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Research Article

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Optimization of cultural conditions for hydrogen production by photosynthetic bacteria isolated from sewage water, Nalgonda, Telangana

Swetha Garimella[1], Vasantha Mittapelli[1], R.Roopa[1], Sabina Harold[2] and Ramchander Merugu[1*]

[1]*University College of Science and Informatics, Mahatma Gandhi University, Nalgonda*
[2]*University College of Commerce and Business Management, Mahatma Gandhi University, Nalgonda*

ABSTRACT

In this study, we have analyzed the hydrogen produced by purple photosynthetic bacterial consortium isolated from sewage water, Nalgonda. In presence of acetate, 6.0 ml of hydrogen was produced followed by benzoate and lactate as carbon sources. Lowest amount of hydrogen was produced in mannitol containing medium. In the presence of sodium nitrate and yeast extract as nitrogen sources more amounts of hydrogen was seen compared to other nitrogen sources. In Ammonium chloride and glutamate as nitrogen sources lowest amounts of hydrogen production was seen. In presence of cyanocobalamine as growth factor more amounts of hydrogen was produced. Riboflavin induced lowest amounts of hydrogen. T-Test was done on two samples assuming unequal variances and the results are presented.

Keywords: Purple non sulphur bacteria, sewage water, hydrogen production, carbon, nitrogen sources.

INTRODUCTION

Photosynthetic bacteria are well known for their biotechnological applications which include hydrogen, PHB, ALA production and bioremediation [1]. Anoxygenic photosynthetic bacteria are distributed widely in natural habitats with large amounts of soluble organic matter [2]. In photosynthetic bacteria, PNSB (Purple Non-Sulfur bacteria) were widely studied as they are most versatile compared to other groups of photosynthetic bacteria. Different cultural conditions have been optimized for enhancing the production of hydrogen form this group of bacteria [3-17]. Environmental factors such as pH, temperature, light, carbon, nitrogen and growth factors are the major factors which play an important role in hydrogen production [18,19]. In this study, consortium isolated from sewage water sample from Nalgonda, Telangana state was studied for their hydrogen producing potential. The results obtained were statistically analyzed using t-test with the predicted standard to be at 33%. The significance of the above results are discussed in this communication.

EXPERIMENTAL SECTION

Purple non sulphur anoxygenic phototrophic bacteria were isolated from water sample from Nalgonda, Telangana State. Sewage water samples were inoculated into BP medium and incubated under anaerobic light conditions (2000lux). Bergey's Manual of Systematic Bacteriology (1994) [20] was used for the identification of these bacteria. Different concentrations of electron donors (1%), nitrogen sources (1%) and growth factors (100µl) were used to study their effect on hydrogen production. Ten days old cultures of phototrophic bacteria of 1% (v/v) concentration

were inoculated into 25 ml vessels which were sealed. After optimizing the parameters the effect of ASN (Acetate and sodium nitrate) medium on hydrogen production was analyzed with different concentrations of carbon and nitrogen. The technique used for hydrogen measurement was water displacement method. Gas Chromatography was used for gas analysis. Statistical analysis was done keeping the predicted amount of hydrogen at 33%.

