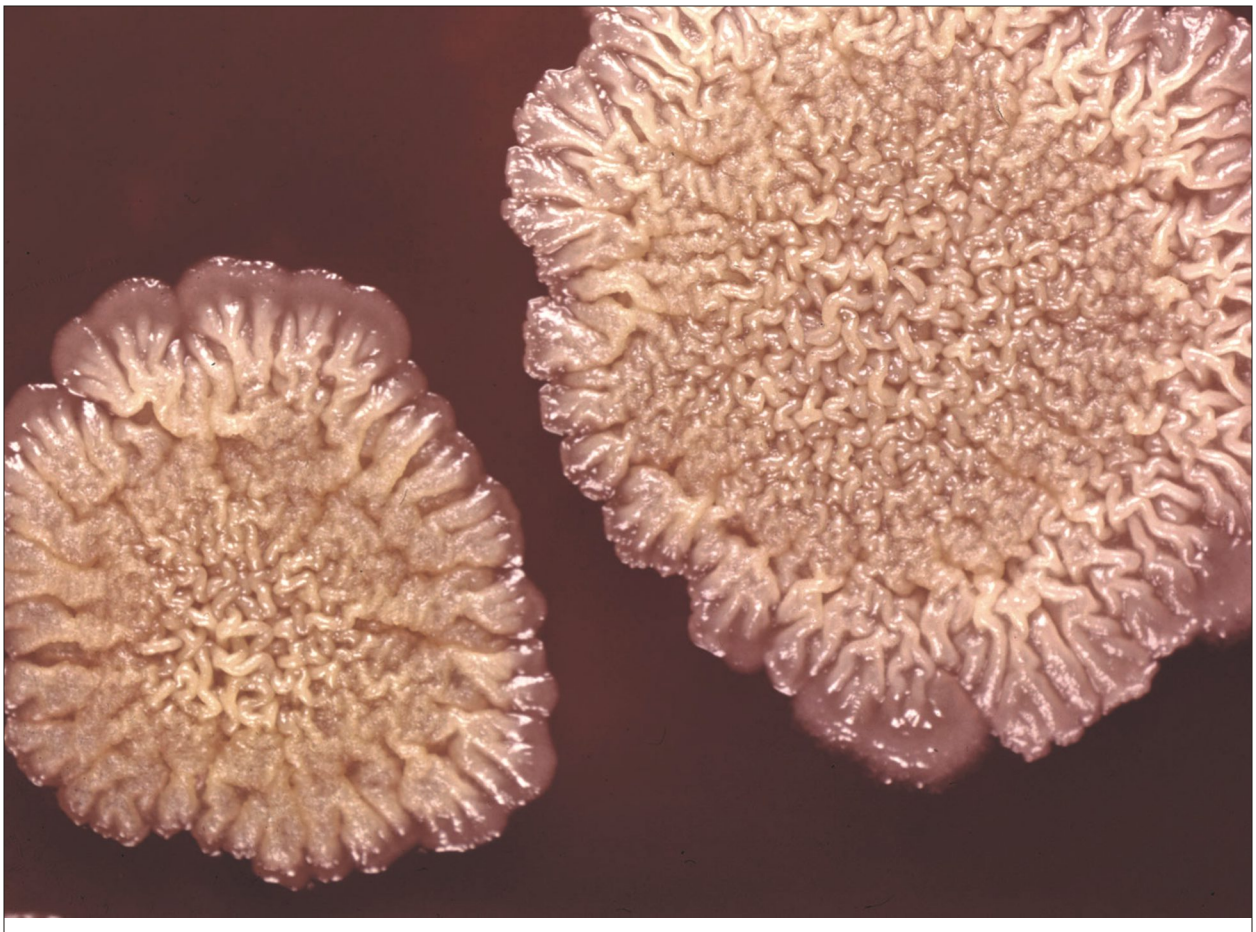

Bacteriology

Journal

ISSN 2153-0211

Academic
Journals Inc.

www.academicjournals.com

Bacteriology Journal 1 (1): 8-15, 2011
ISSN 2153-0211 / DOI: 10.3923/bj.2011.8.15

