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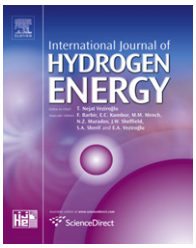

Hydrogen productivity of photosynthetic bacteria on dark fermenter effluent of potato steam peels hydrolysate

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ARTICLE INFO

Article history:
Received 28 July 2010
Received in revised form
20 September 2010
Accepted 26 September 2010
Available online 15 October 2010

Keywords:
Biohydrogen
Photofermentation
Dark fermentation
Potato steam peels
Purple non-sulfur bacteria

ABSTRACT

Hydrogen productivities of different photosynthetic bacteria have been searched on real thermophilic dark fermentation effluents (DFE). The results obtained with potato steam peels hydrolysate (PSP) DFE were compared to glucose DFE. Photobiological hydrogen production has been carried out in indoor, batch photobioreactors using several strains of purple non-sulfur (PNS) bacteria such as *Rhodobacter capsulatus* (DSM1710), *Rhodobacter capsulatus hup-* (YO3), *Rhodobacter sphaeroides* O.U.001 (DSM5864), *Rb. sphaeroides* O.U.001 *hup-* and *Rhodopseudomonas palustris*.

The efficiency of photofermentation depends highly on the composition of the effluent and the PNS bacterial strain used. *Rb. sphaeroides* produced the highest amount of hydrogen on glucose DFE. *Rb. capsulatus* gave better results on PSP DFE. This study demonstrates that photobiological hydrogen production with high efficiency and productivity is possible on thermophilic dark fermentation effluents. Consequently, a sequential operation of dark fermentation and photofermentation is a promising route to produce hydrogen, and it provides a higher hydrogen yield compared to single step processes.

1. Introduction

Hydrogen has been accepted as an energy carrier of the future since it does not release CO_2 and other "greenhouse gasses" into the environment and it is environmentally clean. Its combustion liberates large amounts of energy per unit weight and gives only water as a by-product. Biological hydrogen production by using microorganisms has gained a considerable importance, since it can utilize renewable resources like agricultural wastes, can be operated at ambient temperature and atmospheric pressure and is less energy intensive when compared to the chemical hydrogen production methods [1]. Biohydrogen production can be achieved by using microalgae, cyanobacteria, fermentative or photosynthetic bacteria. Photofermentation by using purple non-sulfur bacteria (PNS) offer some advantages since oxygen which represses hydrogen producing enzymes is not generated. PNS bacteria can utilize a wide variety of organic substrates such as organic acids, sugars, fatty acids or waste products of factories with high substrate conversion efficiencies. They can show a great metabolic diversity so that they can survive in widely differentiated physiological conditions and can use a wide range of wavelengths of the light spectrum [2,3].

A sequential dark and photofermentation is a promising route for biohydrogen production since more hydrogen can be obtained compared to single stage dark fermentation or photofermentation processes. During dark fermentation, carbohydrate containing substrate is converted to organic acids,

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doi:10.1016/j.ijhydene.2010.09.096

CO_2 and hydrogen by mesophilic or thermophilic bacteria. In the second stage, the effluent of dark fermentation containing organic acids such as acetate and lactate is utilized in photofermentation by photosynthetic bacteria for further hydrogen production. Studies on sequential dark fermentation and photofermentation for biohydrogen production show that higher hydrogen yields compared to single stage processes can be obtained with pure sugars [4–6] and cheaper feed stocks like miscanthus hydrolysate [7], molasses [8], wheat starch [9], and cassava starch [10].

In an EU 6th Frame Project, HYVOLUTION (Contract No:019825-SES6), sequential operation of dark fermentation and photofermentation approach has been adapted to achieve a cost effective biohydrogen production from biomass, ranging from energy crops to bio-residues from agro-industries. During dark fermentation, biomass is converted to hydrogen by thermophilic bacteria. Subsequently, the organic acids produced during the thermophilic fermentation are used by PNS bacteria via photofermentation process [11].

