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Production of Clean Fuel from Waste Biomass using Combined Dark and Photofermentation

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Abstract: *Sequential dark and photo- fermentation is a rather new approach in biological hydrogen gas production. In the present work, two-stage fermentation process consisting of dark and photo-fermentation periods was carried out in a batch reactor. The study mainly emphasized on assessing the potential of biological conversion of different substrates to hydrogen by studying various parameters like temperature, pH and cell density to achieve maximum hydrogen production. In the first stage, substrate was fermented in the dark stage using Bacillus licheniformis, Enterobacter cloacae and Halobacterium salinarum to produce acetate, CO_2 and H_2. The acetate produced in the first stage is fermented to H_2 and CO_2 by Rhodobacter sphaeroides for further hydrogen production in the second, illuminated stage. The percentage yield for Bacillus licheniformis, Enterobacter cloacae and Halobacterium salinarum using Rhodobacter sphaeroides was found to be 35.7%, 32%, and 26% respectively and process proficiency was found to be 0.2, 0.35 and 0.16 moles/kg*.

Key Words: *Hydrogen; Dark fermentation; Photo- fermentation; Bacillus licheniformis, Enterobacter cloacae; Halobacterium salinarum; Rhodobacter sphaeroides*

I. INTRODUCTION

Two-stage system has certain advantages over single- stage dark-fermentation or photo-fermentation processes. The effluent of dark fermentation in hydrogen production provides sufficient amount of organic acids for the photo- fermentation. Therefore, the limitation by the organic acid availability would be eliminated. Higher hydrogen production yields can be obtained when two systems are combined [1]. Further utilization of organic acids by photo-fermentative bacteria could provide better effluent quality in terms of COD. However, the system should be well controlled to provide optimum media composition and environmental conditions for the two microbial components of the process [2-3]. The ammonia concentration in the effluent of anaerobic fermentation should not be at the inhibitory level for the photosynthetic bacteria [4]. Dilution and neutralization of dark fermentation effluents are required before photo-fermentation to adjust the organic acid concentration and the pH to 7 for the optimal performance of photosynthetic bacteria [5].

Bio-hydrogen production by co-culture of anaerobic and photosynthetic bacteria in single stage has also been studied. Yokoi obtained higher hydrogen production yield (4.5 mol/mol glucose) by co-culture of *C. butryricum* and *Rhodobacter sp*. as compared to single stage dark fermentation (1.9 mol/mol glucose) and sequential two-step fermentation (3.7 mol/mol glucose) of starch. Similarly, higher hydrogen yields from different substrates were reported by co-cultures of *R. marinum* and *V. fluvialis* as compared to *R. marinum* alone[6]. Better hydrogen yield (60%), was observed in combined fermentation by *Lactobacillus amylovous* and *R. marinum* from algae biomass in comparison to sequential two-stage fermentation (45%). In addition, pH of the mixed fermentation remained around pH=7 which is considered as an advantage over the two- stage fermentation process. However, the differences in organic acid production/consumption rates and therefore, potential accumulation of organic acids in the media are the major problems.

The microbial production of hydrogen by fermentation can be broadly classified into two main categories, one is light- independent and the other is light-dependent. The light- independent fermentation process, commonly known as dark fermentation, employs both strict anaerobic and facultative bacteria for the production of hydrogen from a variety of potentially utilizable substrates, including refuse and waste products. It generally gives a higher rate of hydrogen evolution and does not rely on the availability of light sources[7]. In contrast, in photo-fermentation, small-chain organic acids are used by photo-synthetic bacteria as electron donors for the production of hydrogen at the expense of light energy. Phototrophic bacteria have an advantage over their fermentative counterparts, both in terms of a high theoretical conversion and the ability to use light energy in a wide range of absorption spectra. Moreover, these organisms lack an oxygen-evolving activity, which otherwise might cause oxygen-inactivation problems in different biological systems[8]. The generation of hydrogen by fermentative bacteria accompanies the formation of organic acids (e.g. acetate or butyrate) or solvents (e.g. acetone, butanol) as metabolic products. However, these anaerobes are incapable of further breaking down the acids. The accumulation of these acids results in a sharp drop in culture pH and subsequent inhibition of bacterial hydrogen production [9, 10, and 11] leading to low yield. Theoretically, the maximum hydrogen

