria ever described. When oxygen is present, it is able to produce, by respiration, bioenergy to degrade a variety of compounds such as sugars, methanol, and lignin monomers. *R. palustris* can also grow in the light under anaerobic conditions and fix CO_2 if inorganic electron donors (H_2, SO_3^{-2}, H_2S) are present. *R. palustris* can also degrade organic compounds (acids, alcohol, aromatic substances) including toxic molecules such as 3-chlorobenzoate (Fißler et al. 1995; Gibson and Gibson 1992; England et al. 1991; Trüper and Pfenning 1978). Thus, the metabolic versatility of *R. palustris* makes it a very interesting phototrophic microorganism in bioremediations of many types of waste. Nowadays, using waste as a potential energy resource is an accepted and attractive concept. To achieve this goal, the relatively low photosynthetic efficiency of this microorganism, attainable under environmental growth conditions, should be increased by intensive culture. This can be done using photobioreactors,

which can control waste processes and achieve biotechnological progress in environment cleaning and energy recovery.

In principle, the main products of anaerobic fermentation are acetic and butyric acids (Segers et al. 1981). These organic sources can be used as substrate to support the growth of *R. palustris* under anaerobic conditions and light (Trüper and Pfenning 1978). The conversion of these acids would be advantageous in linking clean energy fuel production (H_2) with wastewater treatment (Eroğlu et al. 2004).

In this work, we studied the growth behavior of *R. palustris* using acetate as a carbon source and ammonia chloride as nitrogen source. Experiments were carried out indoors under artificial light and outdoors under natural light. Relationships between biomass output rates, daily growth rates, and solar radiations (SR) were investigated. In summer time, the photosynthetic efficiency was determined over the daylight period. Three different cell concentrations (0.65, 1.01, 1.54 g l^{-1}) were investigated under a natural light–dark cycle (15L/9D), while two cell concentrations (0.65 and 1.54 g l^{-1}) were investigated under a forced one (10L/14D). Specific growth rates and reactor output rates were investigated as well.

