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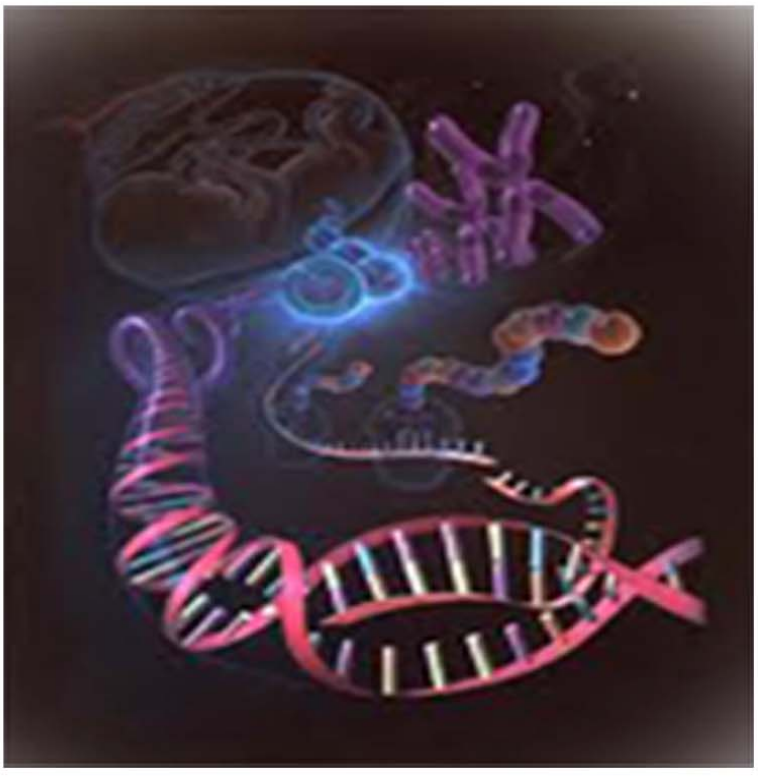

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Research Paper

PRODUCTION OF BIOHYDROGEN FROM DAIRY WASTE USING MIXED CULTURE OF *ENTEROBACTER CLOACEAE* AND *CLOSTRIDIUM PASTEURINUM* IN A BIOREACTOR

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Hydrogen is a valuable gas as a clean energy source; biological processes utilized for hydrogen gas production are bio-photolysis of water by algae, dark and photo-fermentation of organic materials, by bacteria. Dark fermentation process is a new approach, but expensive due to raw material cost. Carbohydrate rich, nitrogen deficient solid wastes including dairy waste can be used for hydrogen production by using suitable bio-process technologies. Mixed microbial consortia of *Enterobacter cloaceae* and *Clostridium pasteurinum* were used for the biohydrogen production. An anaerobic continuous stirred tank reactor with working volume of 12.8L was constructed and operated for 20 days. The temperature of the CSTR was regulated at 37 °C. The pH was controlled at 6.0. A continuous hydrogen gas production was achieved and analysed using gas chromatography.

Keywords: Bio hydrogen production, Dark fermentation, CSTR, Gas Chromatography

INTRODUCTION

Over dependence on fossil fuels today has led to critical environmental problems. Countries like United Arab Emirates, and Egypt which are the major exporters of crude oil would fail to meet the demands by 2015 and 2042, respectively (Kazim *et.al.,* 2001), and the resources would be exhausted within a decade or two (Abdallah *et.al.,* 1999). Combustion of fossil fuel contributes more on greenhouse and release of toxic gases like CO_2, SO_2, NOx and other pollutants leading to global warming.

For these reasons, leading researches are focusing on the exploration of new sustainable energy sources which can be an alternative to fossil fuels. Hydrogen is now being considered as a viable alternative fuel and green energy carrier of future. Since it is a clean fuel with no CO_2 emissions and can be used easily as fuel cells for electricity. Besides, hydrogen has a high

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energy yield of 122 kJ/g, which is 2.75 times greater than those available hydrocarbon fuels. The major problem in utilization of hydrogen gas as a fuel is its non availability in nature (significantly very less concentration) and production is very expensive. Hydrogen gas is also used widely as feedstock for the production of chemicals, hydrogenation of fats and oils in food industry, production of electronic devices, processing steel and also for desulfurization and re-formulation of gasoline in refineries. It has been reported that 50 million tonnes of hydrogen are traded annually worldwide with a growth rate of nearly 10% per year for the time being (Winter, 2005). Based on the National Hydrogen program of the United States, the contribution of hydrogen to total energy market will be 8.10% by 2025 (Armor, 1999). Due to increasing need for hydrogen energy, development of cost-effective and efficient hydrogen production technologies has gained significant attention in recent years.

