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Fed-batch operation for bio-H_2 production by *Rhodopseudomonas palustris* (strain 42OL)

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ABSTRACT

Interest in renewable and clean energies such as hydrogen has increased because of the high level of polluting emissions, increasing costs associated with petroleum and the escalating problems of global climate change. In the presence of a light source, a microbial photosynthetic process provides a system for the conversion of some organic compounds into biomass and hydrogen. Using *Rhodopseudomonas palustris* as a cell-factory, hydrogen photo-evolution was investigated in a photobioreactor (PBR) irradiated either from one or two opposite sides. Irradiating the photobioreactor from only one side, in the presence of malic acid, a reactor hydrogen production of 2.786 l(H_2) PBR^{-1} was achieved. When the PBR was irradiated from two opposite sides, hydrogen photo-evolution increased to 3.162 l(H_2) PBR^{-1}. Experiments were carried out using inoculum from either the retardation or the exponential growth phases. Using the latter, the highest hydrogen photo-evolution rate based on the bacteriochlorophyll (Bchl) concentration was achieved (3295 μl(H_2) mg ($Bchl^{-1}$ h^{-1}). The hydrogen to biomass ratio (r_g) was 1.91 l g^{-1} in the medium containing malic acid and 1.07 l g^{-1} in that containing acetic acid. It was found that the hydrogen production rate was higher with malic than with acetic acid. Although photobiological hydrogen production cannot furnish alone the greater and greater world requirements of clean renewable energy, it is desirable that photobiological hydrogen technology will grow, in the near future, because photobioreactors for bio-hydrogen production can be positioned in fringe areas without competition with agricultural lands.

1. Introduction

The high consumption of fossil fuels via combustion is causing global climate changes because of the emissions of pollutants into the atmosphere. Hydrogen is a clean energy alternative to fossil fuels [1] and may become a primary source of energy in the future [2]. Even if hydrogen is currently more expensive than conventional energy sources, bio-hydrogen technology will most probably play a major role in the future [3].

Methods for the production, uses, and comparative analyses of different biological hydrogenation processes have been noted [2]. Many studies have been carried out on the subject of biological hydrogen production under heterotrophic, photoautotrophic and photo-heterotrophic conditions [4–11]. Under photo-heterotrophic conditions, hydrogen photo-evolution is due to photo-decomposition of organic compounds by photosynthetic bacteria [2,12]. Phototrophic bacteria have been indicated as the most promising microbial system for the biological production of hydrogen [11]. Purple photosynthetic bacteria produce H_2 from organic compounds by an anaerobic light-dependent electron transfer process in which nitrogenase functions as the terminal catalyst [13]. In 1995 the effect of pH on the regulation of hydrogen photoproduction was studied using a nitrogen source like glutamate [14]. In 1998 ammonium-limited chemostat cultures of *Rhodobacter capsulatus* were investigated for their hydrogen photoproduction potential at different dilution rates [4]. In 2000 the effect of light/dark cycle on bacterial hydrogen production by *Rhodobacter sphaeroides* was tested [15]. Recently, resting and/or growing cells have been used for hydrogen production studies [16,17].

In this study, a prolonged bio-H_2 production was investigated using fed-batch cultivations of *Rhodopseudomonas palustris* (strain 42OL). Using inoculum from either the exponential or the retardation growth phases, some experiments were performed with an increasing duration till each one had taken place for 352 h. The photo-decomposition of two organic compounds (malic and acetic acids) was studied. Malic acid was investigated using two different

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Nomenclature

BC_f	final biomass concentration [g(dw) l^{-1}]
BC_0	starting biomass concentration [g(dw) l^{-1}]
Bchl	bacteriochlorophyll (mg l^{-1})
H_2PR_v	hydrogen production rate based on the culture volume (ml l^{-1} h^{-1})
H_2PR_{dw}	hydrogen production rates based on biomass dry weight [(ml g(dw)$^{-1}$ h^{-1})
H_2PR_B	hydrogen production rates based on the Bchl concentration [(μl mg(Bchl)$^{-1}$ h^{-1})]
PBR	photobioreactor
P_R	reactor productivity [g(dw) PBR^{-1}]
r_g	hydrogen to biomass ratio (l g^{-1})
R-H_2P	reactor hydrogen production [l(H_2) PBR^{-1}]
V	working volume (l)

Greek symbols

μ_e	specific growth rate

light intensities. During the investigation, the photobioreactor (PBR) was irradiated either from one or two opposite sides.

2. Materials and methods

2.1. Cultural system

Fig. 1 shows the system used for both bacteria growth and hydrogen photo-evolution. The cylindrical glass photobioreactors (volumes: $V_1 = 1.17$ l and $V_2 = 1.07$ l) were placed in a heat exchanger-Plexiglas water bath of constant temperature and the culture was mixed using a magnetic stirrer. All experiments were carried out in a thermostatic room (22 °C) and atmospheric pressure. Two needles were inserted into the silicone stopper: the first was used to add the substrate into the reactor and the second acted as a gas outlet before the gas was trapped in a graduated glass column.

