Indian J Microbiol (Jan–Mar 2013) 53(1):3–10
DOI 10.1007/s12088-012-0287-6

REVIEW ARTICLE

Integrative Biological Hydrogen Production: An Overview

Sanjay K. S. Patel · Vipin C. Kalia

Received: 14 May 2012 / Accepted: 8 June 2012 / Published online: 22 June 2012

Abstract Biological hydrogen (H_2) production by dark and photo-fermentative organisms is a promising area of research for generating bioenergy. A large number of organisms have been widely studied for producing H_2 from diverse feeds, both as pure and as mixed cultures. However, their H_2 producing efficiencies have been found to vary (from 3 to 8 mol/mol hexose) with physiological conditions, type of organisms and composition of feed (starchy waste from sweet potato, wheat, cassava and algal biomass). The present review deals with the possibilities of enhancing H_2 production by integrating metabolic pathways of different organisms-dark fermentative bacteria (from cattle dung, activated sludge, *Caldicellulosiruptor*, *Clostridium, Enterobacter, Lactobacillus*, and *Vibrio*) and photo-fermentative bacteria (such as *Rhodobacter*, *Rhodobium* and *Rhodopseudomonas*). The emphasis has been laid on systems which are driven by undefined dark-fermentative cultures in combination with pure photo-fermentative bacterial cultures using biowaste as feed. Such an integrative approach may prove suitable for commercial applications on a large scale.

Keywords Biowaste · Dark-fermentation · Hydrogen · Mixed culture · Photo-fermentation

Introduction

Hydrogen (H_2) has been recognized as fuel for the future due to its high efficiency (122 kJ/g) and eco-friendly nature in comparison to fossil fuels [1, 2]. Biological H_2 production (BHP) process has been widely studied under dark- and photo-fermentative conditions. With these approaches the yields of H_2 have been quite low in comparison to the theoretically achievable values of 4 and 8 mol/mol of glucose under dark and photo-fermentative conditions, respectively [2–7]. Quite a few research efforts have been made to overcome the limitations of these processes. It has been realized that in order to recover maximum H_2 from the organic matter, it is necessary to further use the end-products of the dark fermentative process, especially volatile fatty acids (VFA). It is possible to convert VFAs to H_2 by photosynthetic bacteria. The potential of exploiting these processes in various combinations have been reviewed to some extent [7–10]. However a large number of hurdles still seem to persist such as: (i) in the dark-fermentative process—(a) relatively lower H_2 yield (b) the need for strict anaerobic conditions for high H_2 producers, and (c) thermodynamic instability of the process at higher H_2 concentrations, and (ii) during the photo-fermentative process—(a) sensitivity of the H_2 production process to nitrogen content of the feed (b) effect to light intensity and duration of radiation under outdoor (sunlight) and indoor (artificial light sources) conditions, and (c) types of bioreactors required for H_2 production [2, 11–14].

High cost of the feed and operational conditions is the major limiting factor of BHP. Most basic studies have been carried out on simple and complex sugars as feed material [15–21]. For circumventing the issues related to cost of the feed, biowastes of diverse origins especially agricultural, food and fruit processing industries, and those of municipal markets have been suggested as cheap and renewable alternatives [2, 22–28]. Although, the amount of H_2 generated from different biowastes encourages one to pursue this route however, it demands quite a bit of optimization at

S. K. S. Patel (✉) · V. C. Kalia
Microbial Biotechnology and Genomics, CSIR-Institute of Genomics and Integrative Biology, Delhi University Campus, Mall Road, Delhi 110007, India
e-mail: sanjaykspatel@yahoo.co.in; sanjay.patel@igib.in

different stages [29–34]. Instead of dwelling on optimization efforts being made on individual parameters of BHP process, an emerging proposal is to combine the dark- and photo-fermentative H_2 producing organisms [7, 10, 35, 36]. The efforts in this direction have been targeted on the following combinations: (i) using defined dark- and photo-fermentative H_2 producing organisms in a sequential manner in two independent stages (ii) using undefined dark-fermentative H_2-producers along with defined photosynthetic organisms in two stages (iii) using the two types of BHP processes into a single stage, and (iv) using effluent from a dark-fermentative process (not necessarily a H_2 production reactor) and exploiting photo-fermentative bacteria for their H_2 producing abilities [33, 37–48]. In our recent efforts, we have emphasized only on using defined bacterial cultures in a sequential manner and evaluate it with respect to their individual H_2 producing abilities from pure substrates and biowastes [7]. In the present work, we are concentrating our efforts on studies conducted using undefined dark fermentative H_2 producing culture combinations and exploitation of effluent from dark-fermentative process by photosynthetic organisms using biowaste as feed.

