Generation of Hydrogen Gas from Crude Glycerol by Purple Non-Sulfur Photo Fermentative Bacteria, *Rhodobacter Meghalophilus*

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Abstract— *As the world is progressing faster with new technological innovations, the need and demand for energy is also constantly expanding. In the light of conventionally available fossil fuel reserves being exhausted extensively that has left a very deep scar on environment, the urge for alternative environment friendly energy source is the need for energy sustenance. Hydrogen gas is distinct for its high calorific value, clean fuel characteristic and suitability for wide applications. Chemical method likes steam reforming, coal gasification are established technologies available for industrial hydrogen needs but are high in terms of cost and energy input. Biological methods are promising routes for hydrogen gas generation as they can be cost effective and use a variety of organic materials as substrates. The current study is focused on generation of hydrogen gas using Rhodobacter meghalophilus, a mesophilic, and purple non sulfur photo fermentative bacteria. Crude glycerol, byproduct from biodiesel plants is used as carbon substrate because of its rich organic content. Experiments were carried out to study the effect of process parameters viz. volume of crude glycerol, pH and light intensity on generation of hydrogen gas. Crude glycerol in the media was varied from 5 – 15% (v/v), pH between 5.8 – 8.3 and the light intensity at 500, 1000 and 2000 Lx respectively. At 15% (v/v) of crude glycerol, pH of 7.8 and 1000 Lx, the volume of hydrogen gas obtained was 490 ml/L with the substrate to hydrogen gas conversion rate as 0.012 mol/ mol of crude glycerol with light conversion efficiency of 1.16%.*

Keywords— Bio hydrogen, Clean energy, Crude glycerol, Photo fermentation, Rhodobacter meghalophilus.

I. INTRODUCTION

The world is progressing ahead with tremendous improvement in technology and is also facing challenges on energy survival. The world had largely relied on the use of fossil fuels to meet its energy needs. The energy needs have been increasing enormously with advancements in industrial, transport, agricultural sectors, causing rapid depletion of fossil fuels that have been the prime fuel reserve. The liberal use of fossil fuels has also left a serious impact on the ecosystem. The burning of fossil fuels has released exorbitant amount of carbon di oxide that is changing the ecological and geological characteristics of our planet. With the depleting fossil fuel sources, ever rising demand for energy and the hazardous damages caused by fossil fuels, the world's energy requirements rely largely on the search for other alternative resources.

The potential of solar, wind, ocean, geothermal and hydrogen energies are being tapped in for bridging the energy gap. The advantage of these alternative sources is that they are also renewable and environment friendly. Vast amount of research has been carried out to study the potential methods to optimize the energy derivation form these renewable sources. Of these, studies on hydrogen gas as an alternate energy source has gained more momentum due to their advantages that it has high energy content and is eco-friendly, producing only water as it's by product on burning [(1)]. These advantages have proved to show that hydrogen fuel can be seen as a positive substitute to reduce our dependency on fossil fuels.

II. LITERATURE

Unlike solar, wind energy, hydrogen is not freely found in nature. Hydrogen is being used as a raw material in manufacturing plants producing ammonia, plastics, petrochemicals and also in refineries [(2)], which is commercially produced by chemical

methods such as steam methane reforming, coal gasification [3], partial oxidation of hydrocarbons [4] etc. These methods have proven technology but are also energy intensive. Therefore, biological methods are being researched upon to study the production of hydrogen gas by processes such as direct photolysis, indirect photolysis, dark fermentation, photo fermentation, anaerobic fermentation and hybrid fermentation [4] employing microorganisms belonging to the family of green algae [5], cyanobacteria [6], photosynthetic bacteria [7], fermentative bacteria [8]. The use of biological methods will help to generate the hydrogen gas with minimum requirements.