yield is 4 mol hydrogen mol^{-1} glucose when glucose is completely metabolized to acetate or acetone in the anaerobic process[12]. But, it is somewhat difficult to achieve the complete degradation of glucose to carbon dioxide and hydrogen through anaerobic dark fermentation. This appears to be one of the major bottlenecks in the dark fermentation process. The yield of 4 mol H_2 mol^{-1} glucose is too low to be economically viable as an alternative to the existing chemical or electrochemical processes of hydrogen generation[13]. So, the process outlined in the present work takes into consideration the relatively low achievable yield of hydrogen in dark fermentation and the non-utilization of the acids produced therein. Combining fermentative bacteria with photosynthetic bacteria could provide an integrated system for maximizing the hydrogen yield. In such a system, the anaerobic fermentation of carbohydrate (or organic wastes) produces intermediates, such as low-molecular-weight organic acids, which are then converted into hydrogen by photosynthetic bacteria in the second step in a photo-bioreactor. The overall reactions of the process can be represented as:

Stage I. Dark fermentation (facultative anaerobes).
$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ (1)

Stage II. Photo-fermentation (photosynthetic bacteria).
$2CH_3COOH + 4H_2O \rightarrow 8H_2 + 4CO_2$ (2)

So, theoretically it is evident that using glucose as the sole substrate in dark anaerobic fermentation, where acetic acid is the predominant metabolite product, a total of 12 mol hydrogen could be expected in a combined process. Lee et al. [14] studied the combination of purple nonsulfur (PNS) photosynthetic bacteria and anaerobic bacteria for the efficient conversion of wastewater into hydrogen. In their study, effluents from three carbohydrate-fed reactors (CSTR, ASBR, and UASB) were used for hydrogen production. In another study, Kim et al.[15] combined dark fermentation with photo-fermentation to improve hydrogen productivity from food processing wastewater and sewage sludge. In the present study, glucose was considered as a preliminary substrate in the dark fermentation process and the spent medium from this process was used as a substrate for anoxygenic phototrophic PNS bacteria for hydrogen production in photo-fermentation.

I. MATERIALS AND METHODS

Microorganism and culture conditions

Clostridium pasteurianum (MTCC No. 116), *Enterobacter cloacae* (MTCC No. 509), *Bacillus licheniformis* (MTCC No. 429) *and Halobacterium salinarum* (MTCC No.1598) *strains were used for dark fermentation process.*

Rhodobacter sphaeroides strain ATCC 17023, procured from American Type Culture Collection was used in the photo-fermentation study. This is a phototrophic bacterium. The cells are ovoid, 0.5 – 0.7μm wide and 2–2.5μm long, gram-negative and motile. The organism was grown photo-heterotrophically with malate (0.4%) and sodium glutamate (0.15%) as carbon and nitrogen source, respectively (using ATCC standardized media) at 32 ± 2°C and about 3.75 W m^{-2} = light intensity in an anoxic environment. The initial pH of the growth medium was maintained at pH 6.8±0.2[16].

A. Substrate

Waste biomass containing high percentage of cellulose content - sugarcane waste (bagasse) - was selected as substrate. The substrate was pretreated by washing with deionized water and dried (at 120°C) to remove moisture. The dried substrate was macerated into small pieces of approximately 1-2 mm in particle diameter.

B. Experimental Procedure

Hybrid fermentation technology using combined process of dark and photo-fermentation was carried out to study the feasibility of biological hydrogen production. In dark fermentation, microorganisms *Bacillus licheniformis, Enterobacter cloacae and Halobacterium salinarum* was used with photo-fermentative bacteria *Rhodobacter sphaeroides* using bagasse as substrate. The dual process was studied in terms of several process parameters, such as initial substrate volume, initial pH of the medium and temperature, to establish favorable conditions for maximum hydrogen production. Batch reactor experiments were conducted to determine conditions for maximum biological H_2 production yields. A method used to increase the H_2 yield involved bio-reactor. This method was accomplished by in two different ways using batch reactor tests. The experimental procedures were carried out initially in batch tests and later medium batch process using bioreactors were carried out.

C. Hybrid Process

In combined process, the batch test and medium batch test using bio-reactor was conducted using bagasse as the main source of substrate.

