CHEMICAL ENGINEERING TRANSACTIONS Volume 18, 2009
Editor Jiří Klemeš

 ISBN 978-88-95608-04-4 ISSN 1974-9791
DOI: 10.3303/CET0918057

Investigation Of Influencing Factors For Biological Hydrogen Production By *R. Capsulatus* In Tubular Photo-Bioreactors

E. Boran[1], E. Ozgur[1], J.Gebicki[2], J. van der Burg[3], M .Yucel[4], U.Gündüz[4], M.Modigell[2,] I.Eroglu[1]
[1]METU Dept. Chem. Eng. Ankara, Turkey, eboran@metu.edu.tr
[2]RWTH Aachen University Dept. Chem. Eng. Aachen, Germany
[3]Technogrow B.V. Netherlands
[4]METU Dept. Biol. Ankara, Turkey

Biological hydrogen production processes are considered as an environmentally friendly way to produce hydrogen. They offer the chance to produce hydrogen from renewable energy sources, like sunlight and biomass. This study aims the process development for a photo-fermentative hydrogen production by photosynthetic purple-non-sulfur bacteria, *Rhodobacter capsulatus,* in a large scale (80L) tubular photo-bioreactor, in outdoor conditions, using acetate as carbon source. It was shown that *Rhodobacter capsulatus* had a rapid growth (μ_{max} = 0.025 h^{-1}). Moreover, at light intensities below 10000 lx (100W/m^2 solar illumination) the hydrogen production does not occur. It was found that the optimum dry cell weight for hydrogen production was in between 0.8 to 1 g/L in order to increase light penetration. Hydrogen production was not observed for the pH values higher than 8. The productivity in terms of illuminated area and day time was 0.3 mmol $H_2/m^2_{ill}.hr_{ill}$ in December 2008 in a glasshouse. The yield of hydrogen was 19 mg H_2/g acetate input. The acetate utilization was %87.5 at a feed rate of 10L/day and substrate conversion efficiency was %16. As a result, hydrogen production in a pilot scale tubular photobioreactor for continuous hydrogen production was achieved by *R. capsulatus* in outdoor conditions. This study showed that photo-fermentation in a pilot scale tubular photobioreactor would provide hydrogen successfully, even in the low light intensity.

1. Introduction

Hydrogen is seen as the most important energy carrier of the future as having highest energy content per unit weight. Biological hydrogen production provides sustainability to the hydrogen energy as it utilizes various renewable sources like biomass and sunlight. Several types of microorganisms can be utilized for biological hydrogen production through biophotolysis, dark fermentation and photofermentation [Das and Veziroglu, 2001]. The HYVOLUTION (EU 6th Framework Programme) is an integrated project aims to develop a combined process by utilizing the biomass for the production of hydrogen in two steps. In the first step, biomass is utilized for hydrogen production by dark-fermentation using thermophilic bacteria. In the second step, the effluent of dark fermentation is further utilized for hydrogen production by photofermentation with photosynthetic purple non-sulfur bacteria (Claassen and de

Please cite this article as: Boran E., Ozgur E., Gebicki J., Van Der Burg J., Yucel M., Gunduz U., Modigell M. and Eroglu I., (2009), Investigation of influencing factors for biological hydrogen production by rhodobacter capsulatus in tubular photo-bioreactors, Chemical Engineering Transactions, 18, 357-362 DOI: 10.3303/CET0918057

