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Green energy from *Rhodospseudomonas palustris* grown at low to high irradiance values, under fed-batch operational conditions

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Abstract

Rhodopseudomonas palustris was examined under continuous irradiances of 36, 56, 75, 151, 320, 500, and 803 W m⁻², for a co-production of both bio-H₂ and biodiesel (lipids). Cultures were grown under fed-batch operational conditions. The highest overall bio-H₂ produced [4.2 l(H₂) l_{culture}⁻¹] was achieved at 320 W m⁻², while the highest dry biomass (3.18 g l⁻¹) was attained at 500 W m⁻². Dry biomass contained between 22 % and 39 % lipid. The total energy conversion efficiency was at its highest (6.9 %) at 36 W m⁻².

Keywords: green energy; bio-H₂; biodiesel; hydrogen production rate; *R. palustris*

Introduction

Purple non-sulfur photosynthetic bacteria can decompose organic acids by using light energy and nitrogenase in a photofermentation process (Basak and Das, 2007). Green energies (bioH₂ and biodiesel) needs of cultural systems to be produced. Bioreactor design, hydrodynamic aspects and light distribution are key points to improve H₂ yield. Improvements could also be achieved through the genetic modification (Nath and Das, 2004). Chisti (2007) reported: “Biodiesel derived from oil crops is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, biodiesel from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the exiting demand for transport fuels”. A review of biodiesel production from oleaginous microorganisms was recently carried out by Ratledge and Cohen (2008). Purple, non-sulfur photosynthetic bacteria, such as *Rhodospseudomonas palustris*, is able to use irradiance more efficiently than do crop plants, and oil production from bacteria could be improved under adequate or stressed culture conditions.

The purpose of this study is to investigate a co-production of bio-H₂ and rich biomass content of lipids for biodiesel production by means of *Rhodospseudomonas palustris* (strain 42OL) grown at low irradiance (LI) (36, 56 and 76 W m⁻²), at both medium (MI) (151 and 320 W m⁻²) and high irradiance (HI) (500 and 805 W m⁻²). The relationship between light intensity and both hydrogen production rate and the total energy conversion efficiency has also been investigated.

Material and methods

Description of the cultural system

A cylindrical glass photobioreactor [internal diam = 9.6 cm; working volume (V) = 1.07 l] was held at 30 ± 0.2 °C. All irradiance was measured at the surface of the photobioreactor using a flat glass surface (10 x 16 cm) an averaged from nine different location points. The

heat exchanger-Plexiglas basin was not filled of water during the irradiance measurements. The average radial depth of water between the Plexiglas (inside wall) and the photobioreactor (outside wall) was 7.3 cm. The gas produced by bacteria was trapped in a graduated glass column (Carlozzi and Lambardi, 2009).

Organism and culture conditions

Rhodospseudomonas palustris (strain 42OL) was taken from the culture collection of the former Centro di Studio dei Microrganismi Autotrofi of Florence. This centre is now part of the Institute of Ecosystem Study, CNR, Florence - Italy. The bacterium was grown at 30 °C on a medium previously described in the literature (Carlozzi and Sacchi, 2001), but with glutamic acid at 0.865 g l⁻¹ and malic acid at 3.26 g l⁻¹. The initial pH of the medium was 6.8. All experiments were carried out under continuous light, at irradiances of 36, 56, 75, 151, 320, 500, and 803 W m⁻². The photobioreactor irradiated from one side was operated in a fed-batch mode (Carlozzi and Lambardi, 2009). This feeding strategy was used for long-term investigations (max = 408 h); otherwise, the yields produced (biomass and H₂) would have ceased for lack of macronutrients.

Analytical methods

Dry biomass was determined by using the empirical equation of Carlozzi and Sacchi (2001). Growth was determined from the bacteriochlorophyll (Bchl) concentration (Carlozzi et al., 2006). Cultures were irradiated with a 250 W OSRAM Power-star HQI-TS lamp, and the irradiance was measured using a Quantum/Radiometer/Photometer (model LI-185B, LICOR, Lincoln, Nebraska, USA). In order to determine organic acid concentrations in the bacteria cultures, a HPLC was utilised. After disposable syringe filter units (MFS-13 mm, 0.45 µm pore size) were used to remove the cells, the supernatant was tested for malic acid.

The HPLC was equipped with a C18 analytical column (250 mm x 4.6 mm) and run at 25 °C. The mobile phase was 0.1% H₃PO₄, at 1 ml min⁻¹.

Gas and lipid evaluation

The gas mixture produced (CO₂ and H₂) was first made to flow into a basin containing a saline solution absorber of NaOH, which stripped CO₂ and then the hydrogen was trapped in a calibrated column, where it was collected and the volume measured to determine hydrogen production. No CO₂ was found inside the calibrated column. This was checked by sampling 0.1 ml from the calibrate column and injecting it into a Perkin–Elmer Autosystem gas chromatograph equipped with a thermal conductivity detector (TCD) and a Silica Gel 60/80 Grade 12 column (Alltech, Derfield). The carrier gas was He; pure H₂ was used as the standard. Total lipid extraction and the fatty acid profile were determined in accordance with Pushparaj et al. (2008).

