

Status of Biological hydrogen production

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This paper analyzes yields, reaction rates and reactor designs, and compares effectiveness of different substrate-microorganism combinations. Net energy analysis of processes reveals directions for viability and screening of options. Techniques like metabolic flux analysis can be used to establish targets for increased yields. Factors influencing production and possibility of using genetic engineering to increase production rates are discussed. The paper identifies gaps and possible directions for future development of biological hydrogen production.

Keywords: Biohydrogen, Dark fermentation, Photofermentation, Photolysis

Introduction

Hydrogen (H_2), a clean energy source with high-energy yield (122 kJ/g), is considered to be a promising future fuel¹. H_2 is produced by steam reformation of methane, gasification of coal and biomass, and metabolic pathway of special type of microorganisms [commonly known as biological hydrogen production (BHP)]. At present, about 90% of the H_2 is generated by steam methane reforming of natural gas. But this process emits CO_2 and is non-renewable. The main processes for biological hydrogen production are: i) Direct biophotolysis by green algae; ii) Indirect biophotolysis by cyanobacteria; iii) Photofermentation by anaerobic photosynthetic bacteria; and iv) Dark fermentation by anaerobic fermentative bacteria. $p\gamma:H_2$ can also be produced from water gas shift reaction using photosynthesis bacteria as biocatalyst² and biocatalyst assisted photoelectrochemical H_2 production³.

This study analyzes papers published related to BHP on yields, reaction rates and reactor designs, and compares the effectiveness of different substrate-microorganism combinations.

Framework

Classification of Papers

An analysis is carried out of papers published related to BHP, based on www.science-direct.com. About 370

papers have been published since 1976 mainly in 19 international journals (Fig. 1). Three journals with maximum number of papers related to BHP are: i) International Journal of Hydrogen Energy (206 papers); ii) Bioresource Technology (38 papers); and iii) Journal of Biotechnology (18 papers). Papers published in these journals have been analysed and classified into following categories: (A) Papers that deal with experimental work based on different microorganisms, substrates and reaction conditions; (B) Papers, in which experiments have been done on reactor designing and system optimization; and (C) Papers that deal with analytical tools, different techniques of modelling, simulation and system analysis. Review papers are included in category C.

Performance Criteria

Performance of the process has been characterized based on parameters yield, rate and efficiency.

i) Yield

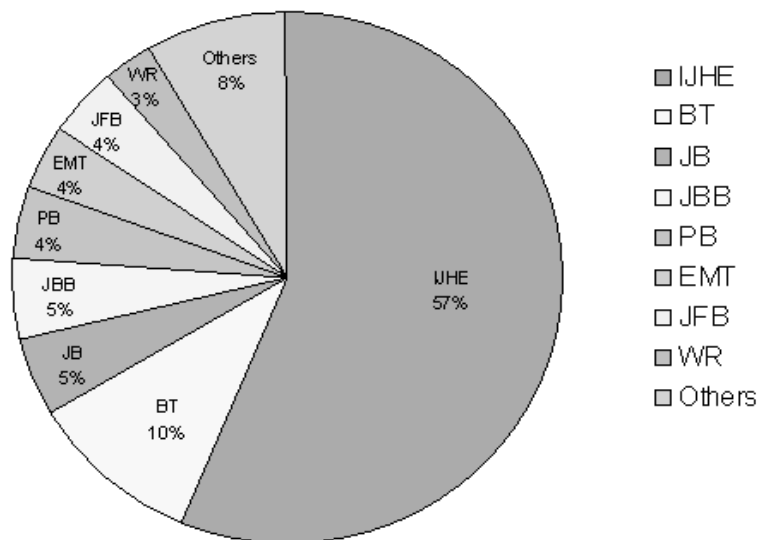
Hydrogen yield (HY) is defined as

$$\text{Yield (HY)} = \frac{\text{Amount of } H_2 \text{ produced (mol)}}{\text{Amount of substrate consumed (mol)}} \quad \dots(1)$$

The common unit of yield is (mol/mol). For viability of these processes yield should be high.

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International Journal of Hydrogen Energy (IJHE), Bioresource Technology (BT), Journal of Biotechnology (JB), Journal of Bioscience and Bioengineering (JBB), Processes Biochemistry (PB), Enzyme and Microbial Technology (EMT), Journal of Fermentation and Bioengineering (JFB), Water Research (WR)

Fig. 1—Number of papers published in different journals

ii) Rate

Rate means H_2 production rate, which are of two kinds: i) Volumetric hydrogen production rate or hydrogen production rate (HPR); and Specific hydrogen production rate (SHPR).