2.1 Microbial substrates for bio hydrogen generation process

Microorganisms depend on carbon as the main source of substrate for hydrogen generation. Simple sugars like glucose, sucrose or lactose are used for bio hydrogen gas generation [9]. However, the use of these sugars as pure source of carbon may not be feasible as the cost of substrate becomes one of the limiting factors while scaling the process. Therefore, to make the process economically viable, cheaper sources of carbon needs to be found as alternative substrates. Runoffs from agriculture [10], dairy [11] and food processing industries [12], brewery industries [13] and municipal solid waste [14] are rich source of carbohydrates such as starch or cellulose. Currently these materials are productively used for biogas generation or simply dumped on landfills and into water bodies. Land or water disposal of these materials are posing severe environmental threats and endangering several life forms in the ecosystem. Therefore, efforts are being made to amalgamate these organic wastes as carbon substrates for hydrogen production through microbial conversion process [15].

2.1.1 Crude Glycerol as a source of carbon substrate for bacteria to generate hydrogen gas

Biodiesel is one of the alternative energy sources that have been a promising substitute for fossil fuels. As such, the number of biodiesel plants is increasing worldwide and the production of biodiesel is also scaled up gradually. In the process of biodiesel production, a byproduct that is equivalent to about 10% (by mass) of biodiesel, known as crude glycerol is produced [16]. Unreacted free fatty acids from vegetable oil and chemicals used in the production of biodiesel such as methanol, sulphuric acid, sodium hydroxide and soap, formed during trans-esterification reaction, find their way along with this glycerol [17]. It is therefore termed crude, for it is highly impure and cannot be used in pharmaceutical, food or cosmetic industries that otherwise use glycerol as one of the raw materials.

Crude glycerol can be chemically treated to remove the impurities and converted to value added products. The conventional treatment steps include a series of unit operations or processes like acidification, neutralization, extraction, adsorption and filtration [18]. But the cost of the treatment processes makes it an unviable option. Therefore, crude glycerol from biodiesel plants are considered as waste material and dumped off. This method of disposal is also hazardous to the environment due to the presence of methanol, sulphuric acid and sodium hydroxide. Therefore, ways to efficiently treat crude glycerol or use them as raw materials to generate value added products can be economically beneficial to the biodiesel industries and also prevent hazard to environment due to their direct disposal.

As chemical treatment of crude glycerol is not cost effective, biological ways of its treatment can be explored as a possible and eco-friendly option due to the rich organic content. Microbial degradation of crude glycerol by fermentation process is an alternative and promising way of treating crude glycerol for its effective usage [19].

Many bacterial species in the mesophilic and thermophilic family, have been studied for their ability to decompose crude glycerol and generate value added products such as lactic acid, propane diol etc [20]. In this regard, microorganisms capable of synthesizing hydrogenase and nitrogenase enzymes have been found to generate hydrogen gas as a metabolic product through bioconversion process via photosynthesis or fermentation [21]. Bacterial species such as *E.coli* [22], purple non sulfur bacteria such as *Rhodobacter sphaeroids* [23], *Rhodobacter palustris* [24], *Enterobacter* [25], *Clostridium* [26] *etc* have the potential to generate hydrogen gas utilizing the rich carbon source available in the crude glycerol.

2.2 Hydrogen generation by photo fermentation

Photo fermentation deals with conversion of organic substrate in the presence of light to produce biomass or metabolic products that have various end uses. A diverse group of photosynthetic bacteria act on the organic substrates under anaerobic condition to generate bio hydrogen by the enzymatic activity of hydrogenase and nitrogenase [27]. The overall reaction of hydrogen production by photo fermentation is according to the reaction (1):

$$C_6H_{12}O_6 + 6H_2O + h_v \rightarrow 12H_2 + 6CO_2 \quad (1)$$

All plants, algae and some bacteria are capable of photosynthesis utilizing, light as the source of metabolic energy. *Cyanobacteria* have been frequently studied for its potential to generate hydrogen by oxygenic photosynthesis [(28)]. However, the purple non sulfur bacteria such as *Rhodobacter* have much higher potential for hydrogen generation by both oxygenic photosynthesis and photo fermentation [(29)].