In this study, photobiological hydrogen production potentials of different PNS bacteria have been tested and compared to each other based on their utilization of the dark fermentation effluents (DFEs) of glucose and potato steam peels (PSP) hydrolysate. DFEs were obtained by anaerobic thermophilic dark fermentation using *Caldicellulosiruptor saccharolyticus*. Photofermentation was carried out by using *Rhodobacter capsulatus* wild type (DSM1710), *Rhodobacter capsulatus* (MT1131) *hup-* strain, *Rhodobacter sphaeroides* O.U.001 (DSM5648) wild type, *Rb. sphaeroides* O.U.001 (DSM5648) *hup-* strain and *Rhodopseudomonas palustris* (DSM127) in indoor batch photobioreactors.

2. Materials and methods

2.1. Bacterial strains and growth medium

Wild type *Rb. capsulatus* (DSM1710), wild type *Rb. sphaeroides* O.U.001 (DSM5648), and *Rp. palustris* (DSM127) were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany. *Rb. capsulatus* (MT1131) *hup-* strain was a gift of Dr Yavuz Öztürk [12] and *Rb. sphaeroides* O.U.001 (DSM5648) *hup-* strain was a gift of Dr Gökhan Kars [13]. Bacteria were activated in modified Bieble & Pfennig medium [14] containing acetate (15 mM), glutamate (10 mM) and 22 mM of potassium phosphate buffer (pH 6.4).

2.2. Dark fermenter effluents

Dark fermentations were carried out with glucose (20 g/L) or PSP hydrolysate (15 g/L of mainly maltodextrines). Starch in PSP was hydrolyzed by α-amylase prior to dark fermentation. Dark fermentation effluents were obtained from the batch cultures of the extreme thermophile *C. saccharolyticus* operated at 72 ± 1 °C [15]. DFEs were produced by Wageningen UR Food & Biobased Research, Netherlands and sent to METU.

The dark fermenter effluent (DFE) of glucose, containing acetate (114 mM), lactate (6 mM), glucose (20 mM) and NH_4Cl (1 mM) was diluted by three times with sterile dH_2O.

The dark fermenter effluent (DFE) of potato steam peel hydrolysate (PSP) containing acetate (102 mM), lactate (28 mM) and NH_4Cl (4.0 mM) was sterilized and diluted by three times with sterile dH_2O. The effluent was supplemented with 20 mM potassium phosphate buffer (pH 6.4), iron (Fe-citrate, 0.1 mM) and molybdenum ($NaMO_4 \cdot 2H_2O$, 0.16 μM).

2.3. Photobioreactors

Prior to the photofermentation experiments, DFEs were sterilized by autoclaving and diluted with sterile dH_2O in order to decrease the ammonium chloride concentration to around 1 mM and acetate concentration to around 30 mM. Photofermentation was carried out in 55 mL glass bottles in batch mode. After bacterial inoculation (10% v/v) of DFEs, the bioreactors were first flushed with argon to obtain an anaerobic atmosphere and then connected to a water-filled graduated glass cylinder by capillary tubes for collecting the evolved gas. The bioreactors were illuminated by 100 W tungsten lamps, with a light intensity of 150–200 W/m^2 at the surface. The experiments were carried out at 30 °C in a cooling incubator (Nüve, ES250) and repeated 3 times using the same source of DFE.

2.4. Analytical methods

The elemental analyses of DFEs were carried out by using an atomic absorption spectrophotometer (Philips, PU9200X). Organic acids were analyzed by high performance liquid chromatography (HPLC) on a MetaCarb 87H column (300 × 7.8 mm, by Varian). Evolved gas was analyzed by a gas chromatograph (Agilent Technologies 6890N) equipped with thermal conductivity detector and a Supelco Carboxen 1000 column (60/80 mesh). The oven, injector and detector temperatures were 140, 160 and 170 °C, respectively. Argon was used as carrier gas at a flow rate of 25 mL/min. The bacterial biomass concentration was determined from the optical densities of the cultures at 660 nm measured by a spectrophotometer (Shimadzu UV-1201). An optical density of 1.0 at 660 nm corresponds to a cell density of 0.55 g dry weight/L of culture (gdw/L_c). The pH was measured with a pH-meter (Mettler–Toledo). The light intensity was measured by a lux meter (Lutron).

