Article

Photofermentative Poly-3-Hydroxybutyrate Production by *Rhodopseudomonas* Sp. S16-VOGS3 in a Novel Outdoor L-Shaped Row Photobioreactor

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Abstract: In the present study, the performance of a 70 L photobioreactor, operating outdoors, was investigated using a purple bacterial strain as *Rhodopseudomonas* sp. S16-VOGS3 for producing poly-3-hydroxybutyrate (PHB). The novel photobioreactor was equipped with 5L-shaped rows; the bottom of every row was placed in a stainless-steel tank containing water with controlled temperature. The photofermentation trials were carried out under fed-batch mode and under a semi-continuous regimen using lactic acid as carbon source. The effect of the irradiance and the C/N ratio on the PHB accumulation was investigated, in order to evaluate the optimal bacterial growth. The results showed the feasibility of the prototype photobioreactor for the production of PHB by *Rhodopseudomonas* sp. S16-VOGS3 under the natural light/dark cycle. During the fed-batch growth (144 h long), the cumulative PHB increased quickly reaching the maximum value of 377 mg/L and decreased to 255 mg/L during the semi-continuous regimen (336 h long).

Keywords: photobioreactor; *Rhodopseudomonas* sp. S16-VOGS3; poly-3-hydroxybutyrate; thermal analysis; C/N ratio

1. Introduction

In 2016, the world petrol-plastic production reached about 335 million tons (Mt) and it is expected to double again over the next 20 years [1]. In 2015, United Nations Environment Program (UNEP) showed that 40% of plastic waste went to landfills, 14% was recycled but 32% entered the ocean and accumulated as debris, representing a global threat to marine ecosystems [2]. Globally, 5 to 13 Mt of plastics end up in the oceans every year [3]. To reduce the problem of recycling of petrol-based plastics and minimize their environmental impact, research efforts are rising in the field of bioplastics (BP) around the world. The biodegradability of bio-based plastics in different natural environments, not only in composting systems, is an important property that makes their life cycle more eco-sustainable than conventional materials. Despite the environmental benefits of BPs, their actual worldwide production is only about 1 Mt/y [1] but their demand is rising and with more applications and products emerging, their market is continuously growing. According to the latest market data compiled by European Bioplastics, global bioplastics production capacity is set to increase from around 2.05 million tons in 2017 to approximately 2.44 million tons in 2022, in absence of government support measures, and to higher values with specific government incentives [4]. The main limitations that penalize the BPs market are the competition for the use of renewable materials for the food and energy sectors and their actual high production costs. Among the BPs available on the market, the polyhydroxyalkanoates (PHAs), polyesters synthesized by numerous bacteria, are attracting a great attention due to their thermoplastic properties similar to that of polypropylene (PP), good mechanical properties and excellent biodegradability in various ecosystems such as fresh water, soil, industrial/domestic compost and sea water. PHAs, with poly-3-hydroxybutyrate (PHB) as the

simplest and most abundant PHA, are synthesized under stressful conditions by higher plants, bacteria (e.g. *Halobacteriaceae* and *Archea*) and many photosynthetic microorganisms such as purple non-sulfur bacteria (PNSB) or genetically enhanced species of microorganisms [5-7].

Due to bioplastic degradability also in seawater, PHAs are among the most promising candidates for the production of biodegradable items for different industrial applications. It has been estimated that the world demand of PHAs is going to increase from the actual 10'000 to 34'000 tons/y in 2018, corresponding to an average growth of about 28% [8]. Nevertheless, their relatively high cost (7–12 €/kg), compared to traditional petroleum derived plastics (polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET)) and to other biodegradable polymers such as poly-lactic acid (PLA), has somehow refrained research activity on their use in commodity applications such as packaging and service items, restricting their use to high-value applications, such as those in medical and pharmaceutical sectors. These high market costs are mainly due to the high costs of used carbon sources (e.g. glucose and sucrose), the low productivity and the low efficiency of extraction process of PHAs from microbial biomasses [9, 10]. Consequently, further research and efforts must be undertaken by scientists in order to reduce the actual production costs of PHAs and increase their industrial sustainability and commercialization.

PHA production processes by means of numerous bacteria (e.g. *Cupriavidus necator*, *Pseudomonas* sp. K, *Ralstonia eutropha* JMP 134 and genera of *Bacillus* sp.) are more studied and known than photosynthetic processes carried out by means of cyanobacteria (*Synechocystis* sp. PCC 6803, *Spirulina platensis* etc.) and/or PNSB (e.g. *Rhodobacter sphaeroides*, *Rhodospirillum rubrum*, *Rhodopseudomonas palustris* etc.). This last family of proteobacteria (*Rhodospirillaceae*) can produce PHA through the photo-fermentation process. The PNSB show a high potential to accumulate intracellular PHB granules as energy and carbon reserves and are active H_2 producers when they are grown under stress conditions [11, 12]. Combined production of PHB and bio-hydrogen through photoautotrophic metabolism could lead to a further advantage for the environment by converting solar energy into alternative sources of energy and bioplastics [12]. The bacterial genera more able to accumulate a substantial quantity of PHB are *Rhodobacter sphaeroides* RV and *Rhodospirillum rubrum*, suggesting that the efficiency of the biochemical pathway depends on the particular species and growth conditions (e.g. some nutrient deficiency and pH value). Knowledge of the mechanism of the aforementioned relationships may provide a clue to an alternative approach of controlling cultivation to achieve the preferred product, PHB or H_2. Khatipov et al. [12] achieved high PHB content (40 % of dry cell weight) into the cells of *Rhodobacter sphaeroides* RV on lactate media increasing the initial pH of the ammonium-free medium to 10 or higher.

In the biological PHB production by photo-fermentative process, the design of the photobioreactors plays an important role. Optimal light utilization and optimal penetration of light is essential to obtain high biomass growth rates and consequent high PHB productivity. In conventional photobioreactors, the light intensity tends to decrease rapidly due to the shielding effect arising from increases in the concentration of cells and products or from formation of biofilm on the surface of reactor vessels [13].

