BIOCELL
2020 44(4): 703-711

Tech Science Press

Optimization of the *in-situ* growth conditions based on a novel photo-microcalorimeter for the sustainable cultivation of photosynthetic microorganisms

Yanjuan LIAO[1,#]; Dongling YU[2,#]; Xiaofang QIN[1]; Jinmei WU[1]; Hui WEI[1]; Hui LI[1]; Xiangying MA[1,*]; Zaiyin HUANG[1,*]

[1] Guangxi Key Laboratory of Utilization of Microbial and Botanical Resources, Guangxi Key Laboratory of Marine Natural Products and Combinatorial Biosynthesis Chemistry, Guangxi University for Nationalities, Nanning, 530006, China
[2] The Teaching and Research Department of Biotechnology, Nanning University, Nanning, 530200, China

Key words: Photosynthetic microorganisms, *In-situ* growth, Photomicrocalorimetry, Thermodynamics, Kinetics

Abstract: Despite the great potential of photosynthetic microbes in the production of renewable fuels, value-adding chemicals, and water treatment, etc., commercial utilization of them is significantly hindered by the lack of techniques to accurately monitor the thermodynamic and kinetic characteristics of the *in-situ* growth of microbes under controlled light illumination for optimal cultivation. Herein, we demonstrated that a newly developed highly sensitive photo-microcalorimetric system successfully captured the impacts of the light wavelength and strength on the thermodynamic and kinetic parameters of the *in-situ* growth of *Rhodopseudomonas palustris*, a representative photosynthetic microorganism. To our best knowledge, this is the first time that highly precise microcalorimetry is employed to monitor exam the *in-situ* growth of photosynthetic microorganisms under controllable photo illumination. We envision this technique can help for the optimization of the growth conditions of photosynthetic microorganisms for scale-up applications.

Abbreviation

P:	heat output
Q:	quantity of heat
K:	growth rate constant
Pt:	heat output at time t in log phase
Pmax:	heat output come to the maximum value
P0:	heat output at t = 0 s
t_G**:**	Generation time.

Introduction

Photosynthetic bacteria are strains of distinct microorganisms that can use their light-absorbing pigments to convert solar energy into chemical energy. For example, in the most important natural processes, photosynthesis, cyanobacteria, and microalgae can use carbon dioxide and water to produce sugar with the help of sunlight. Traditionally, photosynthetic microorganisms have been cultivated for food and feed supplements (Linder, 2019). Photosynthetic bacteria are well-acknowledged as an important source of alternative energy based on their application in the mass production of biofuel (Tiwari *et al.*, 2018) and hydrogen (Lu *et al.*, 2016). Photosynthetic microorganisms have been utilized in the areas of environmental bioremediation (Idi *et al.*, 2015), wastewater treatment (Lu *et al.*, 2019; Talaiekhozani and Rezania, 2017), desalination (Amezaga *et al.*, 2014; Kokabian and Gude, 2015), production of value-adding chemicals (Milano *et al.*, 2019; Zhou *et al.*, 2015), bioenergy (Liu and Choi, 2017; Sekar and Ramasamy, 2015), agriculture (De Godos *et al.*, 2010; Wei *et al.*, 2016) as well as pharmaceutical industry (Jeffryes *et al.*, 2015; Sakaki *et al.*, 2005).

It is believed that photosynthetic microorganisms will have an important impact on the future of human beings (Mazard *et al.*, 2016). The key to the successful commercial application of photosynthetic bacteria is the optimization of the cultivation process of the organisms (Fernandes *et al.*, 2015). Since light illumination is the source of energy for photosynthetic bacteria (Benemann *et al.*, 1973;

*Address correspondence to: Xiangying Ma, mxytgbz@163.com; Zaiyin Huang, huangzaiyin@163.com
#These authors contributed equally to this work
Received: 12 June 2020; Accepted: 22 September 2020

Donia *et al.*, 2011), it is necessary to investigate the detailed influence of different illumination conditions on the growth of the photosynthetic microorganisms.

Due to the lack of techniques for online measurements, the research on the thermodynamic and kinetic of the growth of photosynthetic microorganisms under light illumination is still limited. To our best knowledge, there is no literature reporting the *in-situ* simultaneous quantification of the thermodynamics and kinetics during the cultivation process of these bacteria. Studies on the effects of illumination conditions on the growth of photosynthetic bacteria have mainly relied on intermittent sampling rather than dynamic monitoring due to the absence of tools to precisely regulate light wavelengths and intensities. Carlozzi *et al.* (2006) demonstrated that different wavelengths had distinct effects on the growth of photosynthetic bacteria during the alternating dark–light cycles. Benemann *et al.* (1973) showed that extremely high and low light intensities were unfavorable for the growth of photosynthetic bacteria by measuring the optical density (OD) of bacterial cultures at various time points during growth. Wakayama *et al.* (2000) reported high hydrogen yields (22 L/m^2·d) from photosynthetic bacteria grown under 30-min dark–light cycles by OD measured. Xu *et al.* (1995) used calorimetry to show that the growth kinetics of photosynthetic bacteria followed the Malthusian equation. However, the rate constant (k) was calculated based only on the effect of temperature without considering synergistic photothermal effects. Therefore, it is desired to carry out *in-situ* studies that could reveal the association between thermodynamics and kinetics in photosynthetic bacteria.

Photocalorimetry is an *in-situ*, real-time, non-invasive, direct, and precise method for measuring thermodynamic parameters during biological and photochemical reactions. The principle of photocalorimetry was first described by Magee *et al.* (1939), who measured the efficiency of photosynthesis by *Chlorella vulgaris* as thermal changes per unit time. Since then, researchers have invested a great amount of effort in improving the experimental precision and sensitivity of this technique (Adamson *et al.*, 1978; Dhuna *et al.*, 2007; Dhuna *et al.*, 2008; Gordon *et al.*, 1981; Mukhanov and Kemp, 2009; Olmsted Iii, 1979; Schaarschmidt and Lamprecht, 1973; Seybold *et al.*, 1969; Teixeira and Wadsö, 1990). In a novel photo-microcalorimetric system developed by our group (Fan and Huang, 2011; Wang *et al.*, 2011), a monochromatic light source of a specific wavelength (532 nm) was introduced into the RD496-CK 2000 microcalorimeter through an optical fiber. This design allows for the accurate detection of changes in thermal power and energy without additional requirement on the solvent, spectral, or electrical property of the system under investigation. In addition, the device can precisely regulate the microenvironment of the reaction system by maintaining a temperature of 10^{-4}°C to 10^{-5}°C. Using this device, we have reported results of studies on the *in-situ* growth of nanomaterials as well as photocatalytic studies (Guo *et al.*, 2012; Huang *et al.*, 2015).