Conventional hydrogen gas production methods are steam reforming of methane (SRM), and other hydrocarbons (SRH), non-catalytic partial oxidation of fossil fuels (POX) and auto thermal reforming which combines SRM and POX. Those methods are all energy intensive processes requiring high temperatures (>850°C). Among other methods developed to improve the existing technologies are the membrane processes, selective oxidation of methane and oxidative dehydrogenation (Armor, 1999). Biomass and water can be used as renewable resources for hydrogen gas production. Utilization of wide variety of gaseous, liquid and solid carbonaceous wastes was investigated by Kim (Kim, 2003) as renewable sources for formation of hydrogen gas by steam reforming. Despite the low cost of waste materials used, high temperature requirement (T = 1200°C) is still the major limitation for this process. Electrolysis of water may be the cleanest technology for hydrogen gas production. However, electrolysis should be used in areas where electricity is inexpensive since electricity costs account for 80% of the operating cost of hydrogen production (Armor, 1999). In addition, feed water has to be demineralised to avoid deposits on the electrodes and corrosion. Biological hydrogen production is a viable alternative to the aforementioned methods for hydrogen gas production. In accordance with sustainable development and waste minimization issues, bio-hydrogen gas production from renewable sources, also known as “green technology” has received considerable attention in recent years.

Biological H_2 production are based on biophotolysis of water by algae and cyanobacteria, photodecomposition of organic compounds by photosynthetic bacteria, dark fermentation hydrogen production, acidogenic phase of anaerobic digestion of organic matter and hydrogen systems using two stage dark/ photo-fermentative production of H_2. Key advantages of biological H_2 production are: 1) Process catalysed by microorganisms in an aqueous environment at ambient temperature and pressure; 2) Inexpensive; 3) Low energy requirement; and 4) Well suited for decentralized energy production in small-scale installations in locations where biomass or wastes are available, thus avoiding energy expenditure and costs for transport. Thus biological hydrogen production is a promising and sustainable process where renewable organic waste can be used as energy generating source. Availability of huge quantities of wastewater coupled with anaerobic treatment can be considered to be a useful methodology to

reduce pollution load along with hydrogen generation.

For economical production of hydrogen, carbohydrates and proteins are the main components. It was reported that the hydrogen production potential of carbohydrates is approximately 20 times higher than that of lipids (Bartacek *et al.,* 2007). The fermentative evolution is more advantageous than photochemical evolution for mass production of hydrogen by microorganisms, where various wastewaters can be used as substrates.

Of late, hydrogen production through anaerobic fermentation using wastewater as substrate has been attracting considerable attention (Atif *et al.,* 2005). One such feasible source is Dairy wastewater, which contains complex organics, such as polysaccharides, proteins and lipids, which on hydrolysis form sugars, amino acids, and fatty acids (Hawkes *et al.,* 2002).

Anaerobic digestion of organic substrates to produce methane and carbon dioxide has been a well-developed biological treatment for wastewater and solid waste (Wen-Ming *et al.,* 2005). Being the upstream step to methanogenic pathway, acitogenic processes produce hydrogen and Volatile Fatty Acids (VFA) and are thereby considered an effective and promising means to produce clean energy hydrogen (Levin *et al*., 2004). Fermentative hydrogen production can be achieved by anaerobic acid-forming bacteria such as *clostridium* sp. or facultative anaerobes such as *Enterobacter* sp. (Levin *et al*., 2004 and Das *et al.,* 2001).

The current study is aimed as an attempt to check the feasibility of biohydrogen production from dairy sludge, by constructing an anaerobic Continuous Stirred Tank Reactor (CST) using mixed consortia of *Enterobacter cloacae* and *Clostridium pasteurianum*.