In this study, a wild strain of *Rhodopseudomonas* sp. S16-VOGS3, previously selected as PHB-producer [14], was used for producing PHB in a novel outdoor L-shaped row photobioreactor (L-SRP), under the natural light/dark cycle. The photo-fermentation trial was carried out initially in batch mode and then semi-continuous mode, using lactic acid as carbon source. The aim of this study was to evaluate the performance of the novel photobioreactor in terms of biomass growth and PHB accumulation under the natural sunlight conditions and to investigate the effect of the irradiance (direct and diffuse) and C/N ratio. The extracted PHB from *Rhodopseudomonas* lyophilized-biomass produced was characterized in terms of thermal properties and stability in view of its use as thermoplastic polymer.

2. Materials and Methods

2.1. Photobioreactor

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The photo of the L-shaped row photobioreactor (L-SRP) used for the photofermentative PHB production is reported in Figure 1a. It is equipped with 5 parallel rows (Pyrex® glass make) and the total working volume is 70 L. Every row is a L-shaped loop with 4-pipes laid horizontally in a stainless-steel tank that contains demineralized water at controlled temperature and 8-pipes placed one over the other and connected with U fittings as shown in the Figure 1a. The photobioreactor configuration with the 5 L-shaped loops permitted a good culture temperature control realized by the 4 pipes of each loop submerged in water, reducing the temperature excursion range experienced by bacterial cells. This solution avoided the use of an external refrigeration system.

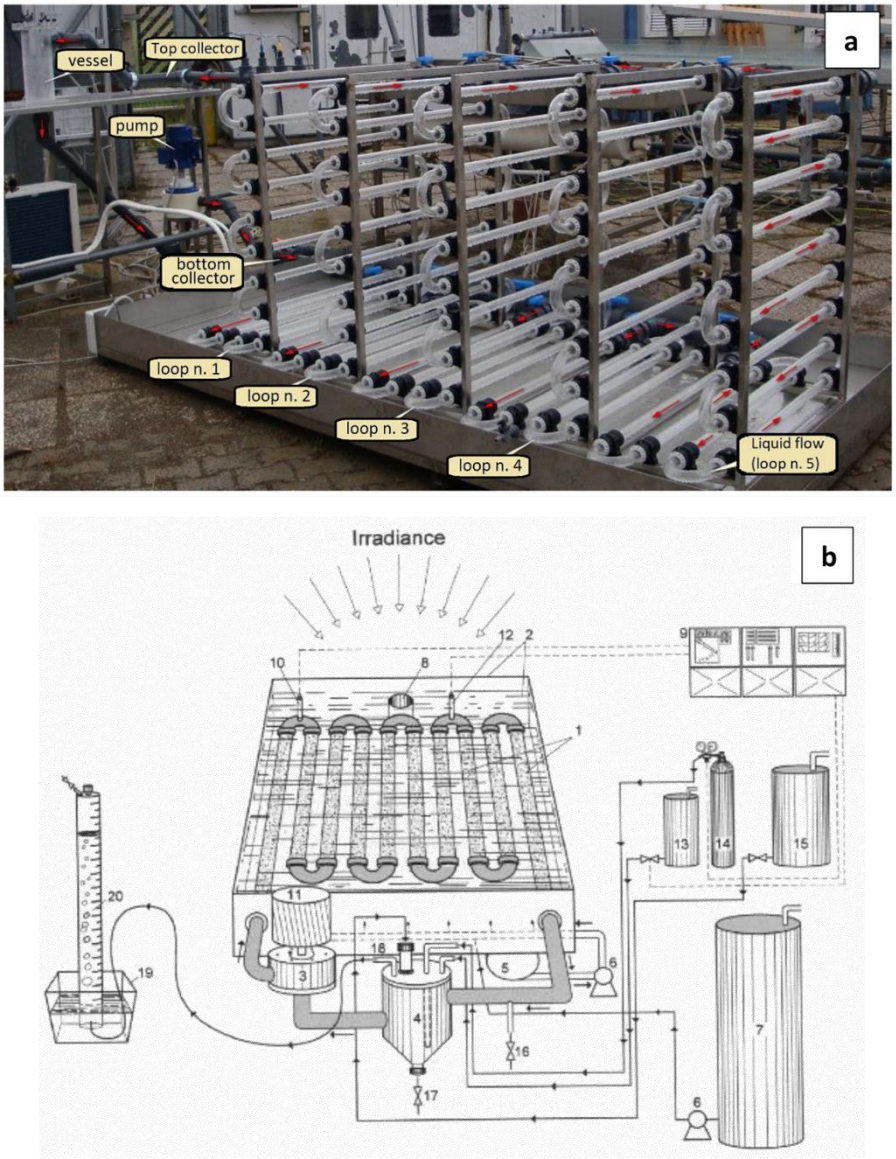

Figure 1. (a) Photo of the novel L-shaped row photobioreactor (L-SRP) operating outdoors for growing *Rhodopseudomonas* sp. S16-VOGS3; **(b)** Schematic diagram of a previous underwater tubular photobioreactor (UWTP) used for growing *Rhodopseudomonas palustris* 42OL [15, 16].

The pipes and U-fittings have inner and outer diameter of 2.6 and 3.0 cm, respectively. All 5 rows are connected with 2 collectors: the first placed at the bottom and the second on the top of the culture system. The photobioreactor is joined with a vessel. A pump, provided with 3 flat blades placed at 120° to one another on the propeller shaft, is used to recirculate the culture in the system: the culture flows into the bottom collector which has 5 pots: one for each loop, then from the bottom up along each line until reaching the top collector. From the top collector, the culture returns to the vessel, where the pH is regulated at 7.0 ± 0.5 by adding 0.5mM HCl. The photobioreactor used in the present

work derives from previous studies [15, 16] on temperature controlled underwater tubular photobioreactor (Figure 1b) operating in outdoor conditions to growth *Rhodopseudomonas palustris* 42OL.

