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Hydrogen Production by *Hup*- Mutant and Wild Type Strains of *Rhodobacter capsulatus* on Dark Fermenter Effluent of Sugar Beet Thick Juice in Batch and Continuous Photobioreactors

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Abstract

The HYVOLUTION project (EU 6th frame) is aimed to develop an integrated process in which biomass is fermented to acetate, lactate, CO_2 and hydrogen followed by photofermentation of acetate and lactate to hydrogen and CO_2 with photosynthetic purple nonsulfur bacteria (PNS bacteria). Growth and hydrogen production of *Rhodobacter capsulatus* was investigated on the dark fermenter effluent of thick juice (processed raw sugar beet juice) which contained acetate and NH_4Cl . In this effluent media, the hydrogen production of wild type bacterium and an uptake-hydrogenase deficient mutant (*hup*-) were compared in small scale (55 ml) batch and large scale (4 L) continuous photobioreactors in indoor conditions under constant illumination of 2000 lux. In continuous operation mode, the overall hydrogen production yields were 1.84 and 1.92 mol H_2 /mol acetate, the maximum hydrogen productivities were 1.29 and 0.89 mmol H_2 /L.h, for the wild type and mutant strains, respectively. On the other hand, in batch operation mode, the overall hydrogen production yields were 1.25 and 1.44 mol H_2 /mol acetate, the maximum hydrogen productivities were 0.28 and 0.52 mmol H_2 /L.h, for the wild type and mutant strains, respectively. The results show that *Rhodobacter capsulatus* is capable of using sugar beet thick juice effluent as substrate for hydrogen production; which makes it a suitable bacterium to be employed in integrated termophilic fermentation-photofermentation process.

1 Introduction

The HYVOLUTION project (EU 6th frame) is aimed to develop an integrated process in which biomass is converted to acetate, lactate, CO_2 and hydrogen by dark fermentation followed by photofermentation of acetate and lactate to hydrogen and CO_2 with photosynthetic purple nonsulfur bacteria (PNS bacteria). In order to combine thermophilic fermentation and photofermentation, it is mandatory to show the suitability of real dark fermentor effluents for the photofermentative hydrogen production by purple nonsulfur (PNS) bacteria.

Previously it was reported that *R. capsulatus* can grow and produce hydrogen successfully on synthetic substrates containing acetate/lactate mixtures [1] as well as dark fermentation

effluents of miscanthus hydrolysate [2], potato steam peels hydrolysate [3] and molasses [4]. Those studies have been carried out at small scale, in batch mode.

In the present study, the effluent solution derived from the fermentation of thick juice (processed raw sugar beet juice) was used as a substrate for hydrogen production in photo-fermentation by wild type *Rhodobacter capsulatus* and its uptake-hydrogenase deficient (*hup*⁻) mutant. The studies were carried out in small scale (50 ml) batch and large scale (4 L) continuous photobioreactors in indoor conditions under constant illumination of 2000 lux.

2 Material and Methods

2.1 Bacteria and culture

Photofermentative bacteria used in this study are wild type and an uptake-hydrogenase deficient mutant (*hup*⁻) strain of *Rhodobacter capsulatus*.

Thick juice dark fermenter effluent (DFE) was obtained from PROFACTOR, Austria. It was centrifuged to obtain a visually clear liquid free from colloidal particles (i.e. bacteria) which may prevent light penetration. The DFE contained 94 mM acetate as the carbon source and 6 mM NH₄Cl as the nitrogen source. The concentration of acetate and NH₄Cl in the medium has a significant impact on the growth lag phase and hydrogen production of *R. capsulatus* [5]. Hence, the thick juice DFE was diluted in 1:2 ratio with distilled water and the acetate and NH₄Cl concentrations were decreased to 31 mM and 2 mM, respectively. In order to keep pH stable at the desired level (6.5-7.5), 20 mM of KH₂PO₄ was added into the diluted media as buffer and the initial pH was adjusted to 6.5. It was previously shown that Fe is an essential element for hydrogen production [6]. In order to enhance the hydrogen production, Fe (0.1 mM Fe-citrate) was added to the effluent solution. Finally, DFE solution was sterilized by autoclave before feeding into the photobioreactors.