Volumetric production rate (HPR) =

$$\frac{\text{Total amount of hydrogen produced}}{\text{Total volume of the culture} \times \text{Time duration}} \quad \dots(2)$$

Specific hydrogen production rate (SHPR) =

$$\frac{\text{Amount of hydrogen produced}}{\text{Mass of substrate is used} \times \text{Time duration}} \quad \dots(3)$$

Common units of HPR are ml/l/h and mmol/l/h and that of SHPR are ml/ g substrate/h and mmol/g substrate/h. But in literature some other units like ml/ g COD/h, ml/g of cell/h, mmol/ mol of chlorophylls/h -etc. have also been used. If rate is high then required size of bioreactor would be less. Rate is directly related to installation or capital cost whereas yield is directly related to operating cost.

iii) Efficiency

In photo processes, efficiency means conversion efficiency of light energy to H_2 energy and is defined as

Conversion efficiency =

$$\frac{H_2 \text{ production rate} \times \text{Energy content in } H_2}{\text{Absorption rate of light energy}} \times 100\% \quad \dots(4)$$

Here, energy content in H_2 = heat of formation of H_2O gas = 241.9 kJ/mol.

In case of fermentative processes (both dark and photo fermentation), in which chemical energy is converted into H_2 energy, efficiency means substrate conversion efficiency and is defined as

Conversion efficiency =

$$\frac{\text{Amount of substrate utilised}}{\text{Total Amount of substrate supplied}} \times 100\% \quad \dots(5)$$

Current Status

Out of 370 papers published on BHP in different journals (Fig. 1, Table 1), majority of papers (64%) deal with experiments based on microorganisms, substrates and reaction conditions (category A). In the last few years, though there has been an increase in papers related to experiments based on reactor designing and optimization (category B). The focus still appears to be on laboratory scale experiments. As the field evolves, it is essential to have a greater emphasis on reactor design, scale up and use of engineering analysis to design better BHP processes.

Table 1—Classification of biohydrogen papers

Year	International Journal of Hydrogen Energy			Bioresource Technology			Journal of Biotechnology			Total
	A	B	C	A	B	C	A	B	C	
Till June 2008	27	16	9	10	10	2	1	0	0	75
2007	17	8	7	4	1	0	4	0	0	41
2006	26	9	2	3	0	0	1	0	0	41
2005	5	2	1	0	1	0	2	0	0	11
2004	12	2	2	1	0	0	2	0	0	19
2003	2	0	0	0	0	0	1	0	0	3
2002	17	6	6	1	0	0	0	0	0	30
2001	3	0	1	2	0	0	1	0	0	7
2000	1	0	1	1	0	0	0	0	0	3
1999	2	1	0	0	0	0	2	0	1	6
1998	1	0	0	0	0	0	0	0	0	1
Before 1998	14	1	5	2	0	0	3	0	0	25
Total	127	45	34	24	12	2	17	0	1	262

Table 2—Status of microorganisms used in different studies in last 4 years

Culture	2008	2007	2006	2005	2004	Total
Fermentative bacteria						
<i>Clostridium sp.</i>	12	5	8	3	5	33
<i>Enterobacter sp.</i>	3	2	0	0	0	5
<i>Escherichia sp.</i>	2	0	2	0	0	4
Thermophilic bacteria	3	0	1	2	1	7
Others	4	3	1	0	0	8
Bacterial consortium	21	9	9	4	4	47
Photosynthetic bacteria						
<i>Rhodobacter sp.</i>	8	2	5	1	0	16
<i>Rhodopseudomonas palustris</i>	1	1	0	0	1	3
<i>Rhodopseudomonas capsulate</i>	0	0	1	0	0	1
<i>Rhodospirillum rubrum</i>	1	0	1	0	0	2
Others	2	0	3	1	0	6
Mixed culture	1	0	0	0	1	2
Mixed culture (photosynthetic + fermentative)						
<i>Enterobacter cloacae</i> + <i>Rhodobacter sphaeroides</i>	1	0	0	0	0	1
<i>Lactobacillus delbrueckii</i> + <i>R. sphaeroides</i>	0	0	1	0	0	1
Cyanobacteria						
<i>Anabaena sp.</i>	1	1	1	0	0	3
Others	1	1	0	0	0	2
Green algae						
<i>Chlamydomonas sp.</i>	5	3	6	1	2	17
Others	1	1	0	0	0	2

Table 3—Substrates used in different studies in different years

Substrates	2008	2007	2006	2005	2004	Total
Glucose	11	6	6	0	4	17
Sucrose	2	2	6	3	3	16
Starch	6	1	1	0	1	9
Cellulose	3	1	1	0	0	5
Solid waste	15	5	3	2	3	28
Wastewater	4	5	3	3	2	17

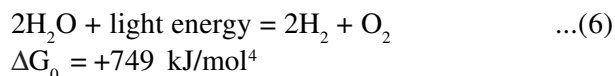
Largest number of studies (Table 2) are available on fermentative bacteria mainly *Clostridium sp.* and bacterial consortium. There have been several papers using photosynthetic bacteria. A range of pure and mutant strains have been used for these two processes. Only a few papers deal with mixed cultures. This likely to increase in the future.

