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REVIEW

Microbial diversity and genomics in aid of bioenergy

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Abstract In view of the realization that fossil fuels reserves are limited, various options of generating energy are being explored. Biological methods for producing fuels such as ethanol, diesel, hydrogen (H_2), methane, etc. have the potential to provide a sustainable energy system for the society. Biological H_2 production appears to be the most promising as it is non-polluting and can be produced from water and biological wastes. The major limiting factors are low yields, lack of industrially robust organisms, and high cost of feed. Actually, H_2 yields are lower than theoretically possible yields of 4 mol/mol of glucose because of the associated fermentation products such as lactic acid, propionic acid and ethanol. The efficiency of energy production can be improved by screening microbial diversity and easily fermentable feed materials. Biowastes can serve as feed for H_2 production through a set of microbial consortia: (1) hydrolytic bacteria, (2) H_2 producers (dark fermentative and photosynthetic). The efficiency of the bioconversion process may be enhanced further by the production of value added chemicals such as polydroxyalkanoate and anaerobic digestion. Discovery of enormous microbial diversity and sequencing of a wide range of organisms may enable us to realize genetic variability, identify organisms with natural ability to acquire and transmit genes. Such organisms can be exploited through genome shuffling for transgenic expression and efficient generation of clean fuel and other diverse biotechnological applications.

Keywords Bacillus · Biodiversity · Biowastes · Biological hydrogen · Genomics

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Introduction

Environmental pollution, global warming, limited fossil fuel reserves, and ever increasing quantities of wastes are a set of issues on the top of organizational and societal agenda [55]. These issues have resulted in renewing our interest in the generation of cleaner energy. The presently available energy sources are thermonuclear energy, nuclear breeders, solar energy, wind energy, hydropower, geothermal energy, ocean currents, tides and waves [181]. Except for fossil fuels, all other forms of energy sources cannot be used directly as fuel. These must be converted to fuel form even for generating electricity [11, 181]. Parallel to these physical and chemical sources, there has been a growing interest in bioenergy: fuels from Fisher–Tropsch synthesis, bio-ethanol, fatty acid (m)ethyl esters, biomethanol, acetic acid, bio-hydrogen (H_2), and methane (CH_4) [23, 29, 48]. The questions arise on the selection of raw materials and bio-fuels which may provide the necessary quantum of bioenergy for a sustainable society. In spite of 50 years of efforts world wide, the solution(s) seem to be far from achieved. Ethanol and CH_4 produced through anaerobic digestion are among the best known microbial products and have been extensively studied [44]. Bio-ethanol can be produced from wheat, sugar-beet, corn, straw and wood [47]. Cellulose, the major raw material for

bio-ethanol production is insoluble in most solvents and has a low accessibility to acid and enzymatic hydrolysis. Hemi-cellulose (pentose sugar) is largely soluble in alkali and more easily hydrolyzed. Sucrose and starch with the aid of invertase and amylase lead to ethanol production [33, 138]. Ligno-cellulose in spite of being the most abundant biological material however, is limited by the delignification step. Bio-diesel via trans-esterification of vegetable oil has over twice the price of petroleum diesel due to cost of feed stock. Most of the bio-diesel is produced from soybean oil, methanol and an alkaline catalyst. The high value of edible oils as food products is a major challenge to make bio-diesel production cost-effective. Alternatively, one may use beef tallow, pork lard, yellow grease [28] and restaurant waste as feed stock but the free fatty acids present in them cannot be converted to bio-diesel using an alkaline catalyst [16, 31]. The global scenario for bio-fuel reflects that a 5% displacement of gasoline requires about 5–8% of available cropland to produce ethanol whereas displacement of diesel to the same extent requires about 13–15% of the available cropland [60]. Displacement of available cropland for bio-ethanol and bio-diesel production may appear lucrative for producing bioenergy; however, this may turn out to be counterproductive especially from the point of view of developing nations, where food has obvious precedence over fuel. Search for efficient cellulases and use of non-edible oil for production of these bio-fuels is likely to make it economical and sustainable.

Potential candidates which may compete as a fuel for the post fossil fuel era are synthetic gasoline, methanol, ethanol and H_2. Electric energy can be used as an alternative as its extremely clean in end use and can be produced from all primary energy sources [46]. For selecting a fuel for the future, the following criteria must be considered: (a) transportation—fuel must be convenient to transport; (b) versatility—must convert with ease to other forms of energy at the user end; (c) utilization efficiency—must be high; (d) environmental compatibility—must not have adverse effect on environment; (e) safety—must be safe to use; (f) economics—must be inexpensive [181]. On comparing all the candidates—fuel oil, methanol, ethanol, H_2 and CH_4–H_2 stands out as the best possible fuel [182]. The main driving force for investigating the production of H_2 instead of CH_4 is the higher economic value of H_2, owing to its wider range of applications in the chemical industry [81]. Among the various unique characteristics are its maximum energy conversion possibilities for a given application. On combustion it gives water and a very small amount of NO_x [11]. H_2 has the best motivity factor of unity, has a maximum energy per unit weight (122 kJ/g) [181] and is easy to collect, store and transport [78, 181]. H_2 has been suggested as a fuel which would eliminate most air pollution problems such as acid rain, health affects, property damage, etc. However, many complications still persist before H_2 can be accepted. One of the reasons for the delayed acceptance of H_2 has been the difficulty of production on a cost effective basis [55, 99]. Just like any other process or technology which is yet to be developed, evaluated and established on commercial scale, there are other associated drawbacks here as well. It is in the incipient stage and the struggle to produce it in large quantities overshadows our attempts and worries to devise mechanisms to develop a safe storage system. Efforts are however, being made in the areas of electrochemical power generation devices (fuel cells) with very promising developments [143]. We will leave these issues here itself and proceed on our journey of biological H_2 production.

Reviews have appeared virtually on all aspects of biological H_2 production: photosynthetic and non-photosynthetic; H_2 producing microbes; simple and complex organic matters including bio-wastes as feed; conditions affecting H_2 production; alternative fuels such as bio-ethanol, bio-diesel, bio-oil etc. [1, 35, 47, 82, 83, 110, 125, 142, 148, 215]. A perusal reveals that individuals have dealt with the problem in their own way. There is thus a need to consolidate the solutions and take a holistic approach to: (1) identify and select (a) the microbes(s) with high H_2 producing abilities from a range of substrates (pure sugars and complex organic matter); (b) the type of feed(s) which are easily biodegradable and available in large quantities (biological wastes or specially grown plants); (c) hydrolytic bacteria and their associates (enhancers and augmenters); (2) select physiological conditions promoting growth of H_2-producers and suppressing H_2-quenchers; (3) maintain the population of H_2-producers optimal for H_2-production and suppressing alternative metabolic routes (ethanol, lactic acid, etc.); (4) look for those microbes which can produce value added products without affecting H_2-yields (such as polyhydroxybutyrate (PHB) production and industrially important enzymes, etc.). In light of this information, it may be desirable to develop consortia of microbes and feed for optimal and economically feasible H_2 production. Since food is constantly required and waste is constantly produced, biowaste may be good feed material. The debate of non-photosynthetic versus photosynthetic H_2 production [24, 99] seems to converge on a consensus of employing the two in a sequential or combined dark-photofermentation manner [72]. Sequential dark and photo-fermentation is rather a new approach in biological H_2 production. A few attempts made in this direction support the view that higher H_2 production yields can be obtained when two systems are combined [196, 199]. Further optimization of the system to provide optimum media composition and environmental conditions for the two microbial components of the process is necessary [41, 97, 196, 198, 199].