Batch test

Bagaase was weighed (35g) and transferred into conical flask. The conical flasks along with the substrate was subjected to pre-treatment,using steam under sterilization in autoclave at 15lbs pressure and temperature greater than 100^{o}C.The minimal media of required pH and the inoculums was prepared. Once the substrate was cooled, 500ml of minimal media and required amount of inoculums *Rhodobacter sphaeroides* (Photosynthetic bacteria) with *Enterobacter clocae, Rhodobacter sphaeroides* with *Bacillus licheniformis* and *Rhodobacter sphaeroides* with *Halobacterium salinarum* was mixed separately to different conical flasks in the laminar air flow chamber under sterile conditions. The experimental study was conducted using one bacterial strain at a time with Photosynthetic bacteria *Rhodobacter sphaeroides*. Conical flasks were then fitted with a stopper having an outlet connected to water displacement jar of 1ltr capacity. The experimental set-up was then incubated at required temperature (26^{o}C, 29^{o}C, 32^{o}C, 38^{o}C and 42^{o}C). The experiments were conducted in under anaerobic conditions using illuminated light source. The volume of water displaced was equal to the volume of gas produced. Each condition studied was duplicated and triplicated and average gas production (ml) was reported.

Medium Batch test

Experimental set up for hybrid hydrogen production

Hydrogen production experiments were carried out in a 500 ml volume column photobioreactor. The reactor had adapted lid. The lid had three outlets: one for H_2 gas outlet, which is connected to the H_2 collection system, the second opening was for removal of argon during bubbling of the culture medium, the third opening had two connections, one connection was for argon inlet, while the other one was for sample collection.The reactor was provided with a water-jacket that allowed continuous water flow provided by a circulatory water-bath which was held at a constant temperature by a thermostat .The jacket also functioned as a filter that prevented the entry of the harmful IR part of the radiation emitted by the light source.

The extensions of the outlet were made by autoclavable tubing. Manipulation of the flow of liquid sample or gas through the tubing was made by ratchet tubing clamps.

The reactor was filled by a culture medium containing minimal media along with substrate bagasse and inoculated with bacterial strains one at a time with *Rhodobacter sphaeroides*. The reactor, then, closed tightly with rubber stopper and together with other connected parts, the setup was constructed and sealed with silicone to prevent any leakage. The gas production was observed after 48 hrs of inoculation. The reactor was illuminated by using a tungsten lamp (150 W) from a distance of 40 cm. The hydrogen gas produced was analyzed by gas chromatography.

D. Gas Collection system

The gas produced from small batch and medium batch processes was collected by water displacement method and in rubber bladder respectively.

Analysis of Biogas

The gas collected from the bio- reactor as was analyzed using a Gas Chromatograph (GC chemito 8610; TCD with porapaq Q column; Temperature: Oven -60^{o}C; Injector - 80^{o}Cand Detector 100^{o}C. Argon was used as carrier gas at a flow- rate of 30 ml/min.

E. Gas Sampling and analysis

The gas sampling was carried out with a syringe of suitable capacity in the liquids sampling port, or with sampling valves with capillaries of different capacity, manually or automatically.

Once the gas sample was injected to the GC, hydrogen peak was observed at the retention time of approximately 2 minutes, indicating the presence of hydrogen percentage produced from each of the substrate. The hydrogen gas percentage was calculated by comparing the sample biogas under investigation with a standard pure hydrogen gas.

II. RESULTS AND DISCUSSION

F. Effect of pH

The effect of pH on hydrogen production enzymes, *Rhodobacter sphaeroides* with *Bacillus licheniformis* is as shown in **(Fig 1)**. The results depicts that the both the organisms show good hydrogen yield at pH ranging between 7 to 8 keeping temperature at 30 ± 2^{0}C .The hydrogen percentage was 35% and the efficiency of hydrogen yield was 0.2mol/kg of substrate. During hydrogen production, the solution's pH

decreased from 8 with time until the pH became 4, after which the pH did not decrease further **(Table 1)**. This phenomenon can be attributed due to the production of secondary metabolites, butyrate and acetate which increase the pH of the broth. The acidic environment created as a result of this will inhibit the production of hydrogen. This indicates that the production of a by-product which is acidic in nature. It was also observed that with decrease in pH of the broth media the relative yield of hydrogen decreased, due to the inhibitory effect of the secondary metabolites. Suitable experiments have to be conducted to ascertain the decrease in the pH of the broth