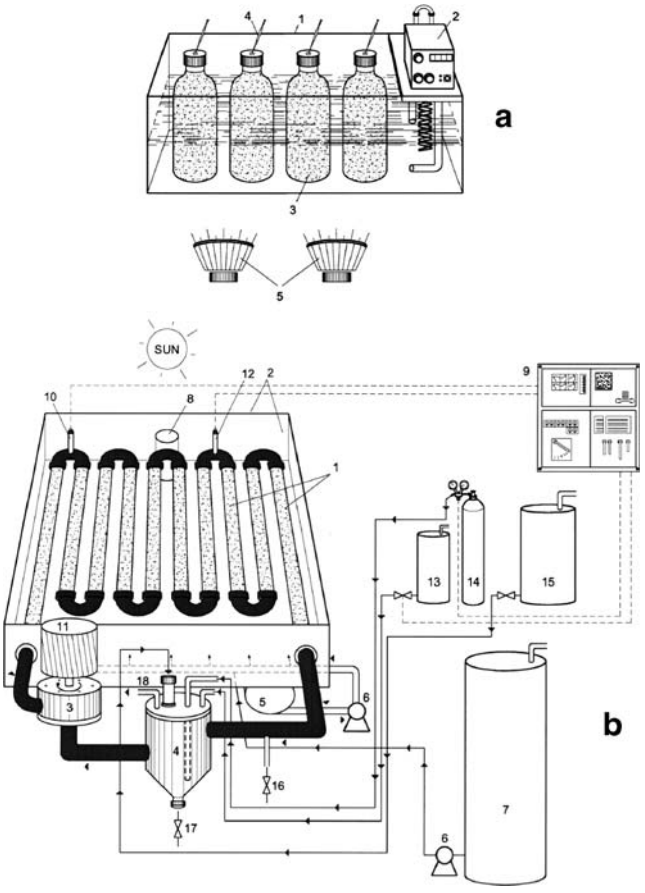

Fig. 1 **a** Schematic laboratory system used to study *R. palustris* grown under artificial light: *1* Plexiglas water bath, *2* thermoregulator, *3* sovirel bottles, *4* syringes, *5* incandescent lamps. **b** Schematic diagram of the anaerobic photobioreactor used to study the growth kinetics of *R. palustris* under a natural light/dark cycle (14L/10D) and a forced light/dark cycle (10L/14D): *1* tubular loop, *2* heat exchanger steel water bath, *3* pump for culture recycling, *4* PVC vessel, *5* PVC water heater, *6* pumps for water recycling, *7* cooling tower, *8* overflow, *9* control unit, *10* pH probe, *11* motor, *12* temperature sensor, *13* acid solution, *14* cylinder argon, *15* fresh medium tank, *16* sampling port, *17* culture outlet port, *18* gas outlet

Materials and methods

Microorganism and culture medium

R. palustris strain 42OL (from our culture collection) was used in this study. It was grown in two media: (1) a first modified van Niel medium with C/N ratio=4.72 (vN-A; see Carlozzi and Sacchi 2001), and (2) a second modified van Niel medium (vN-B). This last medium contained 48.65 mM of $CH_3COONa{\cdot}3H_2O$, 16.65 mM of NH_4Cl, and 0.5 g l^{-1} of yeast extract per liter of medium solution (C/N ratio=5).

Description of growth conditions

During laboratory experiments, *R. palustris* was grown inside four sovirel bottles (500 ml each) closed together and positioned in a row in a water bath (T=30°C) (Fig. 1b). Two bottles contained vN-A medium and the other two bottles contained vN-B medium. We illuminated the culture with an incandescent lamp and the light intensity on the bottle surface was 120 μE m^{-2} s^{-1}.

Outdoor experiments were carried out in a loop tubular photobioreactor immersed in thermostatic water; the bioreactor was located at our experimental site in Scandicci, near Florence, Italy (latitude 43°46′N, longitude 11°15′E). We performed outdoor experiments under a semicontinuous regime: an appropriate culture volume was daily withdrawn from the reactor and replaced with the same volume of fresh medium to maintain the preset initial cell concentration. The culture temperature was kept at 30±1°C, with a fixed pH of 7.0, and the circulation speed of the culture was kept at 0.46 m s^{-1}, producing a Reynolds number of about 27,900 (Carlozzi and Sacchi 2001).

Tubular cultural system description

The underwater tubular photobioreactor (UwTP) is a loop of 20 m in length. The reactor working volume is 0.053 m^3, and the dark to light volume ratio (D_v/L_v) is 0.43. The ten glass tubes, each 2.0 m long with 0.048 m internal diameter, were connected at their extremities by polyvinyl chloride (PVC) U-bends. A pump was provided to stir the

culture and a PVC vessel connected the first and the last tubes, as shown in Fig. 1b.

Analytical procedures

The biomass dry weight (d.w.) was determined every 90 min from sunrise to sunset. The dry weight and the *Rhodopseudomonas* productivity were estimated according to Carlozzi and Sacchi (2001). Elemental analysis of the biomass (C, H, O, N) was performed in triplicate using an elemental analyzer (model 1106, Carlo Erba Instrumentation, Milan). Ash was determined as 100−(C+ H + O + N). We calculated the heat of combustion of the biomass according to Spoehr and Milner (1949). Solar radiation impinging on the horizontal plane was measured with a Kipp and Zonen pyranometer (Model CM 6, Delft, Holland) equipped with a Micros amplification interface (Model IS Treviso, Italy) provided with an electronic data integrator. The specific growth rate (μ) was determined by the following equation:

$$\mu = \frac{ln\mathrm{Cc_{T2}} - ln\mathrm{Cc_{T1}}}{T_2 - T_1}\left(\mathrm{h}^{-1}\right) \quad (1)$$

where Cc_{T2} and Cc_{T1} are the cell concentrations at time T_2 and T_1 during exponential growth.