Effects of Different Initial pH, Argon Gas and Nitrogen Gas on Cell Growth and Hydrogen Production using *Rhodobacter sphaeroides*

Syaripah Za'imah Syed Jaapar, Ehsan Ali, Mohd. Sahaid Kalil and Nurina Anuar
Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment Universiti Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia

Corresponding Author: Mohd Sahaid Kalil, Department of Chemical and Process Engineering, Biotechnology Pilot Plant Laboratory, Faculty of Engineering and Built Environment, University Kebangsaan Malaysia (UKM), 43600 Bangi Selangor, Malaysia Tel: +60389216419, +60193345042 Fax: +60389216148

ABSTRACT

Hydrogen is considered as a promising alternative fuel and energy carrier by virtue of the fact that it does not evolve the CO_2 in combustion. Some non-sulfur photosynthetic bacteria are potent hydrogen producers, utilizing organic acids or alcohols under specific environmental conditions. Effects of different parameters including different initial pH, use of argon and nitrogen gas on hydrogen production were investigated in detail using *R. sphaeroides* NCIMB 8253 in batch culture. The cultures were grown aerobically and anaerobically and hydrogen production was investigated at different initial pH ranging from 6-11. It was found that initial pH 7 can be designated as optimum pH for maximum cumulative H_2 production of 45.03 mL, yield per gram of substrate (225.17 mL g^{-1}) and rate of H_2 production (3.13 mL/L/h) compared to other initial pH within the range of 6-11. Argon gas was found better than nitrogen gas to create more suitable anaerobic conditions for maximum H_2 production using *R. sphaeroides* NCIMB 8253. Culture sparged with argon gas at the beginning produced 45.03 mL H_2 after 144 h incubation; whereas culture sparged with N_2 gas only produced 23.50 mL H_2 after 120 h incubation. The findings reveal that the use of argon gas to create anaerobic environment and the initial pH 7 could favor the enhanced hydrogen production using *Rhodobacter sphaeroides* NCIMB 8253.

Key words: *Rhodobacter sphaeroides*, photofermentation, biohydrogen, biofuel, nitrogen gas, argon gas

INTRODUCTION

Depletion of fossil fuel reserves and emerging environmental problems (Asif and Muneer, 2007) have stimulated the research in the field of new sustainable energy sources that could be environment friendly and a substitute to fossil fuels.

Hydrogen is considered as a promising alternative fuel and energy carrier by virtue of the fact that it does not evolve the CO_2 in combustion. Hydrogen gas is clean fuel with no green house gas emissions and can easily be used in fuel cells for generation of electricity. Hydrogen has a high energy yield of 122 kJ g^{-1} which is 2.75 times greater than hydrocarbon fuels. Due to increasing need for hydrogen energy, development of cost-effective and efficient hydrogen production technologies has gained significant attention in recent years. Photosynthetic bacteria undergo anoxygenic photosynthesis with organic compounds or reduced sulfur compounds as electron donors (Truper and Fischer, 1982). Some non-sulfur photosynthetic bacteria are potent hydrogen

producers, utilizing organic acids or alcohols as electron donors. Since light energy is not required for water oxidation, the efficiency of light energy conversion to hydrogen gas by photosynthetic bacteria is in principle much higher than that by Cyanobacteria. Clostridia such as *C. buytricum* (Yokoi *et al*., 2001), *C. thermolacticum* (Collect *et al*., 2004) and some other anaerobic Clostridia produce hydrogen gas during the exponential growth phase.

Conventional hydrogen production involves non catalytic partial oxidation of fossil fuels and auto thermal reforming which requires high temperature. However, some cost effective techniques have also been introduced including membrane processes, selective oxidation of methane and oxidative dehydrogenation (Armor, 1999). Biological hydrogen from photosynthetic bacteria has advantages because of the ability of the bacteria to utilize a variety of substrates including waste material to produce H_2 with high yield and their ability to act photosynthetically in a wide spectrum of light energy (Fascetti *et al*., 1998; Koku *et al*., 2002).