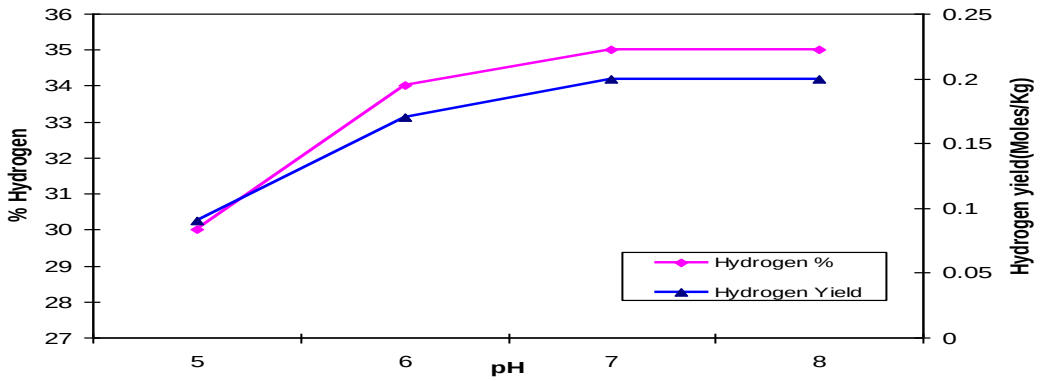

Fig. 1: Study of effect of pH on co-culture of *Rhodobacter sphaeroides* with *Bacillus licheniformis*, at tempreture 30 ± 2^{0}C.

Table 1: Effect of pH on co-culture of *Rhodobacter sphaeroides* with *Bacillus licheniformis*, at tempreture 30 ± 2^{0}C.

pH	Total gas (ml)	Hydrogen %	Hydrogen Yield (Moles\kg)
5	250	30	0.09
6	400	34	0.17
7	473	35	0.2
8	450	35	0.2

G. Effect of Temperature

In **(Fig. 2)** the effect of temperature on yield of hydrogen for *the Rhodobacter sphaeroides* with *Bacillus licheniformis* at pH 7 is represented. After a series of repetitive experiments it was found that the gas yield was high at 32°C **(Table 2)**.The percentage yield was maximum 35.71% and the hydrogen efficiency was found to be 0.2mol/kg of substrate.

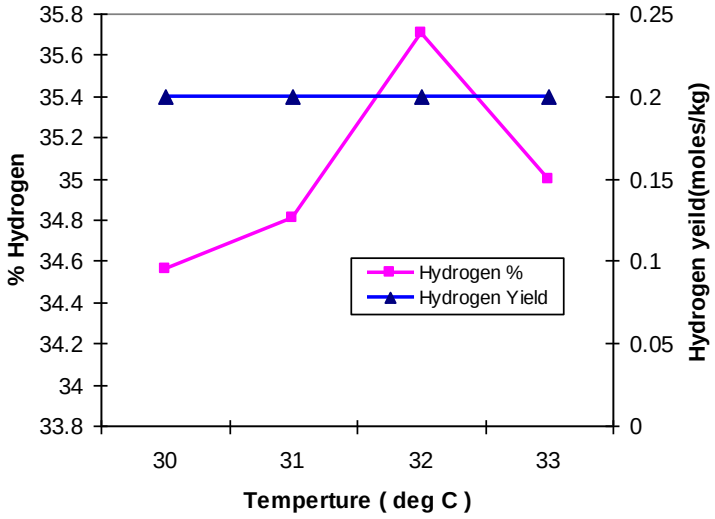

Fig. 2: Study of effect of different temperatures on co-culture of *Rhodobacter sphaeroides* with *Bacillus licheniformis* at pH 7.

Table 2: Effect of different temperatures on co-culture of *Rhodobacter sphaeroides* with *Bacillus licheniformis* at pH 7.

Temperature (^{0}C)	Total gas (ml)	Hydrogen %	Hydrogen Yield (Moles\kg)
30	469.6	34.56	0.2
31	470.5	34.81	0.2
32	473.6	35.71	0.2
33	461.3	35.0	0.2

H. Effect of pH

The results of the effect of pH using coupled culture of *Rhodobacter sphaeroides* with *Halobacterium salinarium* has been given in **(Fig. 3)**. It was found to be that at pH 6 to 7.5, the activity of the cells was high and the yield of hydrogen gas was maximum. The percentage yield of hydrogen was found to be 20 and the efficiency of hydrogen was found to be 0.12mol/kg of substrate. A high pH (8.5-9.0) is unfavorable for photo-fermentation of H_2 because an active uptake hydrogenase functions optimally at this pH **(Table 3)**.