2.2 Photobioreactors and operating conditions

In batch experiments, sealed glass bottles with 55 ml culture volume were used as photobioreactors. In continuous experiments, a photobioreactor made by acrylic sheet and PVC frame with 4L working volume was used. 10% (v/v) bacteria inoculation was made into the bioreactors. Argon gas was used to create anaerobic conditions. The bioreactors were connected to graded cylinders initially filled with water by capillary tubing made of steel. The produced gas amount was measured from the displaced water, escaping at the bottom of the gas collectors.

The photobioreactors were maintained at 30-33°C. The illumination was provided by 100 W incandescent lamps to attain a uniform light intensity of 2000 lux at the surface of photobioreactors. Initial pH in photobioreactors was 6.6-6.8.

During continuous runs, 400ml of media from bioreactor (10% of culture volume of 4L photobioreactor) was replaced by fresh media daily corresponding to the dilution rate of 0.1.

2.3 Analytical methods

Light intensity measurements were made by a luxmeter (Lutron). Evolved gas was analyzed by gas chromatography (Agilent Technologies 6890N) equipped with Supelco Carboxen 1010 column. The bacterial cell concentration was determined spectrophotometrically at

660nm using Shimadzu UV-1201 Spectrophotometer. An optical density of 1.0 at 660nm corresponds to the biomass concentration of 0.54 gdcw/L_c for the wild type strain and 0.47 gdcw/L_c for the mutant strain. The consumptions of organic acids were followed by HPLC (Shimadzu, Alltech IOA-1000 Organic Acid Column). A pH-meter (Mettler-Toledo) was used to measure pH.

3 Results and Discussion

Hydrogen production on thick juice DFE was tested in batch and continuous modes. *Rhodobacter capsulatus* wild type and *Rhodobacter capsulatus* *hup*- mutant (YO3) were employed.

Batch mode study was carried out using 55ml bottle photobioreactors. Figure 1 shows the biomass growth and cumulative hydrogen production by wild type and mutant *R. capsulatus* (*hup*-) strains on thick juice DFE. Wild type cells reached to a higher maximum biomass concentration compared to the mutant cells. Hydrogen production started in the first day in both reactors. All the acetate was consumed in the first two days where logarithmic bacterial growth occurred.

Continuous hydrogen production on the DFE was tested using 4L panel photobioreactors. Daily feeding to the photobioreactors were provided at the 2nd day. Figure 2 illustrates the growth and hydrogen production of wild type and *hup*- mutant strains of *R. capsulatus*. Wild type bacteria grew successfully on DFE and reached to a maximum cell concentration of 1.8 gdcw/L_c. Maximum hydrogen productivity achieved was 1.29 mmol/L_c.h. After 9 days, hydrogen production stopped due to the biomass decrease. *R. capsulatus* *hup*- mutant cell density reached to a maximum of 1.3 gdcw/L_c. Hydrogen production started on the third day and it continued until the end of the experiment (for 23 more days). The productivity was at maximum in 3rd day (0.89 mmol/L_c.h), decreased afterwards but stabilized after 17th day between 0.28 – 0.37 mmol/L_c.h.

