

Photoproduction of Hydrogen by Photosynthetic Bacteria Isolated from Oil Contaminated Soil of Mallapur, Hyderabad, India

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Abstract: In the present study, the ability of hydrogen production by photosynthetic bacteria isolated from oil contaminated soils was studied. Influence of pH, carbon, nitrogen sources and growth factors on hydrogen production by the bacteria was investigated. Photosynthetic bacteria produced maximum amount (6.0 ml/30 ml vessel) of hydrogen under anaerobic light in the presence of the carbon source benzoate, arabinose and nitrogen source ammonium chloride.

Keywords: Hydrogen production, Photosynthetic bacteria, pH, carbon sources, nitrogen sources.

Introduction

Worldwide production of CO₂ leading to climate change due to greenhouse effect requires the use of other sources of energy which do not result in CO₂ emissions. Hydrogen as a energy carrier helps us in mitigating the risk of green house gases. Among the many methods of hydrogen production, photoproduction is considered to be environmental friendly. Photosynthetic bacteria produce more photobiological hydrogen due to their high conversion efficiency[1] and hence more emphasis is laid worldwide on biological hydrogen production[2-4]. Among these bacteria *Rhodospseudomonas palustris*, *Rhodobacter capsulatus* and *Rhodobacter marinus*, *Rhodobacter sphaeroides* and *Rhodospirillum rubrum* [5-12] are being investigated for hydrogen production. Different electron donors and nitrogen sources like acetate, lactate, benzoate, malate, mannitol, starch, nitrates and amino acids are utilized by photosynthetic bacteria for hydrogen production. Optimization of various parameters for hydrogen production has been reported by various researchers[13-16]. In continuation of our earlier work[17-33], hydrogen production by the phototrophic bacterial consortium isolated from Mallapur, Industrial area, Hyderabad, South India was evaluated under different cultural conditions and the results are communicated.

Materials and Methods

The phototrophic bacteria were identified using the Bergey's Manual of Systematic Bacteriology (1994) [34]. Oil contaminated soil was collected from Mallapur, Industrial area, Hyderabad. Growth and effect of pH, carbon, nitrogen and growth factors was determined as reported in our earlier studies [24]. The technique used for hydrogen measurement hydrogen displacement technique.

Results and Discussion

Among the various hydrogen production processes, photoelectrochemical, photochemical, thermo chemical, and photobiological processes are being explored for commercial viability compared to conventional methods of hydrogen production. Photosynthetic bacterial hydrogen production represents a method with

appreciable efficiency for hydrogen evolution when compared to other methods. Ten day grown cultures of photosynthetic bacteria was used to assess their hydrogen potential. Photosynthetic bacterial consortium produced varying amounts of hydrogen under different cultural conditions. Perusal of Table 1 showed that pH of 7.5 induced maximum amount of hydrogen and gradually decreased as the pH increased. Benzoate and arabinose induced equal amounts of hydrogen when compared to other carbon sources (Table 2). Highest production of 6.0 ml /30ml culture of hydrogen was produced in presence of Benzoate and arabinose. In the presence of citrate, the consortium produced the lowest amounts of hydrogen. Intermediate production of hydrogen was seen when tested with other carbon sources. Effect of various nitrogen sources on hydrogen production are listed in Table 3. Ammonium chloride followed by glutamate produced more amounts of hydrogen under anaerobic light. Tryptophan, tyrosine and alanine induced lowest amounts of hydrogen production than other sources. In Other nitrogen sources tested intermediate levels of hydrogen production was tested. Carbon sources showed more production of hydrogen when compared to different nitrogen sources. Effects of growth factors on hydrogen production were tested and the results are presented in Table 3. Vitamin B12 promoted more amounts of hydrogen when compared to other growth factors. Pantothenic acid and riboflavin promoted equal amounts of hydrogen when used as growth factors. Lower amounts of hydrogen were recorded when folic acid was tested.

Table 1: Effect of pH on production of hydrogen by phototrophic consortium

pH	O.D Values	Hydrogen Production (ml/30ml Vessel)
6.5	1.1177	4.5±0.45
7.0	1.1398	5.5±0.65
7.5	1.8778	6.0±0.5
8.0	1.2339	5.5±0.75
8.5	1.2281	5.0±0.70
9.0	1.1537	4.0±0.55
9.5	1.1657	4.0±0.60

Table 2: Effect of carbon sources on production of hydrogen by phototrophic consortium

Carbon Sources	O.D Values	Hydrogen Production (ml/30ml Vessel)
Sodium Benzoate	1.1468	6.0±0.85
Glucose	0.6783	5.0±0.55
Galactose	0.7843	5.5±0.20
Manose	0.7127	5.5±0.30
Arabinose	0.901	6.0±0.60
Lactose	0.9166	5.0±0.75
Manitol	0.5114	5.0±0.20
Malic acid	0.4979	4.5±0.60
Citrate	0.6712	4.0±0.55
Sodium succinate	0.6442	4.5±0.70

