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ORIGINAL ARTICLE

Ecobiotechnological Strategy to Enhance Efficiency of Bioconversion of Wastes into Hydrogen and Methane

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Abstract Vegetable wastes (VW) and food wastes (FW) are generated in large quantities by municipal markets, restaurants and hotels. Waste slurries (250 ml) in 300 ml BOD bottles, containing 3, 5 and 7 % total solids (TS) were hydrolyzed with bacterial mixtures composed of: *Bacillus, Acinetobacter, Exiguobacterium, Pseudomonas, Stenotrophomonas* and *Sphingobacterium* species. Each of these bacteria had high activities for the hydrolytic enzymes: amylase, protease and lipase. Hydrolysate of biowaste slurries were subjected to defined mixture of H_2 producers and culture enriched for methanogens. The impact of hydrolysis of VW and FW was observed as 2.6- and 2.8-fold enhancement in H_2 yield, respectively. Direct biomethanation of hydrolysates of VW and FW resulted in 3.0- and 1.15-fold improvement in CH_4 yield, respectively. A positive effect of hydrolysis was also observed with biomethanation of effluent of H_2 production stage, to the extent of 1.2- and 3.5-fold with FW and VW, respectively. The effective H_2 yields were 17 and 85 l/kg TS fed, whereas effective CH_4 yields were 61.7 and 63.3 l/kg TS fed, from VW and FW, respectively. This ecobiotechnological strategy can help to improve the conversion efficiency of biowastes to biofuels.

Keywords Anaerobic digestion · Biowaste · Biomethanation · Hydrolysis · Mixed bacterial culture

Introduction

Pollution Control Boards and Health Departments are constantly worried about the generation of huge quantities of wastes and the rapidly declining reservoirs of fossil fuels. Uncontrolled fermentation and burning of these wastes and fuels release obnoxious gases [1]. Among the various proposals being explored to solve these problems, anaerobic digestion (AD) appears to be the most lucrative. AD is a metabolically efficient process, but is economically very weak. In order to enhance the economic value of the process, suggestions have been made to derive value added products by diverting the intermediates of the waste solubilizing step to hydrogen (H_2), methane (CH_4), bioplastic, enzymes, etc. [2, 3]. AD is a multi-step process, which involves different bacteria with a wide range of metabolic activities. Although, organic matter of the biowastes can be digested up to 95 % into carbon dioxide and CH_4 [4], however, the whole process is limited by the hydrolytic step. The hydrolysis of organic matters is influenced by its composition, the most difficult to digest are the lignocellulosic biowaste [5]. Another issue which demands attention is the fact that although H_2 is an intermediate of the AD process, however, in nature, it results in CH_4 as the

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final byproduct with little or no H_2 evolution [6]. It is because of the fact that thermodynamically, H_2 production process is not stable and the equilibrium shifts to CH_4 production. This leads to a scenario of interspecies H_2 transfer reactions, where H_2 consumers out number the H_2 producers [7]. Another primary reason for low or no evolution of H_2 during AD is the feedback inhibition of H_2 process by high partial pressure of H_2. Studies to investigate H_2 and CH_4 potential of different biowastes have been evaluated under different physiological conditions [8–13]. It is difficult to produce H_2 from biowaste, since it is invariably accompanied by inherent microflora, which out number the H_2-producing bacteria [8, 14]. Sterilization of biowaste to get rid of contaminating bacteria is a costly proposal. Attempts to produce H_2 from un-sterile wastes have been successful to some extent [6, 15–17]. The need is to look for a robust set of organisms, which can survive under harsh conditions and produce H_2 and CH_4. The ecobiotechnological strategy is based on the concept of using a mixture of bacteria, which have been well defined to carry out the desired metabolic activity. Under a given set of physiological conditions prevailing in a fermenting biowaste, at least one of these well defined bacteria will be able to survive and carry out the process successfully [14, 18].

It has been realized that in all energy generation processes, the major limiting factor is the feed. Biowastes are an obvious choice because of their availability in large quantities and "consistent" supplies on daily basis. Most biowastes are composed of complex organic materials. The very first step in their utilization is the solubilization of macromolecules into simpler and easily metabolizable substrates [15, 19]. Biowastes originating from vegetable markets and food and fruit processing industries, which are rich in fats, carbohydrate and proteins. These macromolecules can be metabolized by bacteria possessing enzymes such as lipases, amylases and proteases [19]. The question is thus, Can an improvement in the hydrolytic process lead to enhancement of the digestion process? In this study, we have used an ecobiotechnological strategy to use well defined bacterial cultures for hydrolysis of unsterile wastes and subject the hydrolysate to another set of H_2-producers and enriched culture of methanogens, independently and in a sequential manner.