Vrije, 2006). Photosynthetic bacteria like purple non sulfur (PNS) bacteria undergo anoxygenic photosynthesis and produce hydrogen using light energy by utilizing simple organic acids. They can grow at a pH of 6-9 depending on the substrate source and have an optimum temperature range between 25 and 35 ^{o}C. The advantages of photosynthetic bacteria are high theoretical yield with different kinds of organic substrates and their resistance to high light intensity and changing environmental conditions (Sasikala et al., 1993). Factors affecting biological hydrogen production from photosynthetic bacteria has been investigated previously (Eroglu et al., 1999). It was found that cell concentration and carbon to nitrogen ratio affect the hydrogen production rate significantly and PNS bacteria should be under nitrogen limitation in order to increase the hydrogen productivity. Later, Uyar et al. (2007) investigated the effect of light intensity and wavelength on hydrogen production. It was shown that near infrared light is necessary to enhance the hydrogen production and light intensity plays an essential role for the photo-fermentation. It was also stated light saturation is an important factor in photobioreactor studies, therefore, certain design criteria have to be met in order to have high surface to volume ratio. Another important factor is continuous stirring which influences the homogenous light and substrate distribution. Zabut et.al. (2006) reported that continuous stirring, consistency in pH and moderate bacterial density enhanced the hydrogen production rate. In addition to these factors, temperature effect is also crucial. Sasikala et al. (1993) investigated the enzymatic behavior of microorganisms and concluded that they have an optimum temperature to sustain their activities. The optimum temperature range for PNS bacteria were reported as 25-35 ^{0}C. A solar panel photobioreactor was operated in outdoor conditions in batch mode and it is found that in the outdoor conditions substrate conversion efficiencies were low as PHB and carotenoid are produced (Eroglu et al., 2008).

The objective of the present work is to develop a continuous process for hydrogen production in a pilot nearly horizontal tubular photobioreactor in outdoor conditions on acetate using *Rhodobacter capsulatus* and to investigate the factors affecting the hydrogen production rate.

2. Materials and methods

2.1 Bacterium and The Reactor Start-Up

Rhodobacter capsulatus wild type (DSM 1710) was obtained from Deutsche Sammlung von Mikroorganismen (DSM, Braunsweig Germany). Bacteria were activated in modified Biebl and Phennig (1981) medium containing 20mM acetate and 10mM glutamate as carbon and nitrogen sources, respectively. The amount of inoculation in activation was 10% by volume of the fresh growth medium. The modified medium of Biebl and Phennig (1981) containing Acetate (40mM) as the carbon source and Na-Glutamate (2mM) as the nitrogen source was used for the hydrogen production. In order to keep the pH stable, buffer concentration of the original B&P medium was increased to 22 mM. Tubular reactor was designed by Technogrow B.V. Netherlands and provided to Ankara by RWTH Aachen. Reactor was settled on a bench with an inclination of 10^{0}. Tubular reactor consisted of 9 tubes, with 60mm of diameter and 2.35 m length and had a total volume of 80L. The total illuminated surface area was 2m^2 and total ground area was 2.88 m^2. Before the startup, tubular reactor was sterilized with the H_2O_2 solution (50 ppm) and washed with distilled water. The inoculation rate was %20 in the tubular reactor. *Rhodobacter capsulatus* wild type (DSM 1710) was grown in the outdoor under anaerobic conditions by flushing the tubular reactor with argon. During

the exponential phase the reactor was illuminated with artificial light source (2 × 500W) continuously and not circulated. At the end of exponential phase, artificial illumination was removed and feeding started (10L/day) with the continuous circulation (210 mL/s) during day time with a rotary pump. The feed contained 40mM of acetic acid and 2 mM of Na-Glutamate which corresponds to a C/N ratio of 45. The hydrogen was collected by the water displacement method. The experiments are carried out in a glass house to protect the reactor from freezing, during winter in Ankara.

2.2 Analytical methods

The organic acid in the effluent was analyzed by HPLC (Shimadzu, Alltech IOA-1000 Organic Acid Column). Gas was analyzed by GC (Agilent Technologies 6890N Supelco Carboxen 1010 column). Growth was followed spectrophotometrically at 660nm (Shimadzu UV-1201 Spectrophotometer) and dry cell weights were calculated by the calibration curve (OD_{660} of 1.0 corresponds to 0.543g/L). The pH, temperature (using Testo 830 T-2) and the light intensity (using Lutron LX-105 Light Meter) were followed.

3. Results and discussion

Nearly horizontal tubular photobioreactor was operated continuously using *R. capsulatus*, in outdoor conditions during December, in Ankara. Bacteria were grown successfully in outdoor conditions with a specific growth rate (μ_{max}) of 0.025 h^{-1} during the exponential phase, which is comparable to the data obtained from the indoor studies at constant temperature. As shown in Figure 1, after the feeding started, the dry cell weight was kept stable around 0.94 g/L throughout the stationary phase. This result is comparable with the previous results (Eroglu et al. 1999, 2008). Higher cell concentrations (above 1g/L) caused an increase in the pH of the reactor above 8.0, which is not tolerable for the hydrogen production from PNS bacteria. These results obey the previous findings of Koku et al. (2002) and Khapitov et al. (1998), where pH values above 8.0 was found to inhibit the hydrogen production. The pH changes throughout the experiment are illustrated in Figure 3.

