

A road map on biohydrogen production from organic wastes



Debabrata Das

Energy crisis is looming the global economy. Our energy requirements are almost fully provided for by carbon-containing fossil sources such as oil, coal and natural gas. The rapid consumption of these fossil resources has caused an accelerated release of the bound carbon as CO₂. The rate at which fossil fuels are depleting, a necessity of alternate fuel has gain importance. The use of fossil fuels for energy is unsustainable and causes build-up of greenhouse gases in the atmosphere leading to global warming. The need of the hour is an efficient fuel with zero carbon footprints and this path can be achieved by using hydrogen. Biofuels store energy chemically that can be harnessed easily. It can also be used in existing combustion engines after blending with petroleum diesel to various degrees. No separate transportation infrastructures would be required for such fuels. Hydrogen having highest energy density (143 kJ/g), is a clean and environment friendly fuel. Fermentative H₂ production using renewable resources (wastes/wastewaters) is a promising way of economical and sustainable energy source as compared to photobiological processes. Dark fermentation of organic wastes may be carried out by mesophilic and thermophilic organisms. The dark fermentation at thermophilic temperatures (60°C) has many attractive advantages. Many industrial organic wastewaters are discharged at elevated temperatures such as palm mill effluent, distillery effluent etc. that can be directly used. Moreover, at higher temperature condition leads to pathogenic destruction, lower risk of contamination by

methanogenic archaea, higher rate of hydrolysis and higher H₂ yield. In addition, during fermentation generates excess heat that requires cooling for mesophilic cultures. Bioprocess engineering lab of Indian Institute of Technology Kharagpur is known for accentuating research on various routes of biohydrogen production in India. Over the years, this lab has developed a comprehensive and refined expertise on the field biohydrogen production (Fig.1). All the domains related to hydrogen production through biological routes have been explored.

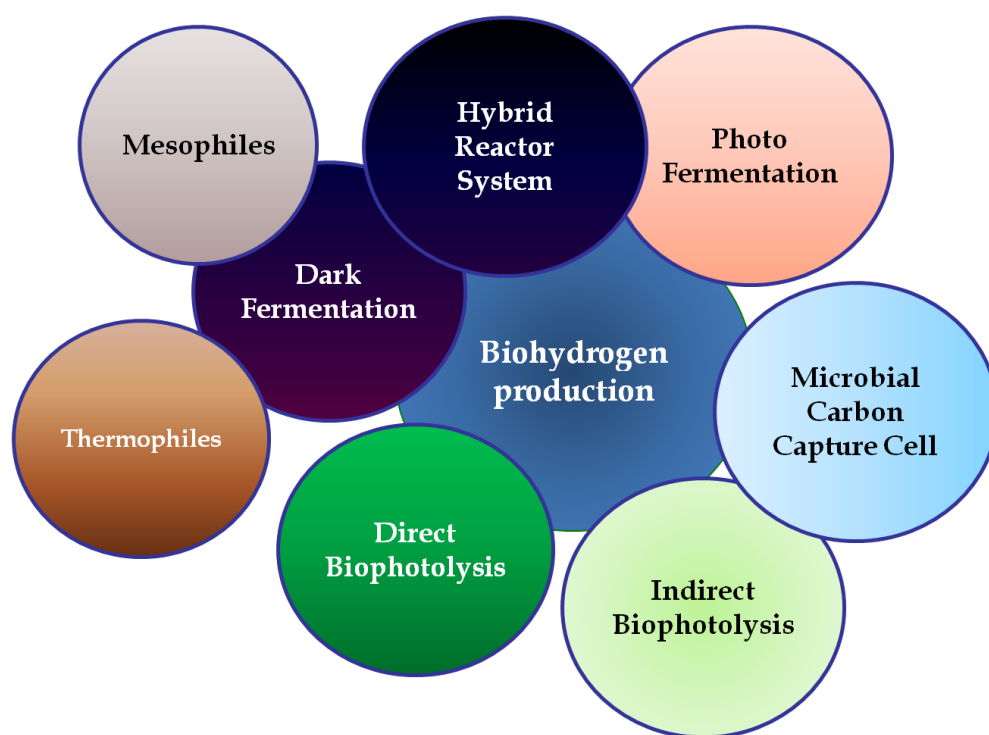


Fig. 1: Biohydrogen production processes

The main objective of the research work is to improve the biohydrogen production process with main emphasis being to increase yields of hydrogen from the existing processes and its generation from organic waste. A wide range of potential H₂ producing microorganisms (which includes thermophiles and mesophiles) have already been identified. One such mesophile potential isolate, *Enterobacter cloacae* IIT BT08 (presently known as *Klebsiella pneumonia*) has been identified. This organism produces hydrogen at high rate as compared