Initially, the focus was mainly on pure strains of culture using glucose as a substrate (Table 3). Recently starch, sucrose, cellulose and waste materials are being used as feedstocks. The emphasis has shifted from laboratory based pure substrates to cheap and easily available natural substrate and waste materials.

Based on the number of papers published, dark fermentation and photo fermentation are the most promising processes. Relatively few papers deal with cyanobacteria and green algae.

Status of Biohydrogen Production (BHP) Processes Direct Biophotolysis

Direct biophotolysis involves photosynthetic reaction where solar energy is converted into chemical energy as



Microorganisms used for this purpose are different species of green algae, a photoautotrophic organism. Fresh water green algae are commonly used for H_2 production but marine green algae that utilised seawater for H_2 production have also been used⁵⁻⁸. *Chlamydomonas reinhardtii* is the most commonly used microbe, besides *Scenedesmus obliquus* and *Chlorella fusca*. H_2 production by microalgae is attractive since it produces H_2 from easily available water using sunlight as energy source, no accumulation of CO_2 and theoretical solar energy conversion efficiency can be more than 80%^{9,10}.

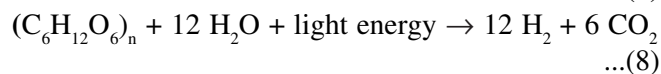
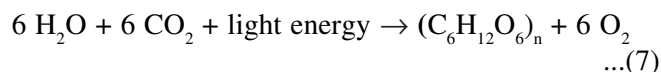
Table 4—Current experimental results for green algae

Microorganism	Light illumination $\mu\text{E}/\text{m}^2/\text{s}$	Rate ml/h	Efficiency %
<i>C.reinhardtii</i> ¹¹	110	2.5	0.125
<i>C.reinhardtii</i> ¹²	100	2.8-2.9	-
<i>C.reinhardtii</i> ¹³	-	2.5	-

But in actual practice, efficiency is low¹¹⁻¹³ (Table 4) due to genetic tendency of green algae to assemble in a large array of light-absorbing chlorophyll antenna molecules in their photosystems. At high solar intensities, rate of photon absorption by chlorophyll antennae of first few layers of cells in culture far exceeds the rate at which photosynthesis can utilise photons, thereby resulting in dissipation and loss of excess photons as fluorescence or heat¹⁴. Though the process has several advantages, but hydrogenases enzymes that produce H_2 are strongly inhibited by oxygen, which is one of the major products of this process. Several steps have been taken to overcome this problem^{12,13,15,16}.

Indirect Biophotolysis

In indirect photolysis, H_2 and O_2 are produced in a two step process that starts with photosynthesis and sugar formation, followed by a light-induced process, in which sugar and water yield H_2 and CO_2 as



Cyanobacteria are ideal candidates for this process since they have simplest nutritional requirements. Commonly used cyanobacteria are various species of *Anabaena*, *Oscillatoria*, *Calothrix* and *Gloeocapsa*¹⁷.

Table 5—Current experimental results on indirect biophotolysis

Microorganism	Light illumination $\mu\text{E}/\text{m}^2/\text{s}$	Rate ml/h	Light conversion efficiency, %
<i>Anabaena</i> PCC 7120 ¹⁹	456	14.9	0.042
<i>Anabaena variabilis</i> ²⁰	32 W/ m^2	7.73 l/kg/h	1.32
<i>Anabaena variabilis</i> ²¹	353	20	1.0
<i>Gloeocapsa alpicola</i> ²²	165	25	-
<i>Synechococcus sp.</i> ²³	25 W/ m^2	-	2.6

Table 6—Current experimental results on photo fermentation

Microorganism	Substrate	Light illumination W/m^2	Rate $\text{ml}/\text{l}/\text{h}$	Conversion efficiency %
<i>R. sphaeroides</i> ²⁴	Organic acids	88	18	1.1
		277	33	0.5
<i>R. sphaeroides</i> ²⁶	Lactate	62	0.88*	4.33.9
		107	1.4*	
<i>R. sphaeroides</i> ²⁷	Lactate	50	-	7.9
		75		6.2
<i>Rhodopseudomonas sp.</i> ²⁸	Acetate	680#	25.2	0.96.2
		43#	10.2	
<i>R. sphaeroides</i> RV +MTP4 ²⁹	Lactate	500	3.64*	2.18
<i>R. sphaeroides</i> +MTPR ³⁰	Lactate	300	2.0*	-
<i>R. sphaeroides</i> RV ³¹	Lactate	300	-	9.23
<i>R. sphaeroides</i> ³²	Butyrate	-	118	-
	Succinate		108	
	Lactate		103	
<i>R. sphaeroides</i> + <i>Halobacterium salinarum</i> ³³	Malate and glutamate	150	17	2.25

*in $\text{l}/\text{m}^2/\text{h}$;
#in $\mu\text{mol photons}/\text{m}^2/\text{s}$

Maximum light conversion efficiency for this process is 16.3%¹⁸. At low light illumination, better conversion efficiency is achieved and with increasing light illumination, light conversion efficiency decreases. In actual practice, efficiency¹⁹⁻²³ is 1-2% (Table 5).