2.2. Microorganism strain and culture conditions

In the present study, *Rhodopseudomonas* sp. S16-VOGS3, belonging to the culture collection of the Istituto per lo Studio degli Ecosistemi (Consiglio Nazionale delle Ricerche (ISE, CNR), Florence, Italy), was employed. The 16S sequence of *Rhodopseudomonas* sp. S16-VOGS3 was deposited in GenBank under the following accession numbers: KU899101-KU899105.

Rhodopseudomonas sp. S16-VOGS3 cells were grown initially indoors and, then, outdoors in the 70-L photobioreactor. The bacterium was cultured in batch mode into 8-Sovirel bottles containing 100 mL medium at constant temperature of 30 ± 0.2 °C under continuous artificial irradiance of 75 W/m^2 in anaerobic conditions. After 7 days of growth, *Rhodopseudomonas* cells were harvested by centrifugation (9000 rpm) and transferred into two photobioreactors with 4 L working volume (not shown here) containing fresh medium. The growing conditions were the following: fed-batch mode, continuous irradiance of 150 W/m^2, constant temperature (30 ± 0.2 °C) and anaerobic conditions obtained by fluxing argon (99.99% pure). A 150 W OSRAM power-star HQI-TS lamp was used to light the culture; the light intensity was measured using a Quantum/Radiometer/Photometer. Successively, cells of *Rhodopseudomonas* sp. S16-VOGS3 were used to inoculate the 70 L photobioreactor positioned outdoors containing fresh medium. 1L of growth medium used for both indoor and outdoor bacterial growth contained 4.0 g lactic acid, 0.5 g NH_4Cl, 1.0 g KH_2PO_4, 0.4 g NaCl, 0.05g $CaCl_2 \cdot 2H_2O$, 0.4 g $MgSO_4 \cdot 7H_2O$, g 0.005 ferric citrate and 0.1 mg para-aminobenzoic acid. Trace elements were provided by adding 10 mL (per 1 L) of a solution containing 30 mg H_3BO_3, 3.0 mg $MnCl_2 \cdot 4H_2O$, 500 mg $Na_2MoO_4 \cdot 2H_2O$, 10 mg $ZnSO_4 \cdot 7H_2O$, 1.0 mg $CuCl_2 \cdot 2H_2O$, 2.0 mg $NiCl_2 \cdot 6H_2O$ and 24.5 mg $CoNO_3 \cdot 6H_2O$. The pH of the medium was adjusted to 6.8 by using NaOH solution. During the outdoor bacterial growth, the pH was adjusted by adding HCl solution.

2.3. Outdoor bacterial growth

The L-SRP was inoculated in the evening by using the total volume of *Rhodopseudomonas* sp. S16-VOGS3 culture grown indoors. No argon was used for obtaining the anaerobic conditions since these conditions were reached two hours and half after the inoculation due to the dark natural conditions. The L-SRP was operated under fed-batch (FB) mode along the 144 h of biomass growth. A second experiment was carried out under a semi-continuous (SC) regimen, adopted to maintain the CDWC of about 4.0 g/L. Moreover, the medium replacement was accomplished every time (culture time of 72 h) adding to the culture an adequate amount of lactic acid to restore its initial concentration of 4.0 g/L. In this way, the unsuitable situation that could have stopped growth of *Rhodopseudomonas* sp. S16-VOGS3 due to lack of nutrients, such as the carbon source, is avoided. The culture samples were collected through a sampling point with respect to time to measure the CDWC, residual substrate (lactic acid) concentration and intracellular PHB concentrations in accordance with the analytic methods reported below. The investigation was carried out under the natural light/dark cycle in the summer period: June 27th – July 18th 2017 at Sesto Fiorentino (Florence, Italy) (Latitude: 43°49′54″ N; Longitude: 11°11′57″ E; Altitude above sea level: 60 m).

3.4. PHB recovery

Bacterial cells were separated from culture suspension by centrifugation at 9000 rpm and, then, the pellet was suspended in a physiological solution (9 ‰) to wash it and, then, the suspension was centrifuged at 9000 rpm. The washing operation was repeated two times before collecting the final biomass that was frozen and, then, lyophilized to be used for the subsequent PHB extraction. The extraction was carried out in accordance with Al-Kaddo et al. [17]: about 2 g of lyophilized cells were added to 200 mL of chloroform ($CHCl_3$) and the suspension was kept under stirring at room temperature for 6 days. Thereafter, the solution was filtered under vacuum utilizing a paper filter (Whatman No.3) to remove cell debris and the filtrate was concentrated in a rotary evaporator to around 25 mL and PHB was precipitate by addition of 200 mL of cold methanol under vigorous stirring. Wet PHB was collected by centrifugation and then it was air dried, weighted and used for the thermal analyses.

3.5. Analytical methods

CDWC and bacteriochlorophyll (Bchl) concentration were determined in accordance with Carlozzi and Sacchi [15]. The PHB content accumulated in the bacterial cells was measured by High-Performance Liquid Chromatography (HPLC) Thermo Finnigan-Spectra System 6000 LP, following the method reported elsewhere [14]. About 6 mg of lyophilized biomass was weighed and utilized for acid digestion to crotonic acid by boiling the biomass in 1 mL of pure sulfuric acid in screw-cap glass test tubes for 30 min. Extracts were appropriately diluted with water, filtered and injected into the HPLC for analysis. Crotonic acid was eluted from a Synergy-Hydro-RP C-18 column (250 x 4.6 mm id.). A mobile phase comprising 15% (v/v) acetonitrile in 0.1 % (v/v) H_3PO_4 in aqueous solution was employed at a flow rate of 1 mL/min. The compound was detected at 214 nm. Peak areas were compared to a calibration curve realized with commercial PHB (Biomer, Krailling, Germany, Europe). The organic acid concentration in the bacteria cultures was determined as reported elsewhere [18] by using the HPLC (Thermo Finnigan-Spectra System 6000 LP) equipped with a C18 analytical column (250 × 4.6 mm) and the column temperature was 25°C. Disposable syringe filter units (MFS-13 mm, 0.45 µm pore size) were used to separate the cells from the culture suspension and the supernatant sample was injected into the C18 analytical column for the analysis of the lactic acid concentration. An aqueous solution 0.1 wt.% H_3PO_4 was used as the mobile phase at a flow rate of 1.0 mL/min. All analyses were carried out in triplicate.