Hydrogen production

H_2 does not exist naturally in earth's crust in uncombined state. There is a need to produce H_2. It can be produced from fossil fuels and biomass [26] via coal gasification, steam reforming and partial oxidation of oil [8, 173]. Although the processes involve renewable sources and involve expensive techniques, these are still practiced due to the "abundant" availability of low cost coal from water (thermal and thermo-chemical processes) or electrolysis and photolysis. A significant portion of biomass sources like straw and wood is poorly degradable and cannot be converted to biofuels by microorganisms [49]. Biomass gasification is well established technique for H_2 production whereas flash pyrolysis is still developing [30]. Biomass gasification is a form of pyrolysis, which takes place at high temperature and produce a mixture of gases containing 6–6.5% H_2 [13, 116]. Thermo-chemical processes involve gasification followed by reforming of Syngas (H_2 + CO) (CO: 28–36%; H_2: 22–32%; CO_2: 21–30%; CH_4: 8–11%; C_2H_2: 2–4%) [115]; or fast pyrolysis followed by reforming of the carbohydrate fraction of bio-oil (CH_4 + CO_2) [27, 36]. Reformed gas through water–gas shift results in H_2, which can be purified by pressure swing adsorption technique. The gasification of waste biomass to produce synthesis gas (or syngas) could offer a solution to this problem, as microorganisms that convert CO and H_2 (the essential components of syngas) to multicarbon compounds are available [49]. Owing to the heavy utilization of fossil and non-fossil fuels and many problems involved, it is difficult to predict the fate of these H_2 production processes [35]. The major drawbacks of the conventional methods are high temperatures of >850 °C [72] (i.e., high energy consumption per ton of H_2 produced [160] and not always environmentally benign and/or fossil fuel processing [118, 127]) and difficulties in handling a relatively un-reactive fuel as a solid and in removing a large amount of ash. In addition, pure oxygen (O_2) is consumed for the process [8]. The capital cost of other cell components in water electrolysis, are highly prohibitive (80% of the operating cost of H_2 is due to electricity) [72].

Biological hydrogen production

Researchers have been investigating H_2 production with anaerobic bacteria since the 1980's, but most of the relevant research used pure bacterial strains as biocatalysts [19]. Production of gases in the human intestine is well known. Evidences of explosive mixture of intestinal gases were reported during electro-surgery and during colonic polyplectomy [55]. Large amount of H_2 is produced as a byproduct of colonic fermentation of dietary fiber and un-adsorbed carbohydrates [100, 144, 150]. Other evidences of H_2 production from carbohydrates like fructans obtained from *Jerusalem artichokes* have been reported from human being [150]. Intake of foods like beans, raisins, bananas, fruit juices was found to increase H_2 production [55].

Some chemotrophic H_2 producing bacteria are symbionts on humans and animals. H_2 producing microbes belonging to Enterobacteriaceae were isolated from sewage treatment plants [19, 109]. H_2 evolution from lake sediments has also been observed [45]. Even the organisms living in deep sea vents where the sun never shines ultimately depend on the oxygen expelled by photosynthetic surface life and on the H_2 given off by the fermentation of photosynthetically produced organic matter [210].

Physiology of hydrogen production

Hydrogen metabolism is basically $H_2 \leftrightarrow 2H^+ + 2e^-$ [187]. Ionization of H_2 results in H_2 uptake whereas reverse reaction leads to H_2 evolution. Ionization of H_2 is perhaps more common and can be found in many biochemical pathways, where ionized H_2 and electrons are carried through electron carries transport system (ETS) by NAD and various cytochromes, eventually combining the O_2 to form water and H_2. H_2 ions are utilized by aerobic organisms to make adenosine triphosphates (ATPs) through ETS. H_2 evolution per se does not confer any advantage to microbes. However, in the absence of an external e^- acceptor (O_2), where the supply of energy is limited, some anaerobes have adapted to use inorganic compounds such as sulfates and nitrates as their terminal oxidants. Hence, for the complete degradation of complex organic matter in nature, H_2 serves as the terminal e^- acceptor for sulphate reducers, nitrate reducers and methanogens [44, 45]. Thus H_2 evolution is obligatory for some members of the microbial community. This natural phenomenon can be exploited for efficient biodegradation. In contrast, photosynthetic organisms, where the energy supply and reducing power can accumulate and be in excess in relation to the overall metabolic scheme, H_2 evolution is strictly for the elimination of excess electrons.

H_2 is produced during microbial growth, through a set of complex biochemical reactions. Many enzymes are involved, which catalyze these reactions. Glucose is a key compound in microbial metabolism. Metabolism of glucose generates energy and intermediates like pyruvate. In general, for every mol of ATP (energy molecule) synthesized 1 mol of protons is formed and 1 mol of H_2 is evolved as result of substrate dehydrogenation. Fermentative bacteria oxidize pyruvate and formate with the help of

hydrogenase and formic dehydrogenlyase enzyme. Strict anaerobes have hydrogenase enzymes while facultative and heterotrophic anaerobes have complex soluble hydrogenase enzymes [169]. Photosynthetic bacteria which have the ability to fix atmospheric nitrogen (N_2) have enzyme nitrogenase, which evolves H_2 by an energy dependent process [209]. Whereas in aerobic N_2 fixing bacteria the H_2 evolved may be recycled by an enzymes uptake hydrogenase, which counteracts the inefficient use of energy in the N_2 fixing process [170]. In particular, hydrogenases are widespread in prokaryotic and lower eukaryotic organisms, although they diverge in their protein structure and in the type of electron carrier they use (e.g. ferredoxins, rubredoxins and quinones etc.) [154].

Fermentation reactions can produce many different end products such as H_2, acetate, ethanol and others. The H_2–acetate couple produces more ATP per mol of substrate than alcohols such as ethanol and butanol and is energetically "preferred" bacterial fermentation product for a sugar [106].

(a) Acetic acid production

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

(b) Butyric acid production

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

What does not favor H_2 production?

