BIO-HYDROGEN PRODUCTION BY *R. SPHAEROIDES* AT AMBIENT ENVIRONMENTAL CONDITIONS USING GLUCOSE, PINEAPPLE PEEL, YAM PEEL AND FERMENTED CORN WASTE WATER

MBACHU, Ifeoma Adaora Chima
Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University
P.M.B. 02 Uli, Anambra State, **NIGERIA**

ABSTRACT

The purple non-sulfur photosynthetic bacteria are very useful organisms for industrial and environmental applications since they can convert agricultural wastes to products which could serve as industrial raw materials. Agricultural wastes accumulation in our environment is alarming however, they are good alternative carbon and energy sources for the autotrophic bacteria, *R. sphaeroides* whose nitrogenase enzyme converts molecular hydrogen to hydrogen gas using organic carbon sources. To determine if *R. sphaeroides* utilizes pineapple peel (PPP), yam peel (YP) and fermented corn waste water (FCWW) for bio-hydrogen production, 6.25ml water sample was used to inoculate 125ml of mineral salts- succinate broth. Isolate cultural characteristics included reddish turbidity in broth culture and reddish colonies on agar plate. Gram stain was negative, motility negative, sulfate assimilation positive, final oxidation product of sulfide was sulfate, confirming the isolate to *R. sphaeroides* by Bergey's criteria. After 160hrs incubation, bio-hydrogen yield started. Glucose, PPP and YP gave a yield of 1.6ml/g, 1.8ml/g and 1.8 ml/g respectively. Yield continued up to 208hrs at which glucose was 9.8ml/g, PPP was 10.9ml/g while YP was 10.2mg/g. Yield stooped for glucose at 208hrs but continued in PPP and YP up to 226hrs with 11.5ml/g from PPP, 10.6ml/g from YP. Production finally stopped at 227hrs with a yield of 11.8ml/g from PPP. In FCWW yield started after 5hrs of incubation with 0.8ml/g obtained after 6hrs. It continued up to 62hrs yielding 7.5ml/g.

Keywords: Ambient environment, Agricultural wastes**,** Bio-hydrogen gas, *R. sphaeroides.*

INTRODUCTION

Many microorganisms have the ability to produce bio-hydrogen gas but some have the capacity for higher production than others even under similar growth conditions. Jeong *et al* (2008) did a comparison of hydrogen production by four representative hydrogen producing bacteria. These bacteria were *Clostridium beijerinkii, Rhodobacter sphaeroides, Bacillus megaterium*, these were collected from American type collection centre (ATCC) and an anaerobic bacteria which they collected from a sludge digester in a waste treatment facility. Their substrate was glucose. Their finding showed *C. beijerinkii* as the best hydrogen producer from glucose, and that *R. sphaeriodes* had some time lapse between glucose consumption and hydrogen production for the simple reason that *R. sphaeriodes* first converts glucose to fatty acids before using the fatty acid to produce hydrogen. Agrawal *et al* (2007) comparing the ability of *R. sphaeriodes* with that of *Bacillus licheniformi, Clostridium pasteuranum* and *Entrobacter cloacae* in converting bagasse to hydrogen under anaerobic conditions reported that hydrogen production started after 15 days of incubation by all the organisms but that *in R. sphaeriodes*, it started after 5days. The maximum rate of production was attained after 48hrs of incubation at optimum pH 7 and temperature 32℃. In the other organisms studied, the maximum rate of production was attained after 24hrs of incubation.

Different studies showed that various agricultural wastes could be applied for bio-hydrogen production by *R. sphaeriodes* (Reungsang *et al* 2004; Gadhaushethy *et al*, 2008). Also those reports showed that electrical lamps were used for their bio-hydrogen production. Essentially, those works

were carried out in the temperate regions where ambient temperatures are rarely above 30℃. If such works are to be done in the tropical regions where temperatures often attain 70℃, will it not be possible to conduct the experiment with sunlight as the only source of illumination. The aim of this research is to investigate bio-hydrogen production by *R. sphaeriodes* by conducting the experiment under natural sunlight only but the substrates are still agricultural wastes, however, the results obtained will be compared with that obtained by the use of glucose as a substrate.

