MICROBIAL HYDROGEN GAS PRODUCTION FROM FERMENTED CORN WASTE WATER BY *Rhodobacter Sphaeroides*

Mbachu, I. A. C.
Department of Microbiology Anambra State University, P.M.B. 02 Uli, Anambra State, Nigeria

Abstract: *Rhodobacter Sphaeroidesw as* isolated from four environmental samples, lake, mud, soil andstream by specific enrichment. Identification of the isolate by cultural characteristics, Gram stain, sugar fermentations and sulfide oxidation showed that it is *Rhodobacter Sphaeroidesby* Bergey'scriteria. *R. Sphaeroidescan* produce hydrogen gas as a product of metabolism, and previous studies have shown that the organism is associated with waste treatment. Therefore, the potentiality of the isolate for hydrogen gas production at ambient environmental conditions using fermented corn waste water and glucose which served as a control was investigated. The effect of light intensity on the potential of the isolate was evaluated. At ambient environmental conditions, the yield of hydrogen gas by the organism was 7.8ml/g with fermented corn waste water and 9.8ml/g **with** glucose. The conversion efficiency values of *R. sphaeroides* for fermented corn waste water and glucose were statistically significant ($p < 0.05$). In the optimization experiments, there was increasing hydrogen evolution withincreasing light intensities. The highest hydrogen gas was recorded at 120W and the least was obtained at 150W while there was no yield of hydrogen gas at 200W. The results show that *R. sphaeroidesis* valuable for the treatment of organic carbon waste and for the realization of economic products.

Key words: *Rhodobacter Sphaeroides, Hydrogen gas production Fermented corn waste water.*

INTRODUCTION

There are two different groups of microorganisms which can produce hydrogen gas. These are the anaerobic bacteria and the photosynthetic bacteria (Jeong*et al* 2008). They both use organic substrates as sole sources of electrons and energy, and convert them into hydrogen. There are some differences between them however the anaerobes do not possess hydrogen evolving enzymes. So they have to be coupled to photosynthetic bacteria if they have to release hydrogen gas. Secondly, they do not require light energy instead they operate a process known as dark fermentation (Levin *et al* 2004).The photosynthetic bacteria on the other hand are of two types, purple-non sulfur bacteria and the cyanobacteria. The cyanobacteria use water only, in the presence of light to produce hydrogen. They do not utilize organic substrates because they photosynthetically produce their own organic carbonintracellularly (Ahoren, 2004, Taton *et al*; 2003). The purple-non sulfur bacteria possess all the attributes required for successful

*Corresponding author:
anaemeneify@yahoo.co ***Mbachu, I. A. C.***

Nigerian Journal of Microbiology 2015, 29: 2946-2959
Published online at www.nsmjournal.org

microbial hydrogen gas production from agricultural wastes. This is because they use organic substrates as energy and electron sources and convert them into hydrogen gas in the presence of light (Todar, 2008). They possess the two enzyme systems, hydrogenase and nitrogenase, needed for hydrogen gas production (Turker*et al;* 2008). The nitrogenase is very oxygen labile but the nitrogenase complex of photosynthetic bacteria can function at oxygen concentrations that are lower than air.(Roberts and Brill, 1981). Ammonia or ammonium salts are inhibitors of nitrogenase complex in *R. sphaeroides* (Jones and Monty, 1979). Glutamine, other fixed nitrogen sources, and molecular nitrogen provoke similar effect. Light strongly stimulates nitrogencase activity in whole cells (Meyer *et al,* 1978). The capability of *Rhodobacter sphaeroides*to produce hydrogen gas has been investigated to some extent by Koku *et al.,* (2002), Eroglu *et al.,* (2002), Zhu *et al.,* (2001), and Fascetti *et al.,* (1998). According to these researchers, the organism prefers organic acids as carbon sources. However, Mohan *et al.,* (2007) said that other carbohydrates and industrial effluents may also be used for hydrogen gas production by the photosynthetic bacteria. The aim of this work therefore, is to use fermented corn waste water and *Rhodobacter sphaeroides* for the production of hydrogen gas at ambient environmental conditions and to optimize those conditions in the case of positive results.