In the present study, we used the photo-microcalorimetric system developed by our group to monitor the *in-situ* growth processes of a representative photosynthetic bacteria. The thermodynamic characteristics and patterns of bacterial growth under various conditions were extracted from the thermograms collected on this unique photo-microcalorimeter. The results provide a theoretical foundation for a better understanding of the *in-situ* growth mechanisms, as well as the optimized growth conditions of the photosynthetic microorganism.

Materials and Methods

The photosynthetic microorganism used in this study was *Rhodopseudomonas (R.) palustris*, CICC 2312, which was purchased from the China Center of Industrial Culture Collection (Beijing, China). The strains were facultative bacteria grown in CM0847 culture media (15.0 g peptone, 5.0 g soya peptone, 5.0 g NaCl, 13.0 g agar, and 1.0 L distilled water), which was adjusted to pH 7.3 ± 0.2 and sterilized at 121°C for 30 min prior to use.

Experiments were conducted using a DHG Series Heating and Drying Oven, pH meter, electronic balance, autoclave (TOMY, Tokyo, Japan), UV-Vis spectrophotometer (model needed), ultraclean bench, PGX-270B programmable multi-wavelength illumination incubator (Ningbo Southeast

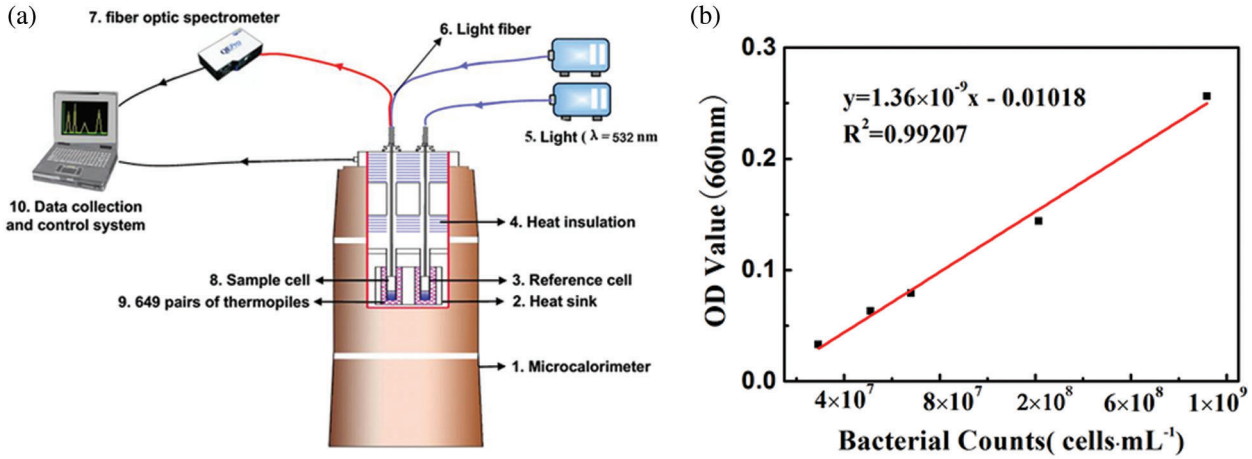

FIGURE 1. (a) Novel photoreaction-microcalorimetric system, which was used to determine thermodynamic parameters during the growth of *R. palustris*. (b) Concentration standard curve of *s R. palustris* by the method of determination of culture concentrations.

Instrument Co. Ltd., Ningbo, China), incandescent lamp, and light-emitting diodes. A novel photoreaction-microcalorimetric system was constructed by combining an RD 496-CK 2000 microcalorimeter (Mianyang Zhongwu Thermal Instrument Co. Ltd., Sichuan, China), a 532 nm light source and optical fiber (Nanjing Chunhui Science and Technology Industrial Co. Ltd., Nanjing, China), and a solar power light intensity meter (TES1333, TES Electrical Electronic Corp., Taipei) (Fig. 1a). A detailed description of this novel instrument can be found in our previous report (Li *et al.*, 2016).

Experimental methods

Determination of culture concentrations

Thawed bacteria were cultured and passaged for three generations. The bacteria were cultured with CM0847 culture media at pH 7.3 for 4 days at 30°C and did not require strict deoxygenation. The resulting culture was used as the bacterial stock solution. Cell counts for photosynthetic bacteria were determined by manual counting with a Petroff-Hausser counting chamber. The concentration standard curve was prepared by plotting OD values of bacterial culture against the number of bacterial cells on blood agar plates (Fig. 1b). The OD value was obtained by measuring at 660 nm on the UV spectrometer. Perform zero adjustments with culture medium and read 5 times for each measurement.

Effect of light source on growth of R. palustris

R. palustris was cultured under blue light (400–520 nm), green light (520–570 nm), red light (620–700 nm), and white light (incandescent light composed of mixed light spectrums) at an intensity of 4 W/m^2 for 84 h, respectively. Aliquots of samples were collected every 12 h for OD measurements at 660 nm on the UV-Vis spectrometer.

Effect of light intensity on growth of R. palustris

R. palustris was cultured under the optimal light source, as determined in the experiment as described above, at 1, 2, 4, 6, and 8 W/m^2 for 84 h. Aliquots of samples were collected every 12 h for OD measurements at 660 nm.