3.6. Thermal analysis

Differential scanning calorimetry (DSC) and thermo-gravimetric analysis (TGA) were used to study the thermal properties of the recovered PHB in view of its use as thermoplastic polymer. DSC was performed using a DSC Q200 (TA Instruments, Waters LLC, New Castle, DE (USA)) calibrated with standard indium. PHB samples (about 12 mg) were first heated from ambient temperature to 200 °C at a heating rate of 10 °C/min in order to erase any previous thermal history. After an isotherm at 200°C for 5 minutes, the samples were cooled until ambient temperature and heated again to 200 °C with an increasing temperature rate of 10 °C/min. The glass transition temperature (T_g), melting temperature (T_m) and enthalpy (ΔH_m) were determined from the second heating thermogram. The degree of crystallinity x_c of PHB was calculated using the following equations:

$$x_c = \Delta H_m / \Delta H_{m,100} \cdot 100$$

where: ΔH_m is the melting enthalpy of the sample (J/g); $\Delta H_{m,100}$ is the enthalpy value for a theoretically 100% crystalline PHB (146 J/g) [19].

The thermal stability of the polymeric samples produced by *Rhodopseudomonas* sp. S16-VOGS3 was determined by TGA using a TA Q-500 (TA Instruments, Waters LLC, New Castle, DE (USA)). About

10 mg of sample was loaded into an aluminum pan and heated from room temperature to 600 °C at a heating rate of 10°C/min under nitrogen atmosphere. The thermal stability of the polymer was determined measuring the decomposition temperature (T_d), defined as a temperature corresponding to a sample weight loss of 5%.

3. Results and discussion

3.1. Photobioreactor performance

The improved geometry configuration of the novel L-SRP tested in the present study is evident and significant respect to some typical horizontal tubular photobioreactors. The verticalization of the cultural system allows the light to reach full pipe surface. By arranging the modules in parallel rows, appropriately spaced, a form of culture lamination is carried out which can be favorably exploited to improve the light distribution inside the culture volume and so dilute the solar radiation (SR). Culture lamination is a way to augment the irradiated surface (Si) and thereby to increase the Si/V ratio of the photobioreactor and control the photic ratio by spacing or narrowing the parallel rows. Table 1 shows the internal diameter (di) and the Si/V ratio of several tubular photobioreactors proposed in literature [16, 20-23]: the lower the internal diameter the higher the Si/V ratio. With a di of 0.010 m, a Si/V ratio of 400 m^{-1} was reached [23]. A recent tubular photobioreactor with a Si/V ratio of 182 m^{-1} was identified as the best culture system among those tested by Palamae et al. [21]. They reported that the Si/V ratio affects the production of biomass and hydrogen differently: a high Si/V ratio favored the hydrogen production over biomass production when growing *Rhodobacter sphaeroides* S10.This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

Table 1. Internal pipe diameter (di) and some important parameters of tubular photobioreactors proposed in literature.

di (cm)	working volume (L)	Si (m^2)	Si/V ratio (m^{-1})	Ref.
8.0	80.0	3.200	40.0	20
4.8	53.0	3.014	56.9	16
2.6	70.0	6.300	90.0	This study
2.2	1.0	0.182	181.7	21
1.6	22.1	2.200	99.5	22
1.0	11.0	4.400	400.0	23

3.2. PHB production

Figure 2 shows the profiles of the daily maximum irradiance (Imax) and solar irradiation (SR) and Bchl, lactate and PHB concentrations in the bacterial culture during the cultivation of *Rhodopseudomonas* sp. S16-VOGS3 in the outdoor L-SRP under fed-batch mode (run1). After a lag-phase growth period, lasted about 48 h, during which Bchl concentration was stable and the irradiance had a fluctuating behavior (Figure 2a, b), it was observed a continuous increase of the Bchl amount up to double its concentration (3.8 $g/L_{culture}$) in the subsequent 96 h. During the fed-batch growth (lasted 144 h), lactic acid was consumed and, consequently, added twice (after 72 and 124 h) to restore its initial concentration to 4 g/L (Figure 2c). The lactate concentration restoring operations caused significant heightening of the C/N ratio in the medium since the concentration of ammonia nitrogen (NH_3-N) in the culture broth decreased progressively over time (Figure 2c). It is known that stressed growth conditions caused by a high C/N ratio and/or nutrient limitation (e.g. Nitrogen and Phosphorus) in the culture medium promote the accumulation of intracellular PHB [24-27]. As expected, the PHB concentration in the culture grew over time and, more significantly, when the

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initial lactate concentration was restored (Figure 2d). In particular, PHB concentration augmented of 32 % in the last period of bacterial growth when the amount of NH_3-N was totally depleted and the growth occurred under nitrogen starvation. After 144 h of batch cultivation, a final amount of 377 mg(PHB)/$L_{culture}$ was reached corresponding to a PHB volumetric productivity of about 63 mg L-1 day^{-1}. Recently, a PHB production of 10.3 mg/L/day was reported by growing (indoors) *Synechocystis* PCC6803 for a period of 28 days [28]. In the present investigation, at the end of *Rhodopseudomonas* sp. S16-VOGS3 fed-batch growth (run 1), the CDWC was 2.92 g/L, which corresponded to the total dry biomass produced of 134.4 g with the 70L photobioreactor; the content of PHB in the dry-biomass was ≥ 19.6 %.