Photo Fermentation

This is a photoheterotrophic process, in which photosynthetic bacteria (*Rhodobacter sphaeroides*, *Rhodopseudomonas capsulate*, *R. palustris*, *Rhodospirillum rubrum* etc.) produce H_2 during metabolism by taking organic acids and alcohols as substrates (normally butyrate, acetate, propionate, lactate, etc). H_2 production by photosynthetic bacteria depends on factors like light intensity, light wavelength and illumination protocol. Rate of H_2 production increases

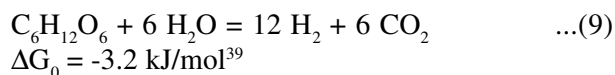
with increasing light intensity and reaches saturation point, which depends upon substrates and microorganisms²⁴⁻²⁶. Lack of infrared light (750-950 nm wavelengths) decreases photoproduction of H_2 by 39%²⁴. Theoretically, H_2 production from one mol of acetic acid, propionic acid and butyric acid are 4, 7, 10 mol respectively. But in actual practice, H_2 yields from one mol of acetic acid, propionic acid and butyric acid are approx. 1.6, 2.8 and 4.0 mol of H_2 ²⁵. Rate increases with increasing intensity but light conversion efficiency decreases^{24,26-28} (Table 6).

Substrates used in this process are mainly organic acids, which are major components of industrial waste and effluent of dark fermentation process. Photo fermentation can be coupled with dark fermentation or used as a wastewater treatment technique. Wastewater

treatment using photosynthetic bacteria gives satisfactory results³⁴⁻³⁷. Another important advantage of this process is that concentration of H₂ in biogas can be as high as 96%³². Erolu *et al*³⁴ got maximum H₂ by using olive oil mill wastewater (OMW); H₂ doubled (from 16 l of H₂ / l of OMW to 31.5 l H₂ / l OMW) by using clay pretreatment.

Dark Fermentation

In these processes, a large range of anaerobic fermentative bacteria (mesophilic, thermophilic and facultative) with pure strain and genetically improved cultures are available for use. Several substrates (glucose, sucrose, starch, cellulose) are also available. By complete conversion from one mole of glucose maximum 12 mol of H₂ can be formed³⁸ (Eq. 9).



But in case of dark fermentation, maximum (4 mol of H₂³⁸) is possible due to production of H₂ containing organic acids (Eq. 10).



$$\Delta G_0 = -206 \text{ kJ/mol}^{39}$$

Woodward *et al*⁴⁰ reported that a maximum of 11.6 mol H₂/mol of glucose is possible. But in actual practice, yield is reported at 2-3 mol/mol of glucose⁴¹⁻⁵⁴ (Table 7).

In dark fermentation, various kind of fermentative reactors have been used but most commonly used reactors are continuously stirred tank bioreactor (CSTR) and unflow anaerobic sludge blanket reactor (UASB). Yield is reported⁵⁵ higher in UASB especially at low retention time under similar reaction conditions⁵⁶⁻⁶⁵ (Table 8). Rate and yield of dark fermentation process are more than others, but the main problem associated with this process is that H₂ concentration is very low (40-60%, v/v). Therefore, fermentative effluent gas cannot be used directly to fuel cell applications and a purification step is required (mechanical/chemical process). A two step gas membrane separation process⁶⁶ gives 73% H₂ in biogas. For yield improvement, some