Hydrogen gas production is more environment friendly by using biological methods as compared to the chemical methods because the chemical methods may cause some unusual environmental and industrial problems, e.g., production of hydrogen gas from the gasification of biomass comes with many problems such as the existence of unacceptable level of tars and low efficiency of the catalysts because of coke deposition (Alalayah *et al*., 2010; Misi *et al*., 2011). Hydrogen can also be generated using *Clostridium saccharoperbutylacetonicum* N1-4 from the different raw material including cellulosic biomass or glucose (Alalayah *et al*., 2009). Hydrogen production by *R. sphaeroides* and some Purple Non Sulfur (PNS) bacteria can be achieved under illumination in the presence of an inert, anaerobic atmosphere, from the breakdown of organic substrates. The culture medium should be under a nitrogen limitation (i.e., a high C/N ratio) which forces the bacteria to 'dump' the excess energy and reducing power by producing H_2. Several individual components constitute the overall production system and these may include the enzyme systems, the carbon flow specifically the TCA cycle and the photosynthetic membrane apparatus. These components are interconnected within the H_2 production scheme by means of the exchange of electrons, protons and ATP. It can be inferred from the preceding description that for the PNS bacteria, H_2 production of any significance occurs under a photoheterotrophic growth mode which is a preferred growth mode for these microorganisms. Yet, PNS bacteria are capable of several alternative metabolic modes such as aerobic or anaerobic respiration, fermentation and photoautotrophy (Koku *et al*., 2002). The studies revealed that PNS bacteria have been investigated for their potential to convert light energy into H_2 using waste organic compounds as substrate (Levin *et al*., 2004). Among all of the photosynthetic bacteria, *R. sphaeroides* has been studied widely for H_2 production (Fang *et al*., 2006). Fermentation is a process which is highly influenced by different environmental parameters (Emily *et al*., 2009) and each strain need to be studied for the optimum values required establishing a set of standard conditions for enhanced hydrogen production. Effects of operating parameters including agitation, aeration and light on hydrogen production using *R. sphaeroides* NCIMB 8253 has already been investigated (Jaapar *et al*., 2009). Effects of age of inoculum, size of inoculum and headspace on hydrogen production using *Rhodobacter sphaeroides* have also been reported by Jaapar *et al*. (2011).

The batch fermentation is a popular strategy to produce value added products using microorganisms. A lot of enzymes and other value added products can be produced by using bacterial fermentation (Fonseca and Antonio, 2007; Ray, 2011).

The proposed study is a continuity of our previous studies regarding important parameters which may contribute well for hydrogen production from *R. sphaeroides* NCIMB 8253 on being

optimized (Jaapar *et al.*, 2011). Influence of different initial pH, argon gas and nitrogen gas on growth and hydrogen production using *R. sphaeroides* NCIMB 8253 was investigated in detail in batch culture. This study may help to establish a set of parameters to enhance the hydrogen production from *R. sphaeroides* NCIMB 8253 in batch culture.

MATERIALS AND METHODS

This study was conducted starting from April 2009 to March 2010 at the Laboratory of Synthetic Biology, Department of Chemical and Process Engineering, University Kebangsaan Malaysia.

Bacterial strain: *R. sphaeroides* NCIMB 8253 was obtained from NCIMB Limited Scotland, in freeze-dried form and the activation of the bacterial strains were performed as previously reported, inoculum of 10% v/v was used throughout this study except unless stated clearly. The culture conditions, strategic use of bacterial medium, growth conditions, analysis and gas collection procedure were also kept same as reported and discussed in our previous study and discussed in this study to relate the parameters under investigation (Jaapar *et al.*, 2011).

Culture conditions: *R. sphaeroides* NCIMB 8253 was grown in modified medium of Biebl and Pfennig. The modified medium of Biebl and Pfennig contains Malic acid (7.5 mM) as the organic carbon source and sodium glutamate (10 mM) as the nitrogen source. Solid agar medium was prepared by using 2% agar Bacteriological No. 1 into 1 L of the modified medium of Biebl and Pfennig. The pH of the medium was adjusted to 6.82 with 1 M sodium hydroxide. The liquid culture medium used for the H_2 production was similar to the growth medium except that the concentrations of malate and glutamate were 15 and 2 mM, respectively. Both medium were sterilized at 121°C for 15 min in the autoclave.

