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Hydrogen Production by Photosynthetic Bacteria *Rhodobacter capsulatus* Hup$^-$ Strain on Acetate in Continuous Panel Photobioreactors

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Abstract

Photobiological hydrogen production from organic acids occurs in the presence of light and under anaerobic conditions. Stable and optimized operation of the photobioreactors is the most challenging task in the photofermentation process. The aim of this study was to achieve a stable and high hydrogen production on acetate, using the photosynthetic bacteria *Rhodobacter capsulatus* Hup$^-$ (uptake hydrogenase deleted strain) in continuous panel photobioreactors.

An indoor experiment with continuous illumination (1500-2500 lux, corresponding to 101-169 W/m^2) and controlled temperature was carried out in a 8 L panel photobioreactor. A modified form of basal culture media containing 40 mM of acetate and 2 mM of glutamate with a feeding rate of 0.8 L/day was used. Stable hydrogen productivity of 0.7 mmol H_2/l_c.h was obtained, however, biomass decreased during the continuous operation. Further indoor experiments with a biomass recycle and different feed compositions were carried out to optimise the feed composition for a stable biomass and hydrogen production. The highest hydrogen productivity of 0.8 mmol H_2/l_c.h and yield of 88% was obtained in the 40 mM/ 4 mM acetate/glutamate continuously fed photobioreactor for a period of 21 days.

1 Introduction

Rhodobacter capsulatus is a phototrophic gram-negative purple non sulfur (PNS) bacteria that is able to produce molecular hydrogen under anaerobic and nitrogen-limiting conditions (brought about by having a high C/N ratio). It uses light as the primary energy source and organic compounds such as acetate, lactate and malate as carbon sources [1]. It grows optimally at 25°C – 35°C temperature range and pH 6-9 [2].

Hydrogen production by the PNS bacteria is mediated by nitrogenase metalloenzyme. The membrane bound [Ni-Fe] hydrogenase, also termed as the 'uptake hydrogenase' primarily consumes hydrogen [3]. The removal of the uptake hydrogenase enzyme by genetic modification has been shown to improve hydrogen production [4].

The C/N ratio in the feed media is a significant factor in photofermentative hydrogen production. Glutamate is a suitable nitrogen source for *R. capsulatus* [5, 6]. Eroglu et al. 1999). Different organic acids such as lactate, malate, acetate and butyrate have been used

in photofermentative hydrogen production studies [7]. Being a major product of the dark fermentation process, the use of acetate provides a possibility of integrating dark fermentation with photofermentation to achieve high hydrogen yield. This is the concept employed by the Integrated Project "Non-thermal production of pure hydrogen from biomass" (HYVOLUTION), a Sixth EU Framework supported programme which aims to produce hydrogen from locally obtained biomass using combined thermophillic dark fermentation and photofermentation [8].

The aim of this study is to determine the optimum feed composition for high hydrogen production and stable biomass growth using *Rhodobacter capsulatus* YO3 (Hup^-) in a continuously operated panel photobioreactor (CPBR). Acetate and glutamate were used as carbon and nitrogen sources respectively.

2 Materials and Methods

2.1 Bacteria, growth and hydrogen production media

Rhodobacter capsulatus YO3 (Hup^-), an uptake hydrogenase-deleted strain of the *Rhodobacter capsulatus* MT1131 developed by Öztürk *et al*. [4], was used in this study. The inoculum was prepared by growing cells in modified Biebl and Pfennig (BP) medium [9] that contained 20 mM acetate and 10 mM sodium glutamate. The hydrogen production media comprised the modified BP medium with acetate (40-80 mM) and sodium glutamate (2-4 mM). Sterilization of the media was done by autoclaving.

2.2 Procedure

The experiments were carried out in 8 L panel photobioreactors made of transparent acrylic sheets (plexiglass). The photobioreactors were chemically sterilized using hydrogen peroxide solution (3% w/v) and thoroughly rinsed off with distilled water. Argon gas was flushed to make the system anaerobic [10]. 25% freshly grown bacterial inoculants were used at the start-up.