The daily growth rate (μ_d) was determined by the following equation:

$$\mu_d = \frac{ln\,\mathrm{Cc_{T2}} - ln\mathrm{Cc_{T1}}}{T}\left(\mathrm{day}^{-1}\right) \quad (2)$$

where T=day.

Photosynthetic efficiency calculation

To determine the photosynthetic efficiency (PE), accounting for the cylindrical geometry of the bioreactor tubes, we used the following equation:

$$\mathrm{PE} = \frac{\mathrm{P} \times 0.955 \times (-\Delta \mathrm{H})}{\mathrm{GLEI} + (-\Delta \mathrm{H}') \times \text{acetic acid consumed}} \times 100 \quad (3)$$

where P is the reactor productivity (grams per reactor per hour), 0.955 is the coefficient to obtain reactor productivity as biomass ash-free (ash = 4.5 % of the biomass d.w.), $-\Delta H$ is the heat of combustion of biomass ash-free (kilocalories per gram), $-\Delta H'$ is the heat of combustion of acetic acid (kilocalories per gram), and acetic acid consumed is measured in grams per reactor per hour. The global light energy input (GLEI, measured in kilocalories per reactor per hour) was determined in accordance with the following equation:

$$\mathrm{GLEI} = \mathrm{TSR} \times 1.57 \times 0.64 \times 0.89 \times c \quad (4)$$

where TSR is the total solar radiation impinging on 1.0 m^2 horizontal surface (megajoules per square meter per hour) and c is the conversion factor from megajoules per square meter per hour to kilocalories per square meter per hour. Other details about PE and GLEI were reported in a previous paper (Carlozzi and Sacchi 2001).

Results

Laboratory experiments under artificial light

The studies under artificial light (incandescent lamp) were carried out to compare *Rhodopseudomonas* growth inside vN-A and vN-B.

We checked the *Rhodopseudomonas* growth determining the bacteriochlorophyll (Bchl) by an empirical equation:

$$\mathrm{Bchl} = (\mathrm{ABS_{805}} - \mathrm{ABS_{830}}) \times \varepsilon \quad \left(\mathrm{mg\,l^{-1}}\right) \quad (5)$$

where ABS represents the absorbance of culture cells in vivo at 805 and 830 nm, and ε is a factor to convert the values to milligrams per liter. Results are shown in Fig. 2. Bchl concentration did not increase during the first 24 h (lag phase) in both cultures (vN-A and vN-B); nevertheless, the lag phase was longer in the vN-B than in the vN-A medium. After the 24th hour, Bchl started to increase in both cultures (acceleration phase) till the 48th hour; after that Bchl started to increase exponentially (exponential phase). After the 72nd hour of growth, exponential growth stopped in both cultures; the slopes progressively decreased

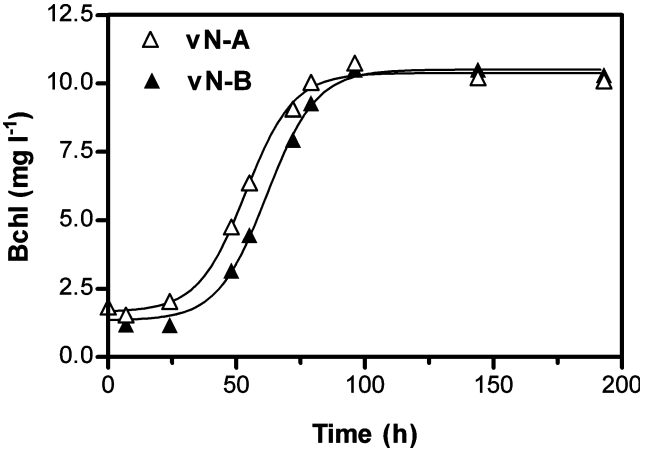

Fig. 2 *R. palustris* growth in two modified van Niel media (*vN-A* and *vN-B*). Cultures were illuminated with a 100-W incandescent lamp (120 μE m^{-2} s^{-1}) and culture temperature was kept at 30°C. An empirical equation was used for Bchl calculation to determine *Rp.* growth after measuring the absorbance at 805 and 830 nm