2.5. Data analysis

The productivity (Q_{H2}) was calculated from the slope of the cumulative hydrogen production versus time graph (mmol/L_c h). The specific productivity (q_{H2}) was estimated by dividing the volumetric productivity to the average cell density at the stationary phase (mmol/g biomass h). The molar hydrogen yield was estimated as the percent of the ratio of mole of hydrogen produced per mole of the theoretical hydrogen that can be produced from the consumed organic acids and sugars. Thermophilic DFEs contained acetate and lactate, as organic acids and glucose as sugar. The stoichiometric equations for hydrogen production from acetate, lactate and glucose are given below:

Acetate: $C_2H_4O_2 + 2H_2O \rightarrow 4H_2 + 2CO_2$ (1)

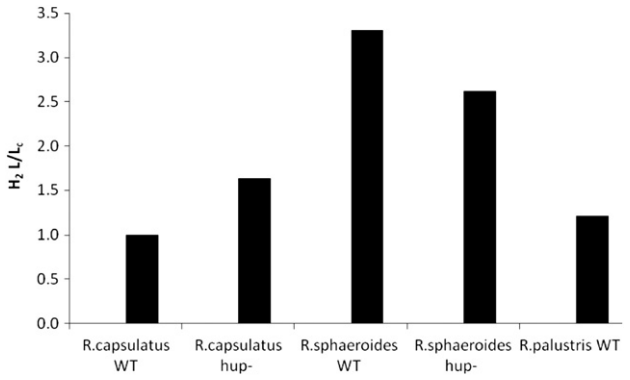

Fig. 1 – Hydrogen production by different strains of PNS bacteria on 3 times diluted glucose DFE. The results are average of 3 independent experiments.

Lactate: $C_3H_6O_3 + 3H_2O \rightarrow 6H_2 + 3CO_2$ (2)

Glucose: $C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2$ (3)

3. Results and discussions

Biological hydrogen production by sequential operation of dark fermentation and photofermentation is a promising route for biohydrogen production as it offers higher yields than a single step process. In this study, hydrogen production by several strains of photosynthetic PNS bacteria has been tested on the thermophilic dark fermentation effluents of *C. saccharolyticus* operated with an artificial medium containing glucose as a carbon source and NH_4Cl as a nitrogen source and on potato steam peels hydrolysate. NH_4Cl concentration is a critical factor for hydrogen production from PNS bacteria as it inhibits nitrogenase enzyme activity [17]. To enable photosynthetic hydrogen production, lower amount of NH_4Cl was used in dark fermentation step. The glucose DFE contained 1 mM of NH_4Cl, which is tolerable for photoheterotrophic hydrogen production. However, the PSP DFE contained 4 mM NH_4Cl, which is high enough to inhibit nitrogenase activity. For this reason, NH_4Cl concentration was reduced by dilution.

The DFEs contained a high concentration of acetate (102–114 mM). Acetate is an intermediary metabolite of bacterial metabolism, and can be used for both biosynthetic and catabolic purposes; therefore, its initial concentration can be an important parameter for optimum hydrogen production [18]. It was stated that the initial concentration of acetate may play an important role for directing the metabolism towards hydrogen production, and 30–40 mM of acetate was found to be the optimum for high yields of hydrogen production from *Rb. capsulatus* bacteria. Similarly, Asada et al. [19] reported the highest hydrogen yield on 21–42 mM of acetate by *Rb. sphaeroides* RV. Hence, we reduced the initial concentration to 30–35 mM by dilution. Undiluted effluent yielded very low amounts of hydrogen by all the strains of PNS bacteria tested in this study (data not shown).

3.1. *Photofermentative hydrogen production on DFE of glucose*

Hydrogen productions on glucose DFE by different PNS bacterial strains are compared in Fig. 1, and the growth, productivities and yields obtained are summarized in Table 1. *Rb. sphaeroides* produced the highest amount of hydrogen on diluted DFE with a productivity of 1.18 mmol H_2/L_c h. The yield attained was 60% of theoretical maximum, which is comparable to the yields obtained with the same strain on artificial medium containing malate and glutamate as carbon and nitrogen sources, respectively [19,20]. Asada et al. [19] reported a similar yield (66%) with *Rb. sphaeroides* RV using artificial medium containing 42 mM of initial acetate. However, the yield on artificial medium containing 30 mM of acetate was reported to be 33% for *Rb. sphaeroides* O.U 001 [20]. Higher yields on glucose DFE might be attributed to the presence of glucose in the effluent which can be fermented by PNS bacteria into volatile fatty acids [21].