MATERIALS AND METHODS

Dairy sludge was collected from Aavin Milk Producers, Chithode, Erode district, Tamil Nadu, India. The sludge can be considered as complex in nature (BOD/COD-0.45) due to the presence of proteins, carbohydrates, and lipids content. After collection, the sludge was transferred immediately to the laboratory and stored at 4°C, and the sludge was not corrected for trace elements deficiency. Sludge was diluted using distilled water to requisite organic loading rate (OLR) prior to feeding and pH adjustment.

PREPARATION OF MEDIUMS FOR CULTURES

Microorganism was obtained from IMTECH, Chandigarh, based on literature being the best hydrogen producers. *Enterobacter cloacae* (MTCC 7079) culture was revived in LB medium as instructed by IMTECH. The media was composed of Tryptone (10 g/L), Yeast Extract (5g/L), NaCl (10g/L), and *Clostridium pasteurianum* (MTCC 116) culture was also revived in Cooked Meat Medium as instructed by IMTECH.

LAB SCALE REACTOR

The dairy sludge was initially tested for the feasibility of hydrogen production in a lab scale reactor. The sludge was pretreated by heating at around 90 °C for 30 minutes and the pH of the sludge was found to be 6.5.Two 500mL serum bottles were taken whose lids had two nozzles each. One was used as the gas collecting chamber and the other as the reactor. The serum bottles were sterilized by passing steam at 121°C

for 30 minutes. Approximately 250mL of dairy sludge was taken in the reactor bottle. To reduce the effect of pH, equal volume of water was added to the reactor. Steam was purged into the reactor to create an anaerobic environment in the reactor bottle. The gas was collected by water displacement method. The continuous production of Hydrogen was monitored for 10 days.

REACTOR DESIGN

A continuous stir tank reactor was designed to study the hydrogen gas production at a small scale. The reactor volume was about 12.5 L. The reactor was designed with a diameter of 20 cm, Height of 40 cm, for which the working volume was measured to be 12.5 L (Figure 1).

REACTOR OPERATION

The fabricated reactor was checked for air tightness by passing steam and was also sterilized suing steam at 121°C for 30 min. the sludge was pretreated by heating at 90°C for 30 minutes. Approximately 2L of dairy sludge was taken and diluted with equal volume of water (Double Distilled and Sterilized), the revived cultures and the medium were added to the reactor. Carbon dioxide was purged into the reactor to create an anaerobic environment in the reactor and also sparged periodically to maintain the pH (measured every 24 hours and maintained at 6.5). Sucrose solution was given as a feed at 100mL/min. on alternate days. The stirrer was run at 110rpm continuously, the reactor was run for 20 days and continuous production of Hydrogen was monitored.

ANALYSIS

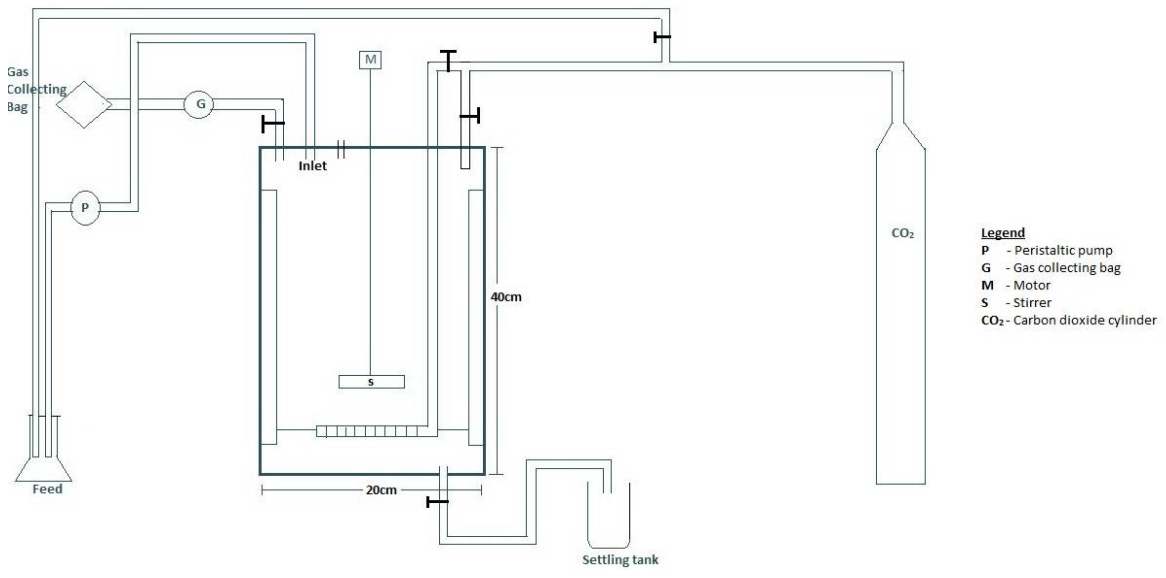
The sample was analyzed using Gas Chromatography (Mayura), carrier gas: Helium, temp 200 °C, at a constant flow with a linear velocity of 5mL/min.