Biological Hydrogen Production

Integrative Two Stage Dark- and Photo-Fermentative Sequential Hydrogen Production

The physiology and metabolic activities of bacteria vary significantly under dark- and photo-fermentative conditions. The efficiency depends primarily on the types of enzymes involved in H_2 evolution. Under dark-fermentative conditions, hydrogenase and nitrogenase are the major enzymes responsible for this process [2, 49]. In the overall conversion of feed to H_2, a few intermediates are also generated, such as VFAs and alcohols. The efficiency of the dark-fermentative H_2 evolution process is governed by VFAs (Eqs. 1–4), such that acetic acid generation can lead to an additional 4 mol of H_2 whereas butyric acid is expected to generate 2 mol of H_2/mol of substrate. Lactic acid and ethanol are considered to be counter-productive to H_2 evolution process [2, 28]. The intermediates of the dark-fermentative BHP, such as acetic and butyric acid can be taken up by photosynthetic organisms to generate additional H_2 (Eqs. 5–6) [45, 46, 50, 51].

$$C_6H_{12}O_6(\text{Hexose}) + 2H_2O \rightarrow 2CH_3COOH\ (\text{Acetate}) + 4H_2 + 2CO_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2CH_2COOH\ (\text{Butyrate}) + 2H_2 + 2CO_2 \quad (2)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH\ (\text{Lactate}) \quad (3)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH\ (\text{Ethanol}) + 2CO_2 \quad (4)$$

$$CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2 \quad (5)$$

$$CH_3CH_2CH_2COOH + 6H_2O \rightarrow 10H_2 + 4CO_2 \quad (6)$$

Using the organisms present in activated sludge enriched for dark-fermentative H_2-producers, along with photosynthetic organisms such as *Rhodobacter sphaeroides*, *Rhodopseudomonas palustris* and undefined photosynthetic bacteria, it has been possible to achieve 2.86–6.07 mol H_2/mol hexose [34, 52], over an incubation period ranging from 1 to 6 days of dark-fermentation followed by 5–14 days of photo-fermentative phase [53, 54]. In most of the cases, the temperature of 31–37 °C has been found to be optimal during the dark phase and 30 °C during the light phase (Table 1). In these cases, starchy wastes have been employed, which had originated from wheat, rice and cassava (Table 1). In other studies, cattle dung, dairy manure and mixed cultures in combinations with *Rhodobacter capsulatus*, *R. palustris*, and *R. sphaeroides*, and their combinations have been shown to yield 3.40–7.15 mol H_2/mol hexose [33, 38, 43, 47, 55, 56]. In these cases, starchy wastes, cheese whey and water hyacinth have been fermented for quite long periods 2–10 days of the dark phase followed by 11–21 days of the light period and exceptionally it was 90/100 days under repeated batch culture [47]. In a few other combinations of dark and photosynthetic bacteria, *Caldicellulosiruptor, Clostridium*, *Klebsiella, Lactobacillus* and *Thermotoga* in association with *R. capsulatus*, *R. sphaeroides*, *Rhodobium marinum* and *R. palustris* have been used for H_2 production (Table 1). These integrative approches of two stage H_2 production have proved effective as most of them have lead to yields up to 7.2 mol/mol hexose [30]. In dark-fermentative BHP, the H_2 yields are quite low in most cases and exceptionally it is posible to achieve a value of 3.8 mol H_2/mol hexose [57]. In contrast, the two stage integrative approach is much more effective and exceptionally only it falls around 2.8–3.9 mol/mol hexose [45, 51, 58]. A summary of the results of yields of ≥7.0 mol H_2/mol hexose reveals that it has been achieved with combinations such as (i) mixed culture—*R. palustris* and water hyacinth (10 g/l) [33], (ii) *Clostridium butyricum—R. sphaeroides*—algal biomass (starch at the rate of 5 g/l) [31], (iii) *C. butyricum* and *Enterobacter aerogenes—R. sphaeroides/Rhodobacter* sp. and sweet potato starch (5–10 g/l) [30, 59].

A perusal of Table 1 allows us to draw a few conclusions on the significance of the roles of photosynthetic organisms in influencing H_2 yields in the integrative BHP process. Here, it can be observed that photo-fermentative organisms can utilize different biowastes to produce high H_2 yields—(i) *R. sphaeroides* could evolve 3.81–8.30 mol H_2/mol hexose (ii) *R. capsulatus* yielded 3.90–6.85 mol H_2/mol hexose, and (iii) *R. palustris* was also effective in

Table 1 Integration of dark- and photo-fermentative bacteria in a sequential two stage hydrogen production from biowastes