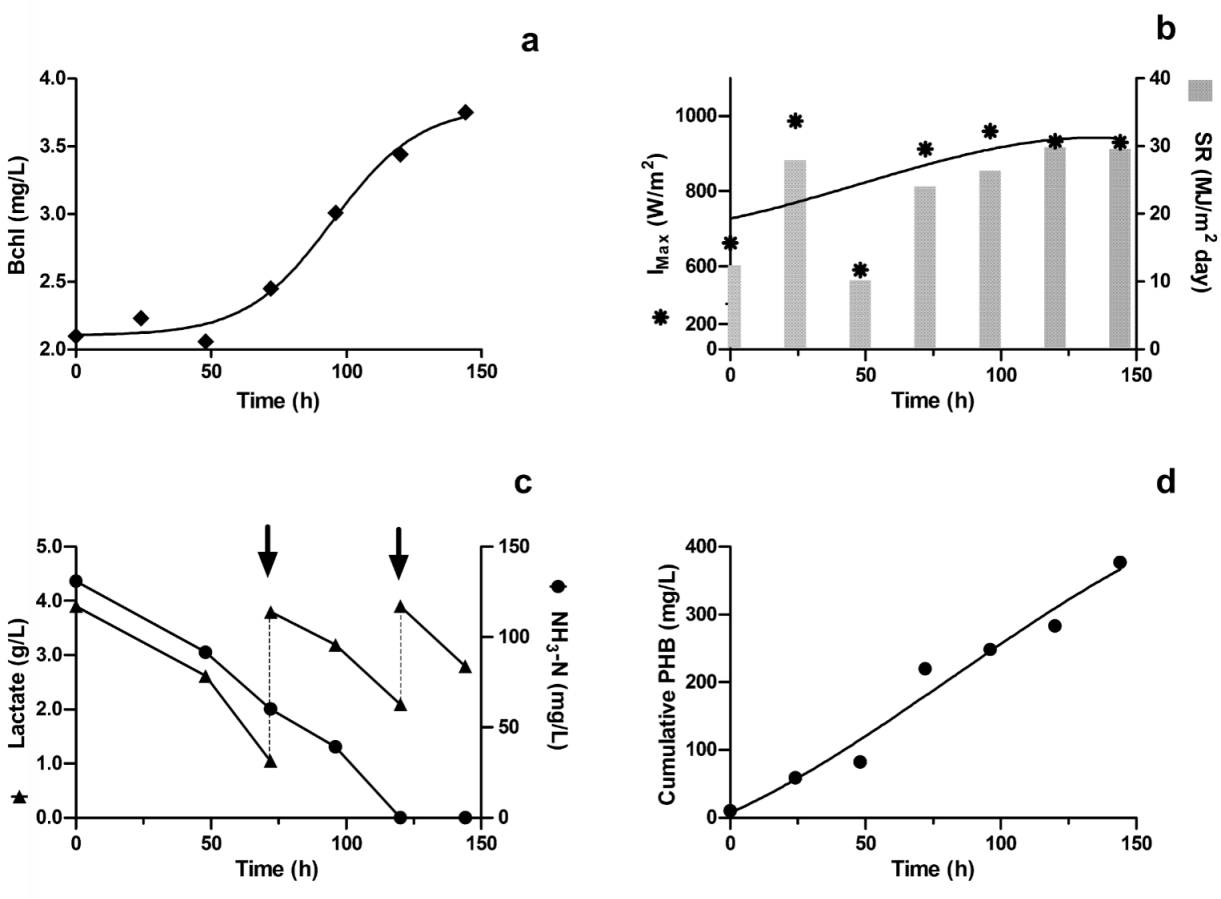

Figure 2. Culture of *Rhodopseudomonas* sp. S16-VOGS3 grown outdoors into a 70 L L-SRP under fed-batch mode (run 1): **(a)** Bchl concentration versus time, **(b)** daily changes of the maximum irradiance (IMax) and solar radiation (SR); **(c)** lactate and ammonia nitrogen (NH_3-N) concentrations versus time and **(d)** intracellular PHB concentration. The arrows indicate the points of addition of lactic acid to restore the initial lactate concentration (4 g/L).

In order to evaluate the effect of the solar light intensity on the productivity of PHB accumulated in the bacterial cells, a further investigation was carried out by covering about 80% of the photobioreactor with a dark cloth as shown in Figure 3a (run 2). In this way, the light intensity impinging on the L-SRP was significantly reduced and the culture was irradiated principally by diffuse irradiance, with the exception of the U-fittings. Figure 3b shows typical daily trends of the direct and diffuse irradiance on a horizontal plane measured at Sesto Fiorentino (Florence, Italy) in the period June-July 2017. The results obtained during the 336 h of *Rhodopseudomonas* growth under diffuse radiation (run 2) are shown in Figure 4a-d. During the first 192 h, the photobioreactor was operated in FB mode; when the Bchl concentration reached the value of 16.4 mg/$L_{culture}$ and the CDWC 4.0 g/L, the regimen of growth was changed from FB to SC mode (Figure 4a). In order to maintain the CDWC ranging from 3.2 to 4.1 g/L, an appropriate culture volume was withdrawn from the reactor

and replaced with an equal volume of fresh medium every 72 h. During the 336 h of cultivation, the maximum light intensity was quite constant and ranged from 900 to 956 W/m^2; on the contrary, the global solar radiation (SR) changed significantly from 22.3 to 29.8 MJ/m^2 day, as shown in Figure 4b. The lowest reached lactate content was about 1.0 g/L and its initial concentration (4.0 g/L) was restored when the fresh culture medium was added in accordance with the SC regimen of growth (Figure 4c). The NH_3-N amount decreased progressively and reached zero after 164 h of cultivation. The accumulated PHB concentration decreased significantly during the first period of bacterial growth; subsequently, in the SC regimen the PHB concentration ranged from a minimum of 154 to a maximum of 286 mg/L (Figure 4d). This second outdoor investigation demonstrated that when *Rhodopseudomonas* sp. S16-VOGS3 was cultured under lower irradiance, the amount of PHB into bacterial cells decreased significantly, indicating the dominant role of irradiance on other parameters such as C/N ratio. In fact, as elsewhere demonstrated [26, 27], stressed growth conditions caused by high C/N ratios should increase the amount of PHB into bacterial cells, but this was true as far as the results of our first investigation (run 1) is concerned, but not for those of the second one (run 2) (Figure 5). In fact, despite the similar increases in the C/N ratio for both cases (Figure 5a), the PHB content into the cells had an opposite behavior (Figure 5b): during the run 1 (direct irradiance), the PHB content rapidly increased reaching the value of 19.6 % of dry biomass weight; during the run 2 (diffuse irradiance), the PHB content decreased reaching a value of about 7 % at the end of the investigation.