(retardation phase) and the stationary phases in both cultures suddenly took over (96th hour). During exponential growth, the specific growth rate of the culture vN-B (μ=0.0434 h^{-1}) was a little higher than vN-A (μ=0.0416 h^{-1}). At the end of the experiment (193rd hour), biomass dry weight was determined. Dry weight values were 0.98 g l^{-1} in vN-A and 1.02 g l^{-1} in vN-B. This last medium was used for outdoor experiments.

Outdoor experiments

In a previous study of *R. palustris* 42OL, we demonstrated that the optimal cell concentration needed to obtain the maximal daily output rate was 1.0 g (d.w.) l^{-1} (Carlozzi and Sacchi 2001). In this study, under a semicontinuous regime and initial cell concentration of 1.02±0.14 g (d.w.) l^{-1}, we investigated outdoors the relationship between biomass output rate and solar radiation. When solar radiation (SR) reached the maximal value, under the climate conditions of central Italy (Tuscany region), the highest biomass output rate was 1.4 g l^{-1} d^{-1} (Fig. 3).

A faithful way to estimate kinetic parameters is to construct a mathematical model for μ_d–SR relationship. If we assume μ_d as the specific daily growth rate attained for different SR concentrations, we can describe the theoretical μ_d–SR relationship. Figure 4 displays the experimental daily growth rates attained to increase solar radiations. The continuous line depicts the theoretical trend of μ_d vs SR; it was of asymptotic type and resolved by the following equation:

$$\mu_d = \frac{1.43 \times \mathrm{SR}}{21.32 + SR} \tag{6}$$

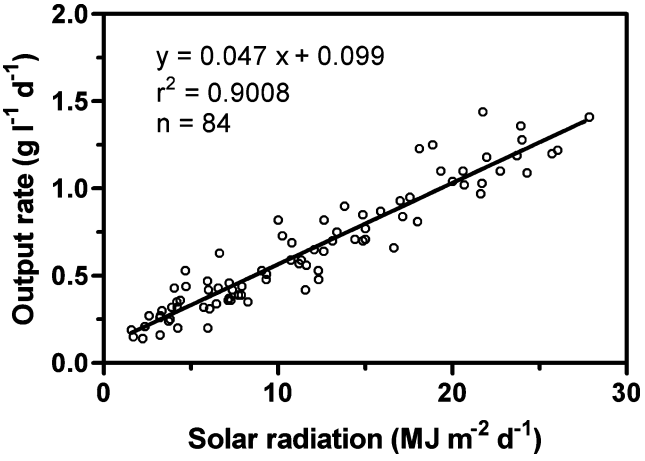

Fig. 3 Volumetric biomass output rate as a function of solar radiation achieved inside UwTP. During the whole experiment, initial cell concentration was 1.02±0.14 g l^{-1}. Data displayed in this figure concern 84-day experiments

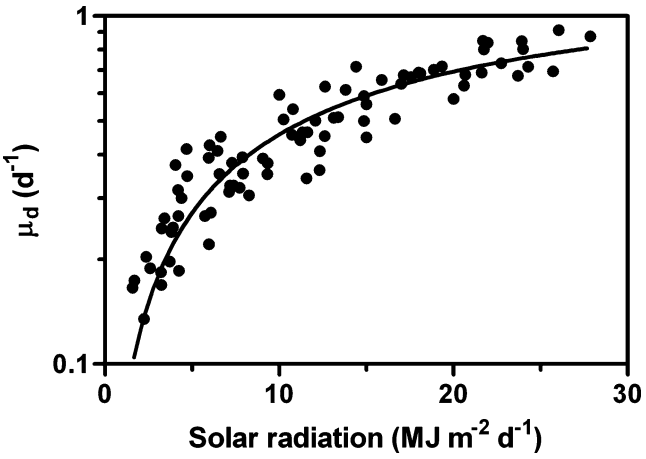

Fig. 4 Experimental data of the daily growth rate (μ_d) as a function of solar radiation and model fit according to Monod's model. Experimental conditions were the same as those quoted in Fig. 3