Organisms		Substrate (concentration: g/l)	Process parameters (in dark/light phase)					H_2 yield (mol/mol hexose)	Reference
Dark-fermentative	Photo-fermentative		Reactor capacity (l)	pH	Temp. (°C)	IP[c] (days)	Culture mode		
Activated sludge	*Rhodobacter sphaeroides*	Wheat starch (20.0)	0.31/0.31	6.8/7.1	37/30	3/8	Batch/Batch	4.55	[20]
	Mixed strains (*R. sphaeroides*)	Wheat starch (20.0)	0.31/0.31	6.8/7.5	37/30	–[d]/14	Batch/Batch	3.81	[53]
	Rhodopseudomonas palustris	Cassava starch (10.0)	0.30/0.30	7.0/7.0	35/30	6/5	Batch/Batch	2.86[h]	[52]
	Mixed PSB[a]	Rice straw (50.0)	0.30/0.10	6.5/7.0	35/30	1/–	Batch/Batch	5.18	[54]
		Cassava starch (10.4)	0.30/0.10	6.3/7.0	31/30	3/8	Batch/Batch	6.07	[34]
Cattle dung compost	*R. sphaeroides*	Cassava starch (18.0)	0.038/0.038	6.8/7.0	37/30	4/8	Batch/Batch	6.51	[38]
		Food waste (20.0)	0.038/0.038	6.8/7.0	37/30	4/8	Batch/Batch	5.40	
Dairy manure microflora	*R. sphaeroides*	Corncob (Sugar, 10.0)	0.60–25/0.32	7.0/7.0	36/35	–/12	Batch and CSTR[e]/Batch	6.59	[43]
Mixed culture	*R. palustris*	Cheese whey (COD, 30.0)	1.0/0.25	7.5/6.9	55/31	–/21	CSTR (HRT[f]–24h)/ Batch	5.00[h]	[55]
		Water hyacinth (10.0)	0.30/0.30	7.0/7.0	35/30	2–5/11	Batch/Batch	7.15	[33]
	Rhodobacter capsulatus	Potato starch (50.0–100.0)	0.25/0.30	6.8/7.0	37/28	10–12/25	Batch/Batch	5.60	[56]
	R. capsulatus and *R. sphaeroides*	Starch (20.0)	0.50/100 cm^3 (dimensions)	6.8/6.4	37/28	90/100	Batch[g]/Batch[g]	3.40–5.30	[47]
Clostridium butyricum	*R. sphaeroides*	Algal biomass (Starch, 5.0)	0.15/0.15	6.8/7.0	37/30	2/15	Batch/Batch	8.30	[31]
	R. palustris	Starch (17.0)	0.20/1.00	7.5/7.1	37/32	6–32/–	Batch/Batch and CSTR/ CSTR (HRT–12/24h)	3.09	[37]
	Rhodobacter sp.	Starch (5.0)	TT[b]/TT	7.0/6.5	30/30	–/–	Batch	3.60	[68]
C. butyricum and *Enterobacter aerogenes*	*R. sphaeroides*	Sweet potato starch (Starch, 5.0)	0.25/TT	5.3/7.5	37/35	10/30	Batch[g]/Batch[g]	7.00	[59]
	Rhodobacter sp.	Sweet potato starch (Starch, 10.0)	0.25/TT	5.3/7.5	37/35	13/30	Batch[g]/Batch[g]	6.70–7.20	[30]
Clostridium acetobutylicum and *Escherichia coli*	*R. capsulatus*	Date palm fruits and sucrose (5.0)	2.00/2.00	7.3/7.0	30/30	3/7	Batch/Batch	3.90	[58]
Thermotoga neapolitana	*R. capsulatus*	*Miscanthus* (Sugar, 10.0)	2.00/0.105	7.0/6.5	80/30	3/10	Batch/Batch	4.50[h]	[57, 60]
Caldicellulosiruptor saccharolyticus	*R. capsulatus*	Potato steam peels (Sugar, 15.0)	2.00/0.055	6.8/6.4	72/30	–/–	Batch/Batch	3.91	[51]
		Sugar beet molasses (Sucrose, 15.0)	1.00/0.055	6.9/6.6	72/30	3/12	Batch/Batch	5.70[h]	[32, 46]
			1.00/0.055	6.9/6.7	72/30	3/7	Batch/Batch	6.85[h]	[32]
			2.00/0.055	6.8/6.4	72/30	–/–	Batch/Batch	5.81	[51]
	R. capsulatus, R. palustris and *R. sphaeroides*	Potato steam peels (Sugar, 15.0)	2.0/0.055	6.4/6.4	72/30	–/6	Batch/Batch	2.87–3.39[h]	[45]
Klebsiella oxytoca	*R. palustris*	Sugarcane bagasse (50.0)	0.14/0.14	7.0/7.0	37/30	–/–	Batch/Batch	4.14[h]	[44]
Lactobacillus amylovorus	*Rhodobium marinum*	Algal biomass (Starch, 4.05)	0.07/0.07	7.0/6.5	30/30	6/6	Batch/Batch	5.40	[29]

[a] Photo synthetic bacteria

[b] Test tube with dimension of 2.4 × 20 cm^2

[c] Incubation period

[d] Values not given

[e] Continuous stirred tank reactor

[f] Hydraulic retention time

[g] Both in batch and repeated batch cultures

[h] Values converted from the original data

generating up to 7.15 mol H_2/mol hexose [31–33, 53, 58]. In view of the effective working of the photosynthetic partners in the integrated BHP process, the observed variations in H_2 yields can be assigned to the dark-fermentative H_2-producers. Dark-fermentative bacteria present in the activated sludge were relatively less effective in producing H_2 in comparison to those present in cattle dung. Among the defined dark fermentative bacteria, *C. butyricum* alone or in association with *E. aerogenes* was quite consistent in yielding 7–8 mol H_2/mol hexose, along with *R. sphaeroides* as the photo-fermentative partner [31, 59]. H_2 yields did not vary when *R. capsulatus* was used in association with a wide range of dark-fermentative H_2-producers [47, 51, 57, 58, 60]. In contrast to *R. sphaeroides,* the combination of *C. butyricum* and *R. palustris* did not prove to be the most effective H_2-producing culture combination [37].