The uptake hydrogenase deficient mutant (*hup-*) strain of *Rb. sphaeroides* O.U.001 showed lower hydrogen production compared to its wild type, although, its performance was 30% higher than the wild type on artificial medium containing malate (15 mM) and glutamate (2 mM) [19]. Compared to malate-grown cultures, significantly lower hydrogen production was observed with *hup-* mutant of *Rb. sphaeroides* when grown on acetate as a sole carbon source [22].

Compared to *Rb. sphaeroides*, *Rb. capsulatus* performed poorly on glucose DFE. Its productivity and yield results were

Table 1 – The maximum biomass concentration, molar hydrogen yield, volumetric and specific productivities and cumulative hydrogen production attained by different PNS bacteria on 3× diluted glucose DFE.

	Biomass Concentration gdw/L_c	Molar H_2 yield (% of theoretical max)	Volumetric productivity (Q_{H2}) mmol $H_2/(L_c$ h)	Specific productivity (q_{H2}) mmol H_2/(g biomass h)	H_2 production mmol H_2/L DFE
Rb. capsulatus	0.8	18	0.43	0.54	147
Rb. capsulatus hup-	0.8	30	0.62	0.78	240
Rb. sphaeroides	0.9	60	1.18	1.31	484
Rb. sphaeroides hup-	0.7	48	0.84	1.2	384
Rp. palustris	0.3	22	0.33	1.1	179

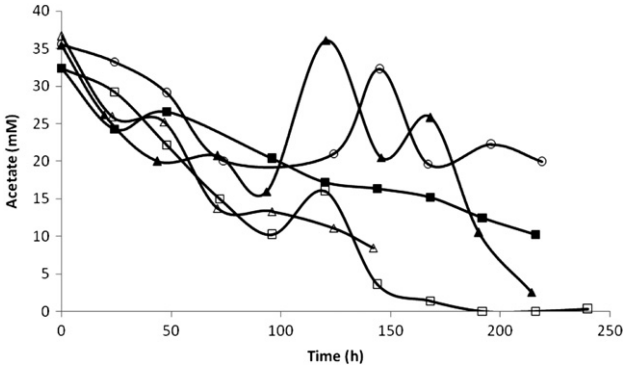

Fig. 2 – Acetate consumption by (△) *Rb. capsulatus* (DSM1710) wild type, (▲) *Rb. capsulatus hup-* strain (MT1131), (□) *Rb. sphaeroides* O.U.001 (DSM5648) wild type, (■) *Rb. sphaeroides* O.U.001 (DSM5648) *hup-* mutant, (○) *Rp. palustris* (DSM127) on glucose DFE.

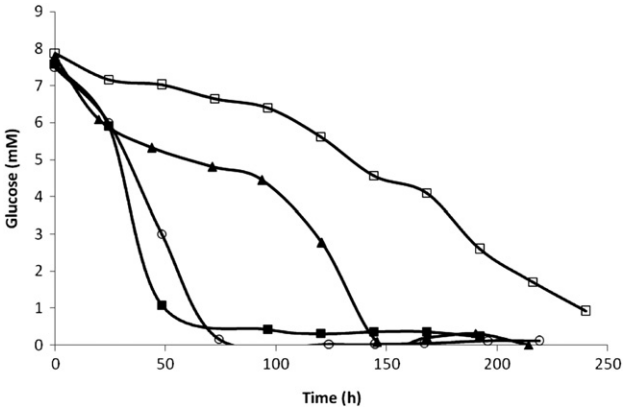

Fig. 4 – Glucose consumption by (▲) *Rb. capsulatus hup-* strain (MT1131), (□) *Rb. sphaeroides* O.U.001 (DSM5648) wild type, (■) *Rb. sphaeroides* O.U.001 (DSM5648) *hup-* mutant, (○) *Rp. palustris* (DSM127) on glucose DFE.

lower than the ones obtained on synthetic BP medium containing 30 mM of acetate and 2 mM of glutamate, where productivity and yield achieved were 0.76 mmol H_2/L_c h and 2.02 L/L_c, respectively [18]. The values obtained with *hup-* strain of *Rb. capsulatus* were higher than the wild type strain. Among the strains tested, the lowest productivity and yield results were observed by *Rp. palustris* (Table 1).