The theoretical μ_d fitted well with experimental data for every solar radiation tested. Approaching the maximal SR, reachable under Tuscan climate conditions, the theoretical μ_d values are fully into the range checked experimentally (Fig. 4).

Elemental biomass composition, together with elemental molar composition and combustion heat of *Rhodopseudomonas* cells harvested at sunset in July, are reported in Table 1.

The stoichiometric equation for bacteria cells is the following:

$$0.57CH_3COOH + 0.17NH_3 + h\nu \rightarrow CH_{1.92}O_{0.41}N_{0.17} + 0.14CO_2 + 0.45H_2O \tag{7}$$

indicating that 87.7% of the carbon of acetic acid was converted to biomass and only 12.3% was lost as CO_2.

We used the combustion heat reported in Table 1 to calculate the PE for the daylight period. Results achieved under natural light–dark cycle are reported in Table 2. At a low biomass concentration (L_{BC}=0.65 g l^{-1}), the reactor output rate increased till 4:00 pm, after which it began to decrease gradually. The specific growth rate was always high

Table 1 Elemental biomass composition, molecular formulae, and heat of combustion of *Rhodopseudomonas* cells harvested at sunset in July

Ash-free biomass composition	
Carbon	52.11%
Hydrogen	8.41%
Nitrogen	10.66%
Oxygen	28.82%
Elemental molar composition	$CH_{1.92}N_{0.17}O_{0.41}$
Heat of combustion	5.98 kcal g (d.w.)$^{-1}$

Analyses were performed in triplicate; mean values are reported.

Table 2 Reactor output rates and specific growth rates attained with *R. palustris* grown outdoors inside the UwTP at low biomass concentration (L_{BC}=0.65 g l^{-1}), optimal biomass concentration (O_{BC}=1.01 g l^{-1}), and high biomass concentration (H_{BC}=1.54 g l^{-1})

Time (h)	Reactor output rates [g (d.w.) R^{-1} h^{-1}]			Specific growth rates(h^{-1})		
	L_{BC}	O_{BC}	H_{BC}	L_{BC}	O_{BC}	H_{BC}
06:15–08:30	2.59	2.12	1.41	0.070	0.038	0.017
08:30–10:00	4.24	4.24	3.53	0.098	0.069	0.061
10:00–11:30	4.59	6.36	4.24	0.092	0.091	0.068
11:30–13:00	5.65	9.91	9.86	0.098	0.121	0.095
13:00–14:30	6.36	2.81	3.50	0.095	0.031	0.031
14:30–16:00	7.79	8.48	7.05	0.101	0.085	0.058
16:00–17:30	4.93	9.17	7.05	0.057	0.081	0.053
17:30–19:00	3.87	3.55	3.55	0.041	0.029	0.025
19:00–20:00	3.18	3.18	2.12	0.032	0.025	0.015