Materials and Methods

Preparation of Hydrolytic, H_2 Producers and Methanogens

We isolated 1,000 bacteria from soils, river sediments and cattle dung. These were screened for those having high activities for the following enzymes: amylase, lipase and protease by method described previously [19, 20]. Fifty bacteria with high hydrolytic activities were evaluated for their performance at pH range 5.0–9.0. A set of 11 bacterial strains were selected and identified through 16S rRNA gene [20]. These bacteria were employed for preparing 11 mixed hydrolytic bacterial cultures (BC1–BC11) (Table S1), designed on the basis of Plackett–Burman method [21] (Tables S2). Similarly, for H_2 production another set of mixed microbial culture (MMC4), previously screened on glucose was used [21]. MMC4 was composed of the following 6 strains: *Enterobacter aerogenes* EGU16, *Proteus mirabilis* EGU21, *Bacillus cereus* EGU43, *B. thuringiensis* EGU45, *B. pumilus* HPC 464, *Bacillus* sp. HPC459, which were previously established to be effective as mixed H_2 producers [21]. Each mixed culture was prepared by combining 6 different microbes in equal proportions amounting to a final cell protein concentration of 10 μg/ml [19]. Enrichment of methanogens was done by incubating 3 % total solids (TS) cattle dung slurry at 37 °C for 20 days [22].

Total Solids and Organic Solids

Samples of vegetable waste (VW) and kitchen food waste (FW) have been analysed for parameters like TS, and volatile solids, which were estimated by heating a sample at 110 °C for 24 h and at 600 °C for 4 h, respectively [22].

Hydrolysis of Biowastes

The biowaste slurries (250 ml) were hydrolysed with 11 mixed microbial cultures namely BC1–BC11. The hydrolysis of waste was carried out for 5 days at 37 °C. Hydrolysis was monitored through the production of volatile fatty acids [19].

Hydrogen Production

Biowaste feed (250 ml) at 3, 5 and 7 % TS was inoculated with MMC4 at the rate of 10 μg cell protein/ml of slurry. pH of the slurry was adjusted to 7.0 prior to incubation and the bottles were made air tight using glass stoppers. pH was adjusted to 7.0 using 2 N NaOH or 2 N HCl and flushed with argon, on a daily basis. The evolved gases were collected by the water displacement method. Gas collection and analysis of the samples were carried out until H_2 evolution ceased [19, 20]. The values presented here are based on three replicates.

Methane Production

Biowaste feed (250 ml) at 3, 5 and 7 % TS was inoculated with methanogens 10 % (v/v). pH of the slurry was

adjusted to 7.0. The reactor bottle was flushed with argon to make the conditions anaeronbic. Biogas production was monitored daily for 15 days and it was observed that biogas production stopped by 10 days except in controls [22]. The values presented here are based on three replicates.

Analytical method

Gas Analysis

The composition of the biogas produced during fermentation processes was determined using gas chromatograph (Nucon GC5765) equipped with Porapak-Q and molecular sieve columns using thermal conductivity detector [19, 21].

Volatile Fatty Acid Estimation

VFA analysis was carried out from 1.0 ml sample taken in 1.5 ml vials. 2–3 drops of ortho-phosphoric acid (25 % v/v) were added to each vial for sample preservation. VFA concentrations were determined using gas chromatograph (GC 6890 N) equipped with flame ionization detector. A capillary column, DBWAXetr (30 m × 53 µm × 1 µm ID) was used for analysis. The oven, injector and detector temperatures were 140, 220 and 230 °C, respectively.