MATERIALS AND METHODS

1. Sample collection

The sample from which the organism was isolated is water, collected from Agulu Lake in Anambra State, Nigeria, with transparent plastic bottle that was disinfected with 100ppm sodium hypochlorite according to (Mahakhan *et al* 2002).

2. Isolation of *R. sphaeriodes*

3. The organism was isolated by selective enrichment sing sodium succinate yeast extract (SSYE) broth according to Lindquist (2000). The medium composition comprised,

1. basal medium, consisting of KH_2P04 0.33g, $MgS0_4.7H_20$ 0.33g,Nacl 0.33g, NH_4cl 0.5g, $CaCl_2.2H_20$ 0.05g, sodium succinate 1.0g, and yeast extract 0.02g. These were dissolved in IL of distilled water, pH was 6.8. It was autoclaved at 121℃ for 15mins and 151bs pressure

2. Trace salts solution. This consisted of $ZnS0_4.7H_20$ 0.01g. $MnCl_2.4H_20$ 0.003g, H_3B0_3 0.03g, $CoCl_2.6H_20$ 0.02g, $CuCl_2.2H_20$ 0.0lg, $NiCl_2.6H_20$ 0.002g and $Na_2M_0 0_4$ 0.003g. These were dissolved in IL of distilled water pH was 4.0

3. Sterile solutions added after autoclaving the basal medium above, consisted of 1.0ml of the above mentioned trace salts solution and 0.5ml of 0.02% feS04.$7H_20$ solution 80ml of this enrichment broth was dispensed into 125ml volume capacity, transparent glass culture bottle with stoppered lid and autoclaved at 121℃, 151b pressure and 15mins. After cooling, it was inoculated with 6.25ml of the water sample and then filled to the neck of the culture bottle with the broth in order to ensure that air bubbles are not trapped within the culture bottle. Sterile Vaseline oil was placed on the top of the culture medium in order to create anaerobic condition within the culture medium according to (Kantachote *et al* 2005) before it was covered with foil paper and paper tape. The incubation was on the laboratory bench at room temperature under illumination by 150w tungsten lamp placed at 90cm from the culture bottles. For the purpose of identification of the isolate, a solid agar medium of the broth was prepared by adding 1.5g agar-agar to 250ml of it. The sterilization and incubation are the same as with the broth culture.

Identification and Characterization of the Isolate

The identification was by Gram staining test, motility, sulfate assimilation, sulfide oxidation, vitamin requirement and aerobic dark growth. The characterization was by sugar fermentation tests. The sugars used were citrate and glucose. Other carbohydrates tested were manitol, ethanol, gluconate, glycerol and succinate.

Bio-hydrogen Production under Ambient Environmental Condition

The substrates used in this work were organic food wastes generated from kitchen. The peels of yam and pineapple were each ground to powder with manual grinding machines to allow for easy mixing in the mineral salts medium. The fermented corn waste water was also prepared in the kitchen. The medium used for the bio-hydrogen production was the mineral-salts solution used for the isolation of the organism but without the addition of succinate, ammonium salt and nitrogen source. This was to allow for nitrogenase activity according to Bollinger *et al* (1985). The photo reactor consisted of 400ml conical flask with a working volume of 250ml closed

with a rubber stopper to which a pneumatic membrane was inserted so that its distal end does not touch the medium in the flask. This pneumatic membrane was passed through distilled water to an inverted burette, filled to the tip with water and the burette was immersed in the water. This was to collect the gas by displacement of water in the burette according to (Mahakham *et al*, 2005; Ugar *et al*, 2007; Salih and Maleek 2010). The reactor was illuminated externally by tungsten lamp placed at 90cm distance. Initial runs of this experiment were first conducted in order to determine the acceptability of the substrates by the organism for growth. This was monitored by turbidity and increase in cell number. For this initial runs, varying percentages of the substrates were evaluated in corresponding volumes of the mineral salts medium. These values are 1%, 2%, 3%, 4% and 5% respectively. Finally after this confirmation, a singular quantity only was used for the rest of the experiment and this was 3%, of 250ml which was 7.5g of the substrates.

