Hydrogen Production from Dairy Waste by Fermentation using Thermophilic Bacteria Thermatoga Maritima

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***Abstract* - The use of alternative and renewable sources of power production is now a mainstream concept in the global energy discussion, and the concept of transportation fuels, such as ethanol, from non-petroleum sources is becoming familiar to a majority of consumers. Hydrogen has the potential to provide energy for stationary conversion devices such as fuel cells, as well as for transportation needs. This paper focuses on production of bio-hydrogen by fermentation of organic waste sourced from dairy industry using a hyper thermophilic bacteria *Thermatoga maritima* and to study the bacterial growth conditions for optimizing the H_2 yield. A laboratory scale batch fermentation unit was set-up and inoculation of the bacteria was done under sterile conditions with strict anaerobic conditions. *T. maritima* was cultured at an optimum temperature of 70 ^{0}C. The gas collected at the end of fermentation process was analyzed using gas chromatography. A yield of 25.95% (v/v) of hydrogen gas was reported with standard ATCC medium and a yield of 19.46% (v/v) is reported with medium formulated with dairy waste.**

Key words: Hydrogen, Organic Wastes, Thermophilic Bacteria, fermentation

I. INTRODUCTION

The importance of an alternative fuel was recognized in early 1900s by Dr. Rudolph diesel. Development and promotion of alternative fuels such as vegetable oils for compression ignition engines is gaining sustained attention. Global demand for hydrogen is expected to increase by around 6.5% in the next 5 years [1]. During the last few decades, several relevant technologies related to alternative fuel have been developed and deployed in many areas of our country including rural and remote areas. Most of the energy, we use today is from nonrenewable energy sources such as oil, natural gas, and coal. About 70% of India's energy generation capacity is from fossil fuels, with coal accounting for 40% of India's total energy consumption followed by crude oil and natural gas at 28% and 6% respectively[2]. India is largely dependent on fossil fuel imports to meet its energy demands by 2030, India's dependence on energy imports is expected to exceed 53% of the country's total energy consumption[2]. The growth of electricity generation in

India has been hindered by domestic coal shortages and as a consequence, India's coal imports for electricity generation increased by 18% in 2013[3]. Non-conventional energy sources consist of those energy sources that are infinite, natural, and restorable, such as tidal energy, solar energy, and wind energy. Currently, some of the important and widely used non-conventional sources of energy are tides, wind, solar geothermal heat, and biomass comprising animal waste, agricultural waste, and human body waste. Hydrogen is believed to take over fossil fuels to meet up future energy demands.

Currently hydrogen gas is produced by Steam Methane Reforming (SMR), Coal Gasification, Partial Oxidation of Hydrocarbon, and Electrolysis of water. These methods are expensive and energy intensive as they are carried out at high temperatures and pressures [4][5].

Biofuels

The origin of all fuel and biofuel compounds is ultimately the sun, as solar energy is captured and stored as organic compounds through photosynthetic processes. Certain biofuels, such as oils produced by plants and algae, are direct products of photosynthesis. These oils can be used directly as fuel or chemically transesterified to biodiesel. Other biofuels such as ethanol and methane produced as organic substrates, are fermented by microbes under anaerobic conditions. Hydrogen gas can be produced by both routes, that is, by photosynthetic algae and cyanobacteria under certain nutrient or oxygen-depleted conditions, and by bacteria and archae utilizing organic substrates under anaerobic conditions. A comparison of biofuel energy contents reveals that hydrogen gas has the highest energy density of common fuels expressed on a mass basis as shown in table 1. For liquid fuels, biodiesel, gasoline and diesel have energy densities in the range of 40 to 46 kJ/kg. Biodiesel fuel contains 13 percent lower energy density than petroleum diesel fuel but combusts more completely and has greater lubricity [6].

Table 1. Energy density values for common fuels at standard temperature and pressure [7]

Fuel Source	Energy density (kJ/g)	Density (kg/m³)	Energy content (GJ/m³)
Hydrogen	143.0	0.0898	0.0128
Methane	54.0	0.7167	0.0387
Diesel	46.0	850	39.9
Gasoline	44.0	740	32.6
Soybean oil	42.0	914	38.3
Soybean biodiesel	40.2	885	35.6
Coal	35.0	800	28.0
Ethanol	29.6	794	23.5
Methanol	22.3	790	17.6
Softwood	20.4	270	5.5
Hardwood	18.4	380	7.0
Rapeseed oil	18.0	912	16.4
Bagasse	17.5	160	2.8
Rice hulls	16.2	130	2.1
Pyrolysis oil	8.3	1280	10.6

Biological methods of hydrogen production [4]:
Direct biophotolysis, Indirect photolysis, Photo fermentation and dark fermentation are the methods used for production of hydrogen by biological routes. A new hybrid method involves combining both dark and photo-fermentation and is found to be most efficient method to produce hydrogen.