The bacterium was grown anaerobically at 30°C in 100 mL serum bottle containing 100 mL liquid medium under 100 W tungsten lamp (3.0-3.8 klux) or 30 W fluorescent lamp having light intensity of 3.8-4.5 Klux. Argon gas was used to create anaerobic conditions by purging it into the medium. The agar cultures were incubated in anaerobic jar at 30°C with tungsten lamp (100 W) as the light source.

Hydrogen production: H_2 gas production was performed in batch culture systems using 100 mL serum bottle containing 100 mL medium using 10% (v/v) inoculum. The temperature was adjusted to 30°C under the light of a tungsten lamp (100 W) with light intensity of 3.8 klux. For all H_2 production experiments, the reactor was flushed with pure argon to provide an anaerobic atmosphere to the cultures. After flushing with argon gas, 10% v/v inoculum of the pre-activated bacteria (in minimal medium of Biebl and Pfennig) was transferred into the H_2 production medium. During the experiments, the produced gas was collected and measured volumetrically in syringes directly connected to the reaction vessel without any water trap.

Analysis: Growth of the culture was monitored by measuring the Optical Density (OD) at 660 nm using a Thermo Spectronic UV-visible spectrophotometer (Model: Genesys 10 UV). Fresh medium was used as reference solution. Cell's dry weight was obtained by centrifuging 10 mL of cells suspension at 13,000 rpm for 10 min and then the pellet was washed twice with deionized water and dried in an oven at 105°C until constant weight was gained. A relationship of cells dry weight

and OD was obtained by plotting a graph of OD versus dry weight of cells. For Gas Chromatograph (GC) analysis, 1 mL gas sample was taken from collected gas by H_2 fermentation. The H_2 gas produced was analyzed by GC equipped with a Thermal Conductivity Detector (TCD) and packed column (SRI 8610C GC, USA). Helium was used as the carrier gas. Oven and detector temperatures were 50 and 150°C, respectively. Percentage of H_2 in total gas by GC analysis was used to determine the total of H_2 gas collected from each experiment. The pH of the culture medium was measured with Eutech Instruments pH meter (Model: pH 510; pH/mV/°C; Cyberscan). Light intensities were measured by a luxmeter (Model: LX-103; Digital Instruments; Lutron).

RESULTS AND DISCUSSION

Growth and pH profile in aerobic and anaerobic photofermentation: Absorbance at 660 nm at specific time intervals was monitored to evaluate the growth of *R. sphaeroides* NCIMB 8253 in aerobic and anaerobic conditions. It is evident from Fig. 1, the growth of the cells in aerobic culture reached exponential phase faster as compared to the culture in the anaerobic condition. The maximum OD_{660nm} of 1.243 was achieved after 160 h incubation in the aerobic culture and declined on further incubation. The decline of the OD of the culture might be attributed to the cell lysis. In case of the anaerobic culture, the maximum OD_{660nm} of 1.033 was achieved after 240 h incubation with a quite low level growth as compared to aerobic culture. According to the previous study of Can *et al.* (2006) and Kars *et al.* (2006), higher cell masses were reported for the non H_2 producing bacterial cells, on the other hand lower cell masses were related to the enhanced H_2 gas production. Therefore, the H_2 production was targeted in anaerobic conditions, as aerobic conditions were unable to produce enough H_2. This phenomenon could be attributed to the total amount of energy and electron spent either to H_2 production or cell components. Comparison of the percentage of H_2, total H_2 produced, yield of H_2 produced per gram of the substrate consumed ($Y_{H_2/S}$) and the rate of H_2 produced from inoculums grown in 24 and 48 h are shown in Table 1. The results demonstrate that the age of inoculum has a strong relation with the healthy growth of the culture and H_2