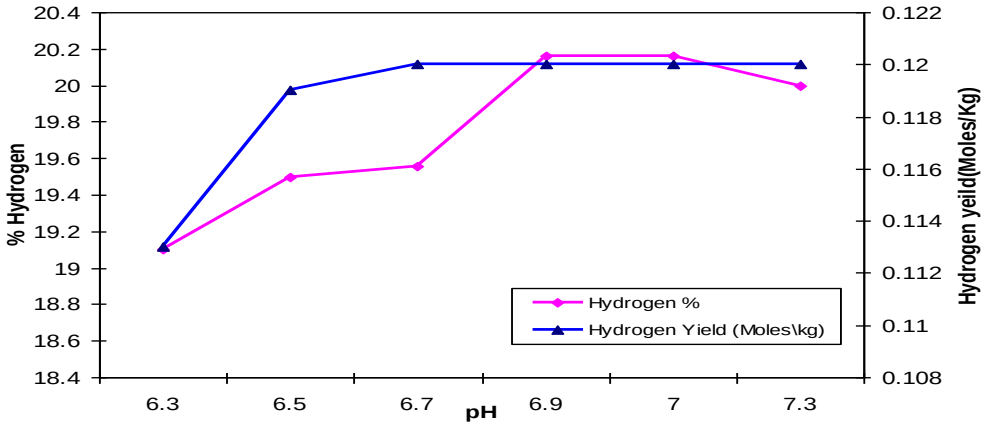

Fig. 3: Study of effect of different pH on co-culture of *Rhodobacter sphaeroides* with *Halobacterium salinarium*

Table 3: Effect of different pH on co-culture of *Rhodobacter sphaeroides* with *Halobacterium salinarium*

pH	Total gas (ml)	Hydrogen %	Hydrogen Yield (Moles\kg)
6.3	462.0	19.1	0.113
6.5	477.5	19.5	0.119
6.7	481	19.56	0.12
6.9	485	20.16	0.12
7	483.5	20.16	0.12
7.3	480	20.0	0.12

I. Effect of Temperature

Fig. 4 represents the effect of temperature on yield of hydrogen for the *Rhodobacter sphaeroides* with *Halobacterium salinarium* at temperature 30. The maximum hydrogen production was observed at this temperature. The hydrogen percentage was found to be 20 and the efficiency of hydrogen was found to be 0.12mol/kg of substrate **(Table 4)**. The main draw- back in this coupled reaction is that the bacteriorhodopsin has low photoactivity under low ionic strength and at temperatures more than 30^0C, so it is recommended to use salt tolerant strain *Rhodobacter sphaeroides* that can work efficiently at 25^0C, which is best temperature for photoactivity of bacteriorhodopsin[17].

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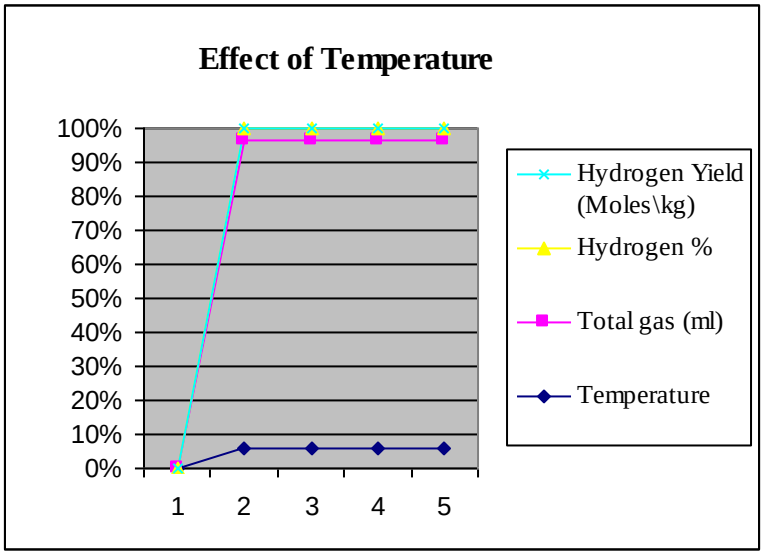

Fig. 4: Study of effect of different temperatures on co-culture of *Rhodobacter sphaeroides* with *Halobacterium salinarium* at pH 7.

Table 4: Effect of different temperatures on co-culture of *Rhodobacter sphaeroides* with *Halobacterium salinarium* at pH 7.