RESULTS AND DISCUSSION

LAB SCALE REACTOR

The pretreated sludge was taken in the serum

Figure1: Schematic Diagram of the Bioreactor (CST)



bottle and kept for gas production for 10 days. Two 500mL serum bottles were taken whose lids had two nozzles each were taken. One was used as the gas collecting chamber and the other as the reactor. 250mL of dairy sludge was taken in the reactor bottle. To reduce the effect of pH, equal volume of water was added to the reactor. Steam was purged into the reactor to create an anaerobic environment in the reactor bottle. The gas was collected by water displacement method (Figure 2).

EFFECT OF pH ON HYDROGEN PRODUCTION

The initial pH of the dairy sludge was 6.0. The optimal pH for anaerobic hydrogen production reported in literature was essentially within the range of 5.5–6.7 (Hawkes *et al.,* 2002 and Fang *et al*., 2002). Initially, the pH was at 6.0; then the pH started decreasing. It may be because of the higher concentration of acids produced during digestion. After five days the pH started increasing. This indicates the conversion of VFA into hydrogen (Fang *et al*., 2002).

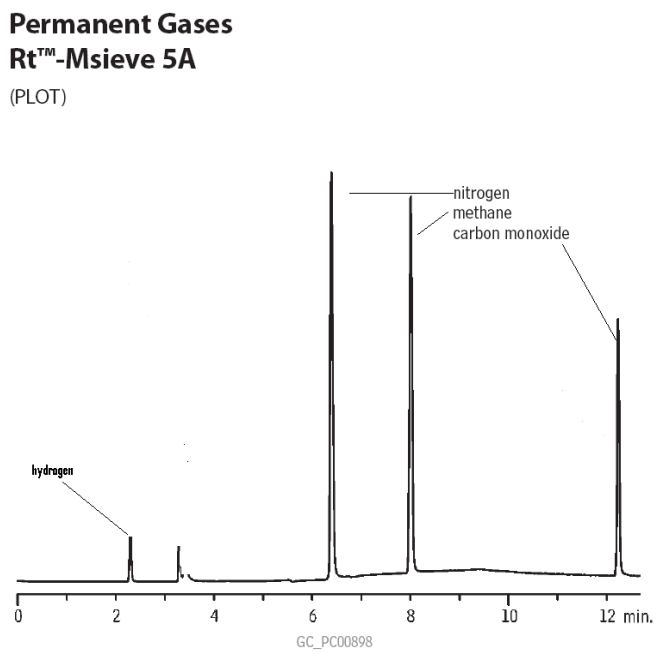
VOLATILE FATTY ACIDS

It is inferred that during the initial days, the Total Volatile Fatty Acids (TVFA) concentration was very high and later, it started to decrease. Production of various VFA in the acitogenic stage may be the reason for the increased TVFA concentration in the earlier days (Shin *et al*., 2004; Han *et al.,* 2005). Then the reduction in TVFA may be due to their conversion into gaseous products (Girija and Kurian, 2004).