from sunrise to afternoon, and its maximum value of 0.101 h^{-1} was reached during the time 14:30–16:00; after which it decreased steadily. At optimal biomass concentration (O_{BC}=1.01 g l^{-1}), the reactor output rate and the specific growth rate increased step-by-step until noon, but both reached the minimum values [RO_R=2.81 g (d.w.) R^{-1} h^{-1}; μ=0.031 h^{-1}] during the period 13:00–14:30. We observed a similar growth behavior at high biomass concentration (H_{BC}=1.54 g l^{-1}). During the period 13:00–14:30, we achieved very low values, RO_R=3.50 g (d.w.) R^{-1} h^{-1} and μ=0.031 h^{-1}. Figure 5 shows how the photosynthetic efficiency changes from sunrise to sunset. At sunrise, PE was 7.8% at L_{BC}, 6.5% at O_{BC}, and 4.4% at H_{BC}. When the initial biomass concentration was low (0.65 g l^{-1}), the photosynthetic efficiency decreased from sunrise to noon, after that PE increased progressively and reached the highest value of 11.2% at sunset. At optimal and high initial biomass concentrations (O_{BC}=1.01 g l^{-1} and H_{BC}=1.54 g l^{-1}), we achieved an oscillatory behavior of PE. Photosynthetic

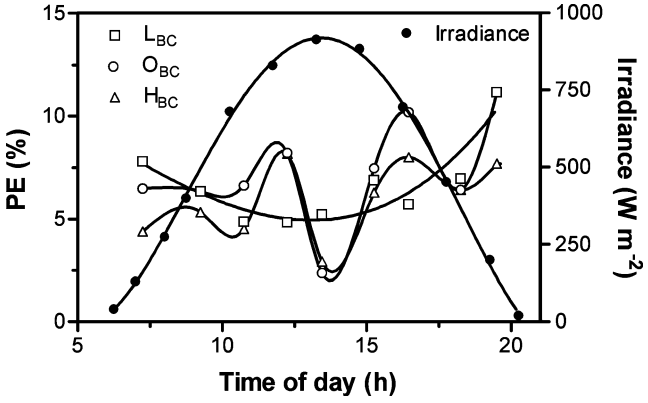

Fig. 5 Daily trends of PE obtained from sunrise to sunset, under a natural light/dark cycle. Three different cell concentrations (L_{BC}=0.65 g l^{-1}; O_{BC}=1.01 g l^{-1}; H_{BC}=1.54 g l^{-1}) were tested. The investigation was performed in July on days with typical, clear sky

efficiency was very low at noon (2.4% at O_{BC} and 2.9% at H_{BC}), just when the irradiance reached the highest value (900 W m^{-2}).

When a light/dark cycle (10L/14D) was imposed on the outdoor culture, we achieved the results reported in Table 3. Low and high biomass concentration cultures (L_{BC} and H_{BC}) reached the highest reactor output rates of 7.42 and 9.19 g (d.w.) R^{-1} h^{-1} respectively, in the period 13:00–14:30. In the same period, we obtained the highest specific growth rate of 0.141 h^{-1} in the reactor with L_{BC}. In the morning, μ was rather high (average of 0.08 h^{-1}) in both cultures and decreased gradually in the afternoon. Figure 6 shows the irradiance and photosynthetic efficiency trends from 9:00 to 19:00, when a light–dark cycle of 10/14 was imposed on *Rhodopseudomonas* cultures. In the morning at H_{BC}, PE was higher than at L_{BC}; in the evening, the PE values at L_{BC} were slightly higher than those obtained at H_{BC}.

Discussion

Before testing the growth behavior of *R. palustris* outdoors, we carried out an experiment under artificial light to study the growth behavior of *Rhodopseudomonas* cells inside two modified van Niel media (vN-A and vN-B). The concentrations of CH_3COONa, NH_4Cl, and yeast extract were increased in the vN-B to prevent their deficiency. This deficiency causes slowing, followed by arrest of growth. Since the results, reported on Fig. 2, did not show any significant differences of *Rhodopseudomonas* growth, we chose to use the more concentrated modified van Niel medium (vN-B) for outdoor experiments.