MATERIALS AND METHODS

Samples Collection

The samples from which the organism was isolated are water, soil and mud. The water samples were collected from different fresh water habitats. These are Oguta Lake in Imo State, Ubahudara stream at Uli, Agulu Lake and Amanssea stream, all in Anambra State. Transparent plastic bottles that were disinfected with 100ppm sodium hypochloride were used to collect the water samples according to Mahakhan *et al.,* (2005).

The soil samples were collected from rice paddy farms according to Montano *et al.,* (2009), at Nise, NnamdiAzikiwe University (NAU) permanent site, Awka and Umuayom village, Awka all in Anambra State. Surface soil samples were randomly collected within a rice field with spoon, then pooled together in a transparent plastic plate and immediately transported to the laboratory for use.

The mud samples were collected the same way the water samples were collected from ponds at Court Road Awka, along the express road in Awka and behind Microbiology Laboratory in NAU.

The sampling was done at two different seasons, dry and rainy for two years. The choice of two seasons is to ascertain whether the habitation of the samples by the organism is seasonal. At each batch, the samples were processed within 24hrs of collection.

Selective Enrichment *for Rhodobacter sp*

The medium used for the selective enrichment of *Rhdobacter sp* was sodium succinate yeast extract broth according to Lindsquit (2000). This medium composition is in three parts.

(1) Basal medium, consisting of KH_2PO_4, 0.33g; $MgSO_4.7H_2O$, 0.33g; NaCl, 0.33g; NH_4Cl, 0.5g; $CaCl_2.2H_2O$, 0.05g; Sodium succinate, 1.0g; and yeast extract,

0.02g. These were dissolved in 1l of distilled water; pH was 6.8. It was autoclaved at 121°C for 15mins and 15lbs pressure.

(2) Trace salts solution consisted of $ZnSO_4.7H_2O$, 0.01g; $MnCl_2.4H_2O$, 0.003g; H_3BO_3, 0.03g; $COCl_2.6H_2O$, 0.02g; $CuCl_2.2H_2O$, 0.01g; $NiCl_2.6H_2O$, 0.002g; and Na_2MoO4, 0.003g. These were dissolved in 1l of distilled water; pH was 4.0

(3) Sterile solutions added after autoclaving the based medium above, consisted of the above mentioned trace salts solution, 1.0ml and 0.02% $FeSO_4.7H_2O$ solution, 0.5ml

Procedure for the Enrichments and Incubation of the culture

The culture bottles used for the enrichment were transparent glass bottles with stoppered lids and 125ml in volume. Into each culture bottle, an 80ml volume of the enrichment broth was ascetically dispensed and autoclaved at 121°C, 15lb pressure for 15mins. Then varying volumes of the water samples (2.5ml, 6.25ml, 8.75ml 12.5ml and 18.75ml) representing 2%, 5%, 7%, 10% and 15% respectively, of the 125ml volume of the culture bottle, were added into the broth medium . For each percentage, a triplicate number of bottles were prepared. Also, varying weights of the soil samples (1.25g; 2.5g; 3.75; 5g and 6.25g) representing 1%, 2%, 3%, 4% and 5% respectively were added into the culture bottles containing the enrichment broth for organism. This was also done with the mud sample. After this inoculation, each of the culture bottles was filled to the neck of the bottle with the broth medium in order to ensure that air bubbles are not trapped in the culture bottle. Some sterile vaseline oil was placed on the top of culture medium in the culture bottles before they were covered with both foil paper and paper tape. This was to create anaerobic condition within the culture bottle according to Kantachote *et al.*, (2005). All the culture bottles were properly labeled for ease of identification and then placed on the laboratory bench to incubate at room temperature under illumination by 150W tungsten lamp, placed at a distance of 90cm from the culture bottles.

Determination of phenotypic characteristics

- **Gram staining, For Cell Shape and Gram reaction.**

  Using a sterile wire loop, a drop of the suspension in the sterile normal saline was taken and used to make a smear, on a glass slide. This smear was allowed to air-dry properly and was heat fixed by rapidly passing the slide, with smear uppermost, three times through the flame of a bunsen burner. This was allowed to cool and then was covered with crystal violet solution for one minute. This stain was rapidly washed off with clean water and after all the water was tipped off the slide, it was covered with Lugol's iodine for 30secs. Then washed off and decolorized rapidly for ten seconds with alcohol, and washed immediately with clean water. A counter stain, Safranin was applied for two minutes, and then washed off. After the staining, the slide was allowed to air-dry by standing it on a rack and was later examined with the light microscope

first with the 40x objective to check the staining and to see the distribution of material and later with the oil immersion 100x objective to report the bacteria and cells.