Determination of thermodynamic parameters during growth of R. palustris

The scheme of the photoreaction-microcalorimetric system for the microcalorimetry experiments is shown in Fig. 1a. In a typical test, blank media and *R. palustris* liquid culture (1.5 mL) were added to the reference cell and sample cell, respectively. The OD value of the bacterial culture was measured prior to each microcalorimetric measurement, and the bacterial count was adjusted to approximately 1.96 × 10^8 cells/mL based on a standard curve (Fig. 1b). The temperature of the system was set to 30°C, and the 532 nm light source was turned on after the baseline was stabilized. The *in-situ* growth of *R. palustris* was monitored at intensities of 2, 4, and 6 W/m^2 for a duration of 290,000 s.

Results

Effect of wavelength and light intensity on R. palustris *growth*

Using a single-factor experimental design, the OD of bacterial cultures grown at four different wavelengths was plotted over time (Fig. 2a). It is evident that the growth rate of the photosynthetic microorganism is affected by the wavelength, and it follows the order of green light > blue light > white light ≅ red light. Therefore, green light (520–570 nm) is the optimal wavelength range for the growth of *R. palustris*. Cultures were then grown at green light, and the OD of bacterial cultures grown under five different light intensities were plotted over time (Fig. 2b). The results suggested that the fastest growth rate of *R. palustris* was achieved after 12 h of culture under a light intensity of 4 W/m^2, followed by 6 W/m^2, and were lowest under a light intensity of 2 W/m^2. OD of the bacterial cultures at the end of the experiment was highest at a light intensity of 4 W/m^2 (0.665), followed by 6 W/m^2 (0.649) and lowest at 2 W/m^2 (0.182).

Construction of R. palustris thermograms using the novel photoreaction-microcalorimetric system

The power-time (*p-t*) curve of the *in-situ* growth of *R. palustris* in the photoreaction-microcalorimetric system, set at 30°C under 523

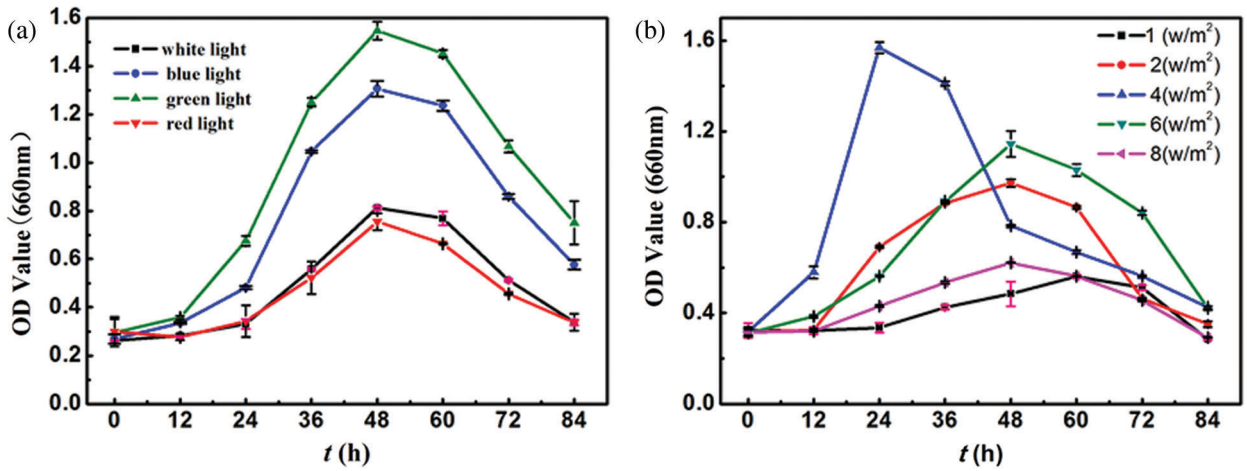

FIGURE 2. (a) OD value of *R. palustris* cultures over time under different light sources with the light intensity of 4 W/m^2. (b) OD value of *R. palustris* cultures over time under different light intensities at green light. The presented OD value and the error bar represent the mean and standard deviation of three measurements, respectively.

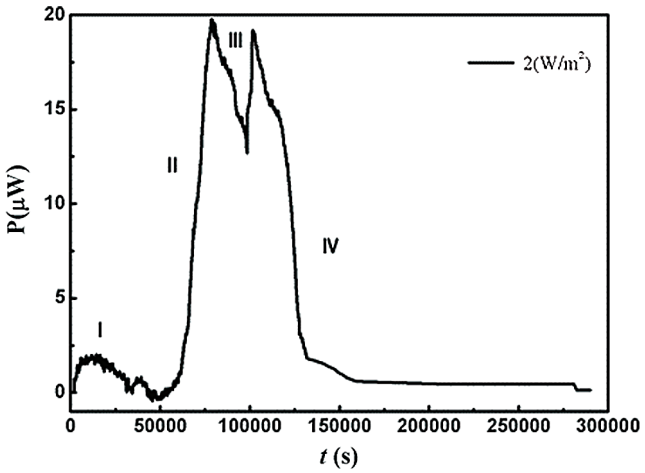

FIGURE 3. Heat power-time curves of *R. palustris* at 30°C under 523 nm light at an intensity of 2 W/m^2 and the four phases of the growth of the photosynthetic bacteria were the lag phase (I), log phase (II), stationary phase (III), and decline phase (IV).

TABLE 1

Growth rate constant (*k*) of *R. palustris* determined by photomicrocalorimetry (N = 5)

Experiment	Growth rate constant k (s^{-1})	R
1	1.191×10^{-4}	0.9999
2	1.190×10^{-4}	0.9975
3	1.191×10^{-4}	0.9993
4	1.191×10^{-4}	0.9989
5	1.192×10^{-4}	0.9999

nm light at an intensity of 2 W/m^2, is shown in Fig. 3a and Tab. 1. As indicated by the *p-t* curve, the growth of the photosynthetic bacteria included four phases: The lag phase (I), log phase (II), stationary phase (III), and decline phase (IV). The average k of *R. palustris* from five replicate experiments was $1.191 \times 10^{-4} \pm 7.071 \times 10^{-8}$ min^{-1} with a correlation coefficient R > 0.9975, indicating that the results were highly reproducible and correlated.