Integrative Single Stage Dark and Photo-Fermentative Hydrogen Production

In contrast to subjecting feed material to dark- and photo-fermentative bacteria under two different sets of conditions, attempts have been made to combine the two (Table 2). Combination of activated sludge (as source of dark-fermentative H_2-producers) with *R. sphaeroides* has resulted in H_2 yield of 0.3–3.4 mol/mol hexose [39, 61–63]. The variation in H_2 yields could be assigned to differences in substrate concentration, inoculum ratios, light intensities, etc. [39, 63]. In most of the reports, batch and fed-batch mode of reactors have been employed. The best results of 3.4 mol H_2/mol hexose were reported when wheat starch was used at the rate of 5 g/l with an inoculum ratio of 1:3 (of dark/photo-fermentative bacteria) in continuous mode (periodic feed) [63].

In other experiments, low H_2 yields in the range of 1.05–1.16 mol/mol hexose were recorded on substituting *R. sphaeroides* with *Rhodobacter* sp. and *R. palustris* combination, along with activated sludge and wheat starch (as feed) [64, 65] and quite high yield of 2.76 mol/mol hexose with pure culture [66]. It allowed one to conclude the superiority of *R. sphaeroides* as a photo-fermentative partner. The high H_2 yielding capacity of *R. sphaeroides* was negatively affected when combined with *Clostridium beijerinckii* as the dark-fermentative partner–resulting in low H_2 yield of 0.6 mol/mol hexose [41]. *R. marium* proved to be an effective H_2-producer, which resulted in high yields of 7.3 mol/mol hexose with *Lactobacillus amylovorus* and 6.2 mol/mol hexose with *Vibrio fluvialis* [29, 67]. Incidentally, in spite of being such highly effective H_2-poducers, *L. amylovorus* and *V. fluvialis* have not been pursued since their initial reports.

Perspectives

Among the different worries which loom large are the pollution due to burning of fossil fuels and their limited resources. Although biohydrogen has been identified as a clean alternative to ever polluting fossil fuels, however, in order to establish biohydrogen as a non-polluting energy carrier it is imperative to carry out innovative research. At present, the struggle is on to look for cheap sources of feed and robust microbes for commercial scale H_2 production. The need stems from the fact that BHP is regarded as inefficient due to low yields. Theoretically 12 mol of H_2 can be generated from each mol of glucose. However, in practice, H_2 yields are stagnant, such that a maximum of 3.8 mol/mol glucose has been shown as the achievable limit with either dark- or photo-fermentative routes by a limited number of bacteria. It was however realized quite soon that H_2 yields can be enhanced by combining the two metabolic routes. Here, VFAs especially acetic acid and butyric acid generated as the end products of dark fermentative H_2-production process can be subjected to photo-fermentative bacteria. Theoretically, acetic acid can be converted to generate 4 mol of H_2 [50]. Such that a H_2 yield of 12 mol/mole glucose can be achieved by employing an integrative approach–dark followed by photo-fermentation [7]. The need is to optimize the various process parameters and thus improve the efficiency of the organisms. Since, bacteria exist largely as complex communities, they create conditions such that ecological selection persists and the most productive system prevails. Taking advantage of the abilities of the bacteria to occur as mixed cultures and as consortia, it is desirable to select bacteria which are compatible to each other and exploit their natural abilities to accomplish our purpose. Facultative anaerobes such as *Bacillus* and *Enterobacter* have abilities to produce H_2 in quantities which are quite comparable to those produced by strict anaerobic (*Clostridium*). They however offer additional advantages in terms of their abilities to survive in the presence of O_2 during the initial stage of anaerobic biodegradation and produce H_2 efficiently. They also offer an added feature by quenching O_2 in cases where *Clostridium* may be the associated H_2-producer [68, 69]. In case of photo-fermentation, light intensity is a major requirement for most metabolic activities. During photo-fermentative BHP, nitrogenase enzyme requires energy for the H_2 production, which is provided by the light energy conversion to ATP [70]. It has been shown that increase in the light energy does enhance BHP [39], although exceptionally it may not prove effective [71]. We can design complex communities consisting of robust and self stabilizing populations. This syntrophic association must be managed for the sustainable development. It is envisaged that the feasibility of these two stage processes

Table 2 Integration of dark- and photo-fermentative bacteria in a single stage hydrogen production from biowastes