Temperature (^{0}C)	Total gas (ml)	Hydrogen %	Hydrogen Yield (Moles\kg)
30	470.5	19.6	0.11763
31	477.6	20.0	0.12
32	485.0	20.16	0.12
33	482.0	20.16	0.12

J. Effect of pH *Rhodobacter sphaeroides* with *Enterobacter cloacae*

The results of the effect of pH using coupled culture of *Rhodobacter sphaeroides* with *Enterobacter cloacae* has been given in **(Fig. 5)**. It was found to be that at pH 9 to 10, the activity of the cells was high and the yield of hydrogen gas was maximum. The percentage yield of hydrogen was found to be 32 and the efficiency of hydrogen was found to be 0.35mol/kg of substrate **(Table 5)**.

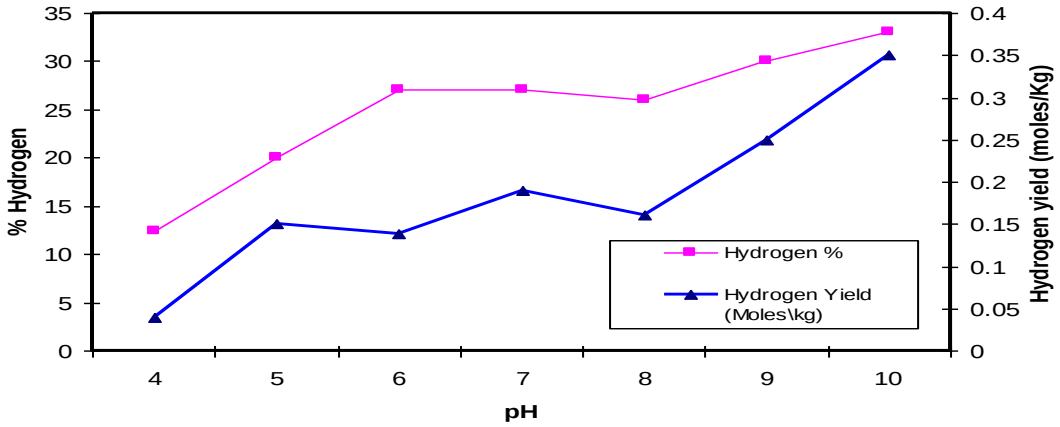

Fig. 5: Study of effect of different pH on co-culture of *Rhodobacter sphaeroides* with *Enterobacter cloacae* at 30 ± 20C.

Table 5. Effect of different pH on co-culture of *Rhodobacter sphaeroides* with *Enterobacter cloacae* at 30 ± 2^{0}C.

pH	Total gas (ml)	Hydrogen %	Hydrogen Yield (Moles\kg)
4	315	12.36	0.04
5	400	20	0.15
6	405	27	0.139
7	562	27	0.19
8	500	26	0.16
9	670	30	0.25
10	850	32.99	0.35

K. Effect of Temperature

Fig. 6 represents the effect of temperature on yield of hydrogen for the *Rhodobacter sphaeroides* with *Enterobacter cloacae* at temperature 32 the maximum hydrogen production was observed at this temperature. The hydrogen percentage was found to be 27 and the efficiency of hydrogen was found to be 0.19mol/kg of substrate **(Table 6)**.

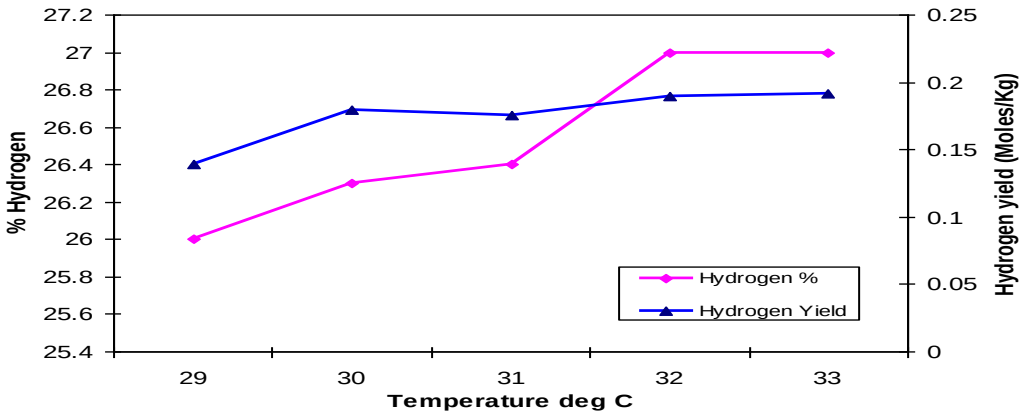

Fig. 6: Study of effect of different temperatures on co-culture of *Rhodobacter sphaeroides* with *Enterobacter cloacae* at pH 7.