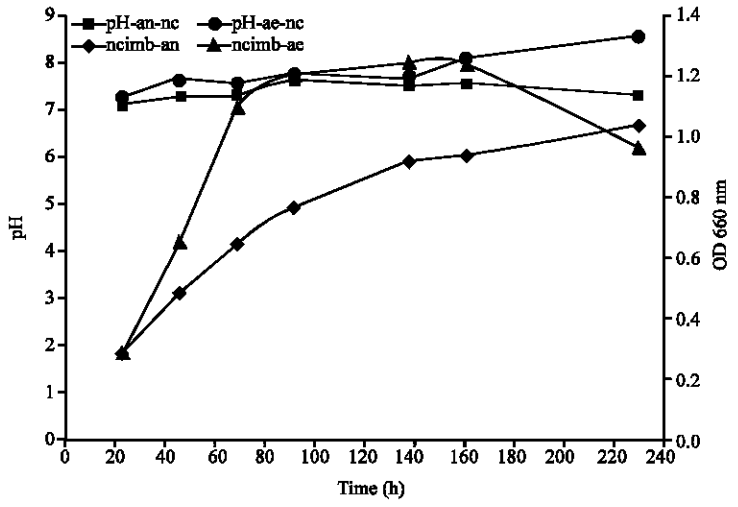

Fig. 1: pH and growth profile of *R. sphaeroides* NCIMB 8253 in aerobic and anaerobic conditions, $^{(pH\text{-}an\text{-}nc)}$ pH in anaerobic condition, $^{(pH\text{-}ae\text{-}nc)}$ pH aerobic condition, $^{(ncimb\text{-}an)}$ growth in anaerobic condition, $^{(ncimb\text{-}ae)}$ growth in aerobic condition

Table 1: Growth conditions and hydrogen production using *R. sphaeroides* NCIMB 8253

Inoculum medium	Production medium	Total h	Total gas (mL)	%H_2	Total of H_2 (mL)	$Y_{H2/S}$ (mL g^{-1})	Rate of H_2 production (mL/L/h)
H_2M[a]	H_2M	144	67.20	18.64	12.53	125.26	0.87
GrM[b]	H_2M	144	66.50	20.76	13.81	138.05	0.96
H_2M	H_2M	144	1.00	0.00	0.00	0.00	0.00
GrM	H_2M	144	60.50	14.73	8.91	89.10	0.62

[a]Hydrogen production medium, [b]Growth medium, ^{H2M} hydrogen production medium, ^{GrM} Growth medium

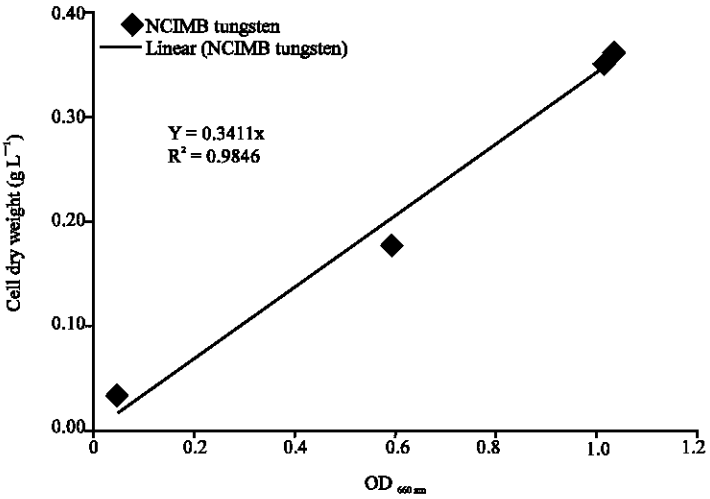

Fig. 2: Calibration curve of dry cell weight versus OD_{660nm} by *R. sphaeroides* NCIM 8253