Photosynthetic biomass productions are generally limited by light (Goldman 1979). We found that the daily biomass output rate is a function of solar radiation availability, as shown in Fig. 3. In general, the kinetics of microbial growth and substrate utilization is described by

Table 3 Reactor output rates and specific growth rates attained with *R. palustris* grown outdoors (under a forced light/dark cycle of 10/14 h) inside the UwTP at low biomass concentration (L_{BC}=0.65 g l^{-1}) and at high biomass concentration (H_{BC}=1.54 g l^{-1})

Time (h)	Reactor output rates [g (d.w.) R^{-1} h^{-1}]		Specific growth rates (h^{-1})	
	L_{BC}	H_{BC}	L_{BC}	H_{BC}
09:00–10:00	2.65	6.36	0.088	0.075
10:00–11:30	2.15	7.07	0.072	0.076
11:30–13:00	3.89	8.48	0.088	0.081
13:00–14:30	7.42	9.19	0.141	0.078
14:30–16:00	5.30	5.65	0.085	0.044
16:00–17:30	3.89	3.89	0.054	0.029
17:30–19:00	3.53	3.53	0.047	0.025

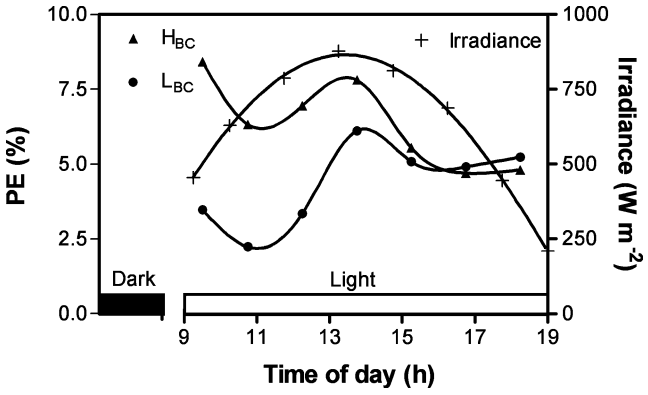

Fig. 6 Daily trends of PE obtained under a forced light–dark cycle of 10L/14D. Experiments were performed at low and high biomass concentration (L_{BC}=0.65 g l^{-1}; H_{BC}=1.54 g l^{-1}). The investigation was performed in July on days with typical, clear sky. Results are the average values of two repetitions for each cell concentration tested

inhibitory or noninhibitory growth: the first is known as Haldane kinetics (D'Adamo et al. 1984),while the second as Monod function (Monod 1949). This last model kinetics is characterized by an increasing growth rate with increasing substrate concentration with an asymptotic approach to a maximum growth rate (Rozich and Colvin 1986). Assuming SR concentrations in the same way of substrate concentrations, our experimental results displayed in Fig. 5 are in accordance with Monod's model: increasing growth rate with increasing solar radiation was of asymptotic type and resolved by Eq. 6. This last could be written as the following:

$$\mu_d = \frac{\mu_{d\,max} \times \mathrm{SR}}{K_S + SR} \tag{8}$$

Equation 8 describes the Monod's model to determine kinetic parameters under noninhibitory conditions. Consequently, the values of Eq. 6, 1.43 and 21.32, correspond respectively to the theoretical maximum growth rate (μ_{dmax}) per day, and the half-saturation constant (K_s) expressed in megajoules per square meter per day. If solar radiation higher than that achieved under our climate conditions of Florence (Italy) was available, we could increase the biomass output rate because *Rhodopseudomonas* cells were not saturated yet, such as shown in Fig. 4. In the past, the primary goal of photosynthetic microorganism mass culture systems was to maximize the production of biomass; the second and often equally important goal was to optimize the production of specific biochemicals (Sukenik et al. 1987). Nowadays, optimization of the production of specific biochemicals is surely the most important goal. By scaling up the photobioreactor, we can produce biomasses as potential sources of livestock and fish feed. Alternatively, we could produce a specific bioactive substance (*cis*-vaccenic acid), a thermoplastic polymer (PHB) or a biofuel (H_2), which can be more economically successful than biomasses for animal feed.