CONTINOUS STIR TANK REACTOR

After inoculating with selectively enriched mixed consortia, the bioreactor was initially operated with dairy sludge of 2L of volume by adjusting the pH to 6.0 for a period of 20 days. Subsequent to

Figure 2: Gas Chromatograph of Sample from Lab Scale Reactor



stable operation, the reactor was supplied with sucrose solution 10ml/min on alternative days. The experimental data depicted the feasibility of molecular hydrogen production by utilizing dairy sludge as substrate. Hydrogen production was first observed from 2 days after start-up, and subsequently increased gradually, and approached maximum on 16th day, it remained more or less uniform after that. The system showed cumulative hydrogen yield of 26.6 ml of hydrogen/day at the end of 20 days.

BIOPROCESS EVALUATION

Throughout the experiments pH was maintained in the acidic range varied between 4.0 and 6.0. The acidic pH was considered to be ideal for effective hydrogen production due to repression in methanogenic activity thus indirectly promoting the hydrogen producers within the system (Zhu and Be´land, 2006). However, highly acidic pH is also considered to be detrimental to hydrogen production as it inactivates the hydrogen producing bacteria (Bahl *et.al.,* 1986 and Zhu and Be´land, 2006). A sharp decline in pH along with lower hydrogen and high VFA generation was documented during the initial hours (12 h). Relatively low hydrogen yield was observed at lower pH values (3.98) could be the result of VFA accumulation was observed during the same time interval. After 48 h a sharp rise in pH due to low VFA accumulation or utilization was observed probably due to lower production of VFA or its higher utilization or both resulting in increase of pH as well as hydrogen generation (Figure 3).

The pH was maintained at 6.0 to create a favorable environment for effective functioning of the selected microbial consortia and to inhibit methanogenesis, which facilitate hydrogen production. The adopted HRT of 24 h further helped to control the methanogenic reaction. Sequencing batch operation mode of the reactor used might also have influenced the hydrogen evolution. The sequencing/periodic discontinuous batch mode operation facilitates controlled unsteady-state conditions and exposure time, frequency of exposure and substrate concentration can be set independent of inflow condition (Vijayaraghavan *et al.,* 2006; Yang *et al*., 2006).

Dairy sludge normally composed of higher concentrations of carbohydrates along with proteins (casein) and lipids. Sugar contributes to 97% of the total COD present in the dairy waste. High sugar concentration in sludge generally inactivates the proteolytic enzymes, thereby, decreasing the protein degradation (Fang and Yu, 2000). Recently, anaerobic degradation of proteins and effects of ammonium on the anaerobic mechanism were investigated in detail (Pavlostathis and Giraldo-Gomez, 1991; Gallert *et.al.,* 1998 and Gavala *et.al.,* 2003). In the present study, after 18 h of fermentation, a significant reduction in sugar concentration along with VFA production followed by utilization was observed. From this point (after 48 h), due to activation of proteolytic enzymes visualized by ammonification, the protein degradation pathway instigated along with concomitant increase in total alkalinity (buffering capacity) due to ammonia generation as the end product from protein degradation.

Since there was a high production in volatile fatty acids and a high rate of methanogenic activity, the hydrogen gas production was very low in the initial period of reactor operation. After 48h of initiation period the production of hydrogen gas was found to be around 20% of the gas

produced. Then the hydrogen gas production was gradually increased and it reaches the maximum of 68% of concentration of the gas collected in the 20th day of reactor operation (Figure 4).