Acetate and lactate consumptions were not complete in some of the reactors, and there were fluctuations in the acetate and lactate concentrations during the process (Figs. 2 and 3). This was attributed to the presence of glucose in the effluent which might yield some acetate and lactate during utilization (Fig. 4). Glucose consumption was more rapid by *Rp. palustris* and *hup-* strain of *Rb. sphaeroides* compared to the other strains tested. For high yields in two-step fermentation, it is important that along with short chain organic acids, the PNS bacteria can also consume glucose, which may remain unfermented at the end of first step fermentation. The energy stored in sugar substrates can also be utilized for biomass growth or for hydrogen production by PNS bacteria.

The pH of the culture was varied in between 6.5 and 7.8 during the course of hydrogen production. Hydrogen production does not occur at pH values below 6.5 or above 8.0. The optimal pH range for various PNS bacteria is 6.8–7.5 [23]. Addition of buffer may further improve the hydrogen yield.

3.2. *Photofermentative hydrogen production on PSP DFE*

The PSP DFE contained 102 mM acetate, 28 mM lactate and 4.0 mM NH_4Cl. In order to reduce the acetate and NH_4Cl concentrations, the DFE was diluted by 3 times. As opposed to glucose DFE, addition of buffer (20 mM potassium phosphate buffer, pH 6.4) was found to be necessary for keeping the pH stable during operation. The elemental analysis of PSP DFE is compared with the synthetic Bieble & Pfennig medium in Table 2. The Fe and Mo, two important co-factors of

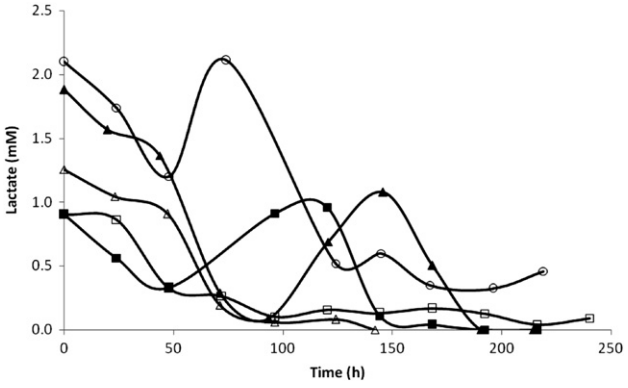

Fig. 3 – Lactate consumption by (△) *Rb. capsulatus* (DSM1710) wild type, (▲) *Rb. capsulatus hup-* strain (MT1131), (□) *Rb. sphaeroides* O.U.001 (DSM5648) wild type, (■) *Rb. sphaeroides* O.U.001 (DSM5648) *hup-* mutant, (○) *Rp. palustris* (DSM127) on glucose DFE.

Table 2 – Comparison of the composition of PSP DFE and Bieble & Pfennig synthetic medium.

	BP Medium	PSP DFE
Total C (M)	0.070	0.79
Total N (M)	0.002	0.04
C/N molar ratio	35	21.3
Acetate (mM)	30	102
Lactate (mM)	–	26
NH_4Cl(mM)	–	1.2
Fe (mM)	0.1	0.017
Mg (mM)	2	1.5
Zn (μM)	0.51	7.58
Mn (μM)	0.51	2.3
B (μM)	0.97	6.67
Co (μM)	0.84	0.94
Cu (μM)	0.12	1.06
Ni (μM)	0.084	0.31
Mo (μM)	0.16	0.001

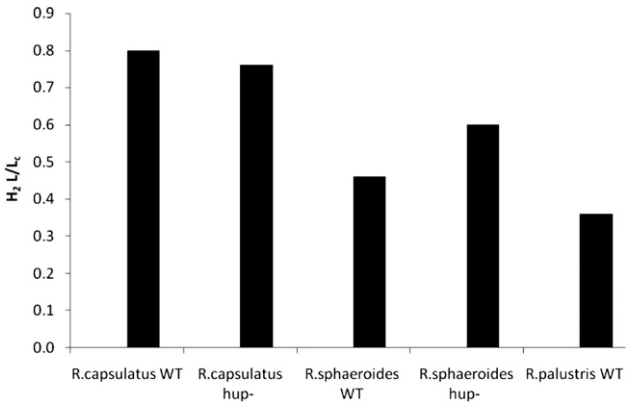

Fig. 5 – Hydrogen production by different strains of PNS bacteria on 3 times diluted and adjusted PSP DFE. The results are average of 3 independent experiments.