Organisms	Substrate (concentration: g/l)	Process parameters							H_2 Yield (mol/mol hexose)	References
		Inoculum ratio[a]	Light intensity[c]	Reactor capacity (L)	pH	Temp. (°C)	IP[h]	Culture mode		
Activated sludge + *Rhodobacter sphaeroides*	Wheat starch (5.0)	1:3	5,000 lux	0.25	7.0	30	–[i]	Continuous[k] (HRT[l]–8)	3.40	[63]
	Wheat starch (5.95)	1:2	10,000 lux	0.31	7.5	30	8	Batch	1.45	[39]
	Wheat starch (10.0)	1:2	5,000 lux	2.00	7.5	30	10	Fed-batch	1.32[o]	[61]
	Wheat starch (12.8)	1:2	6,000 lux	0.31	7.5	30	12	Batch	0.28–0.36[o]	[62]
Activated sludge + *Rhodobacter* sp. and *Rhodopsedomonas palustris*	Wheat starch (2.5)	1:7	9,500 lux	0.31	7.5	30	8	Batch	1.05	[64]
	Wheat starch (5.0)	1:7	9,500 lux	0.31	7.3	30	13	Batch	1.16	[65]
	Wheat starch (20.0)	1:2	9,500 lux	2.00	7.5	30	11	Combined fed-batch	0.43[o]	[42]
Activated sludge + *Rhodospirillum rubrum*	Cassava starch (COD, 20)	1:1	6,000[d] candela/m^2	0.075	7.2	30	–	Batch	340[p]	[71]
Lactobacillus amylovorus + *Rhodobium marinum*	Algal biomass (Starch, 4.05)	0.5:0.6	330 W/m^2	0.07	6.5	30	16	Batch	7.30	[29]
Clostridium beijerinkii + *R. sphaeroides*	Wheat starch (5.0)	1:3.9	10,000 lux	7.63	7.3	32	–	AHB[m] (HRT-6)	0.60	[41]
	Wheat starch (12.8)	1:2	6,000 lux	0.31	7.5	30	12	Batch	0.12–0.15[o]	[62]
Clostridium butyricum + *Rhodobacter* sp.	Starch (5.0)	2:3	5,000 lux	TT[f]	6.5	30	8–40[j]	Bacth and fed-batch[n]	4.50–6.60	[68]
Citrobacter freundii and *Enterobacter aerogenes* + *R. palustris*	Sugar cane effluent (Sugar, 7.9)	1:1:1[b]	NA[e]	10–100[g]	7.0	37	2	Batch	2.76	[66]
Vibrio fluvialis + *Rhodobium marinum*	Algal biomass (Starch, 4.05)	2:1	330 W/m^2	0.07	7.0	30	9	Batch	6.20	[67]

[a] Dark/light organisms

[b] Co-cultures

[c] Continuous light

[d] Dark and light periods of 12 h each also

[e] Not applicable

[f] Test tube with dimension of 2.4 × 20 cm^2

[g] Reactor dimensions in m^3

[h] Incubation period in days

[i] Values not given

[j] Different sets of experiment

[k] Periodic feed

[l] Hydraulic retention time in days

[m] Annular-hybrid bioreactor

[n] Repeated

[o] Values converted from the original data

[p] ml H_2/g COD

can be established by combining it with microalgae photosynthesis processes, which is likely to enhance overall H_2 production by utilizing CO_2 produced in the previous stages [37]. From a commercial point of view, it may be necessary to integrate other processes such as bioplastic and methane production in it [4, 7, 17, 72–75].

Acknowledgments The authors wish to thank Director of CSIR-Institute of Genomics and Integrative Biology and Department of Biotechnology (DBT) Biology for providing the necessary funds, facilities and moral support. SKSP is thankful to CSIR for granting Research Associate Fellowship.