Results

In natural conditions, the biowaste containing biomacromolecules like carbohydrates, fats and proteins can be degraded by bacteria producing hydrolytic enzymes. Screening of 1,000 bacteria allowed us to select 50 having high activities for amylase, lipase and protease. Further evaluation of the enzymatic activities at a wider pH range enabled us to select 11 having at least one of these enzymatic activities in the pH range 5.0–9.0. Finally eleven bacteria so selected were identified as: *Bacillus aryabhattai* MBG46 (KJ563237); *Acinetobacter* sp. MBG50 (KJ563241) and *A. haemolyticus* MBG52 (KJ563243); *Exiguobacterium* sp. MBG53 (KJ563244) and *E. indicum* MBG54 (KJ563245); *Pseudomonas mendocina* strains MBG51 (KJ563242), MBG57 (KJ563248), MBG58 (KJ563249) and *P. pseudoalcaligenes* MBG45 (KJ563236); *Stenotrophomonas koreensis* MBG44 (KJ563235) and *Sphingobacterium daejeonense* MBG47 (KJ563238) (Table S1). Of the 11 mixed bacterial cultures: BC6, BC7, BC8 and BC10 were found to be effective for hydrolyzing VW as indicated by the total volatile fatty acid composition, over a period of 5 days of incubation (Tables S3). On the other hand, mixed bacterial culture designated as BC1, BC6, BC8 and BC9 were found to be effective for hydrolyzing FW under similar incubation period.

Hydrogen Evolution

H_2 evolution was observed from vegetable waste slurry (VWS) and food waste slurry (FWS) using defined mixed microbial culture of H_2-producers (MMC4) [21]. Unhydrolysed waste (control) was observed to generate 160–390 ml of biogas from 250 ml of VWS. Here, H_2 constituted 39.7–44.4 % of the total biogas, amounting to a net observed volume of 65–155 ml/250 ml slurry. The effective H_2 yield was in the range of 6–9 l/kg TS fed. Pretreatment of VW with hydrolytic bacterial cultures was very effective. Of the 11 mixed bacterial cultures, BC6, BC7, BC8 and BC10 were found to be effective in improving H_2 yield (Table 1). At 3 % TS VWS, biogas evolution increased up to 285 ml with BC7. It was accompanied by a substantial enhancement in H_2 evolution up to 130 ml, i.e., a 2-fold increase over control. At 5 % TS VWS, maximum H_2 evolution was observed with BC7. Although H_2 component of the biogas did not change much, however, BC7 resulted in 2.6-fold increase in H_2 yield. Further increase in the concentration of TS in the VWS to 7 % led to increase in the net evolution of biogas (up to 530 ml), however, it was not accompanied by a proportional increase in H_2 evolution. H_2 evolution was almost similar to that recorded with control. In fact, it has been reported previously that H_2 evolution process is negatively influenced by the increase in carbohydrate concentration in the slurry [23]. It may also be remarked that high TS also influence the metabolic process of H_2 evolution since the H_2 component of the biogas was also reduced compared to those observed at 3 and 5 % TS slurries. It may be reasonable to conclude that BC7 is effective in hydrolyzing the VW resulting in 2.0- to 2.6-fold enhancement in H_2 yield (Table 1).

In contrast to VW, the fermentation process was more effective with FW, which may be due to easily digestible components of the waste. Here, 250 ml of FWS without any hydrolysis resulted in the net evolution of 685 ml biogas at 3 % TS. It contained 225 ml H_2, equivalent to 32.8 % of the total biogas (Table 1). The effective H_2 yield was 30 l/kg TS fed. Hydrolysis with different bacterial cultures resulted in gain in H_2 yields, ranging up to 85 l/kg TS fed with BC6. It was accompanied by a higher H_2 component of 62.1 %. Hydrolysis of FW resulted in 2.8-fold enhancement in H_2 yields. Further increase in TS of the slurry did not prove helpful in improving the H_2 production process. H_2 yields of 28–38 l/kg TS fed from 5 % TS pretreated with BC1, BC6 and BC8 and 24–36 l/kg TS fed from 7 % TS slurries were higher than their respective controls. BC6 and BC8 were the most effective mixtures of hydrolytic bacteria, which enhanced H_2 yield. (Table 2).

Table 1 Hydrogen producing abilities of mixed H_2-producers[a] from prehydrolyzed biowastes

Mixed bacterial culture	Biogas volume (ml)	H_2			Biogas volume (ml)	H_2			Biogas volume (ml)	H_2		
		Vol (ml)	%	Yield[b]		Vol (ml)	%	Yield		Vol (ml)	%	Yield
	Vegetable Waste											
	3 % TS[c]				5 % TS				7 % TS			
Control[d]	160	65	40.6	9	180	80	44.4	6	390	155	39.7	9
BC6	180	70	38.9	10	270	85	31.5	7	465	125	26.9	7
BC7	285	130	45.6	17	475	210	44.2	17	530	150	28.3	9
BC8	260	115	44.2	16	380	165	43.4	13	350	75	21.4	4
BC10	250	90	36.0	12	250	100	40.0	8	240	105	43.7	6
	Food Waste											
	3 % TS				5 % TS				7 % TS			
Control	685	225	32.8	30	635	205	32.3	16	620	295	47.6	17
BC1	535	230	43.0	30	735	345	46.9	28	650	265	40.8	15
BC6	1,030	640	62.1	85	800	395	49.4	32	885	420	47.4	24
BC8	485	250	51.5	33	875	470	53.7	38	945	625	66.1	36
BC9	635	290	45.7	39	525	225	42.8	18	515	190	36.9	11