1. Lactic acid production, which may take place via three different pathways [44]

   (a) Homofermentative pathway

   $$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH \quad (3)$$

   (b) Heterofermentative pathway

   $$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + CH_3CH_2OH + CO_2 \quad (4)$$

   (c) Bifidum pathway

   $$2C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH + 3CH_3COOH \quad (5)$$

2. Ethanol production: [44]

   $$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (6)$$

3. Acetic acid production without H_2 production: [23, 44]

   $$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad (7)$$

4. Acetic acid production and H_2 consumers: [23, 44]

   $$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad (8)$$

Maximum H_2 yield from fermentative H_2 production is 4 mol/mol glucose (H_2 productivity, HP: 33%), which can be achieved when only volatile fatty acids (VFAs) are produced and no microbial growth occurs [2]. When butyric acid is produced 2 mol H_2/mol glucose (HP: 17%) is produced and when ethanol is produced zero mol H_2/mol glucose (HP: 0%) is produced. H_2 production from sewage sludge was most efficient when butyric acid production was predominant and propionic acid was a minor component of VFAs [19]. Higher propionic acid was a signal of inefficient H_2 fermentation. Current H_2 productivities are in the range of 10–20%, which is equivalent to 1.17 to 2.34 mol H_2/mol glucose [2, 10, 107]. Under mesophilic conditions at a hydraulic retention time (HRT) of 4–6 h, butyric acid and acetic acid were high. At longer HRT, D-L-lactic acid accumulated and at 6 h of HRT, ethanol was produced. Under these conditions, propionic acid and isobutyric acid were not detected [44]. A continuous-flow stirred tank reactor (CSTR) at thermophilic conditions produced 5–10-fold higher H_2 and lower biomass and ethanol [208]. Incidentally, in another study, H_2-producing anaerobic sequencing batch reactor (ASBR) had a higher H_2 production rate, compared with that produced using CSTRs. This study suggested that the H_2-producing ASBR is a promising bio-system for prolonged and stable H_2 production particularly if enriched H2-producing bacterial populations are achieved [22].

Biodiversity of hydrogen producing microbes and their associates

In nature, microbial communities grow on a wide range of substrates. This co-operation results in a stable, self-regulatory and sustainable system that convert complex organic matter content into a wide range of intermediates, with the final production of CH_4 and CO_2 (Fig. 1). In fact, what is needed is a group of hydrolytic microbes, which will solublize the insoluble complex components (carbohydrates, fats and proteins) of the organic matter. Here we may also look for those microbes, which may act as stimulants or enhancers for the hydrolytic bacteria and consequently for H_2 producers as well. These solublized intermediates then act as feed for H_2 producers, which may be photosynthetic or non-photosynthetic and may operate singly or as consortia (of similar types) or as mixed cultures. Since H_2 production alone cannot account for more than 33% of the energy present in the organic matter [2], we may also need to look for those organisms which may have the ability to utilize the partially digested feed for producing PHB and CH_4. It will ensure better utility and complete degradation.

It will be necessary to evaluate the type of feed suitable for H_2 production. Although a wide range of pure sugars, complex carbohydrates and biological waste have been employed as feed for microbial H_2 production, the issue of the "best" source is still open. In fact, the process has some

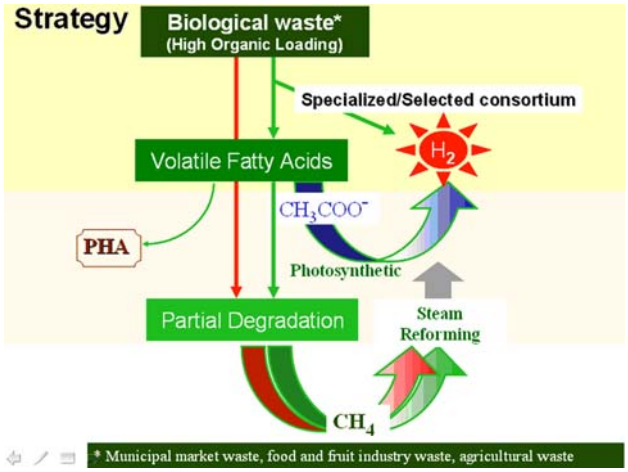

Fig. 1 Strategy for efficient degradation of biological wastes

major limitations such as physiological conditions such as repression by nitrate, sulfate and fumarate [169], acetone [205], molecular nitrogen [90] and accumulation of H_2 causes feed back inhibition [64] and high partial pressure of H_2 inhibits microbial growth [95]. The phenomenon is more prominent when anaerobic degradation of organic compounds like ethanol, fatty acids, etc., is related to H_2 evolution [169]. The presence of H_2 quenchers is also an additional limitation. In nature, low partial pressure of H_2 is maintained by the presence of H_2 consumers. Such an association of H_2-producers (Table 1) and H_2-consumers (Table 2), also known as syntrophic association or inter species H_2-transfer is observed in many ecosystems [204]. In such mixed populations, H_2 is produced by *Ruminococcus* sp., *Selenomonas ruminantium*, *Clostridium cellobioparum*, *Citrobacter freundii*, *Acetobacterium woodii*, *Trichomonas brockii* and *Syntrophomonas wolfei* [169] (Table 1).

Biological hydrogen producers

Unicellular algae like *Scenedesmus*, *Chlamydomonas* and *Chlorella* are capable of liberating H_2 in the presence of light. It involves the transfer of reductant from oxidative carbon metabolism through the photosynthetic ETS to release H_2. The other mechanism involves the photo-oxidation of water and electron transport. Algae can also metabolise glucose for liberating H_2 (Table 1). In photosynthetic bacteria, H_2 evolution occurs when N_2 gas is absent, ATP from phosphorylation and reductant from acetate, succinate, fumarate or malate oxidation are in excess. H_2 evolution is achieved through oxidative decarboxylation of pyruvate via an adaptive H_2-producing enzyme system [203]. Although extensive reviews of algae and photosynthetic microorganisms have been published,

Table 1 Biodiversity of hydrogen producers

Archaea		
Methanobacterium	*Methanococcus*	*Methanosarcina*
Methylotrophs	*Pyrococcus*	*Thermococcus*
Actinobacteria		
Mycobacterium		
Cyanobacteria		
Anabaena	*Aphanocapsa*	*Calothrix*
Gloeobacter	*Gloeocapsa*	*Halobacterium*
Lyngbya	*Mastidocladus*	*Microcyctis*
Nostoc	*Oscillatoria*	*Phormidium*
Spirulina	*Synechococcus*	*Synechocystis*
Firmicutes		
Acetobacterium	*Bacillus*	*Butyrivibrio*
Caldicellulosiruptor	*Clostridium*	*Eubacterium*
Frankia	*Peptostreptococcus*	*Ruminococcus*
Sarcina	*Selenomonas*	*Streptococcus*
Thermobacteroides	*Veillonella*	
Bacteroidetes/Chlorobi		
Acetomicrobium	*Bacteroides*	*Chlorobium*
Pelodictyon		
Thermotogae		
Thermotoga		
Fusobacteria		
Fusobacterium		
Proteobacteria Alpha		
Azospirillum	*Rhizobium*	*Rhodobacter*
Rhodomicrobium	*Rhodopseudomonas*	*Rhodospirillum*
Proteobacteria Beta		
Alcaligenes	*Rubrivivax*	
Proteobacteria Delta		
Desulfovibrio	*Syntrophobacter*	
Proteobacteria Epsilon		
Campylobacter		
Proteobacteria Gamma		
Aeromonas	*Azomonas*	*Azotobacter*
Chromatium	*Citrobacter*	*Enterobacter*
Escherichia	*Hafnia*	*Klebsiella*
Pseudomonas	*Salmonella*	*Serratia*
Thiocapsa		
Thermotogales		
Thermotoga		
Eukarya		
Ciliophora		
Dasytricha		
Parabasalidea		
Trichomonas		
Viridiplantae, Chlorophyta		
Ankistrodesmus	*Chlamydomonas*	*Chlorella*
Chrondrus	*Codium*	*Corallina*
Kirchneriella	*Porphyridium*	*Scenedesmus*