References

1. Kalia VC (2007) Microbial treatment of domestic and industrial wastes for bioenergy production. Appl Microbiol (e-Book). National Science Digital Library NISCAIR, New Delhi, India. http://nsdl.niscair.res.in/bitstream/123456789/650/1/DomesticWaste.pdf
2. Kalia VC, Purohit HJ (2008) Microbial diversity and genomics in aid of bioenergy. J Ind Microbiol Biotechnol 35:403–419
3. Kalia VC, Lal S, Ghai R, Mandal M, Chauhan A (2003) Mining genomic databases to identify novel hydrogen producers. Trends Biotechnol 21:152–156
4. Angenent LT, Karim K, Al-Dahhan MH, Wrenn BA, Domíguez-Espinosa R (2004) Production of bioenergy and biochemicals from industrial and agricultural wastewater. Trends Biotechnol 22:477–485
5. Levin DB, Pitt L, Love M (2004) Biohydrogen production: prospects and limitations to practical application. Int J Hydrogen Energy 29:173–185
6. Lee HS, Vermaas WFJ, Rittmann BE (2010) Biological hydrogen production: prospects and challenges. Trends Biotechnol 28:262–271
7. Patel SKS, Kumar P, Kalia VC (2012) Enhancing biological hydrogen production through complementary microbial metabolisms. Int J Hydrogen Energy. doi:10.1016/j.ijhydene.2012.04.045
8. Hallenbeck PC, Ghosh D, Skonieczny MT, Yargeau V (2009) Microbiological and engineering aspects of biohydrogen production. Indian J Microbiol 49:48–59
9. Hallenbeck PC, Ghosh D (2009) Advances in fermentative biohydrogen production: the way forward? Trends Biotechnol 27:287–297
10. Argun H, Kargi F (2011) Bio-hydrogen production by different operational modes of dark and photo-fermentation: an overview. Int J Hydrogen Energy 36:7443–7459
11. Kotay SM, Das D (2008) Biohydrogen as a renewable energy resource—prospects and potentials. Int J Hydrogen Energy 33:258–263
12. Hallenbeck PC (2009) Fermentative hydrogen production: principles, progress and prognosis. Int J Hydrogen Energy 34:7379–7389
13. Dasgupta CN, Gilbert JJ, Lindblad P, Heidorn T, Borgvang SA, Skjanes K, Das D (2010) Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. Int J Hydrogen Energy 35:10218–10238
14. Hallenbeck PC, Abo-Hashesh M, Ghosh D (2012) Strategies for improving biological hydrogen production. Bioresour Technol 110:1–9
15. Carere CR, Kalia V, Sparling R, Cicek N, Levin DB (2008) Pyruvate catabolism and hydrogen synthesis pathway genes of *Clostridium thermocellum* ATCC 27405. Indian J Microbiol 48:252–266
16. Pan C-M, Fan Y-T, Zhao P, Hou H-W (2008) Fermentative hydrogen production by the newly isolated *Clostridium beijerinckii* Fanp3. Int J Hydrogen Energy 33:5383–5391
17. Porwal S, Kumar T, Lal S, Rani A, Kumar S, Cheema S, Purohit HJ, Sharma R, Patel SKS, Kalia VC (2008) Hydrogen and polyhydroxybutyrate producing abilities of microbes from diverse habitats by dark fermentative process. Bioresour Technol 99:5444–5451
18. Ren N-Q, Liu B-F, Ding J, Xie G-J (2009) Hydrogen production with *R. faecalis* RLD-53 isolated from freshwater pond sludge. Bioresour Technol 100:484–487
19. deVrije T, Budde MAW, Lips SJ, Bakker RR, Mars AE, Claassen PAM (2010) Hydrogen production from carrot pulp by the extreme thermophiles *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana*. Int J Hydrogen Energy 35:13206–13213
20. Patel SKS, Purohit HJ, Kalia VC (2010) Dark fermentative hydrogen production by defined mixed microbial cultures immobilized on ligno-cellulosic waste materials. Int J Hydrogen Energy 35:10674–10681
21. Ghosh D, Hallenbeck PC (2009) Fermentative hydrogen yields from different sugars by batch cultures of metabolically engineered *Escherichia coli* DJT135. Int J Hydrogen Energy 34:7979–7982
22. Kalia VC, Jain SR, Kumar A, Joshi AP (1994) Fermentation of biowaste to hydrogen by *Bacillus licheniformis*. World J Microbiol Biotechnol 10:224–227
23. Kalia VC, Joshi AP (1995) Conversion of waste biomass (pea-shell) into hydrogen and methane through anaerobic digestion. Bioresour Technol 53:165–168
24. Sonakya V, Raizada N, Kalia VC (2001) Microbial and enzymatic improvement of anaerobic digestion of waste biomass. Biotechnol Lett 23:1463–1466
25. Kapdan IK, Kargi F (2006) Bio-hydrogen production from waste materials. Enzyme Microb Technol 38:569–582
26. Balat H, Kirtay E (2010) Hydrogen from biomass present–scenario and future prospects. Int J Hydrogen Energy 35:7416–7426
27. Levin DB, Chahine R (2010) Challenges for renewable hydrogen production from biomass. Int J Hydrogen Energy 35:4962–4969
28. Patel SKS, Singh M, Kumar P, Purohit HJ, Kalia VC (2012) Exploitation of defined bacterial cultures for production of hydrogen and polyhydroxybutyrate from pea-shells. Biomass Bioenergy 36:218–225
29. Kawaguchi H, Hashimoto K, Hirata K, Miyamoto K (2001) H_2 production from algal biomass by a mixed culture of *Rhodobium marinum* A-501 and *Lactobacillus amylovorus*. J Biosci Bioeng 91:277–282
30. Yokoi H, Maki R, Hirose J, Hayashi S (2002) Microbial production of hydrogen from starch-manufacturing wastes. Biomass Bioenergy 22:389–395
31. Kim M-S, Baek J-S, Yun Y-S, Sim S-J, Park S, Kim S-C (2006) Hydrogen production from Chlamydomonas reinhardtii biomass using a two-step conversion process: anaerobic conversion and photosynthetic fermentation. Int J Hydrogen Energy 31:812–816
32. Ozgur E, Mars AE, Peksel B, Louwerse A, Yucel M, Gunduz U, Claassen PAM, Eroglu I (2010) Biohydrogen production from beet molasses by sequential dark and photo-fermentation. Int J Hydrogen Energy 35:511–517
33. Su H, Cheng J, Zhou J, Song W, Cen K (2010) Hydrogen production from water hyacinth through dark- and photo-fermentation. Int J Hydrogen Energy 35:8929–8937

