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EFFECT OF GROWTH FACTORS ON HYDROGEN PRODUCTION BY *RHODOPSUEDOMONAS PALUSTRIS* KU 001 AND *RHODOPSUEDOMONAS PALUSTRIS* OU 001

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ABSTRACT

Anoxygenic photosynthetic bacteria *Rhodopsuedomonas palustris* KU 001 and *Rhodopsuedomonas palustris* OU 001 were isolated and the hydrogen production abilities were analysed in the present study. Studies on optimization of cultural conditions for production of hydrogen revealed that the maximum hydrogen production took Place at 48th hours of incubation in both the strains isolated. More hydrogen production was seen when biotin, thiamine and Cyanocobalamine were used as growth factor sources for *R.palustris* KU001 while high hydrogen production was seen in thiamine, cyanocobalamine followed by biotin in *R.palustris* OU001. pH of 6.8 was amenable for the production of hydrogen and high biomass, hydrogen production two *Rhodopseudomonas palustris* KU001 and *Rhodopseudomonas palustris* OU001.

KEYWORDS: Hydrogen production, *R.palustris*, sewage water, growth factors.

INTRODUCTION

Photosynthetic bacterial presence has been reported in various aquatic environments.[1] Light dependant hydrogen production by anoxygenic photosynthetic bacteria[2], [3] and[4] was found to be superior to other methods of hydrogen production.. The hydrogen produced would be clean yielding water. Photobiological hydrogen generation by photosynthetic purple non sulphur bacteria[5-9] is desirable and also remediates waste water.[10-12] Growth factors are required in small amounts by bacteria and results from either a blocked or missing metabolic pathway in the cells. The growth factors are assimilated by cells to fulfill their specific role in metabolism. Growth factors have a complex chemical structure while some bacteria need them in the media others can synthesis from their precursors namely thiazole and pyridine residues. The capacity to synthesize microbial growth factors can determine the character of the interrelationships between bacteria. Biotin growth factor is involved in the process of CO_2 fixation. Some bacterial Species such as *Acetobacterium*, *Lactobacillus*, Pseudomonas, *Rhizobium*, *Streptococcus* and *Xanthomonas* are known to produce cyanocobalamine. Some require it as grwot factor and play a crucial role in microbial metabolism. Considering these facts, the role of growth factors on hydrogen production by two strains of *R.palustris* was investigated and presented in the work.

MATERIALS AND METHODS

The phototrophic bacteria were isolated by enrichment technique.[13] The chemicals and glassware employed were of AR grade and Borosil make respectively. Double distilled water was employed. Beibl and Pfennig's medium (in mg/L) KH_2 PO_4: 500; $MgSO_4.7H_2O$: 200; NaCl: 400; NH_4Cl: 400; $CaCl_2.2H_2O$: 50; Organic carbon: 1000; Yeast extract: 200; Ferric citrate solution (0.1gm/100ml): 5.0ml; trace element solution, 1 ml and cyanocobalamine (1mg/100ml) : 5.0 ml. The composition of trace element solution was (mg/L) $ZnCl_2$:70; $MnCl_2.4H_2O$:100; H_3BO_3: 60; $CoCl_2.6H_2O$: 200; $NiCl_2.6H_2O$: 20; $CuCl_2.2H_2O$: 20; $NaMO_4.2H_20$: 40 and HCl (25%v/v): 1 ml. pH was maintained at 6.8. The phototrophic bacteria were isolated from the effluent samples by enrichment techniques by inoculating into the medium and incubated anaerobically in the light (2000 lux). Bacteria thus isolated were identified with the help of cultural characteristics (colour, size and shape), carbon and nitrogen requirement, vitamin requirements, absorption spectra analysis, bacteriochlorophylls and carotenoids. Identification keys provided in Bergey's manual of systematic bacteriology[14] was adopted. Absorption spectra of the whole cells was measured by.[15] Liquid culture (1 ml) was taken and 200µl of three growth factors (Biotin, Thiamine and Cyanocobalamine) was added into the media. hydrogen electrode was used for measuring hydrogen production.

The techbiwues were similar tp those of Rajani *et al* and Sasikala *et al*.[16 and 17] To test the hydrogen production activity, the washed cell suspension was inoculated into 10ml of the medium in 15 ml capacity rimless test tubes sealed with subaseals under anaerobic conditions.