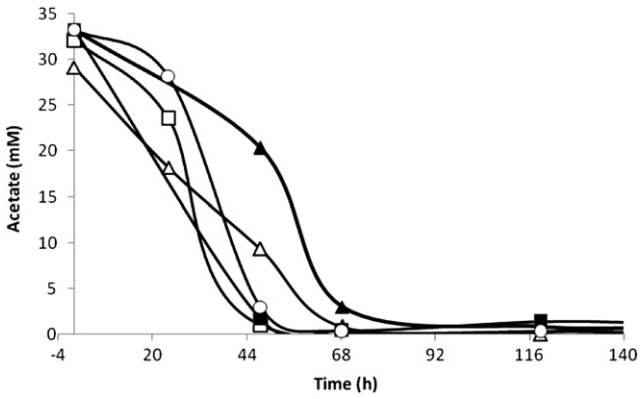

Fig. 6 – Acetate consumption by (△) *Rb. capsulatus* (DSM1710) wild type, (▲) *Rb. capsulatus hup-* strain (MT1131), (□) *Rb. sphaeroides* O.U.001 (DSM5648) wild type, (■) *Rb. sphaeroides* O.U.001 (DSM5648) *hup-* mutant, (○) *Rp. palustris* (DSM127) on PSP DFE.

nitrogenase enzyme, were much lower in PSP DFE. For this reason, the effluent was also supplemented with Fe and Mo, as previously suggested by Özgür et al. [16]. Non-supplemented effluent did not produce hydrogen. The highest productivity (0.55 mmol H_2/L_c h) and yield (24%) values were achieved by *Rb. capsulatus* wild type (DSM 1710).

Hydrogen production on PSP DFE by different strains of PNS bacteria is given in Fig. 5. Table 3 summarizes the growth, hydrogen productivities and yields achieved by different PNS bacteria on PSP DFE. The highest cumulative hydrogen production, productivity and molar yields were obtained by *Rb. capsulatus* wild type and *hup-* strain, followed by *Rb. sphaeroides hup-*, *Rb. sphaeroides* wild type and *Rp. palustris*. This can be attributed to a slightly higher biomass concentration of *Rb. capsulatus* wild type compared to the other strains grown on PSP DFE (Table 3).

Acetate and lactate were consumed completely by all PNS strains tested (Figs. 6 and 7) within the first three days of experiment. Unlike glucose DFE, a linear consumption of both substrates was observed in PSP DFE.

All the strains tested produced lower amounts of hydrogen on PSP DFE than that achieved on glucose DFE. Moreover, the strains acted differentially on different DFE. On glucose DFE, *Rb. sphaeroides* yielded the highest hydrogen, while on PSP DFE, *Rb. capsulatus* gave better results. The reason might be the difference in the carbon source of two DFE, as glucose DFE contained significant amount of glucose (20 mM), while PSP DFE contained no glucose. Furthermore, PSP DFE contained a higher concentration of lactate. The preference for the carbon source may differ significantly from strain to strain. In addition, glucose DFE is a defined feedstock, while PSP DFE was derived from a real biomass feedstock, and contains many molecules, elements, etc. coming from potato peels. These may have affected the hydrogen production. Detailed characterization of the PSP DFE is necessary to determine if any inhibitor is present.

In their recent review about integration of dark- and light-fermentation, Redwood et al. [24] summarized the yields obtained by different microorganisms using different artificial substrates or biomass feed stocks. It was pointed out that results vary significantly from strain to strain and from feedstock to feedstock. The results obtained in this study are in accordance with the literature; hydrogen production on artificial or real DFEs by photofermentation is possible however productivities and yields achieved vary significantly depending on the type of feedstock.

Table 3 – The maximum biomass concentration, molar hydrogen yield, volumetric and specific productivities and cumulative hydrogen production attained by different PNS bacteria on 3× diluted PSP DFE.