34. Cheng J, Su H, Zhou J, Song W, Cen K (2011) Hydrogen production by mixed bacteria through dark- and photo-fermentation. Int J Hydrogen Energy 36:450–457
35. Redwood MD, Beedle MP, Macaskie LE (2009) Integrating dark and light bio-hydrogen production strategies: towards the hydrogen economy. Rev Environ Sci Biotechnol 8:149–185
36. Guwy AJ, Dinsdale RM, Kim JR, Massanet-Nicolau J, Premier G (2011) Fermentative biohydrogen production systems integration. Bioresour Technol 102:8534–8542
37. Lo Y-C, Chen S-D, Chen C-Y, Huang T-I, Lin C-Y, Chang J-S (2008) Combining enzymatic hydrolysis and dark-photo fermentation processes for hydrogen production from starch feedstock: a feasibility study. Int J Hydrogen Energy 33:5224–5233
38. Zong W, Yu R, Zhang P, Fan M, Zhou Z (2009) Efficient hydrogen gas production from cassava and food waste by a two-step process of dark fermentation and photo-fermentation. Biomass Bioenergy 33:1458–1463
39. Argun H, Kargi F (2010) Effects of light source, intensity and lighting regime on bio-hydrogen production from ground wheat starch by combined dark and photo-fermentations. Int J Hydrogen Energy 35:1604–1612
40. Argun H, Kargi F (2010) Photo-fermentative hydrogen gas production from dark fermentation effluent of ground wheat solution: effects of light source and light intensity. Int J Hydrogen Energy 35:1595–1603
41. Argun H, Kargi F (2010) Bio-hydrogen production from ground wheat starch by continuous combined fermentation using annular-hybrid bioreactor. Int J Hydrogen Energy 35:6170–6178
42. Kargi F, Ozmihci S (2010) Effects of dark/light bacteria ratio on bio-hydrogen production by combined fed-batch fermentation of ground wheat starch. Int J Hydrogen Energy 34:869–874
43. Yang H, Guo L, Liu F (2010) Enhanced bio-hydrogen production from corncob by a two-step process: dark- and photo-fermentation. Bioresour Technol 101:2049–2052
44. Wu X, Li Q, Dieudonne M, Cong Y, Zhou J, Long M (2010) Enhanced H_2 gas production from bagasse using *adhE* inactivated *Klebsiella oxytoca* HP1 by sequential dark-photo fermentations. Bioresour Technol 101:9605–9611
45. Afsar N, Özgür E, Gürgan M, Akköse S, Yücel M, Gündüz U, Eroglu I (2011) Hydrogen productivity of photosynthetic bacteria on dark fermenter effluent of potato steam peels hydrolysate. Int J Hydrogen Energy 36:432–438
46. Androga DD, Ozgur E, Eroglu I, Gunduz U, Yucel M (2012) Amelioration of photofermentative hydrogen production from molasses dark fermenter effluent by zeolite-based removal of ammonium ion. Int J Hydrogen Energy. doi:10.1016/j.ijhydene.2012.02.177
47. Laurinavichene TV, Belokopytov BF, Laurinavichius KS, Khusnutdinova AN, Seibert M, Tsygankov AA (2012) Towards the integration of dark- and photo-fermentative waste treatment. 4. Repeated batch sequential dark- and photofermentation using starch as substrate. Int J Hydrogen Energy 37:8800–8810
48. Özkan E, Uyar B, Özgür E, Yücel M, Eroglu I, Gündüz U (2012) Photofermentative hydrogen production using dark fermentation effluent of sugar beet thick juice in outdoor conditions. Int J Hydrogen Energy 37:2044–2049
49. Das D, Dutta T, Nath K, Kotay SM, Das AK, Veziroglu TN (2006) Role of Fe-hydrogenase in biological hydrogen production. Curr Sci 90:1627–1637
50. Fang HHP, Liu H, Zhang T (2005) Phototrophic hydrogen production from acetate and butyrate in wastewater. Int J Hydrogen Energy 90:785–793
51. Özgür E, Afsar N, Vrije T, Yücel M, Gündüz U, Classen PAM, Eroglu I (2010) Potential use of thermophile dark fermentation effluents in photofermentative hydrogen production by *Rhodobacter capsulatus*. J Clean Prod 18:S23–S28
52. Su H, Cheng J, Zhou J, Song W, Cen K (2009) Improving hydrogen production from cassava starch by combination of dark and photo fermentation. Int J Hydrogen Energy 34:1780–1786
53. Argun H, Kargi F, Kapdan IK, Oztekin R (2008) Light fermentation of dark fermentation effluent for bio-hydrogen production by different Rhodobacter species at different initial volatile fatty acid (VFA) concentrations. Int J Hydrogen Energy 33:7405–7412
54. Cheng J, Su H, Zhou J, Song W, Cen K (2011) Microwave-assisted alkali pretreatment of rice straw to promote enzymatic hydrolysis and hydrogen production in dark- and photo-fermentation. Int J Hydrogen Energy 36:2093–2101
55. Azbar N, Dokgoz FTC (2010) The effect of dilution and L-malic acid addition on bio-hydrogen production with *Rhodopseudomonas palustris* from effluent of an acidogenic anaerobic reactor. Int J Hydrogen Energy 35:5028–5033
56. Laurinavichene TV, Belokopytov BF, Laurinavichius KS, Tekucheva DN, Seibert M, Tsygankov AA (2010) Towards the integration of dark- and photo-fermentative waste treatment. 3. Potato as substrate for sequential dark fermentation and light-driven H_2 production. Int J Hydrogen Energy 35:8536–8543
57. de Vrije T, Bakker RR, Budde MAW, Lai MH, Mars AE, Claassen PAM (2009) Efficient hydrogen production from the lignocellulosic energy crop Miscanthus by the extreme thermophilic bacteria *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana*. Biotechnol Biofuels 2:12
58. Abd-Alla MH, Morsy FM, El-Enany A-WE (2011) Hydrogen production from rotten dates by sequential three stages fermentation. Int J Hydrogen Energy 36:13518–13527
59. Yokoi H, Saitsu A, Uchida H, Hirose J, Hayashi S, Takasaki Y (2001) Microbial hydrogen production from sweet potato starch residue. J Biosci Bioeng 91:58–63
60. Uyar B, Schumacher M, Gebicki J, Modigell M (2009) Photoproduction of hydrogen by *Rhodobacter capsulatus* from thermophilic fermentation effluent. Bioprocess Biosyst Eng 32:603–606
61. Ozmihci S, Kargi F (2010) Effects of starch loading rate on performance of combined fed-batch fermentation of ground wheat for bio-hydrogen production. Int J Hydrogen Energy 35:1106–1111
62. Ozmihci S, Kargi F (2010) Comparison of different mixed cultures for bio-hydrogen production from ground wheat starch by combined dark and light fermentation. J Ind Microbiol Biotechnol 37:341–347
63. Sagnak R, Kargi F (2011) Photo-fermentative hydrogen gas production from dark fermentation effluent of acid hydrolyzed wheat starch with periodic feeding. Int J Hydrogen Energy 36:4348–4353
64. Argun H, Kargi F, Kapdan IK (2009) Effects of the substrate and cell concentration on hydrogen production from ground wheat by combined dark and light fermentation. Int J Hydrogen Energy 34:6181–6188
65. Argun H, Kargi F, Kapdan IK (2009) Hydrogen production by combined dark and light fermentation of ground wheat solution. Int J Hydrogen Energy 34:4305–4311
66. Vatsala TM, Raj SM, Manimaran A (2008) A pilot-scale study of biohydrogen production from distillery effluent using defined bacterial co-culture. Int J Hydrogen Energy 33:5404–5415
67. Ike A, Murakawa T, Kawaguchi H, Hirata K, Miyamoto K (1999) Photoproduction of hydrogen from raw starch using a Halophilic bacterial community. J Biosci Bioeng 88:72–77
68. Yokoi H, Mori S, Hirose J, Hayashi S, Takasaki Y (1998) H_2 production from starch by mixed culture of *Clostridium butyricum* and *Rhodobacter* sp M-19. Biotechnol Lett 20:895–899
69. Hung C-H, Cheng C-H, Guan D-W, Wang S-T, Hsu S-C, Liang C-M, Lin C-Y (2011) Interactions between *Clostridium* sp. and other facultative anaerobes in a self-formed granular sludge