Table 3: Effect of nitrogen sources on production of hydrogen by phototrophic consortium

Nitrogen Sources	O.D Values	Hydrogen Production (ml/30ml Vessel)
Ammonium chloride	0.8732	6.0±0.50
Ammonium nitrate	0.4729	4.5±0.65
Glycine	0.5753	4.5±0.75
Sodium glutamate	0.7761	5.5±0.60
Histidine	0.5495	4.5±0.25
Tryptophan	0.4832	4.0±0.30
Tyrosine	0.6063	4.0±0.40
Threonine	0.5567	4.5±0.50
Alanine	0.6128	4.0±0.60

Table 4: Effect of growth factors on production of hydrogen by phototrophic consortium

Growth Factors	O.D Values	Hydrogen Production (ml/30ml Vessel)
Pantothenic acid	1.7866	5.5±0.25
Nicotinic acid	1.6464	4.5±0.30
Biotin	1.2577	5.0±0.50
Folic acid	1.5291	3.5±0.60
Riboflavin	1.0279	5.5±0.40
B ₁₂	1.3536	6.0±0.20

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References:

1. Harun Koku, Inci Ero,glu, Ufuk Gunduz, Meral Yucel, Lemi Turker, 2002. International Journal of Hydrogen Energy (2002). 27: 1315 – 1329.
2. Yongzhen Tao, Yang Chen, Yongquian Wu, Yanling He and Zhihua Zhou. International Journal of Hydrogen Energy, ISSN 0360 – 3199. 2007. 32: 200 -206.
3. Chun-Yen Chen, Mu-Hoe Yang, Kuei-Ling Yeh, Chien-Hung Liu, Jo-Shu Chang, International Journal of Hydrogen energy 33 (2008) 4755 – 4762.
4. Chun-Yen Chen <http://www.sciencedirect.com/science/article/pii/S0960852411007899-aff1>, Chia-Hsien Liu <http://www.sciencedirect.com/science/article/pii/S0960852411007899-aff2>, Yung-Chung Lo <http://www.sciencedirect.com/science/article/pii/S0960852411007899-aff2>, Jo-Shu Chang. Bio resource Technology, 2011. Vol 102, 18: 8484–8492.
5. Chen, C.-Y., W.-B. Lu, J.-F. Wu, J.-S. Chang, International Journal of Hydrogen Energy 32 (2007) 940 – 949.
6. Azbar,N., F. C. Dokgoz, International Journal of Hydrogen Energy 35 (2010) 5028 – 5033.
7. Jamil, Z., M. S. M. Annuar, S. Ibrahim, S. Vikineswary, International Journal of Hydrogen Energy 34 (2009) 7502 – 7512.
8. Oh, Y.K., E.-H. Seol, M.-S.-S. Kim, S. Park, International Journal of Hydrogen Energy 29 (2004) 1115 – 1121.
9. Gabrielyan, L., H. Torgomyan, A. Trchounian, International Journal of Hydrogen Energy 35 (2010) 12201 – 12207.
10. Obeid,J., J.-P. Magnin, J.-M. Flaus, O. Adrot, J. C. Willison, R. Zlatev, International Journal of Hydrogen Energy 34 (2009) 180 – 185.
11. Burgess,J.G., R. Kawaguchi, A. Yamada, T. Matsunaga, Microbiology, 140 (1994) 965– 970.
12. Salih, F. M., M. I. Maleek, Journal of Environmental Protection 1 (2010), 426–430.
13. Koku,H., I. Eroglu, U. Gundz, M. Yucel, L. Turker, International Journal of Hydrogen Energy. (2003). 28: 381 – 388.
14. Zheng,G., L. Wang, Z. Kang, Renewable Energy 35 (2010) 2910 – 2913.
15. Hawkes, F.R., R. Dinsdale, D. L. Hawkes, I. Hussy, International Journal of Hydrogen Energy 27 (2002) 1339 – 1347.
16. Melnicki,M.R., E. Eroglu, A. Melis., International Journal of Hydrogen Energy 34 (2009) 6157 – 6170.
17. Ramchander, M., Pratap, M. P., Nageshwari, B., Girisham, S., and Reddy, S. M. (2012). Factors influencing the production of hydrogen by the hydrogen by the purple non-sulphur phototrophic bacterium *Rhodospseudomonas acidophila* KU001. *Microb. Biotechnol.*, 5: 674-678.
18. Ramchander Merugu , Vasantha Mittapelli , M.P. Pratap Rudra , S. Girisham , S.M. Reddy (2012) . Photoproduction of Hydrogen by Alginate Immobilized *Rhodobacter capsulatus* KU002 under Sulphate and Phosphate Limitations. International Journal of Environment and Bioenergy, 4(3): 141-146
19. Ramchander Merugu, Vasantha Mittapelli, Pratap Rudra Manthur Padigya, Girisham Sivadevuni, Reddy Solipuram Madhusudhan (2013). Photoproduction of Hydrogen by Alginate Immobilised