production. It was found that the culture that was inoculated with 24 h inoculum age produced the highest percentage of H_2 (20.76%), highest yields of H_2 per gram of the substrate consumed i.e., $Y_{H_2/S}$ (138.05 mL g^{-1}) and the highest rate of H_2 (9.6×10^{-4} L/L/h) compared to the culture grown for 48 h in this experiment. The pH changes during the growth of aerobic and anaerobic conditions of cells were monitored and illustrated in Fig. 1. The initial pH was buffered to 7.0 and was not controlled during the growth in either aerobic or anaerobic experiments. There was no considerable difference in the pH values in aerobic and anaerobic conditions of culture and the pH values varied between 7.0 and 8.54 during the cultivation. These results are in agreement with the previous research reported that during H_2 production by *R. sphaeroides* in anaerobic fermentation, a slight decrease in the pH of the medium is observed during the cell growth period but the pH increased with the H_2 production (Eroglu *et al*., 1999).

The bacterial cell concentration was analyzed spectrophotometrically at absorbance 660 nm. Dry cell weight versus OD 660 nm curve was obtained from the samples corresponding to the various points of growth curve. It was established that an optical density of 1.0 at 660 nm corresponds to a cell density of 0.3441 g dry weight per liter of culture or 1 OD_{660nm} is equivalent to 0.3441 g L^{-1} of cell biomass (Fig. 2). It has been reported that 1 OD_{660nm} is equivalent to 0.6 g L^{-1} of cell dry weight (Uyar *et al*., 2007), as they have used the same bacterial strain but cells were dried at 55°C. In present study, the drying was continued until the constant dry weight was achieved at 105°C. Some reports are available to perform drying of cells at 105°C to get the constant dry weight (Chang *et al*., 2005). Some other scientists have also used 105°C to get the constant dry cell mass (Bratbak and Dundas, 1984).

Table 2: Effect of pH on hydrogen gas production

pH	h	Volume of gas (mL)	$\%H_2$	Volume of H_2 (mL)	$Yield_{H_2/S}$ (mL g^{-1})	Rate of H_2 production (mL/L/h)
6	144	45	74.52	33.53	167.67	2.33
7	144	66	68.23	45.03	225.17	3.13
8	24	2	34.71	0.69	3.47	0.29
9	72	20	59.38	11.88	59.38	1.65
10	168	0	53.68	0	0	0
11	192	0	12.84	0	0	0

Table 3: Effect of nature of gas to create anaerobic environment on hydrogen production

Gas	Total gas (mL)	Total H_2 (mL)	% H_2
Argon (y)	66.0	45.03	68.22
Nitrogen (x)	36	23.50	65.28
Equation	y = 1.83x	y = 1.92x	

Effect of initial pH: The effect of initial medium pH on the photoproduction of H_2 from modified Biebl and Pfennig medium was studied at varying pH conditions ranging from 6 to 11 at intervals of 1.0 unit. The experiments were conducted without controlling the pH of medium. The results shown in Table 2 indicate that pH 7 gave the highest cumulative H_2 production of 45.03 mL, yield per gram of substrate (225.17 mL g^{-1}) and rate of H_2 production (3.13 mL/L/h) compared to others initial pH. It shows that initial medium pH 7 was the most suitable pH for the highest cumulative of H_2 volume by *R. sphaeroides* NCIMB 8253, followed by the cultures with initial medium pH 6 and 9 and no gas was produced in the cultures with initial medium pH 10 and 11. The studies regarding pH in batch fermentation to produce hydrogen from food waste also present that maximum hydrogen was produced at pH 7 (Nazlina *et al.*, 2009). Some scientists have reported maximum H_2 production by *R. sphaeroides* O.U.001 at pH 7 using spent media (Nath and Das, 2009). Kim *et al.* (1982) have reported maximum biomass growth of *R. sphaeroides* KD131 at pH 6.8- 7.2 using Sistrom's media. Present experimental findings are in agreement with the results reported before regarding a slight decrease in pH occurred during the biomass growth and the pH increased during the H_2 production by *R. sphaeroides* O.U.001 in modified Biebl and Pfennig medium with initial pH 7.5. Eroglu *et al.* (1999) have also described that H_2 production occurred with medium having pH below than 7.5 (Eroglu *et al.*, 1999).