[5, 17, 55, 72, 82, 83, 91, 148, 169, 188]

Table 2 Biodiversity of hydrogen metabolizers

Archaea	
Archaeoglobus fulgidus	*Methanopyrus kandleri*
Methanothermus fervidus	*Pyrodictum brockii*
Aquificales	
Aquifex aeolicus	*Aquifex pyrophilus*
Calderobacterium hydrogenophilum	*Hydrogenobacter thermophilus*
Cyanobacteria	
Aphanothece halophytico	*Prochlorothrix hollandica*
Westiellopsis prolifica	
Firmicutes	
Rhodococcus opacus	*Streptomyces thermoautotrophicus*
Proteobacteria Alpha	
Acetobacter flavidum	*Bradyrhizobium japonicum*
Paracoccus denitrificans	
Proteobacteria Beta	
Acidovorax facilis	*Ralstonia eutrophus*
Rhodocyclus gelatinosus	*Thiobacillus plumbophilus*
Proteobacteria Delta	
Desulfomicrobium baculatus	
Proteobacteria Epsilon	
Helicobacter pylori	*Wolinella succinogenes*
Proteobacteria Gamma	
Pseudomonas carboxydovorans	
Eukarya, Ciliophora	
Nyctotherus ovalis	

[82, 83, 148]

their usage for H_2 production has been limited. The prerequisite for the use of the most plentiful resources—light and water, is the adaptation of the algae to an anaerobic atmosphere. Unfortunately, H_2 production by this process is quite ineffective since the simultaneously produced O_2 inhibits the hydrogenase enzymes involved in H_2 production [191]. Possible photosynthetic organisms require solar collectors and engineering analysis has suggested that solar generators would be too costly [55]. Use of non-photosynthetic bacteria to produce H_2 will eliminate the need for solar collectors.

Chemotrophic H_2 producers include several bacteria such as *Ruminococcus albus*, *R. flavefaciens*, *S. ruminantium*, *Megasphaera elsdensii*, etc. [188] (Table 1). Certain *Trichomonades* and other anaerobic protozoa are also known to produce H_2 [148]. H_2 production through dark fermentation has been observed in *C. freundii* [109], *Enterobacter*, *Escherichia* and *Hafnia* [72]. H_2 evloution was associated with formate degradation through soluble hydrogenase in *Bacteroides clostridiiformis*, *Eubacterium limosum*, *Fusobacterium necrophorum* and *R. flavefaciens* and through non-soluble hydrogenase in the case of *R. albus* [55]. *S. ruminantium* grown with *Methanobacillus omelianskii* evolved H_2 through reduced NADH formed during degradation of glucose, glycerol or lactate [55]. Another approach receiving attention involves a coupled system of halobacteria and marine cyanobacteria [134]. Strict anaerobes need reducing agents such as argon, nitrogen, hydrogen gas, L-cystine-HCl to remove trace amounts of oxygen present in the medium. This is an expensive way to tackle the problem of oxygen. Therefore, utilization of *Enterobacter aerogenes* along with *Clostridium* instead of expensive chemical reducing agents was suggested to be effective in H_2 production by dark fermentation [197, 198]. Non-endospore forming H_2-producers are enteric bacteria such as *Enterobacter* spp. [124] or/and *Citrobacter* sp. [129]. H_2 production has been recorded at 3.9 mol/mol glucose by *Enterobacter cloaceae* DM11 [113], which is extremely high considering the enteric bacteria, which usually produce <1 mol H_2/mol glucose [126]. *E. cloaceae* IIT BY08 produced 6 mol H_2/mol sucrose, the highest among all carbon sources tested [91].

Although *Clostridium* spp. are among the most widely studied H_2-producers and *Bacillus* the least studied [64, 84, 157], *Bacillus* may be a better choice as H_2 producer (Discussed in later section). *Clostridium saccharolyticus*, a mesophile is among the best H_2 producers with the potential to yield 3.0 l/l/h, which is equivalent to 121 mmol H_2/(l × h) [99].

Bioaugmenters, inducers and stimulators

The cost of a biomass-derived fuel depends critically on the yield of sugar conversion to the final products, in particular the pentose sugars (constituting 5–30% of the total carbohydrates) from hydrolysis of hemicellulose. It is for such reasons that much attention has been focused on the engineering of strains to use all sugars released from biomass hydrolysis [161]. Important plant polysaccharides such as cellulose, arabino-xylans, resistant starch, glucans (1,3–1,4-β glucans) components of plant cell walls and endosperm of cereals (barley, rye, sorghum, rice and wheat) constitute a significant proportion of biological wastes. Bacteria are known for hydrolyzing these biomolecules by excreting (1) lichenases: *Clostridium acetobutylicum*; *C. thermocellum*, *Bacillus* spp., *Bacillus macerens*, *B. circulans*, *B. brevis*, *R. flavefaciens*; (2) lumiarinases: *Rhodothermus marinus*; *C. thermocellum*, *Thermotoga neopolitana*, *T. maritima*; (3) lichenin, 1,3-1,4-β glucan (β glucanases): *Clostridium* spp., *Bacteriodes* sp. [184].

Bacillus pumilus expresses a wide range of hydrolytic enzyme activities such as xylanase, amylase, phytase and

pectinase. The multi-component enzymatic secretion by *B. pumilus* leads to extensive and rapid solublization, degradation and breakdown of complex ligno-cellulosic components present in wheat bran. It enhances availability and accessibility of tightly bound lignin complexes and phenolics like ferulic acid, veratric acid and nutrient N for laccase biosynthesis from *Gonoderma* sp. [156].