RESULTS AND DISCUSSION

Metal cofactors such as copper, molybdenum, zinc, nickel, cobalt, and iron plays significant role on Growth, photo hydrogen production and nitrogenase activity of photosynthetic microorganisms.[18] Studies on hydrogen production by the anoxygenic phototrophic bacterial strains revealed that it could produce good amount of hydrogen. However, amount of hydrogen produced varied with the different growth factors used. The bacterium under study could produce hydrogen over a wide range of pH (4.8 to 9.8). Hydrogen production is reported to be influenced by pH which varies with the organism[19] and.[20] pH is known to effect the activity of hydrogenase.[21] Perusal of table 1 and table 2 showed that change in pH induced different amounts of hydrogen in both the strains studied. More hydrogen production was seen when biotin, thiamine and Cyancobalamine were used as growth factor sources for *R.palustris* KU001 while high hydrogen production was seen in thiamine, cyanocobalamine followed by biotin in *R.palustris* OU001 respectively. pH of 6.8 was amenable for the production of hydrogen and high biomass, hydrogen production two *Rhodopseudomonas palustris* KU001 and *Rhodopseudomonas palustris* OU001. In some earlier studies[22-25] B12 induced more amounts of hydrogen compared to other growth factors. Riboflavin and Pantothenic acid induced lowest amounts of hydrogen.

Table 1: Effect of Growth factors on hydrogen (%) produced at different incubation intervals of *Rhodopseudomonas palustris* KU 001.

S.No	Growth factors (µg/ml)	Different pH Medium	PERCENTAGE OF HYDROGEN PRODUCED AT DIFFERENT INCUBATION TIME				
			6h	12h	18h	24h	48h
1	**Biotin**	4.8	-	-	-	-	100ppm
		5.8	-	100ppm	100ppm	200ppm	200ppm
		6.8	400ppm	500ppm	500ppm	700ppm	800ppm
		7.8	200ppm	200ppm	400ppm	400ppm	500ppm
		8.8	100ppm	100ppm	200ppm	300ppm	300ppm
		9.8	100ppm	200ppm	200ppm	300ppm	400ppm
2	**Thiamine**	4.8	-	-	-	-	-
		5.8	-	-	-	100ppm	200ppm
		6.8	300ppm	600ppm	700ppm	700ppm	800ppm
		7.8	100ppm	100ppm	200ppm	300ppm	500ppm
		8.8	100ppm	200ppm	200ppm	400ppm	400ppm
		9.8	-	100ppm	100ppm	200ppm	200ppm
3	**Cyanocobalamine**	4.8	-	-	-	-	-
		5.8	-	-	-	-	100ppm
		6.8	200ppm	400ppm	500ppm	700ppm	700ppm
		7.8	100ppm	100ppm	100ppm	100ppm	200ppm
		8.8	100ppm	100ppm	200ppm	200ppm	300ppm
		9.8	100ppm	100ppm	200ppm	300ppm	300ppm

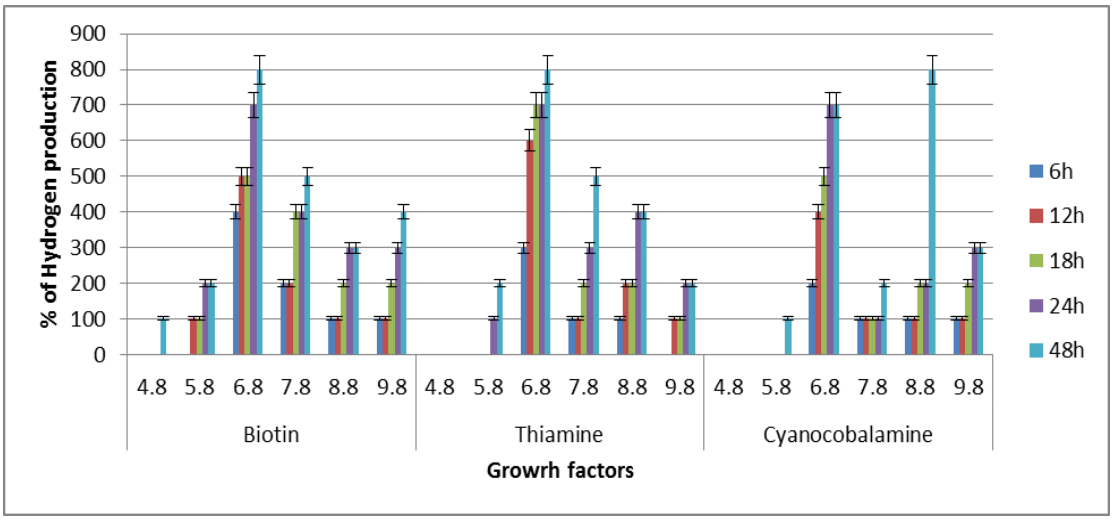

Figure: 1. Graphical representations of % of hydrogen production different pH and Time intervals