Characterization of Isolate

The sugars used are glucose, citrate, succinate, mannitol, ethanol, glycerol and gluconate. The indicator was bromothymol blue. Following the manufacturer instructions, 4.2g of nutrient broth was prepared in 1000ml of distilled water and 9mls of this prepared broth was dispensed into various sterile test tubes aseptically. 0.2% w/v of indicator was prepared in 100mls of distilled water. 1ml of this prepared indicator was dispensed into the test tubes containing 9mls of prepared nutrient broth. 10% of each of the sugars was prepared in 100mls of distilled water. Both the test tubes containing the nutrient broth, indicator and the beakers containing the prepared sugars were sterilized and allowed to cool. After cooling, the nutrient broth test tubes were inoculated with the organism and 1ml of the sterilized sugar solution was dispensed into those test tubes and incubated under illumination for 48 – 72 hrs.

Preparations for biohydrogen production

A number of preparations were made for the experiments involving biohydrogen production. These include:

Substrate selection

The substrate used in this work was fermented corn waste water (mili akamu) generated from a kitchen and glucose. The fermented corn waste water was prepared by soaking the corn grains after picking out the stones and other particles from it. The soaking lasted for three days. Then, it was ground to a paste with an electric grinding machine at Eke market in Awka After this, the paste was sieved with a mesh cloth, and was allowed to settle. The supernatant was decanted and the sediment which is the Akamu was transferred to a clean salt bag. In this bag, it was allowed to completely drain out the water from the akamu so that the akamu will be hard. That water that drained out was mixed with the supernatant, and was used for biohydrogen production.

Medium

The medium used for biohydrogen production (sodium succinate yeast extract broth and BG 12 broth) was the basal medium used for the isolation of the organism. No ammonium salt was added and nitrogen was not added. This was to allow for nitrogenase activity according to Bolliger *et al* (1985). The carbon source used was the ones being investigated.

Inoculum Development

The inoculum was a 5 days old culture of the isolate, which was still in log phase of growth (because their numbers were very high). To get the number of cells inoculated, optical density was used. This was done by first plotting a graph of viable cell counts against absorbance. Then the subsequent optical densities of dilutions were read off on the plotted graph, and the cell number that corresponded with the absorbance at any wavelength was taken as the optical density of that number of cells. For this work, the cell number of 10^3 per ml corresponding to OD 640nm was used.

Construction of photobioreactor

This consisted of 400ml conical flask with a working volume of 250ml closed with a rubber stopper. A pneumatic membrane was inserted through that stopper 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 water. This is to collect the gas by displacement of water in the burette (Salihand,Maleek, 2010; Uyar*et al.*, 2007; Mahakhan*et al.*, 2005). The reactor was illuminated externally by tungsten lamps of various intensities, placed at 90cm from the reactor.

Experimental Procedures

The experiments were batch experiments, conducted in the above described photoreactor. Initial runs of these experiments were first conducted in order to determine the acceptability of the substrate by the isolate for growth. This was monitored by turbidity and increase in cell number. For this initial runs, varying percentages of the substrate were evaluated, in corresponding volumes of mineral salts medium. These values are 1%, 2%, 3%, 4% and 5% respectively. Finally after the confirmation, a singular quantity was used only, for the rest of the experiments and this value was 3% of 250ml which is 7.5g of the substrate. The substrate at the selected quantity of 7.5g was evaluated for biohydrogen production by the photosynthetic bacterial isolate under ambient environmental conditions. Then the optimization experiments were conducted. Three variables were evaluated for the optimization, which are light intensity, pH and vitamins. These are independent variables and for the light intensity, six values were evaluated, 40w, 60w, 80w, 120w, 150w and 200w. For the pH, three values were evaluated 5.5, 6.8 and 7.0. These values were chosen because it is expected that most bacteria will survive within those pH values. For the vitamins, three types were evaluated, p – ABA, thiamine and niacin. The readings were taken every hour. The gas produced was confirmed to be hydrogen by a spark test with lighted match stick.