Light conversion efficiency

The total energy conversion efficiency (η') was determined using the following equation:

$$\eta' (\%) = \frac{33.61 \rho_{H_2} V_{H_2} + (P_B (-\Delta P_B))}{I A t_{H_2} + (MA_c (-\Delta MA)) + (GA_c (-\Delta GA))} \times 100 \quad (1)$$

where ρ_{H_2} is the concentration of H₂ (g l⁻¹); V_{H₂} is the volume of H₂ produced (l); P_B is the total biomass produced (g) (ash = 4.5% of dry biomass; (- ΔP_B) is the heat of combustion of ash-free biomass (dw) (kcal g⁻¹); I is the irradiance (W m⁻²); A is the irradiated area of the photobioreactor (m²), which was calculated as being ½ of the cylindrical reactor surface (2 $\pi r_i h$), with r_i and h indicating, respectively, the internal radius and the height of the cylindrical reactor; t_{H₂} is the evolution time (h); MA_c is the malic acid consumed (g); (- ΔMA) is the heat of combustion of MA (kcal g⁻¹); GA_c is the glutamic acid consumed (g), and (- ΔGA) is the heat of combustion of GA (kcal g⁻¹).

Results and discussion

Growth of *R. palustris* and H₂ production are shown in Fig. 1. Both bio-H₂ and dry biomass productivities and the total energy conversion efficiency versus the irradiance are shown in Fig. 2. The total H₂ produced in the photobioreactor (working volume: 1.07 l) increased in accordance with the irradiance up to 4.2 l(H₂) l_{culture}⁻¹ at 320 W m⁻². By increasing the irradiance further, the H₂ yield decreased by about 7.5%. The average H₂ production rate (HPR_{av}) was 12.3 ml l⁻¹ h⁻¹ 320 W m⁻². Chen et al. (2008) predicted an overall H₂ photoevolution rate of 12.6 ml l⁻¹ h⁻¹ by using acetate and butyrate as dual carbon substrates. The yield of H₂ achieved in this study was about 33 % higher than that reported by Carlozzi and Lambardi, 2009, and the H₂ photoevolution rate was within the range of those cited in the literature for use of the same microorganism species (Chen et al., 2006; Eroğlu et al., 2008). The highest cumulative H₂ was achieved at 320 W m⁻², while the highest dry biomass was attained at 500 W m⁻². This was because the biomass production competed with the H₂ photoevolution.

Fig. 1

Fig. 2

The total energy conversion efficiency was at its highest (6.9 %) at 36 W m⁻², and went progressively down as the irradiance increased. In 2009, Obeid et al. wrote: "The conversion efficiency of light energy to hydrogen is the key factor in the development of a biological process devoted to hydrogen production". The η' of 6.9% is among the highest values cited in the literature (Kondo et al. 2006; Basak and Das, 2009).

Biomasses of *R. palustris* harvested at the end of each fed-batch cycle, during bio-H₂ photoevolution, were analysed in order to determine the total lipid content. The results are shown in Fig. 3. The lipid content 36-39 % of the dry biomass when the irradiance was within the 56-151 W m⁻² range. A further increase in the irradiance caused a relevant and progressive drop in the lipid content in the *R. palustris* biomasses: the minimum content (22 %) was reached when the irradiance was at its highest (803 W m⁻²). With an irradiance of less

Fig. 3

than 56 W m^{-2} , the lipid content of the biomass dry weight also decreased. The major fatty acid of *R. palustris* grown inside a tubular underwater photobioreactor (UwTP) (Carlozzi and Sacchi, 2001) were 16:0 (17 %); 16:1 (7 %); 18:1 (73 %); 18:0 (3 %). Our data are similar to the four *R. palustris* strains cited by Imhoff and Bias-Imhoff (1995). Co-production of both bio- H_2 and biodiesel, as biofuels, is proposed in this study. For biodiesel production, triglycerides are transesterified with methanol. The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides, and lastly to glycerol + methyl esters (biodiesel) (Chisti 2007). The author listed fourteen microalgal species with their respective oil contents. The oil content we found in *R. palustris* biomasses can be included in the group of the five microalgal species, with the highest oil content, reported by Chisti (2007). Bacteria accumulate fewer lipids than do microalgae, and the average oil content is about 20-40% of the dry biomass (Meng et al., 2009). Microorganisms that can accumulate lipids at more than 20 % of their biomass are defined as oleaginous species (Ratledge and Wynn, 2002). Since the lipid content of the *R. palustris*, that we investigated was within the range of 22-39 % of the dry-biomass, we could define our bacteria strain as began an oleaginous species. Carlozzi and Sacchi (2001) reported, outdoors, a production of *R. palustris* dry biomass of $64 \text{ g m}^{-2} \text{ day}^{-1}$. On the basis of Carlozzi's investigations, a projection of the monthly oil production over the years can be made from *R. palustris* grown outdoors in the climatic conditions of Florence, Italy (latitude $43^\circ 46' \text{ N}$, longitude $11^\circ 15' \text{ E}$) (Fig. 4). Projections on bio- H_2 production, outdoors, were not carried out, because we did not have enough experimental results, even though a first, very short investigation has recently been carried out (Carlozzi et al., 2008). To reduce the greenhouse gas effect, oil production (biodiesel) from plants, crops, microalgae, yeast and, recently, bacteria has already been suggested (Ratledge and Wynn, 2002; Chisti, 2007; Rittmann, 2008). Nevertheless, we would remark that this was the first time, which a non-sulfur photosynthetic bacteria (*R. palustris*) has been proposed as a potential oil source for biodiesel production.