2.2. Organism and culture conditions

R. palustris (strain 42OL) was grown to investigate hydrogen photo-evolution. A modified growth medium for bacteria that had been previously described in the literature [18] was used. A nitrogen source (glutamic acid, 0.865 g l^{-1}) and two carbon sources (malic and acetic acids) were investigated to produce hydrogen by a biological system. Glutamic acid and either malic or acetic acid were neutralised with sodium hydroxide, before being added to the medium. The starting pH of the medium was 6.8. All experiments were carried out under continuous light. Three culture temperatures (25, 30 and 35 °C) were investigated to determine the optimal temperature. During hydrogen photo-evolution, the culture temperature was then always kept at 30 ± 0.2 °C. Cultures were irradiated with two light intensities (230 μE m^{-2} s^{-1} or 460 μE m^{-2} s^{-1}). When the light intensity was 230 μE m^{-2} s^{-1} the photobioreactor was irradiated either from one or two opposite sides; when the light intensity was 460 μE m^{-2} s^{-1}, the PBR was irradiated only from one side. All experiments except one were carried out using an inoculum collected during the retardation phase: the exception was carried out using an inoculum from the exponential phase of growth (quoted in the text). During hydrogen photo-evolution, the photobioreactors were managed in a fed-batch mode by periodically restoring the initial concentrations of carbon sources. This happened when the carbon source reached the average value of 1.0 g l^{-1}, such as showed in the graphs. Vice-versa, glutamic acid (0.865 g l^{-1}) was restored every 72 h. This feeding strategy of medium was used to investigate for a long period

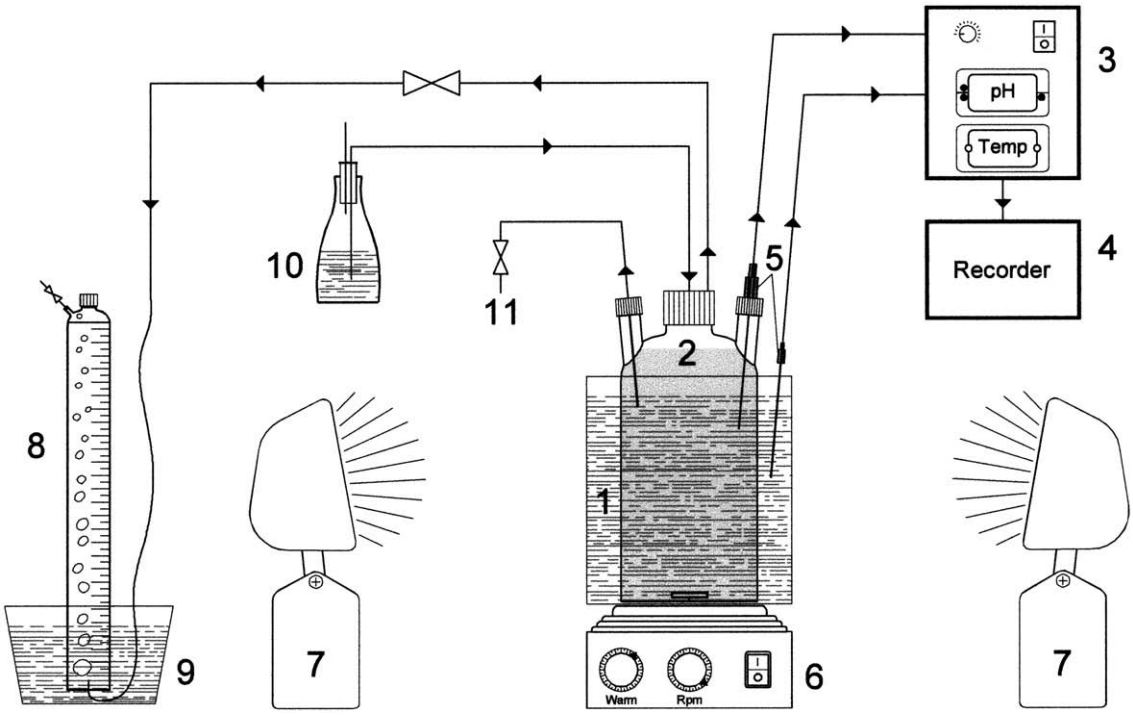

Fig. 1. View of the photobioreactor (placed in a water bath) built for growing *R. palustris* and hydrogen photo-evolution, under artificial light: (1) Heat exchanger water bath (2) Photobioreactor (PBR) (3) Control unit. (4) Recording instrument (5) pH and temperature probes (6) Stirrer (7) Lamps (8) Graduated column trap (9) Saline solution bath (10) sterilised culture medium; (11) sampling.