Table 2: Effect of Growth factors on hydrogen (%) produced at different incubation intervals of *Rhodopseudomonas palustris* OU 001

S.No	Growth factors (µg/ml)	Different pH Medium	PERCENTAGE OF HYDROGEN PRODUCED AT DIFFERENT INCUBATION TIME				
			6h	12h	18h	24h	48h
1	**Biotin**	4.8	-	-	-	-	100ppm
		5.8	-	-	-	100ppm	100ppm
		6.8	100ppm	200ppm	200ppm	400ppm	500ppm
		7.8	200ppm	200ppm	400ppm	400ppm	400ppm
		8.8	100ppm	100ppm	100ppm	300ppm	300ppm
		9.8	100ppm	100ppm	200ppm	200ppm	300ppm
2	**Thiamine**	4.8	-	-	-	-	-
		5.8	-	-	100ppm	100ppm	100ppm
		6.8	200ppm	400ppm	500ppm	500ppm	700ppm
		7.8	100ppm	100ppm	100ppm	200ppm	300ppm
		8.8	100ppm	100ppm	100ppm	200ppm	400ppm
		9.8	-	100ppm	100ppm	100ppm	100ppm
3	**Cyanocobalamine**	4.8	-	-	-	-	-
		5.8	100ppm	100ppm	100ppm	200ppm	200ppm
		6.8	200ppm	200ppm	300ppm	400ppm	600ppm
		7.8	100ppm	100ppm	100ppm	200ppm	400ppm
		8.8	100ppm	100ppm	100ppm	100ppm	200ppm
		9.8	100ppm	100ppm	100ppm	200ppm	200ppm

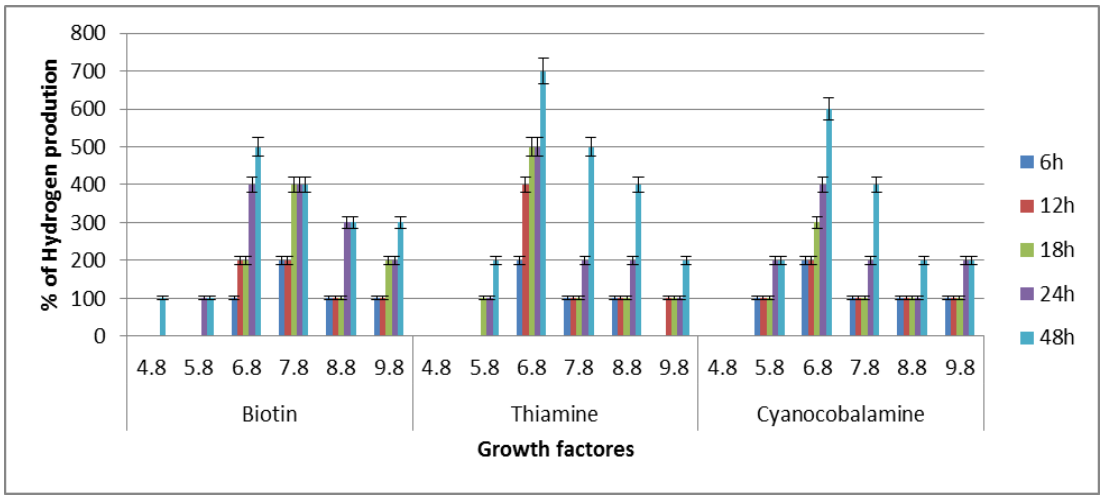

Figure: 2. Graphical representations of % of hydrogen production different pH and Time intervals