Bio-hydrogen production under ambient environmental conditions

The only carbon sources added to the basal media were glucose and fermented corn waste water. The amount of these carbon sources added was 7.5g each at a time. Into the 400ml conical flask, 242.5ml of basal medium was placed. The pH was the pH of the carbon source. This medium was autoclaved at 121^{0}C, 15lb for 15mins. After cooling, the medium was inoculated with 1ml of the isolate suspension with 10^{3}cell per ml. The flask was covered with the stopper described above, and was incubated anaerobically under natural light. The batch was dismantled when it was observed that gas was no longer coming out. This very experiment was conducted with the four substrate types separately for the two isolates whose growth was supported by the carbon sources.

Optimization Experiments

The effects of variable light intensities on biohydrogen production by *R. sphaeroides* using FCWW and glucose assubstrates were evaluated. All were batch experiments. Six

different light intensities were evaluated, 40w, 60w, 80w, 120w, 150w and 200w. The experimental set up consisted of the 242.5ml basal medium, into which 7.5g of any of the carbon sources was added making it 250ml, 1m of 10^3 cell suspension was added. Each of the light intensities was studied at a time. Each of the experiments was terminated when it was observed that the gas was not coming up any longer.

Statistical Analysis

The results (data) obtained from the experiment were statistically analyzed using descriptive statistics.

Table 1: Characterization of Isolate

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	+
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	

RESULTS

Characterization of the Isolate.

The characteristics of the isolate are presented on Table 1. The isolate was Gram negative and exhibited positive for motility, sulfide oxidation, sulfate assimilation, and aerobic dark growth. It fermented citrate, mannitol, ethanol, succinate, glucose and gluconate, but did not ferment glycerol. The colour was pinkish-red and pH range was 6.8 to 7.5.

Hydrogen Production at Ambient Environmental Conditions

Amounts of hydrogen gas produced by *R. sphaeroides* at ambient environmental conditions using glucose are presented on Table 2. Initial output of hydrogen was at 160h with a volume of 1.6ml and the last was at 208h with a volume of 9.8ml.. Table 3 shows the amount of hydrogen gas produced by*R. Sphaeroides* using FCWW at ambient environmental conditions. Initial yield of hydrogen was at 6h with a volume of 0.8ml while the last was at 62h with a volume of 7.8ml.

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

Day	Time/hrs	H_2 ml/g
7	158	
	159	
	160	1.6
8	178	3.1
	179	3.4
	180	3.8
	181	4.3
	182	4.8
	183	5.3
	184	5.8
9	202	7.1
	203	7.5
	204	7.9
	205	8.4
	206	8.9
	207	9.3
	208	9.8
10	226	
	227	

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/mg
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

Hydrogen production on the bases of the different light intensities.

Amounts of hydrogen gas produced by *R. sphaeroides* with glucose at different light intensities are presented on Table 4. The first yield was by 120W with 2.4ml of hydrogen at 134h.. The initial yield by 150W was at 154h with a volume of 2.3ml. With 80W, initial yield was at 156h with a volume of 2.3ml. The yield by 40W and 60W started at the same time, 158h but with different volumes, 1.6 for 40W and 2.2 for 60W. The yield by 120W and 150W stopped at the same time, 208h while for 60W and 80W it stopped at 226h and 227h.

Table 5 shows the amounts of hydrogen gas produced by *R. sphaeroides* with FCWW at different light intensities. The initial yield started at the third hour of incubation by 120W with a volume of 2.0ml. This was followed by 60W and 80W which started at the fourth hour of incubation with volumes of 1.0ml and 1.6ml respectively. The yield by 40W and 150W started at the same time, the fifth hour of incubation with the same volume of 1.0ml for each. Production stopped last by 120W with a volume of 19.9ml. For 80W and 150W it stopped at the same time which was 83h with 16.4ml and 14.2ml respectively. That by 60W stopped at 82h with a volume of 13.8ml.