III. METHODOLOGY

The chemicals used for the experimental study were of analytical grade. The strain JA194^T was obtained from JNTU, Hyderabad. Crude glycerol was sourced from Biofuel Park, GKVK, Bangalore. The media for strain JA194^T was prepared using crude glycerol, yeast extract (0.3 g/L), C_2H_3O (0.5 ml/L), $C_4H_4Na_2O_4$ (1 g/L), $C_2H_7NO_2$ (0. 5g/L), 0.1% of $C_6H_5FeO_7$ solution (5 ml/L), KH_2PO_4 (0.5 g/L), $MgSO_4.7H_2O$ (0.4 g/L), NaCl (0.4 g/L), NH_4Cl (0.4 g/L), $CaCl_2.2H_2O$ (0.05 g/L), vitamin B_{12} solution (0.4 ml/L), trace element solution (1 ml/L). The composition of trace element solution consisted of $ZnSO_4.7H_2O$ (0.1 g/L), $MnCl_2.4H_2O$ (0.03 g/L), H_3BO_3 (0.3 g/L), $CoCl_2.6H_2O$ (0.2 g/L), $CuCl_2.6H_2O$ (0.01 g/L), $NiCl_2.6H_2O$ (0.02 g/L), $Na_2MoO_4.2H_2O$ (0.03 g/L).

Crude glycerol contains unreacted free fatty acids, chemicals such as methanol, sulphuric acid used in transesterification of vegetable oil during biodiesel production process, soap, glycerol and water. The amount of glycerol present in the sample was determined by titrimetric method based on cold oxidation of crude glycerol in a strong acidic medium as given by the equation (2):

$$CH_2OH\text{-}CHOH\text{-}CH_2OH + 2NaIO_4 \rightarrow HCOOH + 2HCOH + 2NaIO_3 + H_2O \quad (2)$$

The mixture was then titrated against a base and glycerol content in the sample (m/m) was calculated by the formula:

$$((V_1 - V_2) \times T \times 0.0921 \times 100)/ m \quad (3)$$

where V_1 and V_2 are the volume of sodium hydroxide used for titration of the reacted sample and the blank respectively. T is the normality of sodium hydroxide used in titration and m is the mass of crude glycerol sample.

The experimental studies on fermentation process for generation of hydrogen gas was carried using a 0.5L, 4 neck jacketed glass reactor. One neck was used for introducing the inoculum and to flush nitrogen into the reactor to maintain anaerobic condition. A K-type thermocouple was inserted through the second neck to monitor the temperature in the reactor. A light source was introduced in to the reactor through its third neck to facilitate the supply of light as *Rhodobacter meghalophilus* is a photo fermentative bacterium. A tube was inserted in the fourth neck of the reactor and the other end of the tube was inserted into an inverted cylinder arrangement to collect the hydrogen gas by water displacement method.

300 ml of the sterilized growth media was used for inoculation in batch fermentative tests. In order to maintain anaerobic condition, nitrogen was flushed in the reactor. 0.25% of L-Cysteine, an amino acid that acts as a reducing agent was also added to maintain anaerobic condition. The parameters such as volume percentage of crude glycerol in the media, pH and the intensity of light source were studied for their effect on *R. meghalophilus* for hydrogen gas production. The gas generated was monitored by the volume of water displaced in the inverted cylinder in a water trough. The percentage of hydrogen in the gas sample was analyzed by GC with TCD and also by a hydrogen gas sensor.

IV. RESULTS AND DISCUSSION

The amount of glycerol present in sample of crude glycerol was determined to be 26% (m/m) by titrimetric test. The amount of crude glycerol added to the nutrient media was varied between 5 to 15% (v/v). As the pH range for bacterial growth is optimum near to neutral range, experimental trials were carried out at pH 5.8, 6.8, 7.8 and 8.3. Effect of light intensity was studied at 500, 1000 and 2000 lx respectively.