[a] Defined mixed microbial culture of H_2-producers (MMC4)

[b] H_2 production in l/kg Total solids fed

[c] Total solids

[d] No mixed bacterial culture added

Table 2 Comparison of methane yields from prehydrolyzed biowastes by direct and indirect biomethanation

Mixed bacterial culture	Direct biomethanation[a]			Indirect biomethanation		
	3 % TS[b]	5 % TS	7 % TS	3 % TS	5 % TS	7 % TS
Vegetable waste						
Control[c]	20.0	26.5	36.4	8.0	13.4	8.6
BC6	17.5	29.3	25.0	7.3	15.2	11.4
BC7	61.7	31.0	15.7	28.3	16.6	10.0
BC8	26.7	42.2	31.4	12.7	13.5	10.7
BC10	17.5	36.4	38.6	6.0	14.7	11.4
Food waste						
Control	55.0	26.0	20.3	26.3	16.7	10.7
BC1	50.0	37.4	32.1	30.0	19.4	12.1
BC6	63.3	54.5	24.3	31.7	17.5	13.6
BC8	46.7	45.2	42.1	21.4	19.2	12.8
BC9	48.3	52.3	37.9	31.5	18.5	12.1

[a] CH_4 production in l/kg Total solids fed

[b] Total solids

[c] No mixed bacterial culture added

Methane Evolution

Biomethanation has been a suitable post H_2 treatment process for effective utilization of biowastes. CH_4 evolution was observed on hydrolysed biowastes through two routes-Direct and Indirect (preceeded by H_2 production). The impact of hydrolysis by mixed bacterial cultures on biomethanation through both the routes was distinctly observed.

Direct Biomethanation

Biomethanation of VWS (250 ml) was observed to vary from 20 to 36.4 l/kg TS fed. It constituted around 61 % of the total biogas produced over a period of 15 days. In contrast, hydrolysis of VW by 11 different mixed bacterial culture (BC1–BC11) having high hydrolytic enzyme activities proved effective in improving the biomethanation process. The four BCs: BC6, BC7, BC8 and BC10 were chosen for further studies as the VFA content of these hydrolysates were quite high and consistent. BC7 proved to be the most efficient with a final CH_4 yield of 61.7 l/kg TS fed at 3 % TS VWS. The net enhancement in CH_4 yield was 3-fold. Although CH_4 yields were higher at 5 and 7 % TS VWS compared to control, however, BC7 treatment resulted in lower CH_4 yields at 5 and 7 % TS VWS, compared to 3 % TS VWS. Hence, we may conclude that hydrolysis of VWS with BC7 is effective at 3 % TS VWS in comparison to untreated VWS.

Direct biomethanation of untreated FWS was more effective in comparison to VWS. Here, the net CH_4 yield was 55 l/kg TS fed. Biomethanation was found to decline

from FWS (control) at higher TS concentrations of 5 and 7 %, where the CH_4 yield were found to be 26.0 and 20.3 l/kg TS fed, respectively. Hydrolysis of FW with well defined mixed bacterial cultures was quite effective with BC6, which enabled us to improve the biomethanation process to yield 63.3 l CH_4/kg TS fed at 3 % TS FWS. Although, the CH_4 yields were higher than the control even at 5 % and 7 % TS FWS, however, these values were relatively lower than those obtained from 3 % TS FWS with BC6.

Indirect Biomethanation

The effect of pretreatment with hydrolytic bacteria was evident even with effluent emanating from H_2 production process. Untreated VWS, resulted in 8.0–13.4 l CH_4/kg TS fed at 3–7 % TS concentrations. In contrast, VWS subjected to hydrolysis by BC7 proved effective even in indirect biomethanation process, with a net gain of 1.16- to 3.53-fold. The best results were observed at 3 % TS VWS. Incidentally, the same combination was the most efficient even via direct biomethanation. On the other hand, FWS slurry was also digested most efficiently by BC6 in the cases of direct and indirect biomethanation. Via indirect biomethanation, a 1.2-fold enhanmcement in CH_4 yield was recorded in comparison to its respective control.