Table 7—Current experimental result on dark fermentation

Microbes	Substrate	Yield mol/mol hexose	Rate ml/l/h
<i>Clostridium sp.</i> ⁴¹	Glucose	2.8 1.7	81 2166
<i>C. cellobilparum</i> ⁴²	Glucose	2.73	-
<i>C. bejerickii</i> ⁴³	Glucose	2.81	-
<i>Escherichia coli</i> ⁴⁴	Glucose	2.0	1.3-5.3
<i>Slostridium sp. Strain 2</i> ⁴⁵	Xylose	2.6	21.1*
<i>Clostridium sp.</i> ⁴⁶	Sucrose	1.62	123
Mixed microflora ⁴⁷	Sucrose	1.8	15.7*
<i>Clostridium CGS2</i> ⁴⁸	Starch	2.03	1500
Mixed microflora ⁴⁹	Starch	1.5	18.8*
Co-culture (<i>clostridium sp.</i> + <i>Thermoana sp.</i> ⁵⁰	Cellulose	1.8	55*
Microflora ⁵¹	Cellulose	2.4	16.1
Bacterial consortium after deactivating methanogenic bacteria ⁵²	Glucose	3.21	364
	Hydrolyzed cellulose	2.2	579
	Natural	2.84	-
	Acidic	3.0	-
<i>Thermoanaerobacterium thermosaccharolyticum</i> ⁵³	-	2.67	12.1*
<i>Ruminococcus albus</i> ⁵⁴	Sweet sorghum	2.5	60 l/kg

*in mmol/l/h

Table 8—Results of different bioreactors

Reactor type	Volume l	Microorganism	Substrate	Yield mol/mol	Rate l/l/h
IBR ⁵⁶	2.5	<i>C. tyrobutyricum</i>	Food waste	223 ml/g hexose	0.3
UASB ⁵⁷	-	<i>Clostridium sp.</i>	Sucrose	1.62	0.12
CIGSB ⁵⁸	0.45-1.3	<i>Clostridium sp.</i>	Sucrose	2.01	9.31
Granule- based CSTR ⁵⁹	6.0	Fermentative bacteria	Glucose	1.84	3.26
AFBR	1.4	Anaerobic fermentative bacteria	Glucose	0.4-1.7	7.6
Biofilm reactor ⁶⁰	1.4	Anaerobic fermentative bacteria	Glucose	0.4-1.7	6.6
AFBR	1.4	Anaerobic fermentative bacteria	Glucose	0.4-1.7	6.6
Granule reactor ⁶⁰	1.4	Anaerobic fermentative bacteria	Glucose	0.4-1.7	6.6
CIGSB ⁶¹	0.88	Mixed bacteria	Sucrose	1.94	7.66
HAIB ⁶²	0.850	Fermentative microflora	Glucose	2.48	-
MBR ⁶³	1	Fermentative culture	Glucose	1.27	1.48
			Sucrose	2.07	1.39
			Fructose	2.75	1.36
GAC-AFBR ⁶⁴	-	<i>Clostridium</i> rich mixed culture	Glucose	1.19	2.36
DTFBR ⁶⁵	8	<i>Clostridium sp.</i>	Sucrose	2.5	2.27

IBR- Immobilised Bioreactor, CIGSB- Carrier-Induced Granular Sludge Bed Reactor, AFBR- Anaerobic Fluidised Bed Reactor, HAIB- Horizontal Flow Anaerobic Immobilised Biomass reactor, MBR- Membrane BioReactor, GAC-AFBR- Granular Activated Carbon Anaerobic Fluidized Bed Reactor, DTFBR- Draft Tube Fluidized Bed Reactor

pretreatment methods had been reported⁶⁷⁻⁷¹ (Table 9). The main reason behind H₂ production improvement is that these pretreatment techniques inhibit methanogenic bacterial activity and due to this H₂ consumption decreases and yield increases.

Integrated Process for Hydrogen Production

During dark fermentation, desired product is H₂, collected in gas phase. But there are some liquid by-products, which contain mainly organic acids (butyric acid, acetic acid, lactic acid, formic acid etc). Major disadvantage of this fermentative broth is disposal problem. On the other hand, fermentative broth can be treated as a carbon source for photofermentation process. Many reports^{25,72,73} show (Table 10) total H₂ yield increase by a considerable amount; maximum yield is 7.1 mol H₂ / mol of glucose⁷³.

Kim *et al*⁷⁴ reported a three step integrated process, in which biomass is produced by *Chlamydomonas reinhardtii* in the first step, then the algal mass (equivalent to starch-glucose) is used as a feedstock for dark fermentation by *Clostridium butyricum* that produces H₂ and organic acids. In the last step, fermentative broth is used for photo fermentation by *Rhodobacter*

sphaeroids to produce H₂. Shi *et al*²⁵ shows steps in the conversion of glucose to H₂ by a combination of dark and photo fermentation (Fig. 2).

Green algae were found to absorb visible light of solar radiation whereas photosynthetic bacterium absorbs some of infrared portion of solar radiation. So combining these two can increase overall light conversion efficiency. Malis *et al*⁷⁵ successfully combined *Chlamydomonas reinhardtii* and *Rhodospirillum rubrum*. Similar integrated processes have also been reported^{76,77}. Such an integrated system would constitute a high-yield, and more economically viable H₂ production (Fig. 3).