Table 6. Effect of different temperatures on co-culture of *Rhodobacter sphaeroides* with *Enterobacter cloacae* at pH 7.

Temperature (^{0}C)	Total gas (ml)	Hydrogen %	Hydrogen Yield (Moles\kg)
29	420	26.0	0.139
30	535	26.3	0.179
31	520	26.4	0.175
32	555	27.0	0.19
33	550	27.0	0.192

L. Effect of Temperature Rhodobacter sphaeroides with Enterobacter cloacae at pH 10

Fig. 7 represents the effect of temperature on yield of hydrogen for the *Rhodobacter sphaeroides* with *Enterobacter cloacae* at temperature 32 and pH 10. The maximum hydrogen production was observed at this temperature and pH. The hydrogen percentage was found to be 32 and the efficiency of hydrogen was found to be 0.35mol/kg of substrate **(Table 7)**.

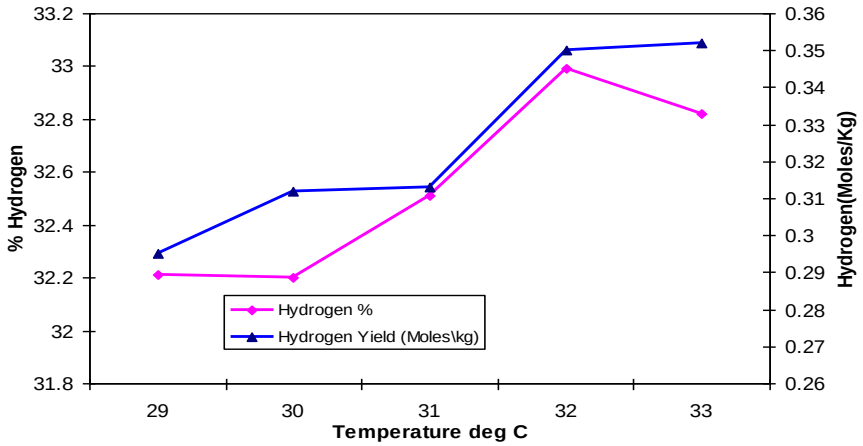

Fig. 7: Study of effect of different temperatures on co-culture of *Rhodobacter sphaeroides* with *Enterobacter cloacae* at pH 10.

Table 7. Effect of different temperatures on co-culture of *Rhodobacter sphaeroides* with *Enterobacter cloacae* at pH 10.

Temperature (^{0}C)	Total gas (ml)	Hydrogen %	Hydrogen Yield (Moles\kg)
29	720	32.21	0.295
30	760	32.2	0.312
31	800	32.51	0.313
32	850	32.99	0.350
33	845	32.82	0.352

III. CONCLUSION

The amelioration of hydrogen productivity by increasing its yield is a challenging area of R&D work in hydrogen biotechnology. Combined dark and photo-fermentation processes, as described in the present work, is an approach to that end. An economic analysis of the process reveals the estimated production cost of hydrogen by the present process is 0.63 Euros l−1 H_2. This estimate is based on reagent-grade pure substrates for media and assumes the cost of biomass as zero. This is considerably higher than the cost of some other bio-hydrogen production processes. However, both the production and unit energy cost of bio-hydrogen could be reduced drastically by using renewable biomass and waste materials as feed stock for fermentation. Moreover, a rigorous techno-economic analysis is necessary to draw a cost-effective comparison between biologically produced hydrogen and the various other conventional fossil fuels. An economic survey, based on fuel cost estimation, appears somewhat complicated when applied in practical terms. This is because of the intervention of several other techno-economic parameters, such as pollution, other short- and long-term environmental costs and direct and indirect health costs, etc. Notwithstanding the economic constraint, the synergy of the present process lies in the maximum utilization of the substrate, which otherwise fails to achieve complete conversion due to thermodynamic limitation.

In dark fermentation, using *E. cloacae*, *B. licheniformis* and *H.salinarum* strains, it is found to have the capacity for the photo-production of hydrogen by *R.sphaeroides* strain in a combined process. The overall yield of hydrogen obtained in the present work using this combined process is higher than either single process. But it is still lower than the theoretical maximum value. The yield could probably have been more if a continuous reactor system was employed, both for the dark and photo-fermentation, instead of a freely- suspended batch culture. Light conversion efficiency poses another limitation to light-driven fermentation processes. This is one of the principal determinants of the cost of a photo-bio-reactor. It was observed that a maximum of only 0.51% light conversion efficiency could be achieved, but at the expense of low light intensity. However, it was difficult to calculate the light conversion efficiency with satisfactory precision, as the experiments were conducted in uncontrollable conditions.