Recently, the same bacterial strain used in the present study (*Rhodopseudomonas* sp. S16-VOGS3) was identified as a potential candidate for employing crude glycerol coming from biodiesel industry [14]. The authors obtained (indoors) a PHB-rich biomass with a content of 18% on dry-weight biomass, demonstrating that a degradable plastic material as PHB could be efficiently produced by feeding *Rhodopseudomonas* sp. S16-VOGS3 with the byproduct coming from biodiesel industry, in order to obtain a significant reduction of PHB production-costs.

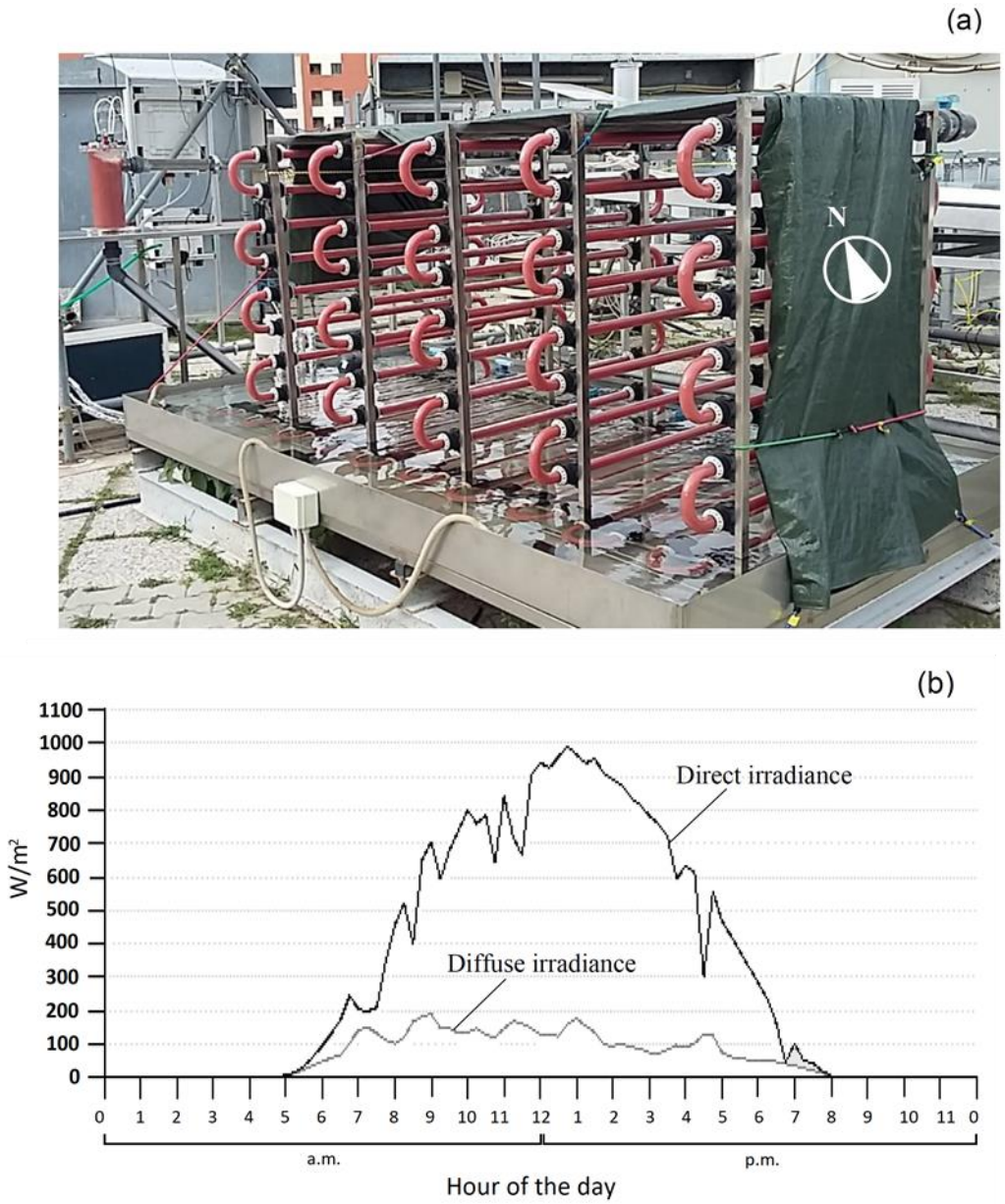

Figure 3. (a) L-shaped row photobioreactor covered with a dark cloth to operate under diffuse irradiance; **(b)** average daily trends of direct and diffuse solar irradiance observed at Sesto Fiorentino (Florence, Italy) in June-July/2017.