RESULTS

Isolation: The isolate had a reddish colour in sodium succinate yeast extract (SSYE) medium and rosette bunch colonies on solid agar medium of SSYE, figs 1a &b

Morphology and identification: the Gran stain was negative, cell shape, rod-shaped and motility was negative. Characterization: the isolate assimilated sulfate, its oxidation product of sulfide and thiosulfate was sulfate. It utilized citrate, mannitol, ethanol, succinate, glucose, gluconate. pH was 6.8-7.5, Table 1

DISCUSSION

The cultural and biochemical characteristics of the isolate were in agreement with Bergey's, description of the isolate and Lindqust report that it is *R. sphaeroides*. Ambient environmental conditions effectively supported the isolate *R. sphaeroides* in its bio-hydrogen production. On glucose, production started at 160hrs and rounded off at 208hrs. The initial volume of hydrogen produced was 1.6ml/g while the final was 9.8ml/g, Table 2. On PPP, the yield of hydrogen stated at 160hrs with an initial volume of 1.8ml/g and rounded off at 227hrs, with the final volume of 11.8ml/g, Table 2.On YP, the yield started at 160hrs with initial volume of 1.8ml/g and rounded off at 6hrs with a final volume of 7.5ml/g Table 2. On FCWW, the yield of hydrogen started at 6hrs with initial volume of 0.8ml/g and rounded off at 62hrs with a final volume of 7.5ml/g, Table 3.The yield of hydrogen from glucose, PPP and YP by *R. sphaeroides* at this ambient environmental conditions was impressive, showing a reliable performance, FCWW gave a poor performance which may be as a result of its low content of carbohydrate since the pap component has been removed for dietary use. It is not advisable to engage glucose in bio-hydrogen production because it not cost effective. PPP

and YP are very suitable for this purpose since they are waste materials and when thrown away constitutes environmental eye sore. The isolate *R. sphaeroides* has very high conversion efficiency since it required small amount of these substrates for the yield of these volumes of hydrogen. And the ambient environmental conditions are without monetary involvement.

CONCLUSION

The adoption of bio-hydrogen production using ambient environmental condition offers promising alternative to the application of electricity to power the isolate, *R. sphaeroides*. However the ambient environmental approach need not necessarily replace the use of electrically powered production but may complement it. The ambient environmental approach will best be sited outdoor not indoors to enable the organism the opportunity of adequate power supply from the sunlight.

REFERENCES

Agrawal, P., Hema, R., & Mahesh, K. (2007). Experimental investigation on biological hydrogen production using different biomass. *Journal Teknologi.* 47 (1), 13-2.

Bolliger, R., Zurrer, L., & Bacholen, R. (1985). Photo production of molecular hydrogen from waste water of a sugar refinery by photosynthetic bacteria. *Applied Microbiol Biotechnol. 23*, 147-151.

Gadhamshetty, V., Sukumaran, A., Nirmaiakhandaz N., & Thein Myint, M. (2008). Photo fermentation of malate for biohydrogen production - A modeling approach. *International Journal of Hydrogen Energy. 33*, 2138-2146.

Jeong, T.Y., Cha, G.C., Yeom, S.H., & Choi, S.S. (2008) Comparison of hydrogen production by four representative hydrogen-producing bacteria. *Journal of Industrial and Engineering Chemistry. 14*, 333 -337. http://www.elsevier com/1ocate/jie

Kantachote, D., Torpee, S., & Umsakul, K. (2005). The potential use of an oxygenic phototrophic bacteria for treating latex rubber sheet waste water. *Electronic journal of biotechnology. 8* (3), *http://ejh.ucv.cl/ content/vds/issues/full/8/index.html.*

Lindquist, J. A. (2000). A summary of photosynthetic bacteria. General Microbiology: A Laboratory Manual McGraw-Hill

Mahakhan, P., Chobvijuk, C., Ngmjarearnwong, M., Trakulnalermsal, S., Bucke, C., Svasti, J., et al.(2002) Molecular hydrogen production by a thermotolerant *Rubrivivax gelatinosus* using raw cassava starch as an election donor. *Science Asia*. 37, 415 - 424.