RESULTS AND DISCUSION

Microbial production of hydrogen involves the enzymes nitrogenases and hydrogenases. Hydrogenases reduce protons to hydrogen. Compared to chemical production of hydrogen biological production is more environmentally friendly. The microbes which involve the production of hydrogen include mesophilic, thermophilic and photosynthetic organisms. In photosynthetic bacteria purple non sulphur bacteria are most widely studied for their hydrogen potentials and other applications. In continuation of our earlier work in this area [21-25] ten day active cultures were used to assess their probability of producing hydrogen. Photosynthetic bacterial consortium produced different amounts of hydrogen with various carbon, nitrogen and growth factors. The pH was maintained at 7.2 under anaerobic light. In presence of acetate, 6.0 ml of hydrogen was produced followed by benzoate and lactate as carbon sources (Table 1). Lowest amount of hydrogen was produced in mannitol containing medium. In the presence of sodium nitrate and yeast extract as nitrogen sources more amounts of hydrogen was seen compared to other nitrogen sources (Table 2). In Ammonium chloride and glutamate as nitrogen sources, lowest amounts of hydrogen production was seen. Among the growth factors, cyanocobalamine as growth factor could induce more amounts of hydrogen. Riboflavin induced lowest amounts of hydrogen (Table 3). T-Test was done on two samples assuming unequal variances and the results are presented in Tables 1a, 2a and 3a. Statistical analysis for the effect of various carbon, nitrogen and growth factors based on the hypothesis of standard at 33%, the results clearly show the deviation between predicted and observed amounts of hydrogen production. Level of significance was 5%. After optimizing the conditions for the production of hydrogen ASN medium was used. The production of hydrogen went upto 6.7ml for 20ml vessel. The pH was 7.2 and growth factor used was cyanocobalamine. In table 5, the effect of light intensity on hydrogen production was measured in the optimized medium of ASN. There was only a marginal increase in the production of hydrogen 2500 lux. Later on even though there was biomass increase with increase in light intensity, enhancement in hydrogen production was not seen.

Table 1: Effect of carbon sources on hydrogen production

Carbon source (1%)	Growth (Optical density at 660nm)	Hydrogen produced (ml/25ml vessel)
Acetate	0.65	6.0±0.4
Benzoate	0.73	5.0±0.5
Mannitol	0.45	2.5±0.2
Cellobiose	0.55	4.2±0.2
Lactose	0.65	3.0±0.3

Table 1a: Statistical analysis of the effect of carbon sources on hydrogen production

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	4.14	8.25
Variance	2.048	0
Observations	5	5
Hypothesized Mean Difference	0	
Df	4	
t Stat	-6.421875	
P(T<=t) one-tail	0.001511257	
t Critical one-tail	2.131846782	
P(T<=t) two-tail	0.003022514	
t Critical two-tail	2.776445105	

Table 2: Effect of nitrogen sources on hydrogen production

Nitrogen source (1%)	Growth (Optical density at 660nm)	Hydrogen produced (ml/25ml vessel)
Sodium nitrate	0.56	5.0±0.4
Glutamate	0.60	3.0±0.2
Ammonium chloride	0.58	3.0±0.3
Yeast extract	0.54	5.0±0.2
Alanine	0.62	3.5±0.2

Table 2a: Statistical analysis of the effect of nitrogen sources on hydrogen production

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	4.75	8.25
Variance	1.583333333	0
Observations	4	4
Hypothesized Mean Difference	0	
df	3	
t Stat	-5.5630359	
P(T<=t) one-tail	0.005730052	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.011460104	
t Critical two-tail	3.182446305	

Table 3: Effect of growth factors on hydrogen production

Growth factors (100µl)	Growth (Optical density at 660nm)	Hydrogen produced (ml/25ml vessel)
Riboflavin	0.60	3.2±0.2
Cyanocobalamine	072	5.0±0.3
Niacin	058	4.0±0.2

Table 3a: Statistical analysis of the effect of growth factors on hydrogen production

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	4.066666667	8.25
Variance	0.813333333	0
Observations	3	5
Hypothesized Mean Difference	0	
df	2	
t Stat	-8.034314216	
P(T<=t) one-tail	0.007570436	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.015140873	
t Critical two-tail	4.30265273	

Table 4: Effect of ASN (Acetate and sodium nitrate) medium on hydrogen production

ASN medium (%)	Growth (Optical density at 660nm)	Hydrogen produced (ml/25ml vessel)
0.5	0.62	5.8±0.4
1.0	0.65	6.0±0.4
1.5	0.72	6.4±0.2
2.0	0.75	6.7±0.3
2.5	0.76	6.5±0.4

Table 5: Effect of light intensity on hydrogen production in ASN medium

Light intensity (lux)	Growth (Optical density at 660nm)	Hydrogen produced (ml/25ml vessel)
2000	0.75	6.7±0.3
2500	0.78	6.8±0.2
3000	0.76	6.4±0.4
3500	0.80	6.2±0.3
4000	0.80	6.0±0.2

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