Hydrogen production started at the late-exponential phase (when dry cell weight is 0.8 g/L) and continued throughout the stationary phase (Figure 1). This result was in accordance with previous findings (Sasikala et al., 1995; Koku et al., 2003). It was observed that, above the bacterial density 1 g/L, hydrogen productivity was greatly decreased (11^{th}-13^{th} December), because of the decrease in light penetration to the deeper sites of the reactor. Hydrogen productivity decreases with decreasing light intensity (Uyar et al, 2007). Illuminated day duration was approximately 9 hours in Ankara during December. During this period the light intensity changes significantly, depending on the weather conditions. In Figure 2 the effect of light intensities on hydrogen production is illustrated. It was observed that when the light intensity is below the average value of 10000 lx (100W/m^2 solar illumination), hydrogen productivity seizes (13^{th}-26^{th} December). This was also observed in solar panel reactors (Eroglu et al. 2008).

Temperature fluctuation is one of the most important factors affecting hydrogen production. In the month December in Ankara, although outdoor temperature fluctuates between -10 and 20 ^{0}C the temperature range is between 5 to 35 ^{0}C in the glass house. Reactor temperature variations are shown in Figure 2, and it is observed that, in the first 15 days of the month, overall temperature of the reactor is higher than the last 15 days.

This is another factor that affect the hydrogen production and it is obvious that in the first period hydrogen productivity higher.

The effluent samples from photobioreactor was analysed daily for its organic acid (acetic, lactic, formic, butyric and propionic acids) composition (Figure 3). The concentration of organic acids other than acetic acid was negligible and the acetic acid concentration was stable around 5mM throughout the experiment. When the feed composition and the effluent composition were considered, utilization of the supplied organic acid (acetate) can be calculated as 87.5%. However, the substrate conversion efficiency (moles of H_2 produced per mole of H_2 that can theoretically be produced if all acetate was used for H_2 production) was calculated as 16%, which shows that most of the substrate was utilized for growth and maintenance.

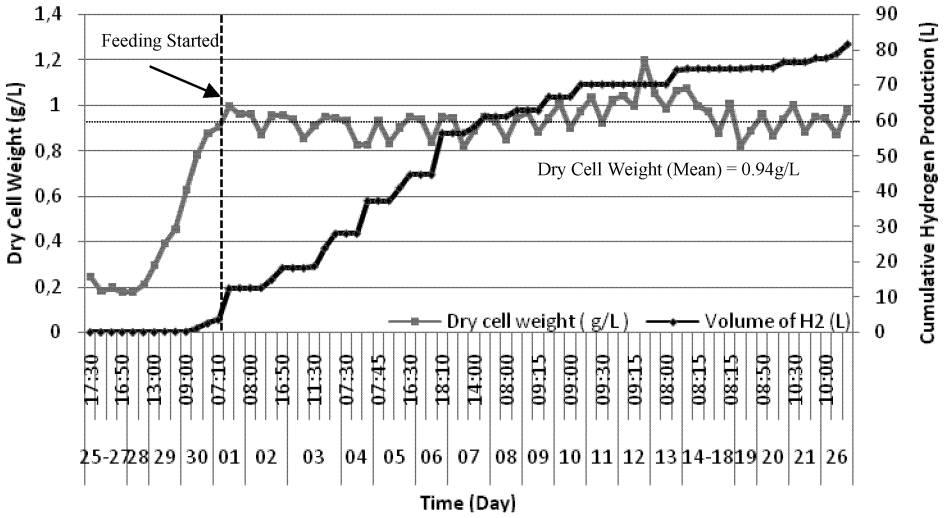

Figure 1: Comparison of the Hydrogen Production (♦) and the biomass growth (■). Feeding was started at the 7th day of growth, and the hydrogen production started at the 6th day. A stable cell concentration of 0.94g/L_c was obtained