Continuous feeding with a rate of 0.8 L/day was started when bacterial cell density reached the stationary phase (O.D≥1.5 or 0.7 gdcw/l_c). The indoor experiments were carried out under continuous illumination (1500-2500 lux / 101-169 W/m^2) provided by four 60 W tungsten lamps and controlled room temperature. The photobioreactor temperature was maintained between 30-34 $^{\circ}$C. Hydrogen gas evolved was collected in a water filled graduated glass column.

2.3 Analytical methods

The bacterial cell concentration, pH, temperature, light intensity, gas and organic acid analysis were measured as described previously [11].

3 Results and Discussion

3.1 Hydrogen production experiment

A panel photobioreactor was operated in indoor conditions using feed medium containing 40 mM acetate and 2 mM glutamate. Stable hydrogen productivity of 0.7 mmol H_2/ l_c.h was

obtained but biomass decreased from 0.85 gdw/L_c to 0.42 gdw/L_c within 31 days (Figure 1 - Phase II).

Organic acid analyses showed that acetic acid was well utilized for growth and hydrogen production by the photosynthetic bacteria. Lactic and butyric acids were formed in very low concentrations while formic acid accumulated. pH in the photobioreactors ranged between 6.9-7.2 and gas analysis showed that the total gas produced comprised 95% hydrogen with the rest being carbon dioxide.

3.2 Biomass stabilization in the continuous panel photobioreactors

The biomass decrease trend observed during continuous operation showed that the 40 mM/2 mM Ac/Glu hydrogen production medium was insufficient for maintaining stable biomass and hydrogen production. Hence, further experiments with biomass recycle and changing the C/N ratio by altering glutamate (2-4 mM) and acetate (40-80 mM) concentrations in the feed media were carried out.

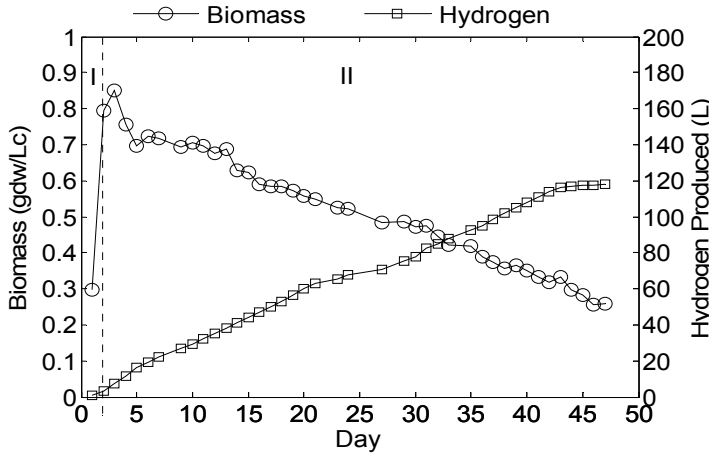

Figure 1: The 40 mM/2 mM Ac/Glu fed indoor CPBR. Phase (I) Startup-Batch, phase (II) Continuous phase.

3.2.1 Biomass recycle

In the biomass recycle experiment, the CPBR effluent was centrifuged, then the pellet was resuspended in hydrogen production medium and fed back into the photobioreactor. Results showed that the biomass recycle was not sufficient to curb the biomass decrease in the CPBR.

3.2.2 Effect of changing C/N ratio

The C/N ratio was decreased by increasing the glutamate concentration in the feed sequentially from 2 mM to 4 mM while maintaining acetate constant at 40 mM. Figure 2 shows the productivities and yields attained. The highest hydrogen productivity (0.8 mmol H_2/ l_c.h) with a stable biomass concentration (0.40 gdw/L_c) was achieved using the 40 mM/4 mM acetate/glutamate containing feed for 21 days. Lactic and butyric acids were formed in very

low concentrations while an increase in formate formation was observed with the increase in glutamate concentration.