Thermograms of R. palustris *growth*

The thermograms of *R. palustris* cultured in the photoreaction-microcalorimetric system under 532 nm illumination at light intensities of 2 W/m^2 (A–E), 4 W/m^2 (A'–E'), and 6 W/m^2 (A"–E") during the lag (AB), log (BC), stationary (CD), and decline (DE) phases are given in Fig. 4. Upon baseline stabilization at t = 2000 s and during the entire lag phase (AB), bacterial growth was slow, and heat release was limited (1.048 J). At t = 5791 s (B), heat release accelerated and peaked at P_{max} = 48.19 μW. This was followed by slow heat release throughout the stationary phase of stable growth (CD). Heat production declined substantially from t = 20,868 s until the end of the experiment.

Energy changes in R. palustris *during different growth phases*

Heat production by *R. palustris* as a result of growth and metabolism was significantly different under the illumination of green light with three different light intensities (P < 0.001). During the lag phase, heat production was highest at 2 W/m^2, followed by 4 W/m^2, and lowest at 6 W/m^2. During the log, stationary, and decline phases, heat production was highest at 4 W/m^2, followed by 6 W/m^2, and lowest at 2 W/m^2 (Fig. 5a, Tab. 2).

Thermodynamic parameters of R. palustris *during the log phase*

The growth rate, maximum heat production, peak time, and generation time of *R. palustris* are summarized in Fig. 5 and Tab. 3. Growth rate constant k (s^{-1}) was greatest under a light intensity of 2 W/m^2, followed by 4 W/m^2, and lowest at 6 W/m^2. The maximum heat production was greatest under a light intensity of 4 W/m^2, followed by 6 W/m^2, and lowest at 2 W/m^2. The peak time was greatest under a light intensity of 6 W/m^2, followed by 2 W/m^2, and shortest at 4 W/m^2. Generation time was longest under a light intensity of 2 W/m^2, followed by 4 W/m^2, and shortest at 6 W/m^2. Although the growth rate at 2 W/m^2 was greater than at 4 or 6 W/m^2, the maximum heat production at 2 W/m^2 was delayed by 10,283 s compared to 4 W/m^2.

Under a light intensity of 2 W/m^2, the decline phase was the longest growth phase, followed by the lag phase, the

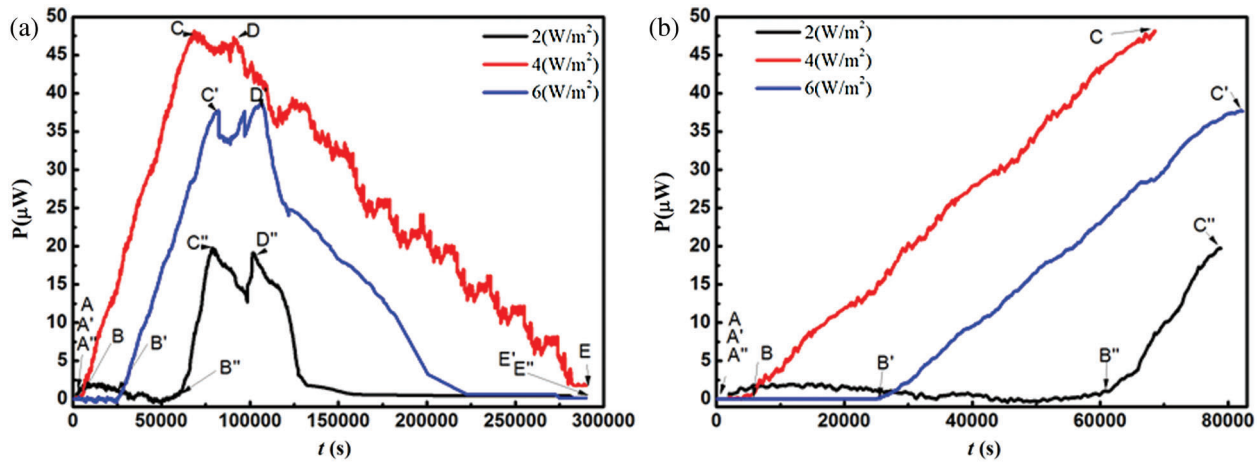

FIGURE 4. Heat production power-time curves of *R. palustris* measured by photocalorimetry.
(a) Growth curves of *R. palustris* under light intensities of 2, 4, and 6 W/m^2 from 0 to 332,701 s. (b) Growth curves of *R. palustris* under light intensities of 2, 4, and 6 W/m^2 from the lag phase to the log phase.