	Biomass Concentration gdw/L_c	Molar H_2 yield (% of theoretical max)	Volumetric productivity (Q_{H2}) mmol $H_2/(L_c$ h)	Specific productivity (q_{H2}) mmol H_2/(g biomass h)	H_2 production mmol H_2/L DFE
Rb. capsulatus	1.27	24	0.55	0.43	117
Rb. capsulatus hup-	1.16	23	0.50	0.43	112
Rb. sphaeroides	1.10	14	0.21	0.19	67
Rb. sphaeroides hup-	1.00	20	0.31	0.31	88
Rp. palustris	1.16	11	0.16	0.14	53

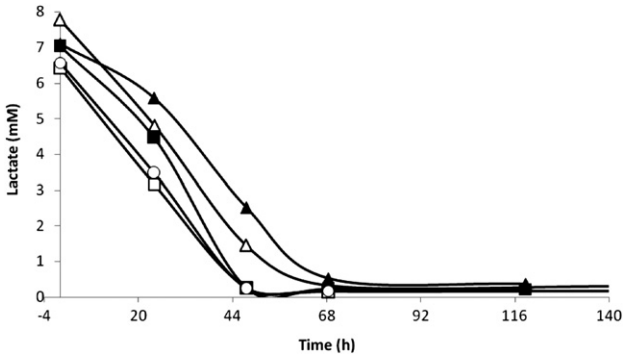

Fig. 7 – Lactate consumption by (△) *Rb. capsulatus* (DSM1710) wild type, (▲) *Rb. capsulatus hup-* strain (MT1131), (□) *Rb. sphaeroides* O.U.001 (DSM5648) wild type, (■) *Rb. sphaeroides* O.U.001 (DSM5648) *hup-* mutant, (○) *Rp. palustris* (DSM127) on PSP DFE.

4. Conclusion

PNS bacteria acted diversely on different sources of DFEs. Hence, use of defined co-cultures might be beneficial to achieve a high efficiency of hydrogen production on different sources of biomass, due to the diverse preference of PNS bacteria for substrates and other metabolites or elements that might be present in the real feedstock. Using co-cultures might be advantageous for overcoming the difficulties of varying feedstock properties, because it is more probable that one of the strains adapts better to the feedstock used. This may also be beneficial for obtaining a more stable operation for continuous cultures.

PNS bacteria are able to ferment glucose into volatile fatty acids which may further be converted to H_2 and CO_2 via photofermentation. For high yields of two-step fermentation, it is important that along with short chain organic acids, the PNS bacteria can also consume glucose, which may remain unfermented at the end of the first step fermentation. The energy stored in sugar substrates can also be utilized for biomass growth or hydrogen production by PNS bacteria.

Acknowledgements

This study was supported by EU 6th Framework Project – HYVOLUTION (Contract No. 19825). We would like to thank to Ms Kerime Güney for the elemental analysis of PSP DFE and Dr. Tamay Şeker from METU Central Laboratory for HPLC analysis.