hydrogen-producing bioreactor. Int J Hydrogen Energy 36:8704–8711
70. Boran E, Özgür E, Yücel M, Gündüz U, Eroglu I (2012) Bio-hydrogen production by *Rhodobacter capsulatus* in solar tubular photobioreactor on thick juice dark fermenter effluent. J Clean Prod 31:150–157
71. Reungsang A, Sangyoka S, Chaiprasert P, Imai T (2007) Factors affecting hydrogen production from Cassava wastewater by a co-culture of anaerobic sludge and *Rhodospirillum rubrum*. Pak J Biol Sci 10:3571–3577
72. Kalia VC, Chauhan A, Bhattacharyya G, Rashmi (2003) Genomic databases yield novel bioplastic producers. Nat Biotechnol 21:845–846
73. Kumar T, Singh M, Purohit HJ, Kalia VC (2009) Potential of *Bacillus* sp. to produce polyhydroxybutyrate from biowaste. J Appl Microbiol 106:2017–2023
74. Singh M, Patel SKS, Kalia VC (2009) *Bacillus subtilis* as potential producer for polyhydroxyalkanoates. Microb Cell Fact 8:38
75. Patel SKS, Singh M, Kalia VC (2011) Hydrogen and poly-hydroxybutyrate producing abilities of *Bacillus* spp. from glucose in two stage system. Indian J Microbiol 51:418–423