Table 4: H_2 output by *R.sphaeroides*with glucose at different light intensities, Incubation was from zero hour

Day	Time/hrs	H_2 ml/g					
		40W	60W	80W	120W	150W	200W
6	134				2.4		
	135				2.8		
	136				3.3		
	137				3.8		
7	154				5.8	2.3	
	155				6.5	2.9	
	156			2.3	7.2	3.4	
	157			2.8	8.0	3.2	
	158	1.6	2.2	3.3	8.9	3.7	
	159	1.9	2.8	3.8	9.6	4.4	
	160	2.1	3.0	4.3	10.3	4.5	
8	178	3.6	4.7	6.3	12.3	5.6	
	179	4.0	4.9	6.9	12.9	6.2	
	180	4.5	5.6	7.5	13.6	6.4	
	181	5.0	6.4	8.3	14.3	6.6	
	182	5.5	7.2	9.0	15.1	7.6	
	183	5.9	8.0	9.7	15.8	8.0	
	184	6.3	8.6	10.3	16.6	8.5	
9	202	8.1	10.1	12.1	18.6	9.5	
	203	8.4	10.7	12.9	19.3	10.5	
	204	8.9	11.4	13.7	20.0	11.2	
	205	9.5	12.1	14.5	20.7	13.0	
	206	10.1	12.8	15.3	21.4	14.0	
	207	11.3	13.2	16.1	21.9	14.9	
	208		13.9	16.7	21.9	15.8	
10	226		15.4	18.9			-
	227			19.8			-
	228						-
	229						-
	230						-
	231						-
	232						-

Table 5: Effect of Light Intensities on *R. sphaeroides* for hydrogen production from FCWW.Incubation was from zero hour

Day	Time/hr	Different Light Intensities					
		40W	60W	80W	120W	150W	200W
1	3				2.0		0.0
	4		1.0	1.6	2.6		0.0
	5	1.0	1.4	2.1	3.2	1.0	0.0
	6	1.3	1.7	2.6	3.7	1.4	0.0
2	34	3.1	3.7	5.1	6.7	3.6	0.0
	35	3.5	4.2	5.7	7.3	4.2	0.0
	36	3.9	4.7	6.4	7.8	4.8	0.0
	37	4.4	5.4	7.1	8.5	5.2	0.0
	38	4.8	6.1	7.8	9.2	5.7	0.0
	39	5.2	6.6	8.4	9.8	6.4	0.0
	40	5.6	7.1	9.0	10.3	7.1	0.0
3	58	7.0	9.3	11.6	13.8	10.6	0.0
	59	7.4	9.8	12.2	14.5	11.1	0.0
	60	7.9	10.5	12.8	15.2	11.6	0.0
	61	8.5	11.2	13.5	15.8	12.2	0.0
	62	9.1	11.8	14.2	16.5	12.9	0.0
	63	9.6	12.3	14.7	17.0	13.5	0.0
	64	10.0	12.7	15.1	17.4	13.9	0.0
4	82		13.8	16.4	19.4	14.2	0.0
	83			16.4	19.9	14.2	0.0
	84				19.9		0.0
	85				19.9		0.0

DISCUSSION

Effectiveness of Glucose as a substrate for biohydrogen production by *R.sphaeroides*.at ambient light intensity, substrate pH and yeast extract.

Hydrogen production started at 160hrs with *R. sphaeroides*and rounded off at 208 hrs. This was at ambient light intensity, substrate pH ie 7 and yeast extract. The initial volume was 1.6 ml/g while the final was 9.8ml/g Table 2.

The amount of glucose consumed by *R.sphaeroides*for the production of 9.8ml of hydrogen was 0.96g. This shows that *R. sphaeroides* has very high conversion efficiency. Also the pH of the medium which was the substrate pH., ie 7, may be responsible for the low yield by the organism whose optimum pH is 6.8. The use of yeast extract as source of vitamin was supportive of the growth of the organism.

Effectiveness of FCWW as a substrate for biohydrogen production by *R. sphaeroides*at ambient light, substrate pH and yeast extract.

The experimental set up was completed by 9.00am on day 1 and that represented the zero hour. Six hours after incubation ie 2.00pm, hydrogen production commenced with initial output of 0.8ml as shown in Table 3. The production stopped on day 3 at 62nd hour. On the other hand, the experimental set up for the use of glucose as a substrate was completed at about the same 9.00 am on day 1. After 160h of incubation corresponding to day 7, hydrogen production started with an initial volume of 1.6ml and rounded off after 208hrs which represented day 9 with a volume of 9.8ml as show on Table 2. From these results, production started earlier in FCWW and also ended earlier but with lower volumes of hydrogen gas than those of glucose. The incidence of early output of hydrogen observed in this substrate may be attributed to the fact that the substrate was fermented and so, was in the form of organic acids which the organism could utilize conveniently.Also corn is a cellulose carbohydrate consisting of lignin according to Agrawal*et al.*, (2007) which will require an organism with a cellulolytic enzyme to degrade in order to release the hydrogen content. FCWW has the capability of yielding hydrogen gas by *R. sphaeziodes* though at a lower volume than with glucose at ambient light intensity.