REFERENCE

1. Yokoi H., Mori S., Hirose J., Hayashi S., and Takasaki Y, H_2 production from starch by a mixed culture of *Clostridium butyricum* and *Rhodobacter sp*. M-19, *Biotechnology Letter., 20, 890– 895*, (1998)

2. Yokoi H., Maki R., Hirose, J., Hayashi S., Microbial production of hydrogen from starch manufacturing wastes., *Biomass Bioengineering, 22, 89-39*, (2002)

3. Yokoi H., Saitsu A., Uchida H., Hirose J., Hayashi S, Takasaki Y., Microbial hydrogen production form sweet potato starch residue, J. *Bioscience Bioengineering., 91, 58 – 63*, (2001)

4. Fascetti E., D. Addario, E. Todini, O. Robertiello, A.: Photosynthetic hydrogen evolution with volatile organic acid derived from the fermentation of source selected municipal wastes, *International J. of Hydrogen Energy, 23, 753-760*, (1998)

5. Kawaguchi H., Hashimoto K., Hirata K., Miyamoto K., H_2 production from algal biomass by mixed culture of *Rhodobium marinum* A-501 and *Lactobacillus amylovorus*, *J. Bioscience Bioengineering, 91, 277-282*, (2001)

6. Ike A., Murakawa T., Kawaguchi H., Hirata, K., Miyamoto K., Photoproduction of hydrogen from raw starch using a halophilic bacterial community, *J. Bioscience Bioengineering., 88, 72-77*, (1999)

7. Das D. and Veziroglu TN., Hydrogen production by biological processes: a survey of literature. *International J. Hydrogen Energy, 26, 13– 28*, (2001)

8. Akkerman I., Janssen M, Rocha J. and Wijffels RH., Photobiological hydrogen production: photochemical efficiency and bioreactor design, *International J. Hydrogen Energy, 27, 1195–1208*, (2002)

9. Oh Y-K., Seol E-H., Yeol Lee E., and Park S., Fermentative hydrogen production by a new chemolithotrophic bacterium *Rhodopseudomonas palustris* P4. *International J. Hydrogen Energy, 27, 1373–1379*, (2002)

10. Oh Y-K., Seol E-H., Kim JR. and Park S., Fermentative biohydrogen production by a new chemoheterotrophic bacterium *Citrobacter sp*. Y19. *International J. Hydrogen Energy 28, 1353–1359*, (2003)

11. Namita Khanna, Shireen Meher Kotay, J. Jose Gilbert and Debabrata Das., Improvement of biohydrogen production by Enterobacter cloacae IIT-BT 08 under regulated pH, *Journal of Biotechnology., 152, 9–15*, (2011)

12. Chin H-L., Chen Z-S., and Chou CP. Fedbatch operation using *Clostridium acetobutylicum* suspension culture as biocatalyst for enhancing hydrogen production, *Biotechnology Program, 19, 383– 388*, (2003)

13. Hallenbeck P.C. and Benemann JR., Biological hydrogen production; fundamentals and limiting processes. *International J. Hydrogen Energy, 27, 1185 – 1193*, (2002)

14. C. M. Lee, P. C. Chen, C. C. Wang, and Y. C. Tung, *International J.Hydrogen Energy, 27, 1308*, (2002)

15. Kim M. S., Moon K. W. and Lee SK, Hydrogen production from food processing waste water and sewage sludge by anaerobic dark fermentation combined with photofermentation. In: Miyake J., et al (eds) *Biohydrogen II. Elsevier, Amsterdam, 263–272*, (2001)

16. Sasikala C., Ramana C.V., Rao PR., Regulation of simultaneous hydrogen photoproduction during growth by pH and glutamate in *Rhodobacter sphaeroides* O.U. 001. *International J. Hydrogen Energy., 20, 123–126*, (1995)

17. Xu Li, Zhen-Zhen Dai, Yong-Hong Wang, and Si-Liang Zhang, Enhancement of phototrophic hydrogen production by *Rhodobacter sphaeroides* ZX-5 using fed-batch operation based on ORP level, *International Journal of Hydrogen Energy, 36, 12794–12802*, (2011).