- Cultures of *Rhodobacter capsulatus* KU002 Isolated from Tannery Effluents. Journal of Biofuels. 4 (2) : 56-60
20. Ramchander Merugu, M. P. PratapRudra, B.Nageshwari, A. Sridhar Rao, and D.Ramesh. Photoproduction of Hydrogen under Different Cultural Conditions by Alginate Immobilized *Rhodopsedomonas palustris* KU003. ISRN Renewable Energy. Volume 2012, Article ID 757503, 5 pages doi:10.5402/2012/757503
 21. Ramchander Merugu, M.P.Pratap Rudra, S.Girisham and S.M.Reddy (2013). Bioproduction of hydrogen by alginate immobilized *Rhodopsedomonas acidophila* KU001. International Journal of Chemical Engineering and Applied Sciences 3(1): 7-9
 22. Ramchander, M., M.S.K.Prasad, Vasavi,D S. Girisham and S.M. Reddy (2007). Bioremediation of waste water by two Anoxygenic Phototrophic bacteria *Nat.Acad. Sci. Lett.*30:223-227
 23. Ramchander Merugu, M.S.K. Prasad, S. Girisham and S.M.Reddy (2008). Effect of trace elements on the growth of two Anoxygenic phototrophic bacteria *Ecol. Envi. Con.* 14; 367-369
 24. Ramchander Merugu, M. P. Pratap Rudra, A. Sridhar Rao, D. Ramesh, B. Nageshwari, K. Rajyalaxmi, S. Girisham, and S. M. Reddy (2011). Influence of Different Cultural Conditions on Photoproduction of Hydrogen by *Rhodopseudomonaspalustris* KU003. *ISRN Renewable Energy* , 328984-90
 25. Ramchander Merugu, M.P.Pratap Rudra, Atthapu Thirupathaiah and Veerababu Nageeti (2011). Hypocholesterolemic effect of the anoxygenic phototrophic bacterium *Rhopseudomonas palustris* MGU001 in hen laying eggs. International Journal of Applied Biology and Pharmaceutical Technology. 2 (2): 463 to 466.
 26. Ramchander Merugu, M.P.Pratap Rudra, Atthapu Thirupathaiah, S.Girisham and S.M.Reddy (2011). Optimisation of Indole Acetic Acid Production by two Anoxygenic Phototrophic bacteria Isolated from Tannery effluents. Journal of pure and applied Microbiology. 5(2) : 34-37
 27. Ramchander Merugu, M.P.Pratap Rudra, Atthapu Thirupathaiah, S.Girisham and S.M.Reddy (2011). Chromate Reduction by a Purple non Sulphur Phototrophic Bacterium *Rhodobacter capsulatus* KU002 Isolated from Tannery Effluents. Journal of Pure and Applied Microbiology. 5(2): 66-69
 28. Ramchander Merugu, M.S.K. Prasad, S. Girisham and S.M. Reddy (2010). Bioproduction of hydrogen by *Rb.capsulatus* KU002 isolated from leather industry effluents. International Journal of Hydrogen energy, 35 (18) : 9591-9597
 29. Ramchander Merugu, M.S.K. Prasad, S. Girisham and S.M. Reddy (2008). Phosphate Solubilisation by Four Anoxygenic Phototrophic Bacteria Isolation from leather Industry. *Nat. Env. Pol. Tech.* 7; 597-599
 30. Ramchander Merugu, M.S.K. Prasad, S. Girisham and S.M.Reddy (2008). Production of Indole acetic acid and free amino acids by two Anoxygenic phototrophic bacteria. *Bioinfolet* 5; 82-84
 31. Ramchander Merugu, S. Girisham and S.M.Reddy (2010). Production of PHB (Polyhydroxybutyrate) by *Rhodopseudomonas palustris* KU003 under nitrogen limitation. *International Journal of Applied Biology and Pharmaceutical Technology* 2:686-688
 32. Ramchander Merugu, S. Girisham and S.M.Reddy (2010). Production of PHB (Polyhydroxybutyrate) by *Rhodopseudomonas palustris* KU003 and *Rhodobacter capsulatus* KU002 under phosphate limitation. *International Journal of Applied Biology and Pharmaceutical Technology.* 3: 746-748.
 33. Ramchander Merugu, Y.Prashanthi, T. Sarojini, Nageshwari Badgu. Bioremediation of waste waters by the anoxygenic photosynthetic bacterium *Rhodobacter sphaeroides* SMR 009. International Journal of Research in Environmental Science and Technology 2014; 4(1): 16-19
 34. Bergey's Manual of Systematic bacteriology (1994). "Enrichment and isolation of purple non sulphur photosynthetic bacteria". Eds:J.T.Staley, M.P.Byrant, N.Pfennig and J.C.Holt.