The oscillatory photosynthetic efficiency achieved under a natural light/dark cycle assumed a nontypical trend over daylight with the exception of that attained at L_{BC} (Fig. 5). The reason why *Rhodopseudomonas* cells exhibited this growth behavior was not clear. Exposure of algae to bright light can result in a photo-inhibitory reduction in the number of functional Photosystem II (PS II) reaction centers and consequent decrease in the maximum quantum yield of photosynthesis (Behrenfeld et al. 1998). During the past few decades, damage to PS II action centers was recognized as the fundamental mechanism responsible for photo-inhibition (Kyle 1978; Barber 1991; Barber and Andersson 1992; Prasil et al. 1992). Photo-inhibition is a state of physiological stress that occurs in all oxygen-evolving photosynthetic organisms exposed to light (Adir et al. 2003).

A large number of studies have reported the sensitivity of PS II to photodamage and much is known about this mechanism of damage and repair (Prasil et al. 1992; Andersson and Aro 1997; Melis 1999; Burnap 2004). In contrast, much less is known about photodamage to Photosystem I (PS I), which is quite resistant to typical light stress. Moreover, the physiological relevance of PS I photodamage is not well-understood (Sheller and Haldrup 2005). PS I is the only photosystem employed by green sulfur and purple bacteria (Pfennig 1978). The reaction center of purple bacteria is highly homologous to that of PS II with regard to protein structure and acceptor side redox components. In spite of the homology to PS II, purple bacteria are not sensitive to photo-inhibition (Tandori et al. 2001). The oscillatory behavior of PE attained under natural light–dark cycle (Fig. 5) was achieved also when *Rhodopseudomonas* cells were exposed under a forced light–dark cycle (10L/14D) (Fig. 6). This last investigation was performed to expose suddenly *Rhodopseudomonas* cells at a relatively high irradiance of 450 W m^{-2} without changing excessively the natural light–dark cycle (15L/9D) that may be found in July (sunrise 5:46–sunset 20:56) under Tuscan (Italy) climate conditions. The large discrepancy of PE achieved in the morning at H_{BC} (PE=8.4%) and L_{BC} (PE=3.4%) was due to a scanty reactor output rate attained at L_{BC}, which was 2.4 times smaller than that achieved at H_{BC}. This happened in spite of the specific growth rate 1.2 times higher at L_{BC} than that at H_{BC} (Table 3). Under a forced light–dark cycle (10L/14D) displayed in Fig. 6, we can see that when the irradiance was at its highest (885 W m^{-2}), we achieved high PEs (7.8% at H_{BC} and 6.1% at L_{BC}). On the contrary, with the natural light–dark cycle (15L/9D), we achieved the lowest PEs (4.8% at L_{BC}, 2.4% at O_{BC}, and 2.9% at H_{BC}) when the irradiance reached the

highest value of 914 W m^{-2} (Fig. 5). Moreover, we noticed that during the period 13:00–14:30, the μ breakdown (until 0.031 h^{-1}) was achieved in both O_{BC} and H_{BC}; it was in agreement with the breakdown of reactor output rates (Table 2). When the investigation was performed forcing a light/dark cycle (10/14), we did not notice any μ breakdown; it increased in the morning and decreased in the afternoon (Table 3). We believe that PE was not correlated with the irradiance trends exhibited in Figs. 5 and 6, but other physiological culture conditions could have regulated growth metabolism inside *Rhodopseudomonas* cells. Studies on *R. palustris* (Imai et al. 1984) showed that under anaerobic light conditions, glycolysis gradually replaces photometabolism with the aging of the cells. When this happened, the PHB stored during previous hours was consumed. It was also concluded that the more PHB the cells have as a reservoir, the more actively glycolysis occurs. In conclusion, the biomass output rate was not a regular function of the daylight irradiance trend and PE had an oscillatory behavior during daylight. The reason why *Rhodopseudomonas* cells had this growth behavior was not fully clear, and further studies are necessary to better explain these physiological aspects.

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