to other mesophilic microbes. Redirection of the biochemical pathways of *K. pneumonia* IIT-BT08 is carried out for the improvement of the hydrogen production. It is done by blocking alcohol and some of the organic acids formation in *K. pneumonia* IIT-BT08 during their metabolism. The principle being that NADH is usually generated by catabolism of glucose to pyruvate through glycolysis. The conversion of pyruvate to ethanol, butanediol, lactic acid and butyric acid involves oxidation of NADH. The concentration of NADH would be increased if the formation of these metabolites could be blocked. Double mutants of *K. pneumonia* IIT-BT08 with defects in both alcohol and organic acid formation pathways are able to enhance H_2 yield as compared to wild type strain (3.8 mol H_2 /mol glucose). Recently, acidogenic microbial consortium obtained from the anaerobic digester is also found suitable for hydrogen production.

To make biohydrogen production renewable, its feedstock should be renewable in nature and should be widely available. Suitability of different organic feed stocks for hydrogen production has been studied. Attempts have been made to explore the use of algal biomass, de-oiled cakes, starchy wastewater, lignocellulosic biomass, cane molasses etc. as feedstock. Usually wastewaters have lack of nutrients for the growth of the microorganisms. Effort has been made to identify cost effective nutrient rich supplement for the improvement of hydrogen production using wastewater as substrate. For that purpose, use of de-oiled cakes as nitrogen supplements has been explored. The maximum cumulative hydrogen production and hydrogen yield on using de-oiled cakes are 3.2 L L^{-1} and $11.2 \text{ mol H}_2/\text{kg COD}_{\text{removed}}$, respectively. Groundnut and coconut de-oiled cakes appear most promising as a substrate as well as nutritional supplement in the hydrogen production process. The suitability of cane molasses as substrate for continuous biohydrogen production has been demonstrated using a 20 L bioreactor. The maximum rate of hydrogen production and yield achieved are 67 L h^{-1} and $18.54 \text{ mol H}_2/\text{kg COD}_{\text{removed}}$, respectively. As H_2 is the product of dark fermentation, its accumulation inhibits the product formation which is in accordance with Le Chatelier's

principle. This increase in partial pressure also contributes towards metabolic shift during fermentation. It leads to formation of reduced end products such as ethanol, propionate, lactate, butanol and acetone. Many strategies are used for the removal of H₂ from the fermentation system. A sophisticated 'Automatic Logic Control System' has been developed for the operation of continuous hydrogen production under reduced partial pressure conditions. Reduced partial pressure always helps in improving the kinetics of hydrogen production. Maintenance of a reduced partial pressure on the overhead space of a reactor has been made automated by implementation this system. This has made the process easy to operate and also helped in improving the overall H₂ yield.

A substantial research has been carried out on development of continuous hydrogen production process especially in customized bioreactors. A prototype 20 L packed bed reactor has also been developed for continuous hydrogen production. Such type of packed bed reactor uses cheaper agro-residues as matrix for whole cell immobilization. The live demonstration of the process has been uploaded in the website: <http://www.bioh2iitkgp.in>

Pilot plant study has been successfully carried out using an 800 L reactor.

MNRE, Government of India has already prepared a Hydrogen road map in India. IIT Kharagpur is the leading Group in India working on "Hydrogen production through biological routes" under this programme. Our endeavour with large scale biohydrogen production has motivated us to commercialize biohydrogen production process for decentralized energy solution. One of our current major activities is to design 10 m³ of bioreactor for commercialization of biohydrogen production process from organic wastes.

Mathematical modeling and simulation on the biohydrogen production processes has been carried out. The efficiency of biohydrogen production can be analyzed by defined mathematical models. These models also increase understanding the effect of substrate concentration, feedback inhibition, and effect of different substrate on hydrogen production. The kinetic parameters determined from unstructured mathematical models could help in designing and scaling up of bioreactors. During H₂ production, kinetics of biomass formation and products (hydrogen, VFA, solvents, etc.) are needed to be studied. Our potent microorganism, *K. pneumonia* IITBT08 has been extensively studied for biohydrogen

production. Mathematical modeling using Lineweaver–Burk equation showed that it has maximum specific growth rate, $\mu_{\max}$ and saturation constant, K_s of 0.28 h^{-1} and 0.98 g L^{-1} , respectively. Biohydrogen production by *K. pneumonia* IITBT08 is growth associated product and this fact has been established by using Luedeking–Piret model. The kinetics of hydrogen production by *K. pneumonia* IITBT08 has been analyzed using modified Gompertz model. It reveals that this organism has maximum hydrogen production potential of 2.6 L/L with lag phase of 45 min .