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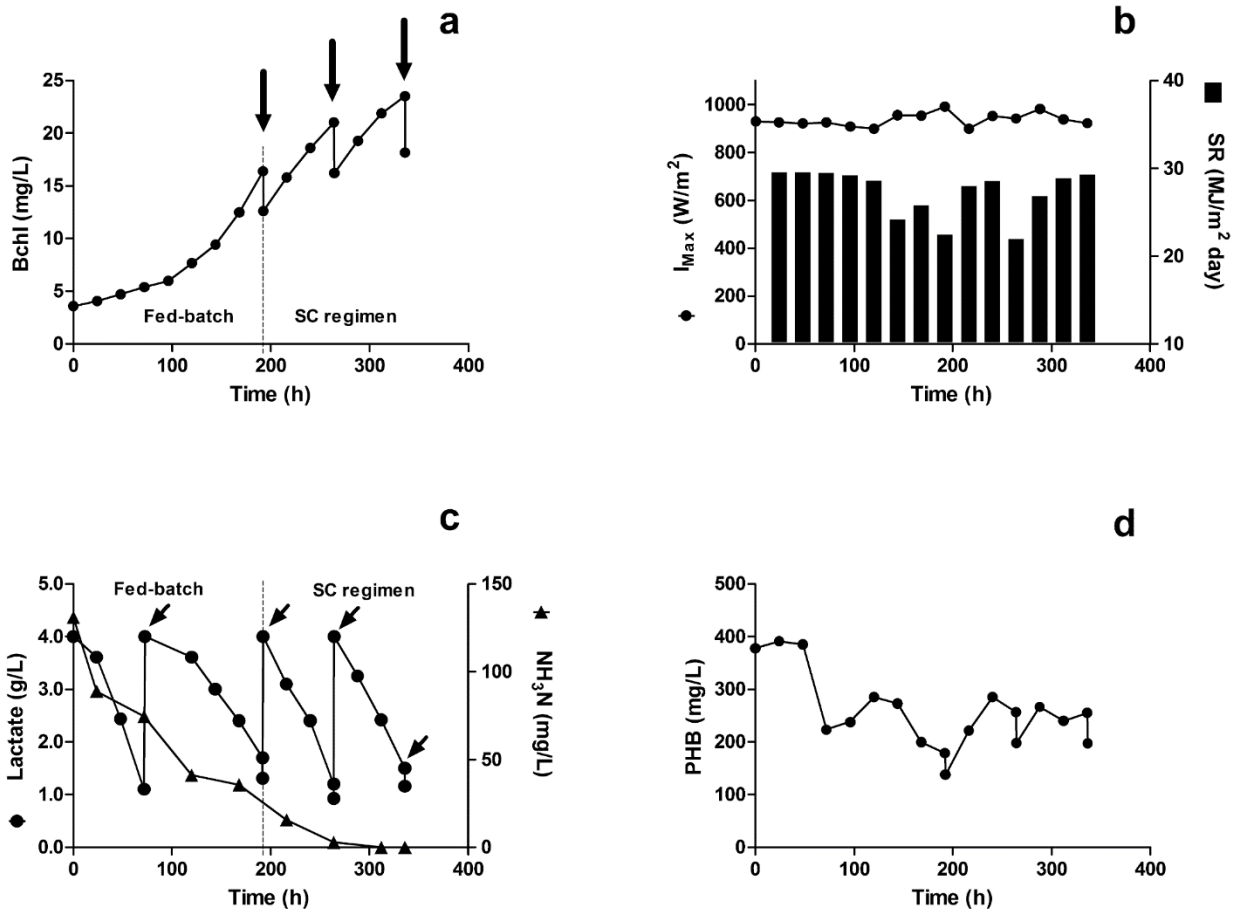

Figure 4. Growth of *Rhodopseudomonas* sp. S16-VOGS3 under fed-batch mode (lasted 192 h) followed by a SC regimen (run 2): **(a)** Bchl concentration versus time, arrows indicate culture dilution times; **(b)** changes of IMax and SR versus time; **(c)** lactate and ammonia nitrogen concentrations versus time; **(d)** intracellular PHB concentration versus time. Arrows indicate the times at which the initial lactate concentration was restored.

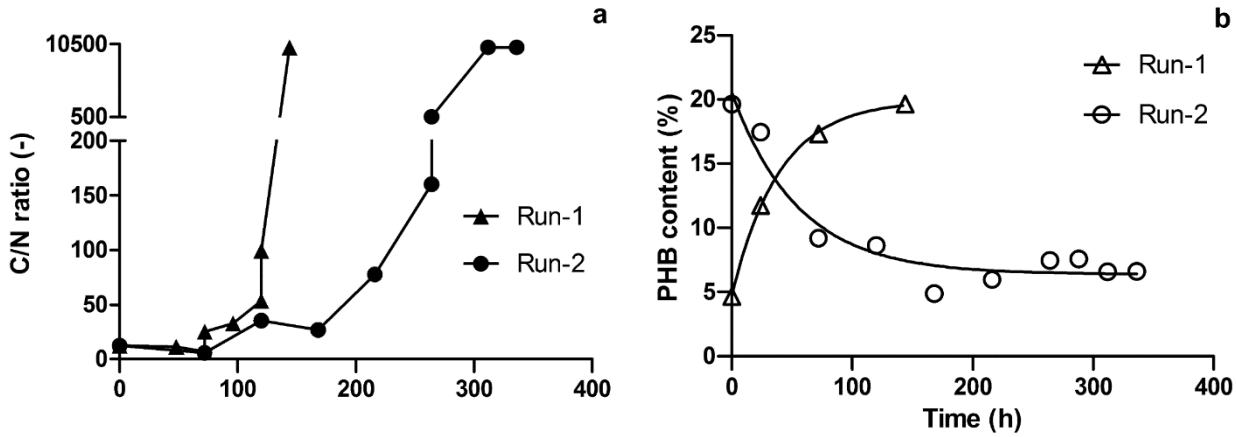

Figure 5. (a) Increase of C/N ratio in the bacterial culture versus time and **(b)** PHB content into *Rhodopseudomonas* cells grown under fed-batch mode (run 1) and semi-continuous regimen (run 2).