Lignocellulosic biomass has long been recognized as a potential sustainable source of mixed sugars for fermentation to biofuels and other biomaterials [52]. At present, purified hydrolytic enzymes are still too expensive and not as potent with real pretreated lignocellulosic feedstock. Nature's most efficient systems to biodegrade lignocellulose are mixed cultures in insect and mammalian guts that have evolved with the host [3]. For the hydrolytic step, laccase has emerged as one of the most sought after enzymes and is being used successfully to delignify wood tissues [1, 157]. Lignin mineralization and solublization can help in the release of cellulose from ligno-cellulosic wastes reportedly available in large quantities [88] by *Aneurinibacillus*, *Azotobacter*, *Bacillus* sp., *Bacillus megaterium*, *Paenibacillus* sp., *Serratia marcescens* [18, 121, 135]. *Sinorhizobium fredii* [54] produces carboxymethyl cellulase (CM-cellulase EC 3.2.1.4) and polygalacturonase (pectinase EC 3.2.1.15) for cleaving glycosidic bonds in plant cell wall polymers. Another ezymatic activity at the solublization stage, which has gained importance, is a specific protease (keratinase) [131] because of the use of feathers as feed for H_2 production [7]. Keratinolytic activity has been observed in *Bacillus* sp., *B. licheniformis* K-508, *Streptomyces* sp., *Thermoactinomyces* sp., *Vibrio* sp., [7, 112], *Aspergillus* sp., *Alternaria radicina*, *Trichorus spiralis*, *Stachybotrys atra*, *Onygene* spp., *Absidia* spp., *Trichophyton mentegrophytes*, *T. rubrum*, *T. sallinae*, [41]; *Microsporum canis*, *M. gypseum* [186], *Streptomyces pectum*, *S. albus*, *S. thermoviolaceus*, *S. fradiae*, *Bacillus* spp., *Fervidobacterium pennovorans*, *B. licheniformis* PWD-1 and *Bacillus* sp. FK46 [164].

The addition of metabolic analogues like amino-acids and their analogues and vitamins have been reported to stimulate the production of enzymes, DL- serine resulted in 3.8 fold increase in polygalacturonase production by *Bacillus* sp. [156]. Stimulation in pectinolytic enzyme synthesis by *Sclerotinia sclerotiorum* occurred by the addition of histidine, glutamate, alanine, asparagine, aspartate, glutamine, arginine and proline to the fermentation media [156]. Addition of DL-norleucine, L-leucine, DL-isoleucine; L-lysine monoHCl and DL-B-phenylalanine resulted in 2.78 fold increase in pectinase production by *Streptomyces* sp. [9]. Biotin, riboflavin and pyridoxine HCl induced laccase production from *Cyathus bulleri* [32]. Vitamins (pyridoxine HCl), L-ascorbic acid, thiamine HCl, nicotinic acid, riboflavin and biotin stimulated laccase production from *Gonoderma* sp. [156].

Microbial treatment systems for the degradation of organic matter need an optimal microbial community and property to enhance the desired output [120]. The importance of bioaugmentation in degradation processes by introducing microbes in the system can be illustrated by the following examples. The efficiency of the 2–4-DCP degrading mixed culture in an activated sludge was enhanced [139] by *Comamonas testosterone*, which had an ability to degrade 3-chloro-aniline [12]. Similarly, a resin acid-degrading bacterium, *Zoogloea resiniphila* HdhA-35 was exploited to counteract pH stress in an aerated lagoon treating pulp and paper mill effluent [202]. Inducers enhance enzyme activity either by expression of the major subtilisin type enzymes in feather degradation, which has been reported as feed for H_2 producers [112], by surfactants known to stimulate bacterial enzyme production [146] or by myo-inositol as a carbon source induces CM-cellulase [54].

Hydrogen production from organic substrates

Cellulose is a major component of carbon fixed by plants. Microbes with ability to degrade cellulose to H_2 are of great importance e.g., *C. cellbioparum*, *S. ruminantium*, *R. flavefaciens*, etc. [101, 174, 188, 204]. Other H_2 producers utilize hemicelluloses, starch, sucrose and other complex carbohydrates [40, 51, 103, 106, 178, 205]. All organic substrates are not directly degraded to H_2 and involve some intermediates. Several other organic compounds [204, 205] including fatty acids [205] may be degraded by anaerobic microbes to produce H_2. Facultative anaerobic bacterium *Klebsiella pneumoniae* and *Azospirillum brazilense* [5, 17] produce H_2 during N_2 assimilation, where hydrogenase and nitrogenase play important roles. Many chemotrophs produce H_2 by using different carbohydrates [169, 204, 205]. H_2 production from simple molecules like glucose [149, 203], xylose [51, 165], maltose [149] and lactose [84] follow pyruvate route. This metabolic pathway is followed in *Clostridium* spp. and several other anaerobes: *T. brockii* [205], *Peptococcus anaerobium*, *E. limosum*, *M. elsdenii*, *Sarcina maxima*, *S. ventriculi*, *R. albus*, *Veillonella alcalescens*, etc [169]. In addition to H_2 producing bacteria, some protozoa also have the ability to oxidize pyruvate.

Theoretically, the maximum H_2 production (mol/mol of substrate) varies from 4.0 from glucose, potato starch and cellulose, 2.0 from lactate and 8.0 from sucrose. However, the reported conversion efficiencies of *Clostridium intermedius* varies up to 38% from glucose, while that of *Clostridium butyricum* varies up to 55% from sucrose and that of *Clostridium* sp. up to 59% from xylose [14, 74, 166].

Cellulose conversion efficiencies of 18% was observed under certain conditions [105]. Highest H_2 yield obtained from glucose is around 2.0 to 2.4 mol/mol [39, 72, 122, 176]. Chen and Lin [21] reported 4.52 mol H_2/mol sucrose and up to 6.0 mol H_2/mol sucrose have been reported with *E. cloaceae* [91].

Hydrogen production from biowastes

In addition to the pure organic substrates, biowastes rich in carbohydrates have also proved to be potential sources of H_2. Fermentation of raw starch of corn, potato and cassava peel results in H_2 generation [15]. On a very small scale, sugarcane, corn pulp and paper waste [149, 177], cheese whey [151, 153], lactic acid factory waste [168] and dairy waste water [180] have also been employed for H_2 generation. H_2 generation by mixed microbial cultures, pure *Bacillus subtilis* and *B. licheniformis* strains from damaged wheat grains [64, 158] and pea-shells [65] have also proved to be potential raw materials. H_2 constituted 30–65% of the total biogas produced, which is equivalent to 50 to 80 L H_2/kg total solids. H_2 production at the rate of 555 ml/g starch waste is among the highest production observed with sugar factory waste water, bean curd manufacturing waste, food waste and sucrose rich waste water (Table 3). Certain microbes such as *Rhodobacter sphaeroides* have been successfully used in production of H_2 from fruit and vegetables waste [87, 117] and have also been tested on sewage with positive results [19, 98, 189]. The efficiency of H_2 production varies with the type of waste employed as feed. The process is currently still at the laboratory stage, and work needs to be done on increasing cost efficiency and application. H_2 from biomass has the potential to compete with H_2 produced by other methods such as from natural gas, which includes catalytic conversion of hydrocarbons, electrochemical or photochemical water splitting [1]. In fact, H_2 generation from sweet sorghum, wheat grains, pea-shells followed by anaerobic digestion of the remaining biomass [4, 65, 158] is a step towards enhancing the efficiency of biological H_2 production.