Analysis Techniques

Metabolic Flux Analysis

Rate of H₂ production from these processes is very slow except dark fermentation. But the major problem associated with dark fermentation is that yield of H₂ production is low. Metabolic flux analysis⁷⁸ (MFA) technique can be used to improve yields. Using this method, intracellular fluxes are calculated from extra cellular fluxes. If measured fluxes are not sufficient, optimization techniques can be used to determine unknown fluxes. Manish *et al*⁷⁹ used MFA to improve

Table 9—Effect of pretreatments

Culture + substrate	Pretreatment used	Result	
		With pretreatment	Without pretreatment (control)
Granule sludge + glucose ⁶⁷	Chloroform added 0.05 %	HPR 135.09 ml/g glucose/h CH ₄ production rate 0.04 ml/g glucose/h	HPR 0.42 ml/ g glucose/h CH ₄ production rate 75.76 ml/g glucose/h
Sewage sludge + glucose ⁶⁷	Chloroform added 0.1 %	HPR 145.32 ml/g glucose/h CH ₄ production rate 0.12 ml/g glucose/h	HPR 124.99 ml/g glucose/h CH ₄ production rate 0.74 ml/g glucose/h
Mixed bacteria + sucrose ⁶⁸	Methanogenic inhibitor - BESA (C ₂ H ₄ BrO ₃ SNa, sodium 2-bromoethanesulfonate Iodopropane	HY 5.28 mol/mol of sucrose	HY 5.17 mol/mol of sucrose
		HY 5.64 mol/mol of sucrose	HY 5.17 mol/mol of sucrose
Mixed bacteria + dairy waste water ⁶⁹	BESA	HY 0.0317 mmol/g COD	HY 0.0018 mmol/g COD
	pH	HY 0.00139 mmol/g COD	HY 0.0018 mmol/g COD
	Heat + pH + BESA	HY 0.0108 mmol/g COD	HY 0.0018 mmol/g COD
Mixed culture ⁷⁰	Sterilization Microwave Ultrasonication	-	A positive effect on hydrogen production
Mixed culture ⁷¹	Sterilization Microwave Ultrasonication Methanogenic inhibitor Freezing and thawing	-	Increase in hydrogen production

Table 10—Results of mixed culture

Microorganisms	Substrate	Yield mol/mol glucose
Microflora + <i>Rhodobacter sphaeroides</i> ⁷²	Sucrose	1.8 (1 st stage) 3.3 (over all)
Microflora + <i>Rhodopseudomonas capsulata</i> ²⁵	Glucose	1.36 (1st stage) 4.56 (over all)
Lactobacillus + <i>Rhodobacter sphaeroides</i> ⁷³	Glucose	7.1 (over all)

H₂ yield for *E. coli* taking glucose substrate and developed a stoichiometric model for *E. coli* with 31 compounds and 27 pathway reactions, giving maximum yield possible at 2 mol/mol of glucose consumed in a particular pathway with maximum production of acetate and ethanol. The prediction based on strains lacking ldh

indicated a possible methodology to increase H₂ production. Similar result was obtained by Oh *et al.*⁸⁰ with *Citrobacter amalonaticus* Y19 giving maximum H₂ yield at 8.7 mol /mol of glucose consumed when metabolism is directed to suggested pathway. So, MFA technique can define the upper limit of H₂ production in microbial fermentation and suggest the most favorable path. This technique can provide apriori directions for genetic modifications towards preferred pathways.

Net Energy Analysis

For new processes, sustainability is of prime importance. Manish *et al.*³⁹ suggested that criteria should be net energy ratio (NER), energy efficiency and greenhouse gas (GHG) emissions. In first step, material and energy balance are computed. Total energy consumption is then classified into renewable and non-renewable energy consumption. An energy conversion process can be a renewable method only when energy