Reungsang, A., Sangyoka, S., Imai, T., & Chaiprasert, P. (2004). 7 Biohydrogen production from cassava starch manufacturing waste water. *The Joint International conference on ''Sustainable Energy and Environment (SEE) ".* 1-3 December, Hua Hin, Thailand.

Salih, P.M., & Maleek, M. I. (2010). Influence of metal irons on hydrogen production by photosynthetic bacteria grown in *Escherichia coli* pre-fermented cheese whey. *Journal of Environmental Protection. I,* 426 -430.

Uyar, B., Erolu, I., Yucel, M., Gunduz, U., & Turker, L. (2007). Effect of light intensity, wavelength and illuminaton protocol on biohydrogen production in photobioreactors. *International Journal of Hydrogen Energy.* 32, 4670 -4677.

Uyar, B., Schumacher, M., Gebicki, J., & Modigell, M. (2008). Photoproduction of hydrogen by *Rhobacter capsulatus* from thermophilic fermentation effluent. *Bioprocess Biosystematic energy.* Doi: 10.1007/s00449-008-0282-9

Fig.1a The isolate in SSYE Broth

Fig 1b The Isolate on SSYE Agar

Table 1: Characterization of Isolates

Characteristics	***Rhodobacter sphaeroides***
Gram reaction	gm –ve
Motility	+
Sulfate assimilation	+
Final oxidation product of sulfide:	
Sulfate	+
Vitamin required p-ABA Thiamine Niacin	-
Thiosulfate oxidized to sulfate	+
Growth on H_2/CO_2	+

Aerobic dark growth	+
Utilization of Citrate	+
Mannitol	+
Glycerol	-
Ethanol	+
Succinate	+
Glucose	+
Gluconate	+
Colour of cultures:	
Pinkish-Red	+
Green	-
Yellowish brown	
Optimum pH:	
6.8 – 7.5	+
5.5 – 6.0	
10.3	-

Hydrogen gas output by *R. sphaeroides* at ambient environmental conditions is presented on tables 2 and 3.

Table 2: H_2 output by *R. sphaeroides* with glucose, PPP and YP at ambient environmental condition, substrate pH and Yeast extract. Incubation was from zero hour

Day	Time/hrs	H_2 ml/g		
		R. sphaeroides		
		Glucose	PPP	YP
7	158			
	159			
	160	1.6	1.8	1.8
8	178	3.1	3.6	3.3
	179	3.4	4.1	3.7
	180	3.8	4.6	4.2
	181	4.3	5.1	4.7
	182	4.8	5.6	5.2
	183	5.3	6.0	5.6
	184	5.8	6.4	6.0
9	202	7.1	8.1	7.4
	203	7.5	8.6	7.9
	204	7.9	9.2	8.5
	205	8.4	9.7	9.0
	206	8.9	10.1	9.4
	207	9.3	10.5	9.8
	208	9.8	10.9	10.2
10	226		11.5	10.6
	227		11.8	

Table 3: H_2 output by *R. sphaeroides* with FCWW at ambient light intensity, substrate pH and yeast extract incubation from zero hour

Day	Time/Hr	H_2/m
1	0[a]	
	1	
	2	
	3	
	4	
	5	
	6[b]	0.8
2	34[c]	2.3
	35	2.4
	36	2.7
	37	3.0
	38	3.6
	39	3.9
	40	4.2
3	58	5.9
	59	6.4
	60	6.8
	61	7.1
	62	7.5