Bioresource Technology 101 (2010) 3115–3120

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Production of bio-fuels (hydrogen and lipids) through a photofermentation process

Pietro Carlozzi [a,*], Arianna Buccioni [b], Sara Minieri [b], Benjamin Pushparaj [a], Raffaella Piccardi [a], Alba Ena [a], Cristina Pintucci [a]

[a] Istituto per lo Studio degli Ecosistemi, Sede di Firenze, Consiglio Nazionale delle Ricerche, Polo Scientifico, Via Madonna del Piano n. 10, 50019 Sesto Fiorentino, Firenze, Italy
[b] Dipartimento di Scienze Zootecniche, Università degli Studi di Firenze, Via delle Cascine n. 5, 50144 Firenze, Italy

ARTICLE INFO

Article history:
Received 27 July 2009
Received in revised form 12 December 2009
Accepted 15 December 2009
Available online 8 January 2010

Keywords:
Bio-H_2
Biodiesel
Hydrogen production rate
Rhodopseudomonas palustris
Boltzmann's sigmoidal regression model

ABSTRACT

The purple non-sulfur photosynthetic bacteria *Rhodopseudomonas palustris* (strain 42OL) was investigated for a co-production of both bio-H_2 and biodiesel (lipids). The investigation was carried out using malic and glutamic acids in a fed-batch cultivation system under continuous irradiances of 36, 56, 75, 151, 320, 500, and 803 W m^{-2}. Boltzmann's sigmoidal regression model was used to determine growth kinetic parameters during hydrogen photoevolution. The upper limit of volumetric hydrogen photoevolution was 15.5 ± 0.9 ml l^{-1} h^{-1}. During the entire cultivation period (408 h), the highest average hydrogen production rate (HPR_{av}) of 11.1 ± 3.1 ml l^{-1} h^{-1} was achieved at an irradiance of 320 W m^{-2}. Biomasses stored at the end of each experimental set were analyzed in order to determine lipid content, which ranged from a minimum of 22 ± 1% to a maximum of 39 ± 2% of biomass dry weight.

1. Introduction

Research for hydrogen production using biological methods (dark-fermentation, photo-biophotolysis and photofermentation), which is evidently attracting attention during the new millennium, has gained much interest all over the world (Das and Veziroglu, 2001; Tao et al., 2007). Hydrogen generated by biological-method technologies could be used directly for the production of electrical energy in fuel cells (Waligórska et al., 2006).

Purple non-sulfur phototrophic bacteria have been proposed for the production of bio-H_2 from a single organic carbon source (Oh et al., 2004), from mixed volatile fatty acids (Shi and Yu, 2006a), from glycerol (Sabourin-Provost and Hallenbeck, 2009) and/or from those found to be contained in waste material (Eroğlu et al., 2008a). The optimization of cultivation conditions, such as physical (pH, temperature, irradiance and its distribution inside microbial cultures) and chemical parameters (nutrient content, their concentrations and C:N ratio), should increase the hydrogen production rate. Improvements in hydrodynamic aspects, bioreactors design, gas separation, light intensity and its distribution inside culture thickness are also key points for improving the hydrogen yield. To produce hydrogen from purple non-sulfur photosynthetic bacteria grown inside photobioreactors, no agricultural land area is required; however, some marginal or desert zones could be devoted to this end. Closed photobioreactors are able to capture solar radiation and convert it into energy as bio-H_2. Photobioreactors can be positioned horizontally or vertically, even in hilly zones, provided that these have a southern exposure. Many studies on photobiological hydrogen production have been carried out in laboratory conditions (Chen et al., 2006; Koku et al., 2003; Oh et al., 2004; Shi and Yu, 2006a; Tsygankov et al., 1998), but very few of these are under natural light (Asada and Miyake, 1999; Carlozzi et al., 2008; Eroğlu et al., 2008b; Modigell and Holle, 1998; Wakayama and Miyake, 2002).

Furthermore, in 2007, Chisti wrote: "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". Like plants, microalgae use sunlight to produce oils but they do so more efficiently than crop plants (Chisti, 2007). Oleaginous microorganisms can be found among different taxonomic groups. The reviews on biodiesel (oil) production from oleaginous microorganisms (microalgae, bacterium, yeast and fungi) were carried out by Ratledge and Cohen (2008) and recently by Meng et al. (2009).

The purpose of this study was to investigate on biofuel production from *Rhodopseudomonas palustris* (strain 42OL) exposed to different levels of irradiance (from 36 to 805 W m^{-2}). Boltzmann's sigmoidal regression model was used to determine the growth kinetic parameters during a co-production of bio-H_2 and biomass rich in oil Table 1.

* Corresponding author. Tel.: +39 055 5225962; fax: +39 055 5225920.
E-mail address: p.carlozzi@ise.cnr.it (P. Carlozzi).