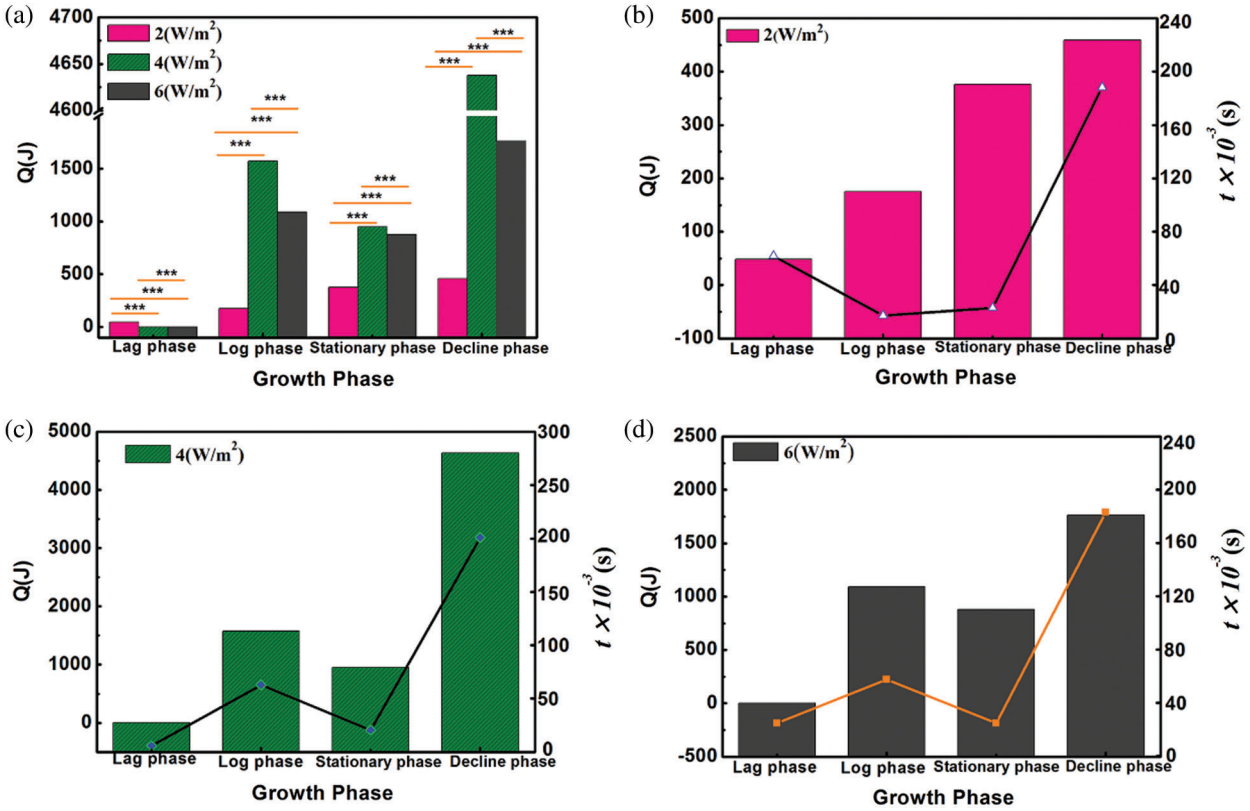

FIGURE 5. Heat production changes in *R. palustris* during different growth phases when cultured under 532 nm light at various intensities. (a) Change in heat production of *R. palustris* at light intensities of 2, 4, and 6 W/m^2. Changes in heat production and growth phase duration at light intensities of (b) 2 W/m^2, (c) 4 W/m^2, and (d) 6 W/m^2. *P < 0.05; **P < 0.01; ***P < 0.001.

TABLE 2

Heat production and growth phase duration of *R. palustris* under different light intensities

Light intensity (W/m^2)	Lag phase		Log phase		Stationary phase		Decline phase	
	t (s)	Q (J)	t (s)	Q (J)	t (s)	Q (J)	t (s)	Q (J)
2	61816	48.26	17058	174.97	22828	376.16	188297	459.32
4	5790	1.048	62805	1573.43	20536	949.77	200868	4637.69
6	24696	0.3865	57634	1090	24612	879.53	183058	1763.05

stationary phase, and the log phase. Under a light intensity of 4 W/m^2, the decline phase was the longest growth phase, followed by the log phase, the stationary phase, and the lag phase. Under a light intensity of 6 W/m^2, the lag phase was the longest growth phase, followed by the decline phase, the log phase, and the stationary phase. The lag phase was longest under a light intensity of 2 W/m^2, followed by 6 W/m^2, and shortest under 4 W/m^2. The log phase was longest under a light intensity of 4 W/m^2, followed by 6 W/m^2, and shortest under 2 W/m^2. *R. palustris* cultured under a light intensity of 4 W/m^2 started the earliest, spent the longest amount of time in the log phase, and had the highest growth efficiency.

Heat release by *R. palustris* intensities during the lag phase was highest under a light intensity of 4 W/m^2, followed by 6 W/m^2, and was lowest under a light intensity of 2 W/m^2.

Discussion

Effect of wavelength and light intensity on R. palustris growth and metabolism

It is important to identify the optimal wavelength and intensity of the light to achieve the desired bacterial growth. In the present study, single-factor experiments assessing different wavelengths showed that growth of *R. palustris* was highest under green light (520–570 nm), followed by blue light, white light, and red light (Fig. 2a). The optimal range of light intensities for the growth of *R. palustris* was 2–6 W/m^2, and growth performance was not enhanced by green light with higher intensities. The cultures reached their maximum OD (1.56) 24 h after inoculation under a light intensity of 4 W/m^2. The growth of the bacteria was reduced under light intensities >6 W/m^2, suggesting that there is a certain threshold value of the light intensity above

TABLE 3

Thermodynamic parameters of *R. palustris* cultured under different light intensities

Light intensity (W/m^2)	Growth rate constant, k ± SD (s^{-1})	Correlation coefficient, *R*	P_{max} (μW)	t_{max} (s)	t_G (min)
2	$1.191 \times 10^{-4} \pm 8.22 \times 10^{-7}$	0.9917	19.78	78878	97.08
4	$7.652 \times 10^{-5} \pm 1.27 \times 10^{-7}$	0.9982	48.19	68595	151.01
6	$6.963 \times 10^{-5} \pm 2.45 \times 10^{-7}$	0.9988	38.56	106942	165.98

which the growth of *R. palustris* was not significantly promoted (Fig. 2b). In fact, high-intensity light may even reduce bacterial growth due to overexcitation of photosystem I by excess energy absorbed by photosynthetic organs, also known as the reports of (Ichii *et al.*, 2003; Luque and Clark, 2010; Melis, 2012; Zhang *et al.*, 2016). In the present study, the light saturation effect was apparent when the light intensity was increased to 8 W/m^2. However, the OD values of *R. palustris* cultured under various light intensities were not significantly different during the four growth phases, indicating that the effect of illumination on bacterial growth is complex. These results will contribute to the development of a theoretical explanation of the mechanisms underlying reproductive and metabolic processes in bacteria.