Conditions affecting biological hydrogen production

Critical factors in biological H_2 production are organic concentration, pH, nutrients, partial pressure of H_2, stirring, H_2 quenchers (Table 2), etc. [77, 128, 130, 160]. Among the various culture conditions which influence H_2 production are: pH, temperature, feed concentration, bacterial population, retention period, etc. [24, 84, 99, 102, 118]. Maximum H_2 production occurs over a pH range of 5.5 to 6.5. One of the most important factors influencing biological H_2 production is temperature. However, mesophilic range of 30–37 °C continues to be optimal for this process [84]. A few attempts of H_2 production in the thermophilic range have also been reported [192]. At high carbohydrate concentration, a metabolic shift occurs from H_2 to alcohols [119, 130]. The impact of organic loading rate on H_2 yield varies. An improvement in H_2 yield was observed at lower organic loading rates of sucrose, glucose and rice winery waste water. On the other hand, similar improvement was also recorded at higher organic loading rates of sucrose, glucose and citric acid waste waters [86]. Sparging the bioreactor with N_2 has been reported to increase H_2 yield [57, 58, 79, 118]. Currently the reasons for increased H_2 production during sparging are not very clear. Sparging was assumed to decrease the dissolved H_2 concentration to alter the activity of H_2 production enzymes [85]. Pyruvate : Ferredoxin oxido-reductase (PFOR) can function at H_2 concentration observed in fermentative H_2 system. NADH : Ferredoxin oxido-reductase (NFOR) can only function for dissolved $H_2 < 0.5$ μM (<60 Pa) [2]. Therefore, higher H_2 is possible by decreasing H_2 for NFOR. In their study, the

Table 3 Microbial conversion of biological wastes in to hydrogen

Substrate	H_2 yield (ml/g substrate)	References
Bean curd manufacturing waste, food waste, sucrose rich waste water, sugar factory waste water, starchy waste	300–555	[57, 58, 72, 101, 104, 119]
Noodle manufacturing waste water, potato starch, pulped sugar beet, rice winery waste water, wheat bran	200–300	[58, 101]
Cabbage, carbohydrate rich high solid organic waste, carrot, chicken skin, dairy wastewater, egg, fat, filtered leachate of waste bio-solids, fruit and vegetable waste, keratin waste, lean meat, molasses, municipal waste, rice bran, sewage, sweet sorghum, wheat grains, wheat starch	<200	[4, 7, 19, 41, 57, 64, 66, 72, 87, 98, 117, 158, 180, 189, 206]

dissolved H_2 could be decreased only at 485 μM (i.e., 10^3 times higher). It appears that impractically high sparging rates would be needed to decrease dissolved H_2 in to the NFOR regulatory zone [85]. Mandal et al. [113] observed double the H_2 yield during batch culture of *E. cloaceae* DM11. The H_2 yield during vacuum operation was 3.9 mol/mol glucose. However, Kraemer and Bagley [86] concluded that no meaningful relationship exists between sparging rates and H_2 yield. Incidentally, CO_2 sparging drastically decreased microbial diversity in a continuous mixed culture [85]. It may thus serve as a warning sign because such conditions may even have an adverse effect on growth and activity of H_2 producing microbes.

Immobilized whole cell technique leads to high reaction rates and thus represents an efficient approach [171] to biocatalysis for carrying out several biochemical reactions including H_2 production [89, 133, 140, 155, 190, 195, 207] and CH_4 production [66]. Most of the solid matrices used for the immobilization of the whole cells are synthetic polymers or inorganic materials. These systems include polyurethane, polyvinyl alcohol, agar gel or porous glass beads, calcium alginate, polyacryl amide gel, k-carageenan or cellulose, banana leaves, wood chips, activated charcoal, baked bricks or clay [6, 89, 110, 155, 195]. Use of immobilized whole cells compared to free floating cells increases the mean cell residence time in the reactor. Studies to improve H_2 yields through immobilization have resulted in up to 1.7 fold increase [80, 93, 189]. A four-fold increase in H_2 production by immobilizing *B. licheniformis* on brick dust has resulted in a H_2 yield of 1.5 mol/mol glucose in batch culture [89]. Lignocellulosic agricultural waste materials such as rice hull, sugarcane bagasse, wheat straw [130], pea-shells [65], banana leaves [66] and coconut coir [92] are regarded as abundant, inexpensive and readily available natural sources. Many countries have imposed regulations to restrict field burning of these wastes in response to restrictions on carbon emission due to global warming [132]. In the past, these waste have been sent to landfills, but in recent years their disposal has become a problem, due to increasing cost of transportation and scarcity of landfill sites for quick disposal [65]. These are potential support materials for retaining large populations of H_2 producers within the reactor. In fact, it has been possible to increase H_2 yield up to 2.36 mol/mol glucose by immobilizing *Bacillus* strains on these lignocellulosic wastes [69].

Another factor which greatly influences the H_2 generation from biowastes is the presence of H_2 quenchers (Table 2) among the mixed microbial populations, primarily sulfate reducers, nitrate reducers and methanogens. In such scenarios, there is thus little or no net evolution of H_2. Different techniques employed to suppress methanogenic activity include heating the waste [129], using specific and non specific inhibitors such as 2-bromoethene sulfonate (BES) or acetylene [159, 160, 177], using microbial and enzymatic pretreatments [158], or low pH and high temperature combinations [20, 122, 129]. Product formation by microflora depends upon dominant populations and selective enrichment of certain microbes can be achieved by inoculations with pure cultures. Increase in H_2 yields at low N_2 content has also been recorded [122]. It seems that although H_2 production can be initiated, for continuous production we may need to further standardize the culture conditions, such as changes in retention time, feed composition, pH, etc.

In search of potential "wonder" bug(s) for hydrogen production

In spite of a large number of reports on H_2 producing microbes, the bioconversion of biological material in to H_2 has been observed to operate at very low efficiencies. The top-rated challenges and technical barriers include no known microorganism capable of naturally producing more than 4 moles of H_2 per mole of glucose, the metabolic pathways have not been thoroughly identified and the reaction is energetically unfavorable. The biomass feed stocks are too costly and there is thus a need to develop low cost methods for growing, harvesting, transporting and pretreating energy crops and or biomass waste products [214]. In the absence of a robust, industrially capable organism, the platform for research to genetically alter the metabolic pathways of the existing microbes is open. As microbiologist and biotechnologists, we need to carefully screen microbial diversity [136] from samples representing a vast diversity of environments, ranging from "normal" environments such as soil, sea water and sediments to extreme environments [108].