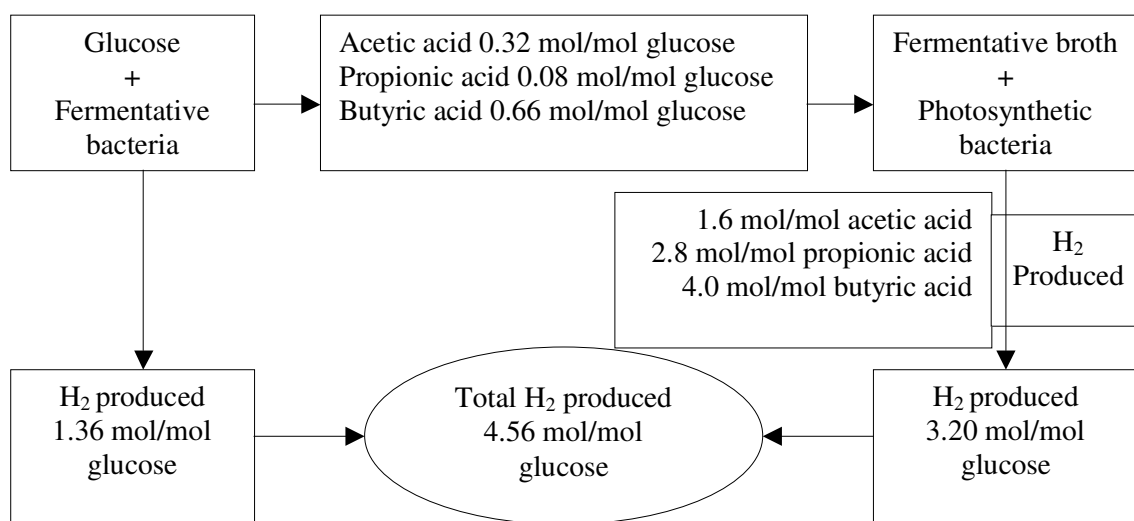


Fig. 2—H₂ production in mixed culture of photo and dark fermentation²⁵

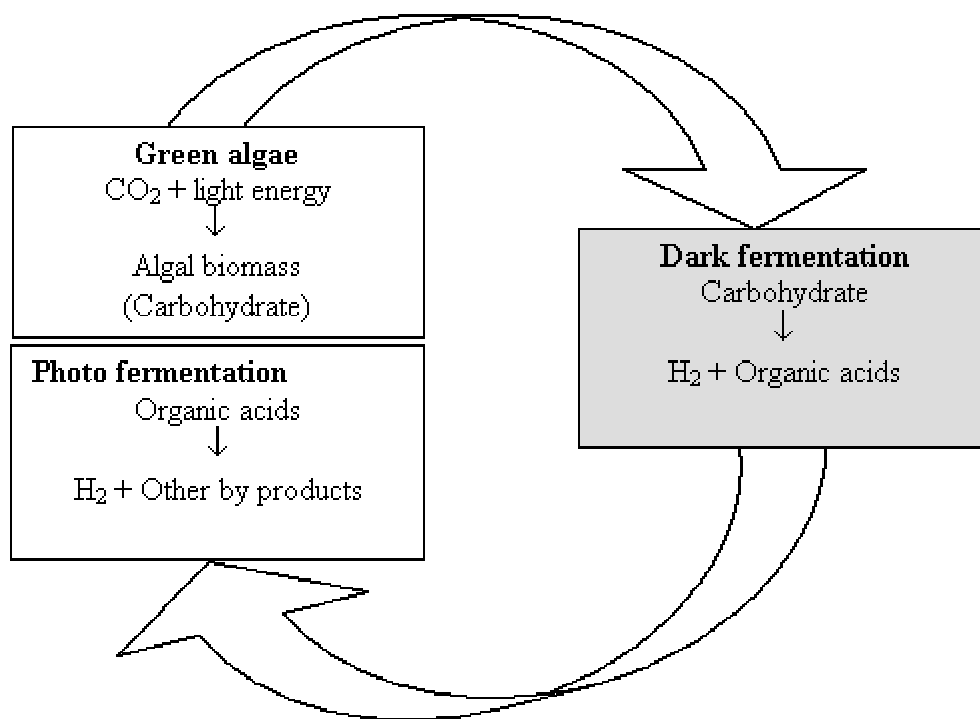


Fig. 3—Combination of two photo and one dark H₂ producing processes

output of the process is more than nonrenewable energy input. So NER is defined as

$$\text{NER} = \frac{\text{Hydrogen output (MJ)}}{\text{Non-renewable energy input (MJ)}}$$

...(11)

An NER value, more than one indicates renewable nature of the process. Using emission factors, GHG emission is calculated. Energy efficiency is defined as

$$\text{Energy efficiency} = \frac{\text{Energy output}}{\text{Energy input}}$$

...(12)

Table 11—H₂ flow rate required for fuel cell⁸¹

Size of PEMFC	H ₂ flow rate required		
kW	g/h	mol/h	Sl/h*
1.0	49	23.9	577.7
2.5	121	59.9	1444.3
5.0	243	119.7	2888.5

*Sl means liter at NTP

Table 12—Size required for a 5 kW fuel cell⁸¹

System	HPR mmol/l/h	Size of bioreactor l
Direct photolysis	0.07	1.71 × 106
Indirect photolysis	0.355	3.37 × 105
Photo fermentation	0.16	7.58 × 105
Dark fermentation	121.0	9.89 × 102

where, energy input indicates energy content in feedstock (chemical energy).

Manish *et al.*³⁹ found that BHP process are viable from net energy and GHG reduction point of view. Integrated process of dark and photo fermentation has maximum efficiency. The result obtained in the same study shows that CO₂ reduction is 7.31-9.37 kg/kg H₂ production (57-73%) and non renewable energy used is 123.2-148.7 MJ/kg H₂ (65-79%) as compared to steam methane reforming (SMR) process. However, when by products from these biohydrogen processes are not utilized, efficiencies of biohydrogen processes are significantly lower than SMR. Hence, by product removal and utilization is an important factor for BHP process. Net energy analysis is a useful tool to determine viability of a process.