Effect of using argon and nitrogen gas to create anaerobic atmosphere: The anaerobic condition for growth and H_2 production by *R. sphaeroides* can be achieved by sparging the culture with argon or N_2 gas before inoculation. This study investigates the effect of argon and nitrogen gas on H_2 production by *R. sphaeroides* NCIMB 8253. Culture sparged with argon gas at the beginning produced 45.03 mL H_2 after 144 h incubation; whereas culture sparged with N_2 gas only produced 23.50 mL H_2 after 120 h incubation (Table 3). The use of argon gas could double the H_2 production by *R. sphaeroides* as compared to the use of N_2 gas. Koku *et al.* (2003) have reported that high nitrogen contents in the medium can inhibit H_2 production (Koku *et al.*, 2003). It was observed that the percentage of H_2 in the total gas produced using argon gas was about 5% higher than the culture with nitrogen. H_2 production by anoxygenic phototrophic bacteria also can be inhibited by NH^+_4 because it represses the synthesis of key enzyme nitrogenase (Zhu *et al.*, 2001). The presence of NH_4^+ ion as nitrogen source may reduce the rate of H_2 production and cumulative H_2 production, since the activity of nitrogenase enzyme is hampered (Basak and Das, 2007).

CONCLUSION

The extract of this study is an established set of information regarding the influence of some common and influencing parameters for H_2 production using *R. sphaeroides* NCIMB 8253. Effects of the culture conditions on the H_2 producing efficiency of the *R. sphaeroides* NCIMB 8253 were investigated to establish the optimized values for a maximum level of H_2 production. The parameters studied were including the effect of pH in aerobic and anaerobic photo fermentation. The highest gas production phase was observed by *R. sphaeroides* NCIMB 8253 at initial pH 7 and by using argon gas instead of nitrogen to create the anaerobic atmosphere. *R. sphaeroides* NCIMB 8253 may grow optimally in growth medium as stated in results and discussion. This report may lead to establish the optimized conditions at laboratory scale and industrial scale for the production of maximum H_2 using *R. sphaeroides* NCIMB 8253.

REFERENCES

Alalayah, W.M., M.S. Kalil, A.A.H. Kadhum, J.M. Jahim, S.Z.S. Jaapar and N.M. Alauj, 2009. Bio-hydrogen production using a two-stage fermentation process. Pak. J. Biol. Sci., 12: 1462-1467.

Alalayah, W.M., M.S. Kalil, A.A.H. Kadhum, J. Jahim, A. Zaharim, N.M. Alauj and A. El-Shafie, 2010. Applications of the box-wilson design model for bio-hydrogen production using *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564). Pak. J. Biol. Sci., 13: 674-682.

Armor, J.N., 1999. The multiple roles for catalysis in the production of H_2. Applied Catal. A: Gen., 176: 159-176.

Asif, M. and T. Muneer, 2007. Energy supply, its demand and security issues for developed and emerging economies. Renewable Sustainable Energy Rev., 11: 1388-1413.

Basak, N. and D. Das, 2007. The prospect of Purple Non-Sulfur (PNS) photosynthetic bacteria for hydrogen production: The present state of the art. World J. Microbiol. Biotechnol., 23: 31-42.

Bratbak, G. and I. Dundas, 1984. Bacterial dry matter content and biomass estimations. Applied Environ. Microbiol., 48: 755-757.

Can, O.T., M. Kobya, E. Demirbas and M. Bayramoglu, 2006. Treatment of the textile wastewater by combined electrocoagulation. Chemosphere, 62: 181-187.

Chang, C.Y., M.Y. Lue and T.M. Pan, 2005. Determination of adenosine, cordycepin and ergosterol contents in cultivated *Antrodia camphorata* by HPLC method. J. Food Drug Anal., 13: 338-342.

Collect, C., N. Adler, J.P. Schwitzguebel and P. Peringer, 2004. Hydrogen production by clostridium thermolacticum during continuous fermentation of lactose. Int. J. Hydrogen Energy, 29: 1479-1485.