REFERENCES

1. Vasavi, D., M .Ramchander, S. Girisham and S.M. Reddy, Bioinfolet., 2007; 4: 139-145.
2. Kumazawa and Shimamura, Kumazawa, S. and K. Shimamura, 1993. J. of Marine Bio-tech., 1993; 1: 159-162.
3. Melis, A., Int. J. Hydrogen Energy, 2002; 27: 1217-1228.
4. Thangaraj, A. and Kulandaivel, G. Bioresource Technology., 1993; 49: 9-12.
5. Melis, A., Int. J. Hydrogen Energy., 2002; 27: 1217-1228.
6. Hoekema, S., M. Bijmans, M. Tanssen, J. Tramper and R.H. Wijffels, Int. J. Hydrogen Energy, 2002; 27: 1331-8.
7. Ramchander Merugu, M.S.K.Prasad, D.Vasavi, S.Girisham and S.M.Reddy. Ecol. Envi. Con., 2008; 14: 485-487.
8. Ramchander, M., M.S.K.Prasad, Vasavi,D S. Girisham and S.M. Reddy, Nat.Acad. Sci. Lett., 2007; 30: 223-227
9. Melis, A., Int. J. Hydrogen Energy., 2002; 27: 1217-1228.
10. Najafpour, G. D, Zinatizadeh, A. A. L, Mohamed, A. R, & Hasnain, M. Isa., H.Nasrol- lahzadeh, High-Rate anaerobic digestion of palm oil mill effluent in an up- flow anaerobic sludge-fixed film bioreactor. Biochemistry, 2006.
11. Truper H.G. and Pfennig N, Characterization and identification the anoxygenic phototrophic bacteria. In: The Prokaryotes. Eds.: M.P.Starr, H.Stolp,

H.G.Truper, A.Balows and H.G.Schlegel. Springer-Verlag, New York.; 1989; 299.
12. Velpula Suresh, Karunakar Rao Kudle, Madhukar Rao Kudle and M. P. Pratap Rudra. Evolution of hydrogen production from photosynthetic bacteria using a single channel hydrogen electrode detector. Journal of Chemical and Pharmaceutical Research, 2015; 7(7): 203-209. ISSN : 0975-7384 CODEN(USA) : JCPRC5
13. Kumazawa ,S. and A.Mitsui,.Int.J.Hydrogen energy., 1981; 6: 339-348.
14. Vincenzini,M., R.Materassi, M.R.Tredici and G.Florenzano, Int. J. Hydrogen Energy., 1982; 7: 231-236.
15. Rajani., Photosynthetic bacteria and their application in hydrogen production. thesis submitted to the department of Botnay Osmania university,Hydreabad, 1992.
16. Sasikala, Ch. and Ch.V.Ramana, Advanced Applied Microbiology, 1995; 41: 173-226.
17. Srinivas Munjam, D. Vasavi, S. Girisham and S.M. Reddy, Science and Culture., 2000; 66: 339-341
18. Dabrock, B., Bahl, H., Gottschalk, G., Appl. Environ. Microbiol., 1992; 58: 1233–1239.
19. Lay, J.-J., Y.-J. Lee, and T. Noike, Water Research, 1999; 33(11): 2579-2586.
20. Ramchander Merugu, M.P.Pratap Rudra , S.Girisham and S. M. Reddy. Hydrogen uptake hydrogenase activities of two anoxygenic phototrophic bacteria isolated from Leather industry effluents. International Journal of ChemTech Research, July-Sept 2012; 4(3): 1108-1110,
21. Srinivas Munjam, D. Vasavi, S. Girisham and S.M. Reddy., Ind. J. Microbiol., 2002; 42: 215-218.
22. Manisha D.R , M.P.Pratap Rudra , Ramchander Merugu Hydrogen production by photosynthetic bacterial consortium isolated from sewage water. International Journal of Research in Biochemistry and Biophysics., 2015; 5(1): 10-14.
23. Swetha Garimella , Vasantha Mittapelli , R.Roopa , Sabina Harold and Ramchander Merugu Optimization of cultural conditions for hydrogen production by photosynthetic bacteria isolated from sewage water, Nalgonda, Telangana. J. Chem. Pharm. Res., 2015; 7(10): 75-78.
24. Raju Nalvolthula, Ramchander Merugu , M.P.Pratap Rudra. Biohydrogen Production by Photosytnhetic Bacteria Isolated from Oil Contaminated Soil of Nacharam, Hyderabad, India. Int.J. ChemTech Res., 2014; 6(11): 4629-4632.
25. Raju Nalvolthula, Ramchander Merugu , M.P.Pratap Rudra. Photoproduction of Hydrogen by Photosytnhetic Bacteria Isolated from Oil Contaminated Soil of Mallapur, Hyderabad, India. Int.J. ChemTech Res., 2014; 6(11): 4625-4628.