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3.2. PHB thermal properties and stability

Figures 6 a, b show the DSC and TG/DTG (thermogravimetric and derivate thermogravimetric) thermograms of the recovered PHB from *Rhodopseudomonas* sp. S16-VOGS3 cells, respectively. The DSC thermogram (second heating curve after cooling from the melt) shows an endothermal baseline shift associated with the glass transition (T_g = 4.2°C) followed by a cold-crystallization exotherm (T_c = 52.8°C) and, then, by a melting endotherm (T_m = 172°C). On the basis of the melting enthalpy of the sample, a PHB crystallinity of 51% was evaluated. These results are in excellent accordance with the results of Martino et al. [29] obtained for PHB produced by cultivation of *Cupriavidus necator* DSM 428, using cooking oil as carbon source, and recovered by an enzymatic and chemically assisted procedure and to those of PHB synthetized by another purple bacteria (*Rhodococcus equi*) investigated by Altaee et al. [30]. Good accordance was obtained also with the results obtained with *Bacillus sphaericus* NII 0838 by Sindhu et al. [31]. The TG and DTG curves of PHB produced with *Rhodopseudomonas* sp. S16-VOGS3 (Figure 6b) show a decomposition temperature (T_d) of 248°C exhibiting a single weight loss with maximum degradation rate at Tmax of 262 °C. Similar results were reported by Martino et al. [29] for the PHB produced with *Cupriavidus necator* DSM 428. In Table 2, the thermal properties and stability of the PHB produced in the present study by *Rhodopseudomonas* sp. S16-VOGS3 are compared with those of PHB produced by another purple bacterial strain (*Rhodococcus equi*) [30]. As shown, the thermal properties are very similar, except a lower crystallinity (51 vs 62 %) and T_d of PHB obtained in this study.

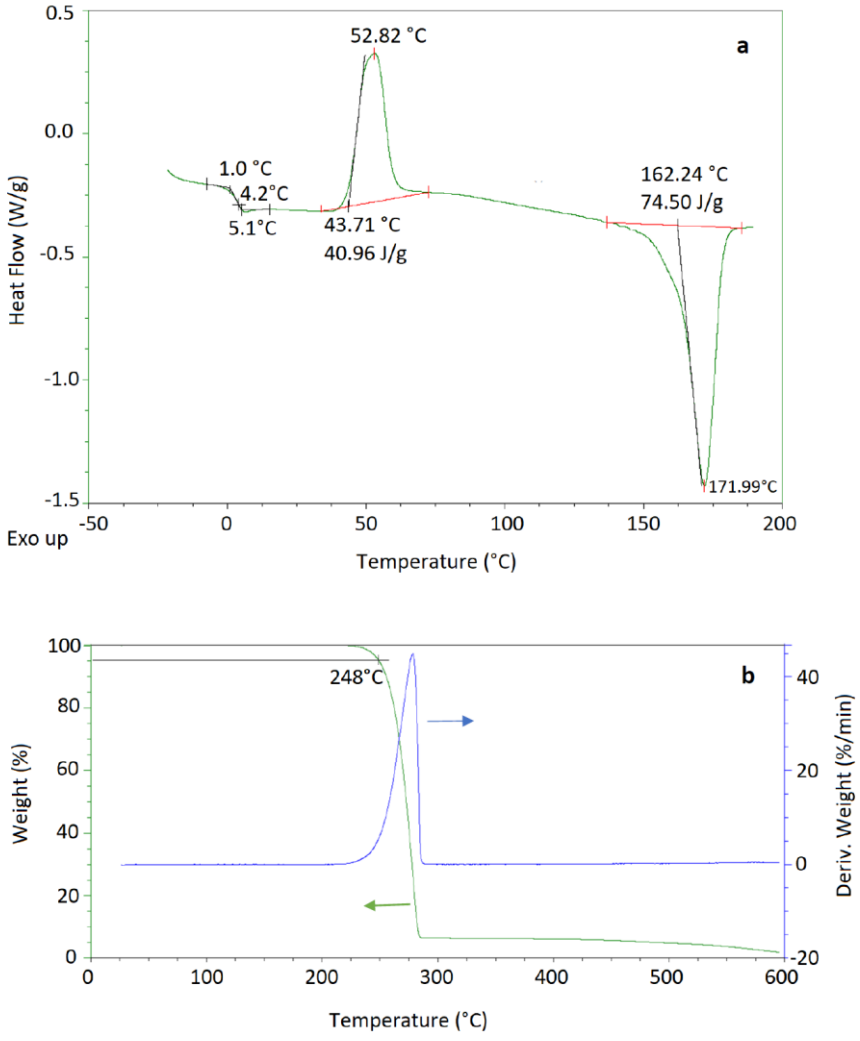

Figure 6. (a) DSC and **(b)** TG and DTG thermograms of PHB produced by *Rhodopseudomonas* sp. S16-VOGS3.

Table 2. Thermal properties of PHB produced by cultivation of purple bacteria.

Microorganism	Biopolymer	T_c (°C)	T_g (°C)	T_m (°C)	ΔH_m (J/g)	T_d (°C)	Ref.
Rhodopseudomonas sp. S16-VOGS3	PHB	52.8	4.2	172	90.7	248	This study
Rhodococcus equi	PHB	nd*	2.8	173	74.5	276	30

*nd: not determined

5. Conclusions

The main aim of the present work was to evaluate the performance of the novel tubular photobioreactor for producing PHB by outdoor cultivation of *Rhodopseudomonas* sp. S16-VOGS3 using lactate as carbon source. The results showed that the peculiar configuration of the photobioreactor permitted an optimal temperature control and solar light capturing for producing PHB-rich biomass. The reduction of the light intensity reduced the cumulative PHB showing the dominant role of this parameter. The thermal characterization of the recovered PHB showed a highly amorphous polymer with good thermal properties and stability, similar to those of PHB obtained in other studies.

Author Contributions: Pietro Carlozzi conceived, designed and carried out the experiments; Pietro Carlozzi e Maurizia Seggiani wrote the paper, Pietro Carlozzi, Maurizia Seggiani, Patrizia Cinelli discussed the data; Norma Mallegni performed the thermal characterizations of the recovered PHBs; Andrea Lazzeri contributed materials and analysis tools.

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