Although the single-factor experiments showed that green light with intensities between 2 and 6 W/m^2 was optimal for *R. palustris* growth, it did not provide information on *in-situ* growth under a single wavelength at specific light intensities. With the addition of experiments that screen a wide range of factors, integration of photocalorimetry allows for the examination of subtle energy changes during bacterial growth. In the present study, thermograms and relevant parameters of *R. palustris* were determined using a highly precise and sensitive photoreaction-microcalorimetric system. Because the bacterial cells in the log phase are viable and consistent in size, they are often used in research or as "seeds" in large-scale production (Pommerville, 2012). Metabolic and enzymatic activities of *R. palustris* are also high and stable during the log phase. In the context of production, the fermentation efficiency of bacteria is often increased by prolonging the log phase. Both cell number and heat production increase exponentially during the log phase, as shown by Eq. (1).

$$\ln P_t = \ln(p_0 + k_t) \quad (1)$$

where P_t is heat output at time t in the log phase, P_0 is heat output at $t = 0$, k is the growth rate constant, and t is the duration of the experiment (Huang *et al.*, 1998). Since the log phase of each *p-t* curve was strongly correlated with Eq. (1), a series of P_t and t values from the log phase were assessed to find the best fit for Eq. (1), and k and growth kinetics equations were then established. Generation time, t_G, was determined based on equation $t_G = \ln 2/k$, and the maximum heat production P_{max} and peak time t_{max} (corresponding to maximum heat production) were obtained from the *p-t* curve. In the present study, *R. palustris* had the fastest growth rate but smallest P_{max} when cultured under a light intensity of 2 W/m^2, indicating that this amount of light was insufficient to activate photosynthesis and that bacteria were unable to obtain enough energy (Idi *et al.*, 2015; Ormerod *et al.*, 1961). Despite the rapid growth of *R. palustris* during the log phase, when generation time was the shortest, and growth rate was the highest, energy release was not at its maximum. Therefore, a light intensity of 2 W/m^2 is not optimal for the growth of *R. palustris*.

In the lag phase, bacteria accumulate energy in order to undergo rapid growth and proliferation during the log phase once the energy level reaches a threshold level. This could explain why *R. palustris* cultured under a light intensity of 2 W/m^2 had the highest growth rate but the shortest generation time. Additionally, although the growth rate of *R. palustris* was reduced to 7.652×10^{-5} s^{-1} and the generation time was extended to 151.01 min under a light intensity of 4 W/m^2, maximum heat output (48.19 μW) was much higher at 2 W/m^2. These results are consistent with the fact that the log phase started earlier under light intensities of 2 and 6 W/m^2 in the single-factor experiment.

Interestingly, it was found that growth rate, generation time, and peak time of *R. palustris* during the log phase, and heat release during the stationary and decline phases, were lower under a light intensity of 6 W/m^2 compared to 4 W/m^2 (Fig. 5a). These results suggested that 6 W/m^2 was also not the optimal light intensity for *R. palustris* growth. Overall, our data demonstrated that light intensities above or below 4 W/m^2 are not optimal for *R. palustris* growth. The growth of *R. palustris* was dependent on a combination of optimal conditions and was not simply proportional to light intensity. This conclusion is in good agreement with established knowledge of photosynthesis bacteria, proving that the information collected on the novel photo-calorimeter reported in the present study. This system can precisely collect the *in-situ*, real-time, and uninterrupted thermodynamic parameters of the growth process of the photosynthesis bacteria, therefore providing accurate information needed to control the growth of the photosynthesis microbes. To our best knowledge, the application of photocalorimetry for this purpose has not been reported.

R. palustris growth characteristcs

Metabolic processes are influenced by changes in the environment, and this can lead to significant changes in the *p-t* curve of the bacteria (Moulisová *et al.*, 2009). Photocalorimetry can reveal details of bacterial growth and metabolism that are undetectable by other methods. Therefore, this novel method is extremely useful for elucidating the growth mechanisms of bacteria, as it provides information on the thermodynamics, kinetic

patterns, and characteristics at different stages of growth. The result also was similar to Sardaro *et al.* (2013), who presented a calorimetric investigation of *R. sphaeroides* life cycle by two different experimental procedures based on the use of a static ampoule and a flow cell, respectively (Sardaro *et al.*, 2013). Although photo-microcalorimeter technology provides accurate *in-situ* thermodynamic information about the growth process of bacteria, during the growth process, each process of bacterial growth does not completely match with the increase of light intensity. Maybe it is related to the photosynthetic system, even because the dimension of the light-harvesting apparatus and with the physical dimension of the cells is possible.

Conclusions

Although photosynthetic microorganisms are suggested to have great applications in the green production of alternative energy and value-adding products as well as for bioremediation of the environment, the industrial cultivation of these important microbes has received limited success partially due to the lack of a precise online measurement tool which can monitor the *in-situ* growth of them. In the present study, we have demonstrated the integration of light source with microcalorimeter provided the unique opportunity to investigate the effect of light illumination conditions on the *in-situ* growth of the photosynthetic microorganisms. The results clearly proved that the photo-microcalorimeter designed in our project can conveniently monitor the processes of bacterial growth and metabolism under light with a desired wavelength and intensity and generate the desired thermodynamic and kinetic parameters of the *in-situ* growth of the photosynthetic microbes. This novel technique can detect tiny differences in the *in-situ* bacterial growth under different growth conditions that cannot be detected by conventional analytical methods. Thus, it is envisioned that the photo-microcalorimeter will become a powerful tool to help the optimization of the industrial cultivation of photosynthetic microorganisms.

Statistical Analysis: Statistical analyses were performed using GraphPad Prism6 software, version 6 (GraphPad Software, Inc.). Statistical significance is calculated using Student's two-tailed unpaired *t*-test or ANOVA with Holm-Sidak's multiple comparisons test. Ns, not significant ($P > 0.05$); $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Author Contributions: Conceptualization, ZYH, YJL, XYM and DLY; methodology, ZYH, YJL and DLY; formal analysis, YJL; investigation, YJL, XFQ, DLY, JMW, XYM, HW and HL; resources, ZYH and XYM; writing—original draft preparation, YJL; writing—review and editing, YJL supervision, XYM and ZYH; funding acquisition, TRL and ZYH.

Availability of Data and Materials: The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Funding Statement: This work was funded by the Chinese National Natural Science Foundation (21873022), the National Natural Science Foundation of Guangxi (2018GXNSFAA138069), and the Middle and Young Teachers Basic Ability Enhancement Project of Guangxi (2017KY0167, 2018KY0165).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

Adamson AW, Vogler A, Kunkely H, Wachter R (1978). Photocalorimetry. Enthalpies of photolysis of trans-azobenzene, ferrioxalate and cobaltioxalate ions, chromium hexacarbonyl, and dirhenium decarbonyl. *Journal of the American Chemical Society* **100**: 1298–1300. DOI 10.1021/ja00472a049.