REFERENCES

[1] Das D, Veziroglu TN. Hydrogen production by biological processes: a survey of literature. Int J Hydrogen Energy 2001; 26:13–28.
[2] Basak N, Das D. The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: the present state of the art. World J Microbiol Biotechnol 2007;23: 31–42.
[3] Kars G, Gündüz U. Towards a super H_2 producer: improvements in photofermentative biohydrogen production by genetic manipulations. Int J Hydrogen Energy 2010;35:6646–56.
[4] Tao Y, Chen Y, Wu Y, He Y, Zhou Z. High hydrogen yield from a two-step process of dark and photofermentation of sucrose. Int J Hydrogen Energy 2007;32:200–6.
[5] Nath K, Muthukumar M, Kumar A, Das D. Kinetics of two-stage fermentation process for the production of hydrogen. Int J Hydrogen Energy 2008;33:1195–203.
[6] Chen CY, Yang MH, Yeh KL, Chang JS. Biohydrogen production using sequential two-stage dark and photo fermentation processes. Int J Hydrogen Energy 2008;33: 4755–62.
[7] Uyar B, Schumacher M, Gebicki J, Modigel M. Photoproduction of hydrogen by *Rhodobacter capsulatus* from thermophilic fermentation effluent. Bioprocess Biosyst Eng 2009;32:603–6.
[8] Özgür E, Mars AE, Peksel B, Louwerse A, Yücel M, Gündüz U, Claassen PAM, Eroğlu I. Biohydrogen production from beet molasses by sequential dark and photofermentation. Int J Hydrogen Energy 2010;35:511–7.
[9] Argun H, Kargi F, Kapdan IK. Hydrogen production by combined dark and light fermentation of ground wheat solution. Int J Hydrogen Energy 2009;34:4305–11.
[10] Su H, Cheng J, Zhou J, Song W, Cen K. Improving hydrogen production from cassava starch by combination of dark and photo fermentation. Int J Hydrogen Energy 2009;34:1780–6.
[11] Claassen PAM, de Vrije T. Non-thermal production of pure hydrogen from biomass: HYVOLUTION. Int J Hydrogen Energy 2006;31:1416–23.
[12] Öztürk Y, Yücel M, Daldal F, Mandacı S, Gündüz U, Türker L. Hydrogen production by using *Rhodobacter capsulatus* mutants with genetically modified electron transfer chains. Int J Hydrogen Energy 2006;31:1545–52.
[13] Kars G, Gunduz U, Rakhely G, Yucel M, Eroğlu I, Kovacs KL. Improved hydrogen production by uptake hydrogenase deficient mutant strain of *Rhodobacter sphaeroides* O.U.001. Int J Hydrogen Energy 2008;33:3056–60.
[14] Bieble H, Pfennig N. Isolation of members of the family *Rhodosprillaceae*. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG, editors. The prokaryotes, vol. 1. New York: Springer; 1981. p. 267–73.
[15] De Vrije T, Mars AE, Budde MAW, Lai MH, Dijkema C, De Waard P, et al. Glycolytic pathway and hydrogen yield studies of the extreme thermophile *Caldicellulosiruptor saccharolyticus*. Appl Microbiol Biotechnol 2007;74(6): 1358–67.
[16] Özgür E, Afsar N, de Vrije T, Yücel M, Gündüz U, Claassen PAM, et al. Potential use of thermophilic dark fermentation effluents in photofermentative hydrogen production by *Rhodobacter capsulatus*. J Clean Prod; 2010;. doi:10.1016/j.jclepro.2010.02.020.
[17] Drepper T, Groß S, Yakunin AF, Hallenbeck PC, Masepohl B, Klipp W. Role of GlnB and GlnK in ammonium control of both nitrogenase systems in the phototrophic bacterium *Rhodobacter capsulatus*. Microbiology 2003;149:2203–12.
[18] Özgür E, Uyar B, Öztürk Y, Yücel M, Gündüz U, Eroglu I. Biohydrogen production by *Rhodobacter capsulatus* on acetate at fluctuating temperatures. Resour Conservat Recycl 2010; 54:310–4.
[19] Asada Y, Ohsawa M, Nagai Y, Ishimia, Fukatsu M, Hideno A, Wakayama T, et al. Re-evaluation of hydrogen productivity from acetate by some photosynthetic bacteria. Int J Hydrogen Energy 2008;33:5147–50.

[20] Uyar B, Eroglu I, Yücel M, Gündüz U. Photofermentative hydrogen production from volatile fatty acids present in dark fermentation effluents. Int J Hydrogen Energy 2009;34: 4517–23.
[21] Eroglu E, Gunduz U, Yucel M, Eroglu I. Photosynthetic bacterial growth and productivity under continuous illumination or diurnal cycles with olive mill wastewater as feedstock. Int J Hydrogen Energy 2010;35:5293–300.
[22] Kars G, Gündüz U, Yücel M, Rakhley G, Kovacs K, Eroglu I. Evaluation of hydrogen production by *Rhodobacter sphaeroides* O.U.001 and its hupSL deficient mutant using acetate and malate as carbon sources. Int J Hydrogen Energy 2009;34: 2184–90.
[23] Sasikala K, Ramana CV, Raghuveer PR. Phototrophic bacteria: physiology and advances in hydrogen production Technology. In: Neidleman S, Laskin AI, editors. Advances in Applied Microbiology, vol. 38. New York: Academic Press; 1993. p. 211–95.
[24] Redwood MD, Paterson-Beedle M, Macaskie LE. Integrating dark and light bio-hydrogen production strategies: towards the hydrogen economy. Rev Environ Sci Biotechnol 2009;8: 149–85.