Effect of Light Intensities on biohydrogen production by *R.sphaeroides*.

Six Light intensity variations were investigated. The pH was still the substrate pH and the source of vitamin was yeast extract. The results showed that hydrogen production increased with increasing light intensity. This is in agreement with Bolliger*et al*, (1985) and Kantachote*et al* (2005). However, with higher light intensities such as 150w evolution was seriously lowered as can be seen in the Table 5. There was no evolution of hydrogen gas at all with 200w.This could be attributed to the fact that the cells were being denatured by the high temperature generated by the 200w,a situation which they described as saturation effect of lightYang *et al* (2002).From the results, hydrogen gas evolution started earlier bythe organism under 40w light intensity than it did under ambient light intensity and the

yield was higher too Tables 4 and 5. The results showed that 120w intensity was the best for the organism as can be seen by the volume of hydrogen evolved. The organism is indeed photosynthetic heterotroph which is able to utilize the carbon sources provided and require light activities.

The material used for the construction of the photobioreactor is also a factor in connection with light intensity. According to Waligorska*et al* (2006), the yields of hydrogen generation process as well as their kinetic parameters are strongly dependent on the kind of microorganisms applied, cultivation conditions, light intensity, light spectrum, and distribution inside the photobioreactor. And light distribution inside the reactor strongly depends on the material applied for photobioreactor construction. Also that the material used for construction of photobioreactor should exhibit low diffusivity for hydrogen and oxygen, indicate high transmissitivity for solar radiation and sterilization possibilities. In their work, they observed that the highest yield of hydrogen was obtained when sodium glass photobioreactors, illuminated with 5klux was applied. And that application of borosilicate glass or polycarbonate photobioreactors reduced the amount of evolved hydrogen by 75%. In this work, pyrex was used.

Conclusion

The objectives of this research were met. The results showed that the waste substrate applied was utilized by *Rhodobactersphaeroides* to produce hydrogen. Fermented corn waste water was fairly comparable to glucose for biohydrogen production.From these findings, FCWW that was before now regarded as waste will now be seen as potential raw material for production processes and therefore will no more be thrown away carelessly to both pollute and deface the environment.Efforts should be vested towards obtaining pure culture of the organism from habitats, which are very much around our environment.There was hydrogen production at ambient light intensity but it was low.Hydrogen evolution was increasing with increasing light intensities but 200w light intensity did not support hydrogen production. The optimum was 120w. The next to it was 150w. The optimum pH was 6.8. The optimum vitamin for *Rhodobactersphaeroides* is thiamine but niacin is equally good.

Contributions

1. Wesucceeded in showing that, fermented corn waste water, can be used to produce hydrogen gas.
2. Axenic culture of *Rhodobactersphaeroides*, can utilize the above mentioned waste under ambient environmental conditions, and optimum conditions to produce hydrogen gas.
3. The optimum Light intensity is 120w.

Recommendation

- Shaker should be used for further experiments.
- Photobioreactors with side outlet should be used in order to evacuate gas head oxygen.
- Burette with larger volume should be constructed to collect more volumes of hydrogen gas or metal cylinders that will be capped with a valve.
- The substrates used should be subjected to fermentation by

other heterotrotrophs other than the photoheterotrophs. This will cut short the lag phase.

- A continuous batch experiment may be attempted as that will allow the organisms the chance to thoroughly exhaust the hydrogen molecules in the carbohydrates.

REFERENCES

Ahoren, O., (2004). A proposal for further integration of the cyanobacteria under the bacteriological code. *International Journal of Systematic Evolutionary Microbiology*.**54** (5), 1895-1902.

Eroglu, E., Eroglu, I., Gunduz, A., Yucel, M., &Turker, L., (2002).Biological hydrogen production from olive mill waste water by *Rhodobactersphaeroides*O.U. 001.*In: Proceedings of International Conference on Environmental problems of Mediterramean region (EPMR) 2002)*.Nicosa, North Cyprus.

Fascetti, E., D'Addario, E., Todini, O., &Robertiell O., A. (1998). Photosynthetic hydrogen evolution with volatile organic acids derived from the fermentation of source selected municipal wastes. *International Journal of Hydrogen Energy*.**23**, 753 – 756.