Figure 3: Time Vs. Hydrogen Gas

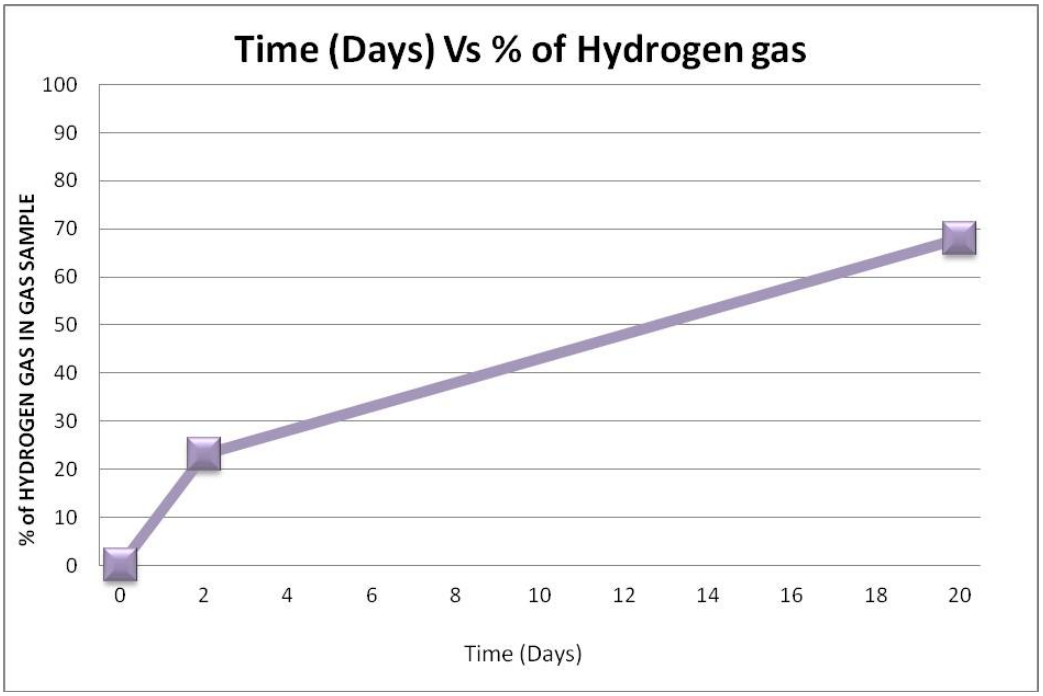
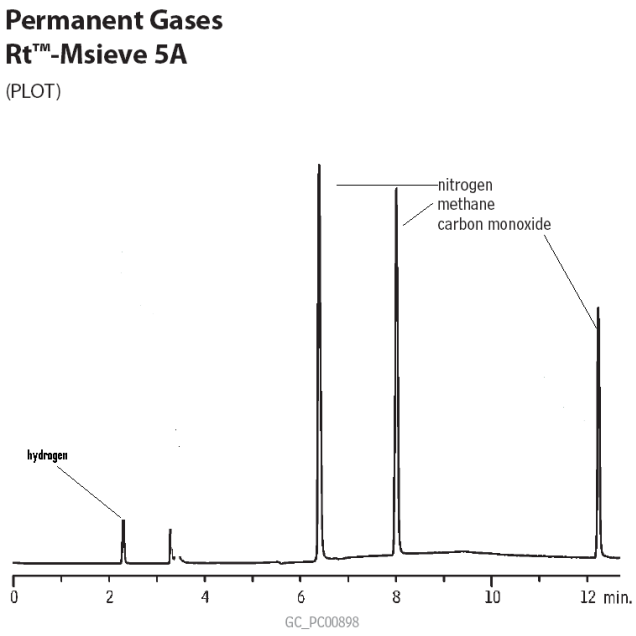


Figure 4: Gas Chromatograph Result for Biohydrogen Production



CONCLUSION

The study demonstrated the feasibility of hydrogen generation from dairy sludge by anaerobic fermentation in a continuous stir tank reactor using anaerobic mixed inoculums. However, the process of hydrogen generation was found to be dependent on the OLR applied. The pre treatment steps adopted for enumerating the hydrogen production from anaerobic inoculum were found to be effective. The selected reactor operating conditions (acidophilic pH 6) were found to be optimum for effective hydrogen yield. Integration of suspended configuration with sequencing periodic discontinuous batch operation was found to be highly flexible, and has a great potential to provide the possibilities of influencing the microbial system by selectively enriching the specific group of micro flora. The system is comparatively easy to operate and cost efficient. Using mixed microbial cultures is considered to be a practical, cost-effective and promising approach to achieve hydrogen production in large scale. The described process has a dual benefit of hydrogen production with simultaneous usage of dairy sludge in an economical, effective, and sustainable way.

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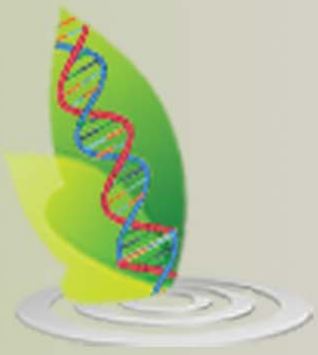

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