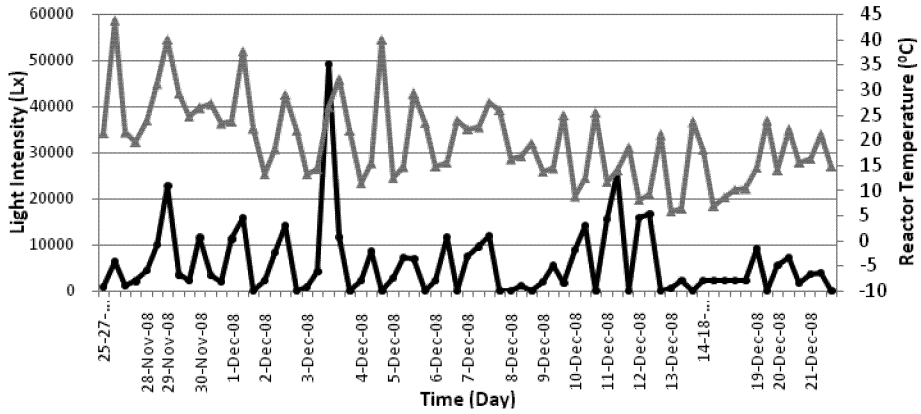

Figure 2: Changes in Light Intensity (●) and Reactor Temperature (▲) (10000lx = 100W/m^2)

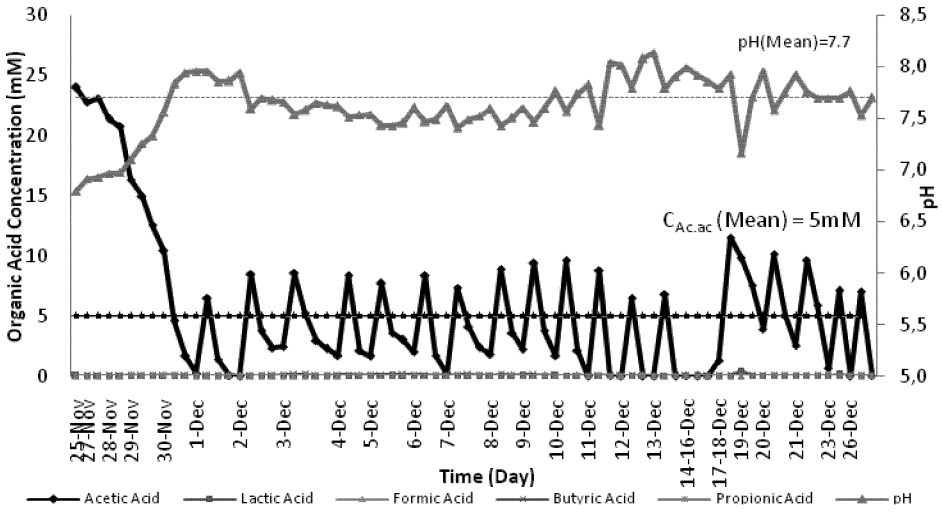

Figure 3: Effluent Composition (●) vs. pH (▲)

Almost no hydrogen production was observed in the last 10 days of experiment. During these days, the average temperature of the reactor was lower relatively and light intensities were below 10000 lx. Table 1 summarizes the productivities and yields obtained over 21 days of high rate hydrogen production. The total illuminated surface area was $2m^2$ and cumulative hydrogen produced was 78 L until 21st of December. Moreover, high dry cell weight resulted high pH affected the productivity between the 11th and 14th of December.

Table 1– Hydrogen Productivity, Yield and Acetic Acid Consumption Data of R.capsulatus in 80L Continuous Tubular Reactor during December 2008 in Outdoor Conditions (The values are the average of three weeks steady operation)

Productivity			*Substrate Conversion Efficiency*	*Yield*		*Conversion*
ml H_2/ $m_{2\ ill.}.hr_{ill.}$	*mmol* $H_2/L_{c.}.hr_{ill.}$	*ml* H_2/ $L_{c.}.hr_{ill.}$	*%*	*mg* H_2 / $g_{Ac.acid\ input}$	*mg* H_2 / $g_{Ac.acid\ utilized}$	*%*
276	0.3	7	16	19	21	87.5