Recently, thermophilic biohydrogen production process has been explored. *Thermoanaerobacterium thermosaccharolyticum* ST1 has been isolated which yielded a maximum of $2.7 \text{ mol H}_2/\text{mol glucose}$. Use of this organism in continuous hydrogen production in packed bed reactor shows higher hydrogen yield and rates as compared to mesophilic system. A detailed continuous hydrogen production using high temperature effluent such as starchy wastewater has shown that with variation in organic loading rate, the hydrogen production improved. Another interesting observation that has been observed is the inverse relationship between NADH/NAD^+ ratio with rate of hydrogen production. NADH is the reducing equivalent that is required to produce molecular hydrogen via Fe-Fe hydrogenase pathway. Mutants have been developed that have suppressed competing pathways and higher pool of NADH for improvement of hydrogen production.

The spent media generated after completion of dark fermentative hydrogen production is generally rich in short chain fatty acids such as acetate, butyrate, propionate etc. The energy trapped in these metabolized can be used to further improve gaseous energy recovery by integrating it with photofermentation process. Many innovative technologies in the field of photofermentative hydrogen production have been developed. One such innovation is development of a rocking flat panel reactor for photofermentation.

Energy calculation for rocking motion and integration with dark fermentation has been one of the achievements. *Rhodobacter sphaeroides* O.U. 001 is capable of producing biohydrogen

via photofermentation. Maximum hydrogen production rate of 11 mL/L h is observed using spent media.

Recently, research work has also been carried out to explore the possibility of integrating biohydrogen production process with biomethanation under the eponym of Biohythane. This process shows promise in improving gaseous energy recovery through biological routes. By implementation of such two stage process, it is possible to improve gaseous energy recovery up to 56% as compared to single stage hydrogen production process.

In recent years, sequestration of atmospheric CO₂ by cyanobacteria and green algae have been receiving increased attention. Algal cultivation for CO₂ sequestration has many advantages such as faster growth rates and the possibility of cultivation on non-arable land areas or in lakes or the ocean. Finally, an integrated approach using microbial fuel cells (MFCs) for generation of electricity from wastes can be a giant step for mankind into a sustainable future. Green algae and cyanobacteria are the vast group of both facultative photoautotrophic and photoheterotrophic microorganisms. Microalgae can be cultivated under aqueous conditions ranging from freshwater to situations of extreme salinity and from controlled closed system to open system. Algae are generally classified into several taxonomic groups on the basis of their nature and properties, type, number and morphology of flagella, reverse food products chemistry and product of photosynthesis, their chemical and physical properties of cell walls, morphological characteristics of cells and thalli, mode of reproduction etc. Their uniqueness that separates them from other microorganisms is due to presence of chlorophyll and having photosynthetic ability in a single algal cell, therefore, allowing easy operation for biomass generation, and effective genetic and metabolic research in a much shorter time period than conventional plants. The algal biorefinery concept has also been explored. The efforts are concentrated on high rate algal biomass generation in controlled photobioreactors with subsequent use of the obtained biomass as a source of food,

feed, biofuels and bioactive compounds. In continuous mode of operation with *Chlorella sorokiniana*, the maximum biomass productivity of $0.11 \text{ g L}^{-1} \text{ h}^{-1}$ is observed at an optimum dilution rate of 0.05 h^{-1} using 5% air–CO₂ (v/v) gas mixture. Biological fixation of CO₂ has been also studied using industrial flue gas. The flue gas emitted from the oil producing industry contains mostly CO₂ and H₂S (15.6 %v/v and 120 mg L^{-1} , respectively) along with nitrogen, methane, and other hydrocarbons.