In other experiments, The C/N ratio was increased by increasing acetate concentration (40 mM, 60 mM and 80 mM) while keeping the glutamate concentration at 4 mM in the feed. Higher biomass concentrations and lower hydrogen productivities and yields were observed with increasing acetate concentrations (Figure 2). The biomass concentrations obtained were 0.40 gdw/L_c, 0.54 gdw/L_c and 1 gdw/L_c in the 40 mM, 60 mM, and 80 mM acetate fed CPBRs, respectively. Accumulation of acetic acid was observed in the 60 mM and 80 mM acetate fed CPBRs. This shows that continuous systems operating at certain stable biomass concentration have an acetate threshold above which accumulation occurs.

3.2.3 Effect of increasing glutamate and acetate concentration in the feed media keeping C/N ratio constant

The CPBRs fed by the 40 mM /2 mM Ac/Glu, 60 mM /3 mM Ac/Glu and 80 mM /4 mM Ac/Glu (all having C/N= 45) are compared. Higher biomass concentrations were obtained with increasing C and N sources concentration in the feed. However, hydrogen productivity and yield decreased (Figure 2). The highest hydrogen productivity of 0.7 mmol H_2/l_c.h was achieved in the 40 mM /2 mM Ac/Glu CPBR. The other two CPBRs had 0.5 mmol H_2/L_c.h of hydrogen productivity.

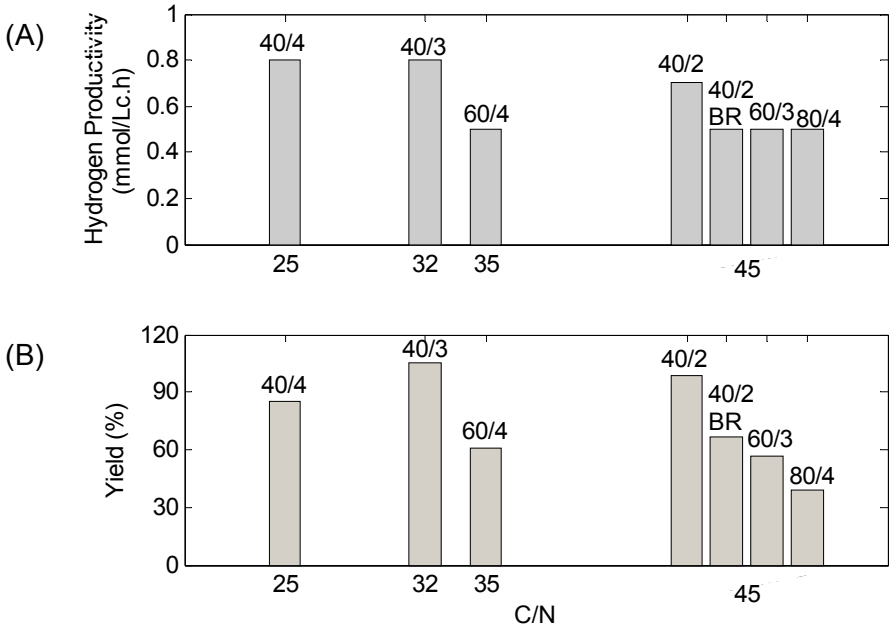

Figure 2: (A) Hydrogen productivity. (B) Yield in the CPBRs operated by different acetate and glutamate containing feeds. (40/4 stands for 40 mM Ac/ 4 mM Glu).

3.3 Light conversion efficiencies in the CPBRs

The light conversion efficiency, which is a percentage measure of the amount of absorbed light energy that is utilized for hydrogen production, was found to range between 0.24-1.21%. Low light conversion efficiency (<10%) has been reported to be the major drawback in photofermentative hydrogen production. Light energy (photons) absorbed is mostly dissipated as heat energy [12].

4 Conclusions

The composition of the feed media is an important factor to be considered in continuous operation. Stable biomass and hydrogen production levels highly depend on the carbon and nitrogen ratio. The optimum C/N ratio of 25 and feed media consisting 40 mM acetate and 4 mM glutamate is recommended in continuously operated photobioreactors with *Rhodobacter capsulatus* YO3 (*Hup*-) strain.

Acknowledgements

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