Most of the biological wastes undergo AD process with no net evolution of H_2. It is primarily because of inter species H_2 transfer phenomenon. Since H_2 generation results in accounting for 35 % of the total energy present in the organic matter content of the feed, it becomes imperative to subject the effluent from H_2 stage to methanogens. Here, we can expect a maximum of 65 % of the energy as CH_4, with respect to CH_4 yield observed via direct biomethanation as 100 %. In VWS (3 % TS) and BC7 combination, we observed 63.3 l CH_4/kg TS fed via direct biomethanation. Via indirect biomethanation, we can expect a CH_4 yield of 41.1 l. Since we could observe VWS (3 % TS) and BC7 combination to generate 31.7 l CH_4, it is equivalent to 77 % of the expected value. On the other hand, with FWS (3 % TS) and BC6 combination, we could generate 61.7 l CH_4/kg TS fed via direct biomethanation and 28.3 l CH_4/kg TS fed via indirect biomethanation. Thus we could recover 70 % of the CH_4 yield expected via direct biomethanation. In both the cases, we could recover 70–75 % of the expected CH_4 yields.

Discussion

Bioprocesses involving single bacterial cultures are always at the risk of getting contaminated [14]. In order to run the process continuously there is a need to maintain conditions which are favorable to the bacteria in question and at the same time prevent others from growing. Invariably, it demands sterile feed material. In the case of fermentation of biowastes, it is difficult to sterilize the feed [19]. Hence, the presence of inherent bacteria continues to pose a threat, as they metabolize the organic matter into undesirable by-products. Ecobiotechnological approach relies on the use of robust bacteria with well defined activities. Mixed defined bacteria as inoculum enhances the chance of survival of at least one or two types of bacteria, which are sufficient to ensure consistency and reproducibility of the process. This approach has been exploited previously for producing polyhydroxyalkanoates [14, 18]. In the present work, the whole process is quite complex. For complete degradation of biowastes, coordinated activities of different set of bacteria are operative: (1) Hydrolytic bacteria (2) H_2 producers and 3) methanogens [1]. The major metabolic limitations are: (1) The hydrolytic process, and (2) H_2 transfer reaction [7]. For hydrolysis of organic matter, the need is to have well defined bacteria with high hydrolytic activities. And such bacteria are present in small numbers in natural populations. The other issue is the fact that H_2 produced by one set of bacteria is immediately quenched by methanogens, such that there is little or no net evolution of H_2 [15]. In the present study, bacteria with high relative enzyme efficiencies were mixed in equal proportions. Of the 11 such mixed bacterial cultures, BC7 and BC6 found to be effective in enhancing H_2 yield from vegetable waste and food waste to the extent of 1.9- and 2.8-fold, respectively, in comparison to control. Hydrolysate generated by BC6 and BC7 were effective in 1.15- and 3.1-fold improvement in CH_4 yield. In the case of hydrolysate initially subjected to H_2 producers and subsequently by methanogens, BC7 resulted in 3.53-fold and BC6 led to 1.2-fold enhancement in CH_4 yields. Thus under all conditions, hydrolytic bacterial mixture proved effective in enhancing the processes for generating bioenergy. Secondly, the split of H_2 stage and CH_4 stage allowed us to overcome the problem of H_2-energy transfer [8, 23]. These findings provide an evidence that hydrolysate of organic matter can be easily converted into bioproducts of high economic values. Combining these metabolic pathways may enable complete and efficient degradation along with sustainability.

Conclusion

Hydrolysis of biowastes with defined bacterial cultures helps to improve H_2 and CH_4 production from VW at 3 % by 1.9- and 3.1-fold, whereas with FW the corresponding enhancements were 2.83- and 1.15-fold, respectively. FW is a better feed for H_2 (5-fold) compared to VW. 3 % TS is the best concentration observed for H_2 and CH_4 generation

with both VW and FW. The effective H_2 yields were 17 and 85 l/kg TS fed, where as effective CH_4 yields were 61.7 and 63.3 l/kg TS fed from VWS and FWS, respectively. Hydrolysis thus proved beneficial in achieving cost-effective conversion of waste to energy.