The search for a robust H_2 producer(s) begins with an important organism, capable of fermenting multiple sugars as feed, withstand adverse environmental conditions, compete with naturally occurring microflora, tolerate "toxic" compounds, produce compounds of economic importance and grow aerobically, even under fermentative conditions, preferably independent of light, etc. Among the potential candidates as "wonder" bug(s) for H_2 production are the facultative anaerobes such as *Aeromonas, Alcaligenes, Bacillus, Campylobacter, Citrobacter, Escherichia, Enterobacter, Klebsiella, Salmonella, Serratia, Streptococcus, Thermotoga* and aerobic chemotrophs such as *Azospirillum, Azotobacter, Mycobacterium, Pseudomonas* and *Rhizobium*. The key microbial groups in the diverse consortia of anaerobic fermenters and those related to the hydrolysis and acidogenic fermentation of the organic matter are Clostridia and Enterobacteriaceae, since they generate H_2 from carbohydrates and other organic

substrates. At present the top contenders as the future H_2 producers among the non-photosynthetic organisms are *Clostridium*, *Enterobacter* and *Bacillus* [84, 125]. On the other hand, among the photo-biological H_2 producers are *R. sphaeroides*, *Rhodobium marinum* and *V. fluvialis* [76].

Facultative anaerobes such as *E. aerogenes* [167] and obligate anaerobes such as *Clostridium* spp. [40] are known to convert sucrose to H_2 at the rate of 1.25 and 2.2 mol/mol hexose, respectively. Lay et al., [96] studied the feasibility of H_2 production from organic fraction of municipal solid waste by *Clostridium* and could evolve 180 ml H_2/g volatile solids. In cow dung cellulose is the most abundant carbohydrate and the amounts of soluble carbohydrates and starch are negligible. *Clostridium cellulosi* decomposes cellulose with the production of H_2, CO_2, acetate and ethanol as fermentation products [201], which may be the reason for low H_2 production from cow waste by *C. thermocellum* [200]. In a mixed microbial population in H_2 producing granular sludge, at least 65% of the species belonged to *Clostridium*. Since most *Clostridium* spp. cannot tolerate O_2, addition of a reducing agent such as cysteine to the medium is common practice [166]. Facultative anaerobes may therefore promote H_2 production by obligate anaerobes, by consuming any traces of O_2 in a reactor. For example, *E. aerogenes* and *C. butyricum* growing on starch yielded 2 mol/ mol glucose without any reducing agent [197]. The exchange of roles between *Clostridium* and *Bacillus* as H_2 producers was observed in an innovative approach to enrich mixed microbial populations of H_2 producers. Here heat pre-treatment and changes in HRT have shown that spore forming bacteria such as *Clostridium tetanomorphum* are predominant in the initial stages (up to day 15) and *Bacillus laveolaticus* as dominant bacteria there after in H_2 producing bioreactors [163]. In another study, increase in H_2 production at reduced HRT was linked to a shift in bacterial population from *Clostridium* sp. and *Bacillus* (most closely related to *B. racemilacticus* and *B. myxolacticus*) to predominantly *Clostridium* sp. [62, 175]. These studies suggested that the shift in microbial population has been probably due to the presence of homo-acetogens at longer HRT and acidogens at shorter HRT. Such studies may provide clues as to why *Bacillus* spp. have not been reported widely among the H_2 producers.

A comparison of the H_2 producing potentials of *Clostridium*, *Enterobacter* and *Bacillus* from a wide range of studies reveal the following:

i) *Clostridium* sp., *C. butyricum* and *C. paraputrificum* have been shown to yield 1.3 to 2.5 mol H_2/mol sugar [38, 50, 74, 165, 195].
ii) *E. aerogenes* and *E. cloacae* have been largely studied for H_2 production from glucose, sucrose and molasses. Here, H_2 yields varied from 0.6 to 3.8 mol/mol sugar [111, 127, 167] and
iii) *Bacillus coagulans, B. licheniformis* and *B. subtilis* have been shown to evolve 1.5 to 2.36 mol H_2/mol glucose [69, 84, 91]. *B. licheniformis* and *B. subtilis* could also generate H_2 from damaged wheat grains at the rate of 45 to 64 L/ kg Total solids [64, 158].

Among the most recent developments is the proposal to run the two systems in sequence or in combination. A mixed culture of *Clostridium* sp. and *Bacillus* sp. yielded 1.52 mol H_2/mol sucrose [163]. Here the mechanism in operation is the utilization of carbohydrates or carbohydrate rich wastes for dark fermentation and fatty acids for photosynthetic process. In a co-culture of *C. butyricum* and *Rhodobacter* sp. higher H_2 yields of 4.5 mol/mol glucose were observed in comparison to single dark fermentation (1.9 mol/mol glucose) and sequential two step fermentation of starch yielding 3.7 mol/mol glucose [196]. Similarly, higher H_2 yields from different substrates were reported by co-cultures of *R. marinum* and *V. fluvialis* compared to *R. marinum* alone [61]. In yet another combination of *Lactobacillus amylovous* and *R. marinum*, higher H_2 yield was linked to lower production rate [75]. A mixed culture of *E. aerogenes* and *R. sphaeroides* resulted in the evolution of 3.15 mol H_2/mol glucose [84].

Genomics in aid of hydrogen production

In order to overcome the metabolic barriers by manipulating electron flux, a single host organism for transgenic expression of H_2 pathways is necessary. With the advent of recent advances through microbial genome projects, a large amount of genetic and metabolic information has been made available in public domains [212, 213]. Genomic data mining approach has been exploited for searching novel H_2 producers [68]. Sequence analysis and pathway alignment of H_2 metabolism glyoxalate and dicarboxylate metabolic pathways (formate dehydrogenase and hydrogenase) in 176 sequenced genomes [213] has led to the identification of potential H_2 producers such as *Wolinella succinogenes*, *Desulfitobacterium dehalogenans*, *Burkholderia fungorum* and *Novosphingopbium aromaticiviorans*, etc. In the past decade, very few new H_2-producing organisms have been reported (e.g. *Caldicellulosiruptor saccharolyticus*, *Gloeocapsa alpicola*, *Rubrivivax gelatinosus* and *Thermotoga elfii*), and there has been little significant improvement in the H_2 yields, which ranges up to 3.3 mol/mol of glucose [114, 172, 179]. In view of these facts, this genomic approach has revealed certain interesting potential H_2 producers. These novel H_2 producers [68] show unique characteristics such as

(1) degradation of industrial wastewaters (perchlorates), remediation of contaminated soil and ground water, (2) bioremediation by dehalogenation of chlorinated phenols, ethenes, polychlorinated biphenyls (PCBs), (3) beneficial plant root colonizers, role in global C-cycle and (4) degradation of aromatic hydrocarbons including toluene, *p*-cresol, xylene, naphthalene, etc. They have ability to grow in a wide range of environments including soil, fresh waters, and marine life. These bacteria thus not only have the potential to produce H_2, but are also known to utilize wastes as feed. This data mining approach can be further extended to detect robust organisms, which are not presently categorized among the H_2 producers because of absence of gene(s) crucial for the H_2 metabolic pathway to be fully functional. Such an approach has been recently suggested for the detection of "non" producers of polyhydroxyalkanoates and antibiotics [70, 71] and possible shuffling of genomes for transforming them to producers [70].