4.1 Effect of crude glycerol on biohydrogen production

The amount of crude glycerol, added as carbon substrate in the nutrient media was varied between 5 to 15% (v/v) to study its impact on the growth of bacteria and hydrogen gas generation. The volume of gas collected during the growth period of bacteria in the fermentation process was recorded as shown in the fig. 1.

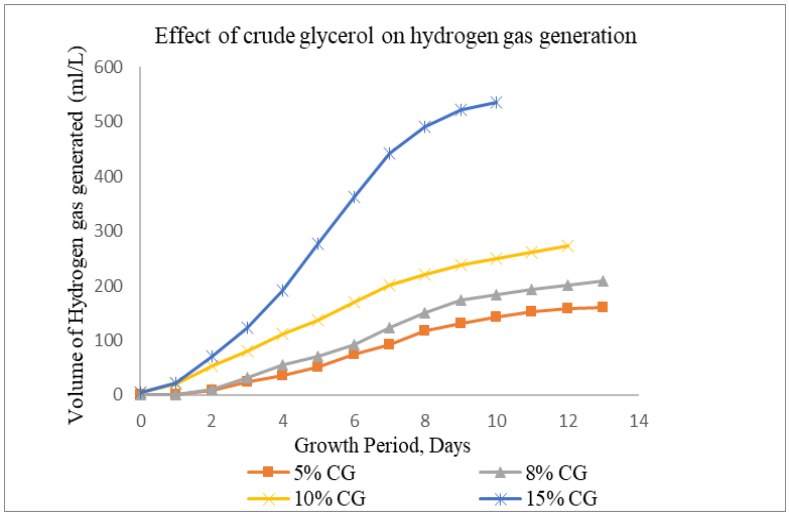

FIGURE 1: Effect of crude glycerol on hydrogen gas generation

It was observed that the growth of bacteria was influenced by the glycerol concentration in the medium. The bacterial growth was influenced by the length of the lag phase that varied with glycerol concentration. It can be seen that the lag phase in the growth of bacteria was more when crude glycerol in the nutrient medium was added in 5% and 8% (v/v) respectively. As the volume of crude glycerol in the nutrient medium increased gradually to 10% and 15% (v/v) respectively, the lag phase was found to be shortened. This shows that enzymatic activity of nitrogenase increases with increase in the volume of crude glycerol in the medium. Thus, increasing the volume of crude glycerol in nutrient medium is beneficial for hydrogen production. But, the soap content in its composition also increases. The effect of increase in soap content in the medium was noticed with foam formation when the crude glycerol was increased above 15% (v/v) in the nutrient medium. Therefore, batch experimental studies were carried out with crude glycerol volume varied between 5 -15% (v/v). The study showed that the volume of hydrogen gas generated was high when 15% (v/v) of crude glycerol was added to the growth medium. In the experimental trials, 490 ml/L of gas was found to be generated with 15% (v/v) of crude glycerol in the medium.

4.2 Effect of pH

Bacteria are sensitive to changes in pH due to which their metabolic pathways are altered that in turn affect the substrate degradation. The pH of the standard growth medium was found to be 6.4. With addition of crude glycerol, the pH of the medium was 6.9 due to alkaline nature of crude glycerol that contains soaps, formed by reaction of free fatty acids with alkali in transesterification reaction in biodiesel production. The pH of the growth medium in batch fermentative trials was varied between 5.8 to 8.3 and the volume of hydrogen gas generated was observed. Fig.2 shows the volume of hydrogen gas collected with pH of the medium at 5.8, 6.8, 7.8 and 8.3 respectively.