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References

1. Kalia VC (2007) Microbial treatment of domestic and industrialwastes 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. doi:10.1007/s10295-007-0300-y
3. Kumar P, Patel SKS, Lee JK, Kalia VC (2013) Extending the limits of *Bacillus* for novel biotechnological applications. Biotechnol Adv 31:1543–1561. doi:10.1016/j.biotechadv.2013.08.007
4. Bouallagui H, Touhami Y, Cheikh RB, Hamdi M (2005) Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. Process Biochem 40:989–995. doi:10.1016/j.procbio.2004.03.007
5. Kalia VC, Sonakya V, Raizada N (2000) Anaerobic digestion of banana stem waste. Bioresour Technol 73:191–193. doi:10.1016/S0960-8524(99)00172-8
6. Kalia VC, Jain SR, Kumar A, Joshi AP (1994) Fermentation of biowaste to H_2 by *Bacillus licheniformis*. World J Microbiol Biotechnol 10:224–227. doi:10.1007/BF00360893
7. Archer DB, Thompson LA (1987) Energy production through the treatment of wastes by micro-organisms. J Appl Bacteriol 63:59s–70s. doi:10.1111/j.1365-2672.1987.tb03612.x
8. Chu CF, Xu KQ, Li YY, Inamori Y (2012) Hydrogen and methane potential based on the nature of food waste materials in a two-stage thermophilic fermentation process. Int J Hydrogen Energy 37:10611–10618. doi:10.1016/j.ijhydene.2012.04.048
9. Chuang YS, Lay CH, Sen B, Chen CC, Gopalakrishnan K, Wu JH, Lin CS, Lin CY (2011) Biohydrogen and biomethane from water hyacinth (*Eichhornia crassipes*) fermentation: effects of substrate concentration and incubation temperature. Int J Hydrogen Energy 36:14195–14203. doi:10.1016/j.ijhydene.2011.04.188
10. Molino A, Nanna F, Ding Y, Bikson B, Braccio G (2013) Biomethane production by anaerobic digestion of organic waste. Fuel 103:1003–1009. doi:10.1016/j.fuel.2012.07.070
11. Patterson T, Esteves S, Dinsdale R, Guwy A, Maddy J (2013) Life cycle assessment of biohydrogen and biomethane production and utilisation as a vehicle fuel. Bioresour Technol 131:235–245. doi:10.1016/j.biortech.2012.12.109
12. Premier GC, Kim JR, Massanet-Nicolau J, Kyazze G, Esteves SRR, Penumathsa BKV, Rodríguez J, Maddya J, Dinsdale RM, Guwya AJ (2013) Integration of biohydrogen, biomethane and bioelectrochemical systems. Renew Energy 49:188–192. doi:10.1016/j.renene.2012.01.035
13. Xiao L, Deng Z, Fung KY, Ng KM (2013) Biohydrogen generation from anaerobic digestion of food waste. Int J Hydrogen Energy 38:13907–13913. doi:10.1016/j.ijhydene.2013.08.072
14. Kumar P, Singh M, Mehariya S, Patel SKS, Lee JK, Kalia VC (2014) Ecobiotechnological approach for exploiting the abilities of *Bacillus* to produce co-polymer of polyhydroxyalkanoate. Ind J Microbiol 54:151–157. doi: 10.1007/s12088-014-0457-9
15. Sonakya V, Raizada N, Kalia VC (2001) Microbial and enzymatic improvement of anaerobic digestion of waste biomass. Biotechnol Lett 23:1463–1466. doi:10.1023/A:1011664912970
16. Patel SKS, Kumar P, Kalia VC (2012) Enhancing biological hydrogen production through complementary microbial metabolisms. Int J Hydrogen Energy 37:10590–10603. doi:10.1016/j.ijhydene.2012.04.045
17. Patel SKS, Kalia VC (2013) Integrative biological hydrogen production: an overview. Indian J Microbiol 53:3–10. doi:10.1007/s12088-012-0287-6
18. Johnson K, Jiang Y, Kleerebezem R, Muyzer G, van Loosdrecht MCM (2009) Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity. Biomacromolecules 10:670–676. doi:10.1021/bm8013796
19. 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. doi:10.1016/j.biombioe.2011.10.027
20. 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. doi:10.1016/j.biortech.2007.11.011
21. 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. doi:10.1016/j.ijhydene.2010.03.025
22. Kalia VC, Kumar A, Jain SR, Joshi AP (1992) Biomethanation of plant materials. Bioresour Technol 41:209–212. doi:10.1016/0960-8524(92)90003-G
23. Kalia VC, Joshi AP (1995) Conversion of waste biomass (pea-shells) into hydrogen and methane through anaerobic digestion. Bioresour Technol 53:165–168. doi:10.1016/0960-8524(95)00077-R