Comparative genomics of sequenced genomes of *Bacillus* revealed that it possesses genes for α, β, and γ subunits of formate dehydrogenase, but genes for the large and small subunits of hydrogenase could not be detected. Hence, it was categorized among "non"-producers. However, a review of published literature reveals that *Bacillus* can produce H_2, [64, 84, 158], which may imply that another hydrogenase might be operative. Incidentally, there are no reports available in public domain on the enzymes involved in H_2 production in *Bacillus*. There are two separate pathways operative in *Clostridium* and *Escherichia*. In *Clostridium*, the genes for Pryuvate Fd/Flavodoxin oxidoreductase [EC.1.2.7.1] and H_2 Fd/Flavodoxin oxidoreductase [EC.1.2.7.2] are involved in H_2 production. In *Escherichia*, Pryuvate formate lyase, PFL [EC 2.3.1.54], Formate dehydrogenase Fdh-α, Fdh-β, Fhh-γ [EC 1.2.1.2] and Hydrogenase (Large and small subunits) [EC 1.18.99.1] are responsible for H_2 evolution. Incidentally, Pyruvate Fd/ Flavodoxin oxidoreductase is highly expressed in *C. acetobutylicum* and *C. perfringens* and is missing in the genomes of *B. subtilis* and *B. halodurans* [73]. On the other hand, Pryuvate Formate lyase is not highly expressed in *C. acetobutylicum* and again is missing in two *Bacillus* genomes, *B. subtilis* and *B. halodurans.* It is however among the prominently expressed genes in enteric proteobacteria and not in other prokaryotes. In such a scenario *Bacillus* apparently uses Pyruvate dehydrogenase complex (*pdh ABCD*) [63], which is highly expressed in *B. subtilis* and *B. halodurans* and is missing in *C. acetobutylicum* and *C. perfringens* [73]. It provides clues that *Bacillus* and *Clostridial* H_2 production systems are under different metabolic controls. The complete pathway of H_2 production in *Bacillus* needs to be elucidated before resorting to genome shuffling approaches can be exploited.

Bacillus can be considered as a strong contender for the future biological H_2 producer because of its unique features. *Bacillus* represents microbes of high economic, medical and biodefense importance, production of biopesticides [53] and biofuels such as H_2 [64, 158], commercial enzymes and probiotics. Among the 175 different *Bacillus* species, a majority of isolates are represented by *Bacillus anthracis*, *B. cereus, B. thuringiensis*, *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. megaterium*, *B. sphaericus*, *B. clausii* and *B. halodurans* [211]. Because of these divergent characteristics of economic importance and intra species diversity, 11 closely related *Bacillus* are among the 29 Bacillales sequenced so far [213].The very basis of the complete nucleotide sequencing of *B. licheniformis* type strain (ATCC 14580) genome was its enormous economic importance [147]. Two gene clusters involved in cellulose degradation and utilization have been reported from *B. licheniformis* which may enable it to utilize biowaste rich in cellulose into cellobiose and ultimately glucose to produce H_2 [147]. Among the various strains of *Bacillus* isolated in our laboratory, *Bacillus* sp. and *B. licheniformis* have been shown to produce H_2 [64, 158]. These two *Bacillus* strains have the capacity to ferment glucose, maltose, fructose, dextrose, sucrose, mannose, etc.

Unlike most other bacilli, which are predominantly aerobic, *B. licheniformis* is a facultative anaerobe with a saprophytic lifestyle, which may allow it to grow in additional ecological niches. *B. licheniformis* is known for its numerous commercial and agricultural uses primarily because of its extracellular products which include several proteases, α-amylase, penicillinase, pentosanase, cyclogucosyltransferase, β-mannanase and several pectinolytic enzymes important for degradation of polysaccharides, proteins, lipids and other nutrients. The proteases are used in the detergent industry, for dehairing and bating of leather [34, 37], and degradation of feather [7, 112], where as amylases from *B. licheniformis* can hydrolyse starch, hence used for desizing textiles and sizing of paper [34], and oil recovery [25, 123]. The ability to transform into an endospore, enhances it ability to survive under unfavourable conditions and even compete with other microbes [162]. In metal contaminated waste-waters, the use of metal (Ni) resistant microorganisms such as *Bacillus* sp. can reduce bio-available metal concentrations via sequestration and may foster enhanced biodegradation [152]. *Bacillus* strains are also bestowed with an ability to even mitigate the affects of fungal pathogens on maize, grasses and vegetable crops [141].

In brief, *Bacillus* has many features which favour it as an organism of choice for H_2 production. Being a spore former, it can survive under unfavourable conditions and even compete with other microbes. It has a large number of enzymatic activities such as lipase, amylase, protease,

urease, cellulose, etc for hydrolyzing cellulose and biological wastes in to simpler soluble compounds. It can produce laccase, which is important for de-lignification of ligno-cellulosic bio-wastes Its ability to produce poly galacturonase is stimulated by addition of amino-acids. *Bacillus* has unique advantage over other microbes, being non- photosynthetic, does not require light for H_2 production. It is capable of converting wastes such as damaged wheat grains, pea-shells, starch, etc. to H_2. Its ability to produce H_2 could be enhanced up to 2.36 mol/mol glucose by immobilization on ligno-cellulosic wastes. The role of *Bacillus* in improving the efficiency of degradation process can be gauged by its ability to produce large number of industrially important products including PHA. *Bacillus* spores are being used as human and animal probiotics, which do not pose any environmental health hazard. In agriculture it holds importance due to its denitrification property.

Conclusion

The economics of biological H_2 production process is quite low at present. To realize the goal of efficient H_2 production, the importance of diverse microbial communities in mineralization of organic matter (biological matter/wastes) occurring in natural ecosystems [185] need not be re-emphasized [145]. Microbial and functional diversity are to be harnessed, including those for bioproducts like volatile fatty acids and bioplastics, CH_4, etc. [2, 56, 86, 145]. Although photosynthetic organisms such as *Rhodopseudomonas*, *Rhodospirillum* and *Rhodobacter* have been shown to produce H_2 and PHB, [59, 98, 183, 193] non-photosynthetic organisms such as *Bacillus* strains have been shown to produce H_2 and PHB albeit in independent studies [84, 94, 158, 194] and more recently even in a single organism [137]. In yet another novel approach, mining of sequenced genomes has revealed certain organisms with potential to produce these two products and have ability to grow on industrial waste waters [67]. For biological H_2 and energy production to become an economically feasible commercial activity, an integrated approach would require the participation of specialists from each aspect of this multi-step process and application of knowledge acquired from diverse areas. Future energy systems require money and energy to build. We may all agree that there are finite supplies of both. Hard decisions must be made about the path forward and must be followed by a sustained and focused effort [173]. The integration of agroenergy crops and biorefinery manufacturing technologies offers the potential for the development of sustainable biopower and biomaterials that will lead to a new manufacturing paradigm [139].

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