Amezaga JM, Amtmann A, Biggs CA, Bond T, Gandy CJ, Honsbein A, Karunakaran E, Lawton L, Madsen MA, Minas K (2014). Biodesalination: A case study for applications of photosynthetic bacteria in water treatment. *Plant Physiology* **164**: 1661–1676. DOI 10.1104/pp.113.233973.

Benemann JR, Berenson JA, Kaplan NO, Kamen MD (1973). Hydrogen evolution by a chloroplast-ferredoxin-hydrogenase system. *Proceedings of The National Academy of Sciences of The United States of America* **70**: 2317–2320. DOI 10.1073/pnas.70.8.2317.

Carlozzi P, Pushparaj B, Degl'innocenti A, Capperucci A (2006). Growth characteristics of *Rhodopseudomonas palustris* cultured outdoors, in an underwater tubular photobioreactor, and investigation on photosynthetic efficiency. *Applied Microbiology and Biotechnology* **73**: 789–795. DOI 10.1007/s00253-006-0550-z.

De Godos I, Vargas VA, Blanco S, González MCG, Soto R, García-Encina PA, Becares E, Muñoz R (2010). A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. *Bioresource Technology* **101**: 5150–5158. DOI 10.1016/j.biortech.2010.02.010.

Dhuna M, Beezer AE, Connor JA, Clapham D, Courtice C, Frost J, Gaisford S (2008). LED-array photocalorimetry: Instrument design and application to photostability of nifedipine. *Journal of Pharmaceutical and Biomedical Analysis* **48**: 1316–1320. DOI 10.1016/j.jpba.2008.09.007.

Dhuna M, Beezer AE, Morris AC, Gaisford S, O'neill MA, Hadgraft J, Connor JA, Clapham D, Frost J (2007). Development of an isothermal heat-conduction photocalorimeter. *Review of Scientific Instruments* **78**: 025105. DOI 10.1063/1.2670220.

Donia MS, Fricke WF, Partensky F, Cox J, Elshahawi SI, White JR, Phillippy AM, Schatz MC, Piel J, Haygood MG (2011). Complex microbiome underlying secondary and primary metabolism in the tunicate-*Prochloron* symbiosis. *Proceedings of The National Academy of Sciences of The United States of America* **108**: E1423–E1432. DOI 10.1073/pnas.1111712108.

Fan G, Huang Z (2011). Simple carbothermal reduction route for Sb_2O_3 submicron rods. *Micro & Nano Letters* **6**: 55–58. DOI 10.1049/mnl.2010.0177.

Fernandes BD, Mota A, Teixeira JA, Vicente AA (2015). Continuous cultivation of photosynthetic microorganisms: Approaches, applications and future trends. *Biotechnology Advances* **33**: 1228–1245. DOI 10.1016/j.biotechadv.2015.03.004.

Gordon H, Palmer R, Smith S (1981). New ideas in calorimetry. *Physica Scripta* **23**: 564–568. DOI 10.1088/0031-8949/23/4A/032.

Guo YX, Chen J, Huang ZY, Fan GC, Sun JL (2012). Investigation of *in situ* growth of SrMoO4 nanoplates by microcalorimetry. *Chemical Research in Chinese Universities* **28**: 1058–1060.

Huang ZY, Li XX, Liu ZJ, He LM, Tan XC (2015). Morphology effect on the kinetic parameters and surface thermodynamic properties of Ag_3PO_4 micro/nanocrystals. *Journal of Nanomaterials* **2015**: 1–9. DOI 10.1155/2015/743121.

Huang ZY, Liu Y, Qu S, Feng Y (1998). A comparative study on half-inhibitory concentration of Schiff base-metal complexes reacting with bacteria. *Thermochimica Acta* **320**: 121–126. DOI 10.1016/S0040-6031(98)00415-8.

Ichii K, Matsui Y, Murakami K, Mukai T, Yamaguchi Y, Ogawa K (2003). A simple global carbon and energy coupled cycle model for global warming simulation: Sensitivity to the light saturation effect. *Tellus B: Chemical and Physical Meteorology* **55**: 676–691. DOI 10.3402/tellusb.v55i2.16709.

Idi A, Md Nor MH, Wahab MFA, Ibrahim Z (2015). Photosynthetic bacteria: An eco-friendly and cheap tool for bioremediation. *Reviews in Environmental Science and Bio/Technology* **14**: 271–285. DOI 10.1007/s11157-014-9355-1.

Jeffryes C, Agathos SN, Rorrer G (2015). Biogenic nanomaterials from photosynthetic microorganisms. *Current Opinion in Biotechnology* **33**: 23–31. DOI 10.1016/j.copbio.2014.10.005.

Kokabian B, Gude VG (2015). Sustainable photosynthetic biocathode in microbial desalination cells. *Chemical Engineering Journal* **262**: 958–965. DOI 10.1016/j.cej.2014.10.048.

Li X, Huang Z, Liu Z, Diao K, Fan G, Huang Z, Tan X (2016). *In situ* photocalorimetry: An alternative approach to study photocatalysis by tracing heat changes and kinetics. *Applied Catalysis B: Environmental* **181**: 79–87. DOI 10.1016/j.apcatb.2015.07.036.

Linder T (2019). Making the case for edible microorganisms as an integral part of a more sustainable and resilient food production system. *Food Security* **11**: 1–14. DOI 10.1007/s12571-019-00912-3.

Liu L, Choi S (2017). Self-sustaining, solar-driven bioelectricity generation in micro-sized microbial fuel cell using co-culture of heterotrophic and photosynthetic bacteria. *Journal of Power Sources* **348**: 138–144. DOI 10.1016/j.jpowsour.2017.03.014.

Lu C, Zhang Z, Ge X, Wang Y, Zhou X, You X, Liu H, Zhang Q (2016). Bio-hydrogen production from apple waste by photosynthetic bacteria HAU-M1. *International Journal of Hydrogen Energy* **41**: 13399–13407. DOI 10.1016/j.ijhydene.2016.06.101.

Lu H, Zhang G, Zheng Z, Meng F, Du T, He S (2019). Bio-conversion of photosynthetic bacteria from non-toxic wastewater to realize wastewater treatment and bioresource recovery: A review. *Bioresource Technology* **278**: 383–399. DOI 10.1016/j.biortech.2019.01.070.