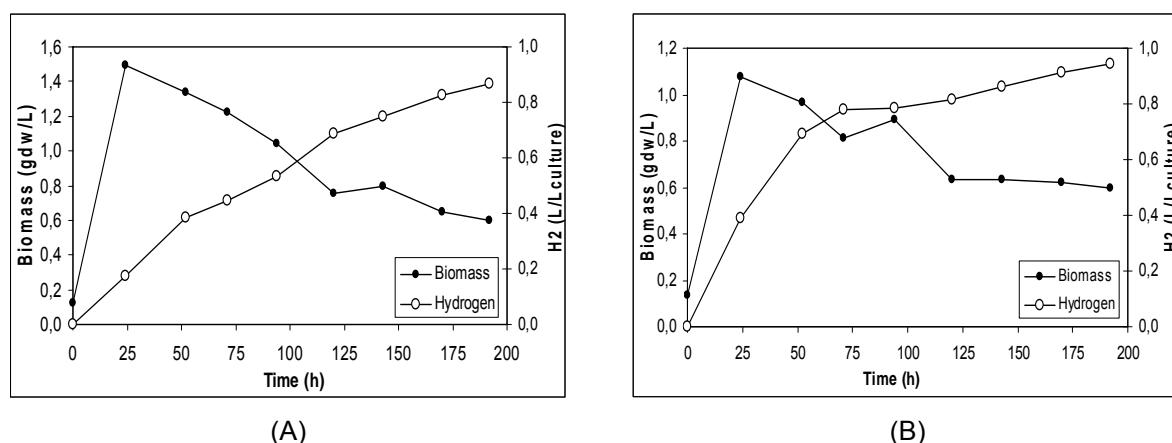


Figure 1: Growth and hydrogen production on DFE by (A) wild type *R. capsulatus* and (B) *R. capsulatus* *hup*- strain in 55mL batch photobioreactors.

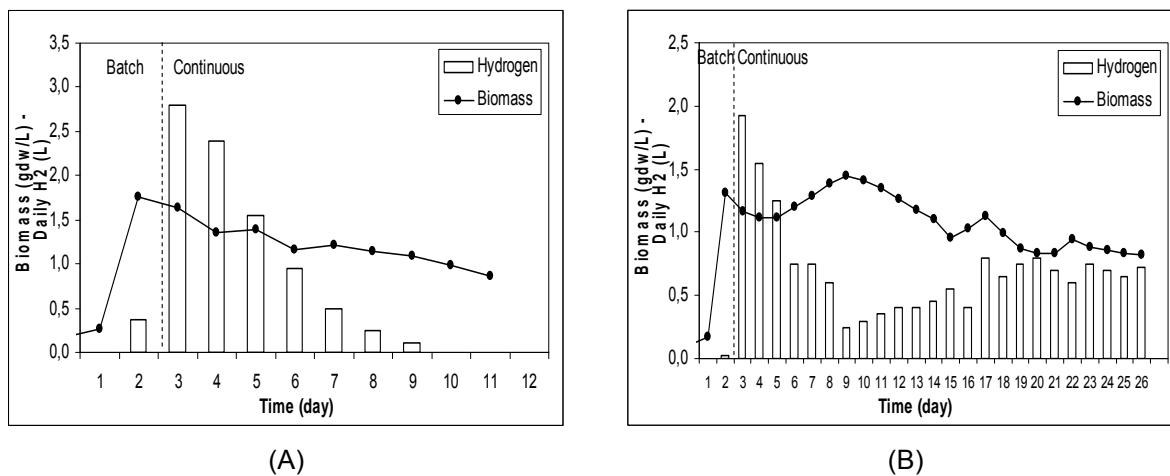


Figure 2: Growth and hydrogen production on DFE by (A) wild type *R. capsulatus* and (B) *R. capsulatus hup-* in 4L continuous panel photobioreactors.

Hydrogen production yields (as percent of theoretical maximum based on consumed substrate) and hydrogen productivities obtained by *hup-* mutant and wild type *Rhodobacter capsulatus* on the DFE in batch and continuous photobioreactors were compared in Table 1.

Table 1: Comparison of the yields and productivities obtained at different operations.

Operating mode	Strain	Duration (Days)	Max Biomass (gdw/L)	Yield (%)	Max Productivity (mmol/L _c .h)
Batch	WT	8	1.54	31	0.28
Batch	Hup ⁻	8	1.09	36	0.52
Continuous	WT	12	1.76	46	1.29
Continuous	Hup ⁻	26	1.44	48	0.89

Rhodobacter capsulatus hup- mutants strains reached to a lower maximum cell concentration but their yield and productivity were higher compared to the wild type cells in both batch and continuous operating modes. The maximum biomass concentration, yield and productivity of hydrogen were higher in continuous mode compared to the batch mode with both bacterial strains.

The presented results show that thick juice DFE is a suitable substrate for the photofermentative hydrogen production, provided that they are supplemented with buffer and nutrients, especially Fe, which is crucial for nitrogenase activity. The dilution of DFEs at the start-up is essential in adjusting the initial acetate concentration to 30 – 40 mM. Sterilization by autoclaving prevents contamination in long term operations. The recommended feeding rate is 10% by the volume of the reactor, daily. In order to control the pH, addition of 20 mM of potassium phosphate buffer to the DFEs was suggested.

Acknowledgement

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