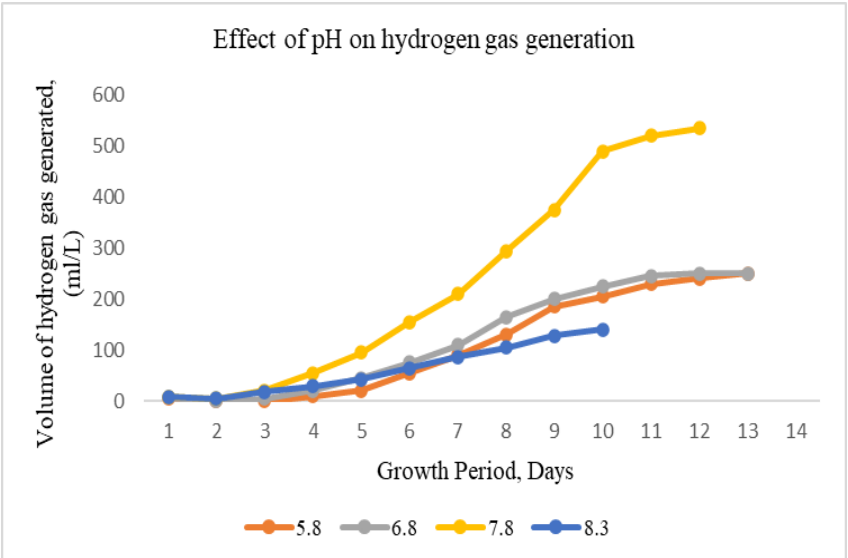

FIGURE 2: Effect of pH on hydrogen gas generation

As pH of the growth medium increased to slightly alkaline, gradual reduction in lag phase was noticed which was reflected by the difference in the volume of gas collected for fermentation trials at different pH values? The volume of hydrogen gas was found to increase with pH of the medium at 5.8, 6.8 and 7.8, but showed a decrease in volume for pH 8.3. The volume of gas produced was observed to be high at 490 ml/L for tests carried out when pH of the medium was increased to 7.8. At 8.3 pH, the volume of gas generation was found to decrease compared to lower pH values. As hydrogen gas is produced as a metabolic product in anaerobic fermentation, the decrease in gas production shows that the substrate degradation by the bacteria started to decrease at pH of 8.3. This could be due to changes in cellular activities of the microorganism.

4.3 Effect of light intensity

R.meghalophilus is a photo fermentative bacteria. In addition to the carbon source in the nutrient medium, the bacteria also derive its energy for growth from light. Hydrogen production rate has been found to exhibit a linear relation with intensity of light. Therefore, the effect of light intensity on hydrogen production was studied at 500, 1000 and 2000 lx in the photo fermentative tests. Fig. 3 shows the effect of light intensity on hydrogen gas generation by photo fermentation.