Fig. 4

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References

- Basak N, Das D (2007) The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: the present state of the art. *World J Microbiol Biotechnol* 23:31-42
- Basak N, Das D (2009) Photofermentative hydrogen production using purple non-sulfur bacteria *Rhodobacter sphaeroides* O.U.001 in an annular photobioreactor: a case study. *Biomass Bioenergy* 33:911-919
- Carlozzi P, Lambardi M, Pushparaj B et al (2008) Indoor and outdoor photobiological hydrogen production by *Rhodospseudomonas palustris*, strain 42OL. *Current Topics Biotechnol* 4:93-100
- Carlozzi P, Lambardi M (2009) Fed-batch operation for bio-H₂ production by *Rhodospseudomonas palustris* (strain 42 OL). *Renew Energy* 34(12):2577-2584
- Carlozzi P, Pushparaj B, Degl'Innocenti A et al (2006) Growth characteristics of *Rhodospseudomonas palustris* cultured outdoors, in an underwater tubular photobioreactor and investigation on photosynthetic efficiency. *Appl Microbiol Biotechnol* 73:789-795
- Carlozzi P, Sacchi A (2001) Biomass production and studies on *Rhodospseudomonas palustris* grown in an outdoor, temperature controlled, underwater tubular photobioreactor. *J Biotechnol* 88:239-249
- Chen CY, Lee CM, Chang JS (2006) Hydrogen production by indigenous photosynthetic bacterium *Rhodospseudomonas palustris* WP3-5 using optical fiber-illuminating photobioreactors. *Biochem. Eng J* 32:33-42

- Chen CY, Lu WB, Liu CH et al (2008) Improved phototrophic H₂ production with *Rhodospseudomonas palustris* WP3-5 using acetate and butyrate as dual carbon substrates. *Biores Technol* 99:3609-3616
- Chisti Y (2007) Biodiesel from microalgae. *Biotechnol Adv* 25:294-306
- Eroğlu I, Tabanoğlu A, Gündüz U et al (2008) Hydrogen production by *Rhodobacter sphaeroides* O.U.001 in a flat plate solar bioreactor. *Int J Hydrogen Energy* 33:531-541
- Imhoff JF, Bias-Imhoff U (1995) Lipids, quinones and fatty acids of anoxygenic phototrophic bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) *Advances in Photosynthesis: Anoxygenic Photosynthetic Bacteria*. Kluwer Academic Publisher. Dordrecht, The Netherlands
- Kondo T, Wakayama T, Miyake J (2006) Efficient hydrogen production using a multi-layered photobioreactor and a photosynthetic bacterium mutant with reduced pigment. *Int J Hydrogen Energy* 31:1522-1526
- Meng X, Yang J, Xu X et al (2009) Biodiesel production from oleaginous microorganisms. *Renew Energy* 34:1-5
- Nath K, Das D et al (2004) Improvement of fermentative hydrogen production: various approaches. *Appl Microbial Biotechnol* 65:520-529
- Obeid J, Magnin J-P, Flaus J.M et al (2009) Modelling of hydrogen production in batch cultures of the photosynthetic bacterium *Rhodobacter capsulatus*. *Int J Hydrogen Energy* 34:180-185
- Pushparaj B, Buccioni A, Paperi R et al (2008) Fatty acid composition of antarctic cyanobacteria. *Phycologia* 47(4):430-434
- Ratledge C, Cohen Z (2008) Microbial and algal oils: Do they have a future for biodiesel or as commodity oils? *Lipid Technol* 20(7):155-160
- Ratledge C, Wynn JP (2002) The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Adv Appl Microbiol* 51:1-51

Rittmann BE (2008) Opportunity for renewable bioenergy using microorganisms. *Biotechnol Bioeng* 100(2):203-212

Figure captions

Fig. 1. Trends of bacteriochlorophyll (Bchl) synthesis versus time attained with *R. palustris* grown at low (A), middle (B) and high irradiance. Values are means of three replications \pm standard deviation.

Fig. 2. Bio-H₂ and dry-biomass productivities and the total energy conversion efficiency achieved at seven different irradiances (36, 56, 75, 151, 320, 500 and 803 W m⁻²). Bars \pm standard deviation.

Fig. 3. Lipid content in dry-biomass of *R. palustris* grown from 36 to 803 W m⁻² during hydrogen photo-evolution. Values are means of three replications \pm standard deviation.

Fig. 4. (A) Solar radiation and (B) **predicted results** of the monthly oil production from *R. palustris* grown outdoors in the climatic conditions of Florence, Italy (latitude: 43° 46' N; longitude: 11° 15' E). Bars \pm standard deviation.