Energetics:
In theory, the microbial fermentation of glucose ($C_6H_{12}O_6$) to hydrogen could occur as

$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \quad \Delta G^{\circ\prime} = -25.9$ kJ/reaction

In this reaction, 99 percent of the energy originally present in glucose is contained in the 12 mole of hydrogen produced. This is calculated by comparing the energy released from glucose combustion vs. hydrogen combustion:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad \Delta G^{\circ\prime} = -2872$ kJ/reaction

$12H_2 + 6O_2 \rightarrow 12H_2O \quad \Delta G^{\circ\prime} = -2846$ kJ/reaction

The standard free energy of formation ($\Delta G^{\circ\prime}$) in the above equation is believed to be too small to allow for microbial growth; therefore, this pathway is not known to occur in microbial systems. Under physiological conditions, Thauer estimated that 42 to 50 kJ/ reaction are required for ATP synthesis at 100 percent efficiency and equilibrium conditions, and approximately 63 kJ/ reaction under non equilibrium conditions.
By considering only microbial pathways that are thermodynamically favorable, Thauer predicted that a maximum of 4 mole H_2 /mole glucose could be produced by microbial fermentation [8] .

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2CO_2 + 4H_2$
$\Delta G^{\circ\prime} = -216$ kJ/reaction

The $\Delta G^{\circ\prime}$ value for this reaction (also reported as –207 kJ/reaction for standard conditions of 25°C, 1 M solutes and 101 kPa total gas pressure) is sufficient for ATP production and microbial growth, and has been found to occur in natural and bioreactor systems. But, the ΔG values reported for actual culture conditions are high and therefore more favorable to H_2 formation. In addition, other research cites a critical value of only 19 kJ/ reaction for $\Delta G^{\circ\prime}$ as the minimum required for microbial growth. Therefore, the maximum ATP production by fermentation of glucose could potentially be greater than 4 mol/mol glucose.

Table 2. Micro-organisms used for hydrogen generation [4]

Broad classification	Name of the micro-organisms
Green algae	*Scenedesmus obliquus* *Chlamydomonas reinhardii* *C. moewusii*
Cyanobacteria Heterocystous	*Anabaena azollae* *Anabaena CA, A. variabilis, A. cylindrica* *Nostoc muscorum N. spongiaeforme Westiellopsis prolifica*
Cyanobacteria Non-heterocystous	*Plectonema boryanum* *Oscillatoria Miami BG7* *O. limnetica, Synechococcus sp., Aphanothece halophytica* *Mastidocladus laminosus, Phormidium valderianum*
Photosynthetic bacteria	*Rhodobater sphaeroides* *R. capsulates, R. sulidophilus, Rhodopseudomonas sphaeroides* *R. palustris, R. capsulate, Rhodospirillum rubrum* *Chromatium sp., Miami PSB, Chlorobium limicola, Chloroexu aurantiacus, Thiocapsa roseopersicina, Halobacterium halobium*
Fermentative bacteria	*Enterobacter aerogenes* *E. cloacae* *Clostridium butyricum, C. pasteurianum Desulfovibrio vulgaris, Magazhaera elsdenii, Citrobacter intermedius* *Escherichia coli*

Hyperthermophilic bacteria [9]
Hyperthermophilic bacteria of the order of thermotogales, consists of one family, Thermotogaceae, and six genera, including *Thermotoga*. There are nine species of *Thermotoga* (*T. maritima T. neapolitana, T. thermarum, T. elfii, T. subterranea, T. hypogea, T. petrophila, T. naphthophila,* and *T. lettingae*), all of which are obligate anaerobic bacteria that ferment glucose to acetate, carbon-di-oxide, and hydrogen. The first two described members, *T. maritima* and *T. neapolitana*, are the most closely related based on 16S rRNA gene sequence analysis. *T. maritima* is investigated for hydrogen production studies in this work.

EXPERIMENTAL SET-UP

The experimental setup is shown in the figure 1. A Buchner's flask of 1 liter capacity was used for fermentation. A heating plate and K- type temperature controller was used to maintain the temperature at 70°C. Copper coils wound around the Buchner's flask were used to provide adequate condensation provisions. A measuring jar inverted in a trough of water was used to collect the hydrogen gas by water displacement.