The highest reduction in the CO₂ content of inlet flue gas is 4.1% (v/v). Cyanobacteria are also used for CO₂ sequestration study. *Anabaena* sp. PCC 7120 is grown in customized airlift photobioreactors. Higher light utilization efficiency and a higher rate of CO₂ biofixation are observed with maximum biomass concentration of 0.71 g L^{-1} using BG11₀ medium under aerated conditions. Another new approach for CO₂ sequestration is microbial carbon capture cells (MCCs). Cyanobacteria have been grown in photo biocathode in dual-chambered flat plate mediator-less MFCs which is separated by an anion exchange membrane. The performance of the MCC with *Anabaena* purged with CO₂–air mixture is compared with that of a conventional cathode purged with air only. It has been established that light can efficiently penetrate up to 3 cm on giving external illumination. Hydrogen production from algal cultivation has been explored via two distinct technologies. Firstly, use of photo biological process of algae for hydrogen production shows maximum hydrogen production rate of 1 mL/L h on using *Chlorella sorokiniana*. Similar studies with cyanobacteria, *Anabena* sp. also showed slower hydrogen production rates. Second strategy of hydrogen production from algal biomass is to use it as feedstock for dark fermentation. The algal biomass are generally rich in carbohydrates such as starch, cyanophecian starch etc. which can be a potential source of fermentable sugars. Highest hydrogen yield of 2.67 mol/mol of hexose is observed by using *C. sorokiniana* biomass as feedstock.

The advent of Microbial fuel cell (MFC)-based technologies have turned out to be promising technologies for direct energy production from various wastewaters. MFCs can utilize organic substrates and subsequently convert their chemical energy to electricity in a single step using microorganisms. MFC is a bio-electrochemical reactors/device typically consists of anode and cathode chambers physically separated by a proton exchange membrane (PEM). A MFC can be divided into three major components, namely an anaerobic anode chamber, an aerobic cathode chamber and an ion exchanger connecting the two chambers. Anode chamber is the microbial growth compartment, which provides all the necessary condition for the growth and the electron extraction from the microorganism. The chamber is fed with growth media named as anolyte, redox mediator (not required in case of mediator-less MFC), microorganism and an electrode that acts as the anode of the MFC. The bacterial growth in this chamber produces the necessary protons and electrons through metabolic reactions. However, numerous hurdles need to be overcome to make this technology economically feasible and suitable for field applications.

Extensive research work has been carried out on MFC process and focused to improve the performance, reduce the construction cost, and expand the application scopes of MFC based technologies collectively known as bioelectrochemical systems. Low cost materials for the anode, cathode and membrane in MFCs are studied to increase the power performance using complex wastewaters as substrate. Catalytic enhancement with MnCo_2O_4 in presence of polypyrrole (PPy) on carbon cloth cathode shows maximum potential reaching power densities of 10.2 W m^{-3} using low cost KOH doped PVA-PDDA anion exchange membrane. The developed MFC system has been explored for hydrogen production under microbial electrochemical system. The developed biofilm on anode during MFC regime would augment towards the poised potential provided to the cathode. At cathode the proton generated during

MFC regime may be converted to molecular hydrogen. The rate of hydrogen production from such system is found to be 10 mL/L h.

Conclusion

Suitability of different biohydrogen production processes has been explored. *K. pneumonia* IIT-BT 08 is found suitable for H₂ production using different carbon sources at 36 °C and pH 6.5. Double mutant strain of this organism and reduced partial pressure of H₂ improved the gas generation significantly. Scaling up studies has been successfully carried out up to 20 L and 800 L. Acidogenic microflora present in the anaerobic digestion process is found suitable for the H₂ production. The spent medium from dark fermentation is found suitable for H₂ photo-production by *R. sphaeroides* O.U. 001 or microalgae like *Chlorella sorokiniana* or MFC in a two-stage batch fermentation process but H₂ yield is less as compared to dark fermentation process. The possibilities of using different organic residues such as coconut de-oiled cake, sewage sludge, cane molasses, distillery effluents, starch wastes, algal biomass etc. for H₂ production are explored. Continuous hydrogen production is successfully demonstrated using immobilized whole cell system using environmental friendly solid matrix. The present immobilized system may overcome some difficulties like gas hold-up, diffusion problem, etc. due to the improvement of reactor configuration. Modeling and simulation studies are carried out for the different bioH₂ production processes. Biohydrogen production has been established as a prospective alternative and integral component of green sustainable energy. However, two major aspects need indispensable optimization viz. a suitable renewable organic wastes/wastewater and ideal microbial consortia that can convert this biomass efficiently to hydrogen. Biohythane process can improve both COD reduction and gaseous energy recovery significantly indicating its potentiality to overcome the energy scarcity problem in future using organic wastes as substrate.