Luque R, Clark J (2011). *Handbook of biofuels production: Processes and technologies*. Woodhead Publishing Limited.

Magee JL, Dewitt TW, Smith EC, Daniels F (1939). A photocalorimeter. The quantum efficiency of photosynthesis in algae. *Journal of the American Chemical Society* **61**: 3529–3533. DOI 10.1021/ja01267a089.

Mazard S, Penesyan A, Ostrowski M, Paulsen IT, Egan S (2016). Tiny microbes with a big impact: The role of cyanobacteria and their metabolites in shaping our future. *Marine Drugs* **14**: 97. DOI 10.3390/md14050097.

Melis A (2012). Photosynthesis-to-fuels: From sunlight to hydrogen, isoprene, and botryococcene production. *Energy & Environmental Science* **5**: 5531–5539. DOI 10.1039/C1EE02514G.

Milano F, Punzi A, Ragni R, Trotta M, Farinola GM (2019). Photonics and optoelectronics with bacteria: Making materials from photosynthetic microorganisms. *Advanced Functional Materials* **29**: 1805521. DOI 10.1002/adfm.201805521.

Moulisová V, Luer L, Hoseinkhani S, Brotosudarmo THP, Collins AM, Lanzani G, Blankenship RE, Cogdell RJ (2009). Low light adaptation: Energy transfer processes in different types of light harvesting complexes from *Rhodopseudomonas palustris*. *Biphysical Journal* **97**: 3019–3028. DOI 10.1016/j.bpj.2009.09.023.

Mukhanov V, Kemp R (2009). Design and experience of using light-emitting diodes (LEDs) as the inbuilt light source for a customised differential photomicrocalorimeter. *Journal of Thermal Analysis and Calorimetry* **95**: 731–736. DOI 10.1007/s10973-008-9468-2.

Olmsted J III (1979). Photon flux measurements using calorimetry. *Review of Scientific Instruments* **50**: 1256–1259. DOI 10.1063/1.1135691.

Ormerod JG, Ormerod KS, Gest H (1961). Light-dependent utilization of organic compounds and photoproduction of molecular hydrogen by photosynthetic bacteria: Relationships with nitrogen metabolism. *Archives of Biochemistry and Biophysics* **94**: 449–463. DOI 10.1016/0003-9861(61)90073-X.

Pommerville JC (2012). *Alcamo's fundamentals of microbiology: Body systems*. Second edition, Burlington, MA: Jones & Bartlett Publishers.

Sakaki K, Watanabe M, Suda Y, Ishizuka A, Noparatnaraporn N (2005). Applications of photosynthetic bacteria for medical field. *Journal of Bioscience and Bioengineering* **100**: 481–488. DOI 10.1263/jbb.100.481.

Sardaro A, Castagnolo M, Trotta M, Italiano F, Milano F, Cosma P, Agostiano A, Fini P (2013). Isothermal microcalorimetry of the metabolically versatile bacterium *Rhodobacter sphaeroides*. *Journal of Thermal Analysis and Calorimetry* **112**: 505–511. DOI 10.1007/s10973-012-2895-0.

Schaarschmidt B, Lamprecht I (1973). UV-irradiation and measuring of the optical density of microorganisms in a microcalorimeter. *Experientia* **29**: 505–506. DOI 10.1007/BF01926809.

Sekar N, Ramasamy RP (2015). Photosynthetic energy conversion: Recent advances and future perspective. *Electrochemical Society Interface* **24**: 67–73. DOI 10.1149/2.F06153if.

Seybold PG, Gouterman M, Callis J (1969). Calorimetric, photometric and lifetime determinations of fluorescence yields of fluorescein dyes. *Photochemistry and Photobiology* **9**: 229–242. DOI 10.1111/j.1751-1097.1969.tb07287.x.

Talaiekhozani A, Rezania S (2017). Application of photosynthetic bacteria for removal of heavy metals, macro-pollutants and dye from wastewater: A review. *Journal of Water Process Engineering* **19**: 312–321. DOI 10.1016/j.jwpe.2017.09.004.

Teixeira C, Wadsö I (1990). A microcalorimetric system for photochemical processes in solution. *The Journal of Chemical Thermodynamics* **22**: 703–713. DOI 10.1016/0021-9614(90)90023-J.

Tiwari B, Chakraborty S, Verma E, Mishra AK (2018). *Photosynthetic microorganisms and bioenergy prospects: Challenges and potential*. John Wiley & Sons. DOI 10.1002/9781119276050.ch5.

Wakayama T, Nakada E, Asada Y, Miyake J (2000). Effect of light/dark cycle on bacterial hydrogen production by *Rhodobacter sphaeroides* RV. *Applied Biochemistry and Biotechnology* **84–86**: 431–440. DOI 10.1007/978-1-4612-1392-5.

Wang LD, Huang ZY, Guo YX, Wang TH (2011). In-situ growth and mechanism of the octahedron BaMoO_4 nanostructures. *Chemical Journal of Chinese Universities* **32**: 2898–2843.

Wei H, Okunishi S, Yoshikawa T, Kamei Y, MaO D, Santander-De Leon SMS, Nonato Nuñal S, Maeda H (2016). Synergistic effect of photosynthetic bacteria and isolated bacteria in their antifungal activities against root rot fungi. *Biocontrol Science* **21**: 173–177. DOI 10.4265/bio.21.173.

Xu X, Yu X, Zheng P, Chen W, Feng X (1995). Studies on kinetics of substrate utilization of hydrogen production from wastewater with immobilized cells of photosynthetic bacteria. *Chinese Journal of Biotechnology* **11**: 69–77.

Zhang B, Wang L, Riddicka BA, Li R, Able JR, Boakye-Boaten NA, Shahbazi A (2016). Sustainable production of algal biomass and biofuels using swine wastewater in North Carolina. *US Sustainability* **8**: 477. DOI 10.3390/su8050477.

Zhou Q, Zhang P, Zhang G, Peng M (2015). Biomass and pigments production in photosynthetic bacteria wastewater treatment: Effects of photoperiod. *Bioresource Technology* **190**: 196–200. DOI 10.1016/j.biortech.2015.04.092.