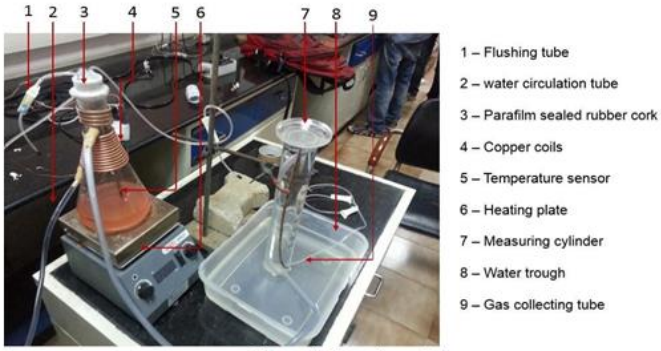


Figure 1: Experimental Set up

METHODOLOGY

Medium formulation

Standard ATCC medium 43589 was prepared to provide the nutritional requirements of the bacteria for its culturing.

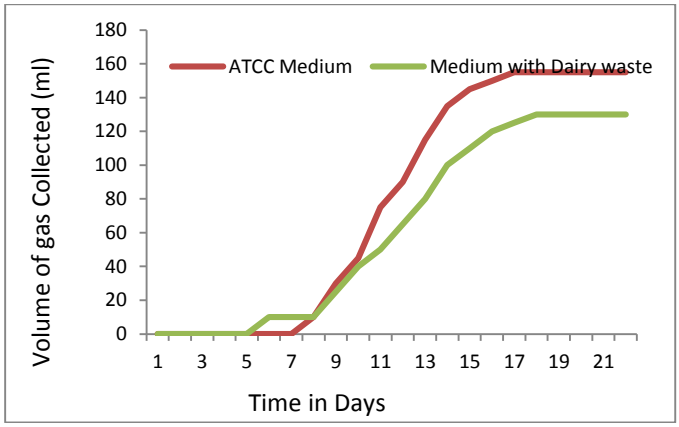
Inoculation

The dairy waste used as substrate for the organism was analyzed to determine the solids and glucose content. 1 ml of the inoculum was drawn from the vacuum sealed vile into the syringe and injected into the medium solution in a laminar air flow chamber. 5 ml of dairy waste was added to the medium solution and the flask was thoroughly sealed with parafilm layers. Anaerobic conditions were established inside the Buchner flask by flushing nitrogen gas into the flask through flushing tubes. The flushing tubes were then clipped to prevent any gas from entering the headspace. The temperature was maintained at 70^{O}C, while monitoring the volume of gas displaced in the measuring cylinder continuously. The volume of gas displaced was continuously recorded and the accumulated gas sample was collected in a 1.0 litre Tedler bag and analyzed for its hydrogen content by gas chromatography.

RESULTS AND DISCUSSIONS

Solids content in dairy waste was 6 g/lt and glucose was found to be 4.6 g/l. Figure 2 shows the data pertaining to generation of Hydrogen gas from the day of inoculation. No increment was observed for the first seven days after inoculating. This is due to the initial lag phase of the microbial growth density, post which the gas accumulation was noticed by the decrease in the water level in the inverted measuring cylinder which synchronizes with the bacterial growth phase. Finally, the gas accumulation was attained constant after seventeenth day of inoculation which depicts the start of the stationary phase in the bacterial growth. The gas sample was later analyzed by gas chromatographic analysis to confirm the production of hydrogen gas and to determine its yield. The report of the gas chromatographic analysis showed the yield of hydrogen in the gas sample as 19.46% (v/v). A similar experiment was carried out culturing the bacteria in the standard ATCC media and the yield of hydrogen gas was reported to be 25.95% (v/v).

Figure 2: Volume of gas collect ed during fermentation



DISCUSSION

The bacteria *Thermatogo maritima* was successfully cultured under anaerobic conditions and at a temperature of 70˚C. The experimental studies showed that the bacterial growth started after an initial lag phase and the hydrogen gas was produced during the growth phase of the organism. A sample of culture was subjected to gram staining test during the growth phase to study the microbial growth. Gas collected was analyzed and the yield obtained using dairy waste was 19.46% (v/v) when compared with standard medium which yielded 25.95% (v/v) hydrogen gas. The yield could further be improved by optimizing the medium composition and the process.

CONCLUSION

Thermatogo maritima shows good potential for biohydrogen production. The study shows that dairy waste can be effectively utilized to generate energy using *T. maritima*. Only 4 moles of hydrogen is produced by fermentation instead of theoretical yield of 12 moles as 8 moles are utilized in metabolism of micro-organisms according to the energetics of the biochemical reaction. To compensate for this loss in hydrogen yield, further studies on medium formulation for minimizing the lag phase and to enhance the growth phase on continuous scale is necessary.

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