4. Conclusion

Growth, hydrogen production and organic acid utilization of *Rhodobacter capsulatus* wild type (DSM 1710) were examined in a large scale (80L) tubular reactor in outdoor conditions during December 2008 in Ankara. It was seen that at light intensities below 10000 lx hydrogen production decreases. An increase in the bacterial density decreases light penetration and also affects the pH which inhibits the hydrogen production rates. Therefore optimum bacterial density range is between 0.8-1 g/L. Moreover temperature fluctuations and decrease in reactor temperature reduced the productivity. Acetic acid concentration was stable around 5mM in the reactor and it is sufficient to obtain a productivity of 7 ml $H_2/L_{culture}.\ hr_{ill.}$ The conversion obtained was 87.5% however; the

substrate conversion efficiency was 16% which shows most of the substrate was utilized for growth and maintenance. As a result of this study, pilot scale tubular photobioreactor development for continuous hydrogen production with acetate containing media is achieved for the first time.

Acknowledgements

This study was supported by EU 6th Framework Project "HYVOLUTION". Thanks to Technogrow B.V, Netherlands and RWTH Aachen University for providing the tubular Reactor materials and special thanks to Dominic Deo Androga for HPLC analysis.

5. References

Biebl, H., and Phennig, N., 1981, The Prokaryotes, vol 1: Isolation of the members of the family *Rhodosprillaceae*, In: Starr, M.P., Trüper, H.G., Balows, A. and Schlegel, H.G., Springer, New York, 267-273

Claassen,P.A.M. and de Vrije, T., 2006, Non-thermal production of pure hydrogen from biomass: HYVOLUTION. Int. J. Hydrogen Energy. 31, 1416-1423

Das, D., Veziroglu, T.N., 2001, Hydrogen production by biological processes: a survey of literature, Int. J. Hydrogen Energy. 26, 13-28

Eroglu, I., Aslan, K., Gunduz U., Yucel M. and Turker L., 1999, Substrate consumption rates for hydrogen production by *Rhodobacter sphaeroides* in a column photobioreactor, Journal of Biotechnology. 70, 103-113.

Eroglu, I., Tabanoglu, A., Gunduz, U., Eroglu, E., and Yucel. M., 2008, Hydrogen production by *Rhodobacter sphaeroides* O.U.001 in a flat plate solar bioreactor, Int. J. Hydrogen Energy. 33, 531-541

Khatipov, E., Miyake, M., Miyake, J., and Asada, Y., 1998, Accumulation of poly-β-hydroxybutyrate by *Rhodobacter sphaeroides* on various carbon and nitrogen substrates, FEMS Microbiol Lett, 162, 39-45

Koku, H., Eroglu, I., Gunduz, U., Yucel, M., and Turker, L., 2002, Aspects of the metabolism of hydrogen production by *Rhodobacter sphaeroides,* Int. J. Hydrogen Energy. 27, 1325-1329

Koku, H., Eroglu I., Gunduz U., Yucel M. and Turker L., 2003, Kinetics of biological hydrogen production by the photosynthetic bacterium *Rhodobacter sphaeroides* O.U.001. Int. J. Hydrogen Energy. 28, 381-388

Sasikala, K., Ramana, C.V., Rao, P.R., and Kovacs, K.L., 1993, Anoygenic Phototropic Bacteria: Physiology and Advances in Hydrogen Production Technology, Advanced Applied Microbiology, 38, 211-295

Sasikala, C., Ramana, C.V., and Rao, P.R., 1995, Regulation of simultaneous hydrogen photoproduction during growth by pH and glutamate in *Rhodobacter sphaeroides* O.U.001, Int. J. Hydrogen Energy. 20, 123-126

Uyar, B., Eroglu, I., Yücel, M., Gündüz. U and Türker, L., 2007, Effect of light intensity, wavelength and illumination protocol on hydrogen production in photobioreactors, Int. J. Hydrogen Energy. 32, 4670-4677

Zabut, B., El-Kahlout, K., Yücel, M., Gündüz. U., Türker, L., and Eroglu, I.,2006, Hydrogen gas production by combined systems of *Rhodobacter sphaeroides* O.U.001 and *Halobacterium salinarium* in a photobioreactor, Int. J. Hydrogen Energy. 31, 1553-1562