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/locate/jie

Jones, B.L; & Monty, K.J.(1979). Glutamine as a feedback inhibitor of the *Rhodopseudomonassphaeroides*nitrogenase system. *Journal of Bacteriology*. **139**, 1007-1013.

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.*

Koku, H., Eroglu, I., Grunduz, U., Yucel, M., &Terker, L. (2002) Aspects of the metabolism of hydrogen production by *Rhodobactersphaeroides.International Journal of Hydrogen Energy*.**27**, 1325-1329.

Levin, D.B., Pitt, L., & Love, M.(2004). Biohydrogen production: prospect and limitations to practical application. *International Journal Hydrogen Energy*. **29**, 173-185.

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

Mohan, S.V., Bhaskar, Y.U., & Krishna, P.M. (2007).Biotydrongen Production from chemical wastewater as substrate by selectively enriched anaerobic mixed consortia: influence of fermentation pH and substract composition. *International Journal of Hydrongen Energy*.**32**, 2286-2295.

Mahakhan, P., Chobvijuk, C., Ngmjareamwong, M., Trakulnalermsal, S., Bucke, C., Svasti, J., et al.(2005). Molecular hydrogen production by a thermotolerant*Rubrivivaxgelatinosu* susing raw cassava starch as an election donor.*Science Asia*.**31**, 415 – 424.

Meyer, J., Kelley, B.C. &Vignais, P.M.(1978). Nitrogenase fixation and hydrogen metabolism in

photosynthetic bacteria. ***Biochimie. 60,*** 246-260.

Montano, G.L., Chan, J.S., Jarabelo, R.E., Pastor, A.B.L. & Delacruz, T.E.E. (2009). Isolation and characterization of purple non-sulfur bacteria (PNSB) from a rice paddy soil in Bulacan, Philippines. *Philippine Journal of Systematic Biology.**111,*** 57 – 67.

Reungsang, A., Sangyoka, S., Chaiprasert, P., & Imai, T. (2007). Factors affecting Hydrogen production from cassava waste water by a co-culture of Anaerobicsluge and *Rhodospirillumrubrum. Pakistan journal of biology cseince.**10*** (20), 3571 - 3577.

Roberts, G.P., & Brill, W.J.(1981). Genetics and regulation of nitrogen fixation. *Annual Review of Microbiology. **34,***183

Salih, F.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. **1,*** 426 – 430.

Taton, A., Grubisic, C., Brambilla, E., DeWit, R., &Wilmotte, A.(2003). Cyanobacterial diversity in natural and artificial mats of Lake Fryxell(Dry Valleys, Antarctica): a morphological and molecular approach. *Applied Environmental Microbiology.**69,*** 5157-5169.

Todar, K. (2008). Important groups of Prokaryotes.Online Textbook of Bacteriology.*Textbook of bacteriology.net/prokaryotes2:html.*

Turker, L., Gumus, S., &Tapan S.(2008). Biohydrogen Production: Molecular aspects. *Journal of Scientific and Industrial Research.**67,*** 994-1016.

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.

Waligorska, M., Moritz, M., &Laniecki, M. (2006). Hydrogen generation by *Rhodobactersphaeroides* O.U.001: the effect of photobioreactor construction material. *World Hydrogen Energy Conference 16 (2006).13 – 16 June. (ie) 16/13 – 16 June 2006.*Lyon France.

Yang, S.P., Wang, Z.W., Zhao, C.G., Qu, Y.B., &Qians, X.M. (2002).Generation of Hydrogen from Photolysis of Organic acid by photosynthetic bacteria.*Chinese Chemical Letters.**13*** (11), 1111 – 1114. http://www.imm.ac.cn/journal/cc.html.

Zabut, B., Sharif, F.A., &Bashiti, T. (2002). Photo production of hydrogen by *Rhodobactersphaeroides* O.U. 001 in a column photoseactor: effect of *Halobacteriumhalobium. Journal of the Islamic University of Gaza.**10***(1), 21 – 32.

Zhu, H.G., Wakayama, T., Asada, Y., and Miyake, J. (2001). Hydrogen production by four cultures with participation by anoxygenicphototrophic bacterium and anaerobic bacterium in the presence of NH_4^+ . *International Journal of Hydrogen Energy.**26,*** 1149-1154.