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.12.049

Nomenclature

BC	biomass concentrations ($g\ l^{-1}$)
BC_0	starting biomass concentration ($g\ l^{-1}$)
BC_f	final biomass concentration reached at the end of each experimental set ($g\ l^{-1}$)
BC_{Max}	maximum biomass concentration ($g\ l^{-1}$)
dw	dry weight ($g\ l^{-1}$)
HI	high irradiance ($W\ m^{-2}$)
HPR	hydrogen production rate ($ml\ l^{-1}\ h^{-1}$)
HPR_{av}	average hydrogen production rate ($ml\ l^{-1}\ h^{-1}$)
HPR_{dw}	hydrogen production rate based on biomass dry weight ($ml\ g\ (dw)^{-1}\ h^{-1}$)
HPR_{Max}	maximum hydrogen production rate ($ml\ l^{-1}\ h^{-1}$)
I	irradiance ($W\ m^{-2}$)
K	constant of exponential growth (h^{-1})
LI	low irradiance ($W\ m^{-2}$)
MI	middle irradiance ($W\ m^{-2}$)
P_{av}	average biomass productivity attained experimentally ($g\ l^{-1}\ h^{-1}$)
PBR	photobioreactor
R^2	correlation coefficient (−)
t	time (h)
t_{50}	time required to reach half BC_{Max} (h)
t_i	interval time between BC_f and BC_0 (h)

Greek symbols

μ	growth rate (h^{-1})
μ_e	specific growth rate (h^{-1})

2. Methods

2.1. Description of the culture system

The culture system used for a co-production of bio-H_2 and biomass rich in oil was a cylindrical glass photobioreactor (internal diameter, 9.6 cm; working volume, 1.07 l) placed in a heat exchanger-Plexiglas water bath at a constant temperature, and the culture was mixed using a magnetic stirrer (Carlozzi, 2009). All experiments were carried out in a thermostatic room and under atmospheric pressure. The gas produced by bacteria cells was first made to flow into a basin containing a saline solution absorber of NaOH, which stripped CO_2 and then the hydrogen was trapped in a calibrated column, where it was collected and the volume measured to determine hydrogen production. The calibrated column was refilled with a saline solution of NaOH every morning.

2.2. Organism and culture conditions

R. palustris (strain 42OL) was grown for the purpose of a co-production of both "bio-H_2 and biomass rich in oils". We used a growth medium for bacteria that had been previously described (Carlozzi et al., 2006), but suitably modified. A nitrogen source (glutamic acid, $0.865\ g\ l^{-1}$) and a carbon source (malic acid, $3.26\ g\ l^{-1}$) were used to produce hydrogen by means of a biological system. The initial pH of the medium was 6.8, and the culture temperature was of 30 ± 0.2 °C. All experiments were carried out under continuous light; at seven different irradiances (36, 56, 75, 151, 320, 500, and $803\ W\ m^{-2}$). The cultures were managed in fed-batch modes by periodically restoring the initial concentrations of both organic substances. This was done when the malic acid reached the average value of $1.0\ g\ l^{-1}$; vice versa, glutamic acid ($0.865\ g\ l^{-1}$) was restored every 72 h. No culture volume was withdrawn from the reactor, but a concentrated stock solution was added to the reactor replacing the small volume withdrawn from the reactor for culture sampling. This feeding strategy was used for long-term investigations (max = 408 h); otherwise, the yields produced (biomass and H_2) would have stopped for lack of macronutrients (Carlozzi, 2009).

2.3. Analytical methods

The growth of phototrophic *R. palustris* (strain 42OL) was determined by means of dry weight (dw) biomass concentration (Carlozzi et al., 2006). Cultures were irradiated with a 250-W OSRAM power-star HQI-TS lamp. The irradiance was measured using a Quantum/Radiometer/Photometer (model LI-185B, LI-COR, Lincoln, Nebraska, USA). In order to determine organic-acid concentrations in the bacteria cultures, a HPLC (Thermo Finnigan – Spectra System 6000 LP) was utilised. The HPLC was equipped with a C18 analytical column (250 × 4.6 mm) and the column temperature was 25 °C. 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 mobile phase was a solution of water + 0.1% H_3PO_4, and the flow was $1.0\ ml\ min^{-1}$. The gas produced (after removing the CO_2 by a saline solution of NaOH) was trapped in a calibrated column, where it was collected and the volume measured to determine hydrogen production. No CO_2 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 TCD detector and a Silica Gel 60/80 Grade 12 column (Alltech, Derfield) (Carlozzi, 2009). The carrier gas was helium; known amounts of pure gases were used to calibrate the instrument.

Total lipid extraction was carried out by grinding 0.25 g of lyophilized bacterial cells in a mortar with sand and by extracting

Table 1
Average biomass productivity and kinetic parameters obtained by means of both Boltzmann's sigmoidal regression and exponential growth models. Data are means ± SE.

Irradiance ($W\ m^{-2}$)	P_{av} ($g\ l^{-1}h^{-1}$)	Boltzmann's sigmoidal regression				Exponential growth	
		BC_{Max} ($g\ l^{-1}$)	t_{50} (h)	K (h^{-1})	R^2 (−)	μ_e (h^{-1})	R^2 (−)
36	0.009	1.75 ± 0.10	79 ± 04	0.0286 ± 0.004	0.9985	0.0166 ± 0.0006	0.9990
56	0.007	2.18 ± 0.13	113 ± 11	0.0205 ± 0.016	0.9862	0.0249 ± 0.0026	0.9927
75	0.006	2.16 ± 0.08	108 ± 12	0.0199 ± 0.016	0.9859	0.0253 ± 0.0004	0.9998
151	0.008	2.26 ± 0.13	125 ± 07	0.0254 ± 0.021	0.9909	0.0241 ± 0.0019	0.9914
320	0.004	2.20 ± 0.11	220 ± 08	0.0113 ± 0.010	0.9965	0.0119 ± 0.0010	0.9872
500	0.007	3.59 ± 0.16	260 ± 08	0.0135 ± 0.012	0.9962	0.0080 ± 0.0004	0.9920
803	0.005	3.09 ± 0.28	302 ± 19	0.0098 ± 0.009	0.9964	0.0052 ± 0.0004	0.9730