Hydrogen and Fuel Cell Application

H₂ can be utilized for electricity generation and transportation using fuel cells. Levin *et al.*⁸¹ estimated amount of H₂ required to run a proton exchange

Table 13—Recalculated size of bioreactor

System	HPR l/h	Temperature of effluent gas, °C	HPR mmol/l/h	Required size of bioreactor, l
Direct photolysis ¹²	0.0029	28	0.117	1.0 × 106
Indirect photolysis ²²	0.025	30	1.006	1.2 × 105
Photo fermentation ³²	0.118	30	4.75	2.5 × 104
Dark fermentation ⁵⁸	9.31	25	380.78	3.2 × 102

Table 14—Experimental results for bioreactor-fuel cell combination

Culture	Conditions maintained	Observation
Photosynthetic bacteria ²⁶ (<i>Rhodobacter sphaeroides</i>)	- Reactor volume, 11 l - Light illumination, 107 W/m² for 100 h - Gas input to fuel cell, 0.6-2.0 l/h	Gas produced 35.7 litre with purity 88.1% - Output power, 1W - Conversion efficiency- Light to hydrogen 4.3% Fuel cell, 44% Overall, 1.9%
Anaerobic fermentative bacteria ⁸² (<i>Clostridium pasteurianum</i> + <i>Klebsiella sp.</i>)	- Reactor volume, 3 l - Sucrose as substrate - Fuel cell fed, 1.72 l/h - Input H₂ purity, 99% - Fuel cell efficiency, 50%	- HPR, 1.5 l/h - H₂ in biogas, 40.6% - Voltage output, 2.28 V - Current out put, 0.38 A - Power output, 0.87 W

membrane (PEM) fuel cell (Table 11) and also estimated bioreactor size required to run a 5 kW PEM fuel cell (Table 12). The methodology of Levin *et al*⁸¹ was used to estimate the size of bioreactor using the best performance data. To power a 5 kW fuel cell required volumetric flow rate was 119.7 mol/h. Table 13 shows that the required size of bioreactor for four processes reduced significantly in the last four years. In few studies^{26,82}, biohydrogen processes have been combined with fuel cells (Table 14).

Cost Analysis of Biohydrogen Production Processes

Yield is directly proportional to the operating cost and rate is directly proportional to the reactor cost or installation cost. H₂ storage cost that is almost same for all kinds of process related to H₂ production. In case of photosynthesis water is used a substrate so operating cost is very low and mainly consists of the maintenance cost. As production rate of this process is very low, required size of reactor would be high and hence installation cost is high. Zabrosky⁸³ reported that reactors (10% light conversion efficiency) should cost about \$50/m² for single stage and \$100/m² for two-stage system, and suggested that near-horizontal tubular reactor (NHTR) might cost \$50/m² and thus projected cost of H₂ will be \$15/GJ. As reported in NREL annual report⁸⁴, an 110000 m² pond type bioreactor (at Phoenix, Arizona.) using green algae had a reactor cost of \$10/m². Total capital investment is \$5,168,000, whereas operating cost including storage cost is \$1480000 and thus H₂ production cost is \$13.5/kg of H₂ (US Department of Energy, DOE set a goal for renewable H₂ production at a cost of \$2.60/kg H₂). Madamwar *et al*⁸⁵ reported that cost of H₂ from cyanobacterial photosynthesis is \$25/m³ that is far less than reported cost of H₂ from photovoltaic splitting of water (\$170/m³)⁸⁶. For viability the costs of these processes have to be reduced to less than 20% of its present cost.

Summary and Conclusions

BHP processes are an active research area. Most of the research has been based primarily on laboratory scale experiments with different microorganisms and substrates. There has been a shift from pure substrates to naturally available substrates and wastes. It is likely that mixed cultures and integrated two-step or three-step processes combining dark and photo fermentation are likely to be viable in the future. Rate and yield are important performance parameters for BHP processes.

Techniques from biochemical engineering like MFA can be used to identify desired genetic modifications to improve yield. However, MFA has not been applied for mixed strains and naturally occurring substrates. It is necessary to evolve appropriate techniques for dynamic modeling to study the effect of process variations on rate and yield. Preliminary cost analysis indicates that existing processes need to have cost reductions to about 20% of its present value. An analysis of bioreactor sizes to operate a 5 kW fuel cell indicates that sizes have reduced to about 30% of values in 2004. BHP processes are still on the technology learning curve and offer significant scope for improvement. Systematic efforts to address scale-up issues and optimized reactor design are likely to result in viable BHP processes in future.

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