Emily, L.W.T., J. Nandong and Y. Samyudia, 2009. Experimental investigation on the impact of aeration rate and stirrer speed on micro-aerobic batch fermentation. J. Applied Sci., 9: 3126-3130.

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

Fang, H.H.P., H. Zhu and T. Zhang, 2006. Phototrophic hydrogen production from glucose by pure and co-cultures of *Clostridium butyricum* and *Rhodobacter sphaeroides*. Int. J. Hydrogen Energy, 31: 2223-2230.

Fascetti, E., E. D'Addario, O. Todini and A. Robertiello, 1998. Photosynthetic hydrogen evolution with volatile organic acid derived from the fermentation of source selected municipal wastes. Int. J. Hydrogen Energy, 23: 753-760.

Fonseca, G.G. and R.V. Antonio, 2007. Polyhydroxyalkanoates production by recombinant *Escherichia coli* using low cost substrate. Am. J. Food Technol., 2: 12-20.

Jaapar, S.Z.S., M.S. Kalil and N. Anuar, 2009. The effect of aeration, agitation and light on biohydrogen production by *Rhodobacter sphaeroides* NCIMB 8253. Pak. J. Biol. Sci., 12: 1253-1259.

Jaapar, S.Z.S., M.S. Kalil, E. Ali and N. Anuar, 2011. Effects of age of inoculum, size of inoculum and headspace on hydrogen production using *Rhodobacter sphaeroides*. Bacteriol. J.,

Kars, G., U. Gunduz, M. Yucel, L. Turker and I. Eroglu, 2006. Hydrogen production and transcriptional analysis of *Nifd*, *Nifk* and hups genes in *Rhodobacter sphaeroides* o.U.001 grown in media with different concentrations of molybdenum and iron. Int. J. Hydrogen Energy, 31: 1536-1544.

Kim, J.S., H. Yamauchi, K. Ito and H. Takahashi, 1982. Selection of a photosynthetic bacterium suitable for hydrogen production in outdoor cultures among strains isolated in the Seoul, Taegu, Sendai and Bangkok areas. Agric. Biol. Chem., 46: 1469-1474.

Koku, H., I. Eroglu, U. Gunduz, M. Yucel and L. Turker, 2002. Aspects of the metabolism of hydrogen production by *Rhodobacter sphaeroides*. Int. J. Hydrogen Energy, 27: 1315-1329.

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

Levin, D., L. Pitt and M. Love, 2004. Biohydrogen production: Prospects and limitations to practical application. Int. J. Hydrogen Energy, 29: 173-185.

Misi, S.E.E., A. Ramli and F.H. Rahman, 2011. Characterization of the structure feature of bimetallic Fe-Ni catalysts. J. Applied Sci., 11: 1297-1302.

Nath, K. and D. Das, 2009. Effect of light intensity and initial pH during Hydrogen production by an integrated dark and photofermentation process. Int. J. Hydrogen Energy, 34: 7497-7501.

Nazlina, H.M.Y., A.R. Nor Aini, F. Ismail, M.Z.M. Yusof and M.A. Hassan, 2009. Effect of different temperature, initial ph and substrate composition on biohydrogen production from food waste in batch fermentation. Asian J. Biotechnol., 1: 42-50.

Ray, R.R., 2011. Microbial isoamylases: An overview. Am. J. Food Technol., 6: 1-18.

Truper, H.G. and U. Fischer, 1982. Anaerobic oxidation of sulphur compounds as electron donors for bacterial photosynthesis. Phil. Trans. R. Soc. London, 298: 529-542.

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

Yokoi, H., A. Saitsu, H. Uchida, J. Hirose, S. Hayashi and Y. Takasaki, 2001. Microbial hydrogen production from sweet potato starch residue. J. Biosci. Bioeng., 91: 58-63.

Zhu, H., T. Wakayama, Y. Asada and J. Miyake, 2001. Hydrogen production by four cultures with participation by anoxygenic phototrophic bacterium and anaerobic bacterium in the presence of NH_4^+. Int. J. Hydrogen Energy, 26: 1149-1154.