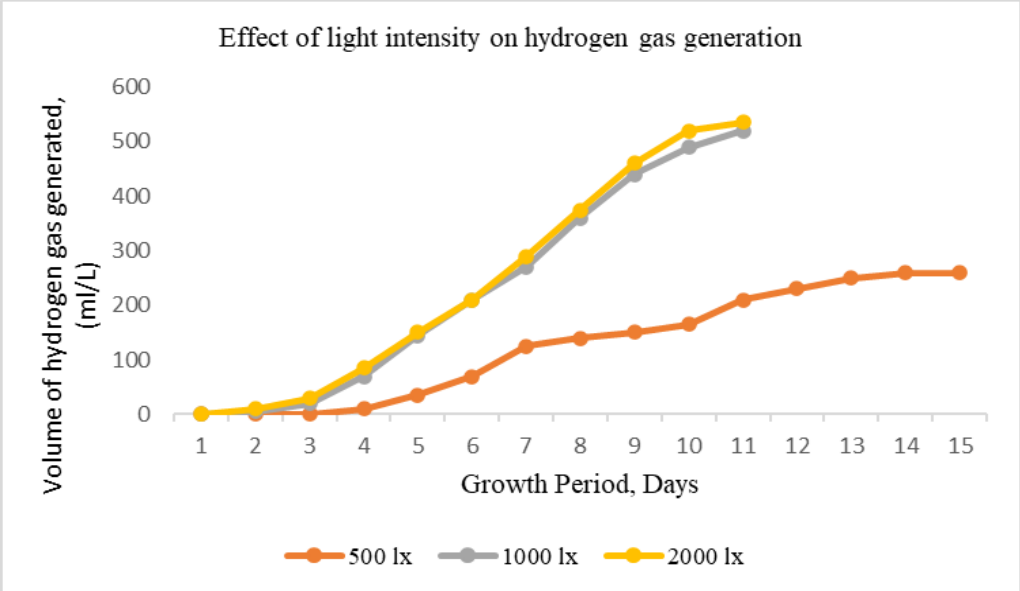

FIGURE 3: Effect of light intensity on hydrogen gas generation

With increase in the light intensity, the lag phase in the bacterial growth was observed to be shorter than lower light intensity. The volume of hydrogen gas increased with increase in light intensity from 500 to 2000 lx. However, the volume of hydrogen gas at 2000 lx was close in range with that obtained at 1000 lx. Light conversion efficiency is significant in photo fermentation and is estimated by comparing the thermal combustion value of hydrogen gas generated with absorption of light intensity as calculated by the formula (4):

$$\mathfrak{E} = [(33.61 \cdot \rho \cdot V_{H2})/(I \cdot A \cdot t)] \cdot 100 \quad (4)$$

Where V_{H2} is the volume of hydrogen generated in L, ρ is the density of the hydrogen gas in g/L, I is the light intensity in W/m^2, A is the irradiated area in m^2 and t, time for hydrogen gas generation.

The Efficiency of light conversion has been found to be 0.55%, 1.16% and 1.13% for 500 lx, 1000 lx and 2000 lx respectively. Therefore, light intensity of 1000 lx is taken as the light saturation point at which a maximum of 490 ml/L of hydrogen gas was collected with light conversion efficiency of 1.16%.

4.4 Hydrogen gas yield

The volume of hydrogen gas collected during the batch fermentation tests was analyzed by a hydrogen gas sensor to determine the concentration of hydrogen in the gas. The sensor was made of SnO_2 whose thermal conductivity varies with the concentration of hydrogen gas. The concentration of hydrogen in the gas sample was found to be 457 mg/L with glycerol concentration of 15% (v/v), pH at 7.8 and light intensity of 1000 lx. The yield of hydrogen gas was calculated to be 0.012 mol/ mol of crude glycerol.

V. CONCLUSIONS

Hydrogen gas can be a promising alternative source of energy that can drive the future world. While the industrial demand for hydrogen gas is currently met by energy intensive, chemical methods of production, they are unsustainable in terms of production cost and environmental pollution. Biological methods can be promising as they do not demand energy and can employ wide family of bacterial species that can act on various organic waste materials to generate hydrogen gas. This is seen as a promising way to meet the future energy demand. In the present study, *Rhodobacter meghalophilus* from the family of *Rhodobacter* was identified and investigated for its capability to generate hydrogen gas. As crude glycerol is used as carbon substrate for the bacterium in the fermentation process, its effect on hydrogen gas generation was studied by varying the volume of crude glycerol in the nutrient medium in 5, 8, 10 and 15% (v/v). pH and light intensity are two other significant parameters that impacts the bacterial growth and the hydrogen gas generation in photo fermentation process. pH of the medium was varied between 5.8 to 8.3 and the light intensity of 500, 1000 and 2000 lx was used in photo fermentation runs.

From the experimental runs carried out with the above study parameters, 490 ml/L of gas was collected with the hydrogen concentration of 457 mg/L by using 15% (v/v) of crude glycerol in the nutrient medium, pH of 7.8 and light intensity of 1000 lx. As the bacteria are photo fermentative, the process needs the presence of light along with a carbon substrate for its energy supplementation and hydrogen gas generation. Thus, to make the process further sustainable, solar cells could be used to power the light source for the photo fermentation process.

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