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Production of biohydrogen gas from dairy industry wastewater by anaerobic fermentation process

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Abstract
Hydrogen is view as a very clean energy source, since its combustion release mainly dihydrogen monoxide as a reaction product additionally it has advantage of having the highest energy density when compared to any other fuel. Biological production of hydrogen gas has consequential advantage over chemical methods. This research article summaries the fermentative biohydrogen production from dairy wastewater. This research deals with the production of biohydrogen gas and removal of pollution load from dairy wastewater. In this study various parameters like contact time, pH value, reactor operating temperature, Chemical Oxygen Demand (COD), Total Solid (TS), Total Dissolved Solid (TDS) and Conductivity are considered for the dairy effluent. An anaerobic batch reactor attached with magnetic stirrer with working volume of 1.5 litres was constructed and operated for 12 days. The temperature of the CSTR regulated at atmospheric temperature. H_2 gas obtained after 12 days about 128 ml at pH value 6.0-4.9, atmospheric temperature 28±2 ^{0}C and colour of the substrate was almond.

Keywords: Dairy wastewater; Biohydrogen; Fermentation; Yeast; Dark-Fermentation.

1. Introduction

Nowadays, global energy requirements are mostly dependent on fossil fuels, which eventually lead to foreseeable depletion due to limited fossil energy resources. In recent times a great deal of attention is being paid to the usage of hydrogen as alternative and eco-friendly fuel throughout the world (Mohan *et al.*, 2007) [5]. Recent reviews on hydrogen indicated that the worldwide need on hydrogen is increasing with a growth rate of nearly 12% per year for the time being and contribution of hydrogen to total energy market will be 8-10% by 2025 (Pandu and Joseph, 2012) [6]. Biological production of H_2 is one of the alternative methods where processes can be operated at ambient temperatures and pressures, and are less energy intensive and more environmental friendly (Mohan *et al.*, 2007) [5]. One of the great challenges in the coming decade is how to get new renewable energy sources that are environmental friendly and to replace high dependency on fossil fuels. Until recently, almost all of the energy needed is mostly derived from the conversion of fossil energy sources, such as for power generation, industrial and transportation equipment that uses fossil fuels as a source of energy. Fossil fuels are source of non-renewable energy and also have seriously negative impacts on the environment (Wahab *et al.*, 2014) [8]. Hydrogen gas is a clean energy source with a high energy content of 122 KJg^{-1}. Unlike fossil, fuels hydrogen does not cause any CO_2, CO, SOx and NOx emissions producing water as its only by-product when it burns reducing green house effects considerably. Hydrogen is considered to be a major energy carrier of the future and can directly be used in fuel cells for electricity generation. Biological method mainly includes photosynthetic hydrogen production (photo fermentation) and fermentative hydrogen production (dark fermentation) (González *et al.*, 2011) [1].

Dairy wastewater contains complex organics such as polysaccharides, proteins and lipids, which on hydrolysis form sugars, amino acids and fatty acids. In subsequent acidogenic reaction, these intermediate products are converted to volatile fatty acids, which are further degraded by acetogens, forming acetate, CO_2, and H_2 Lastly, both acetate and H_2/CO_2, are converted by methanogens to CH_4. To harness H_2 as end product from anaerobic process instead of CH_4, inhibition of methanogenic reaction and enhancement for acidogenic reaction are important prerequisites. Also optimized operating conditions can result in good H_2 yield. An attempt has been made to harvest H_2 from dairy wastewater treatment through anaerobic fermentation in suspended growth bioreactor using anaerobic mixed consortia, by restricting the methanogenic activity and manipulating operating conditions of the reactor (Mohan *et al*., 2007) [5]. Dark fermentation, traditionally known as anaerobic digestion, is considered as a feasible process because it generates bio-hydrogen from carbohydrate substrates including biomass and organic waste materials. However, the yield of bio-H_2 is relatively low, since H_2 is produced as an intermediate and can be further reduced to methane, acetate and propionate by hydrogen-consuming bacteria (HCB) during dark fermentation. To increase the production rate of biohydrogen, more attention needs to be given to developing methods that inhibit the activity of HCB and exclusively enrich hydrogen-producing bacteria. Critical factors in biological H_2 production are pH, temperature, feed concentration, bacterial population, retention period, etc. (González *et al*., 2011) [1].

The major criteria for the selection of waste materials to be used in biohydrogen production are the availability, cost, carbohydrate content and biodegradability. Simple sugars such as g-lucose, sucrose and lactose are readily biodegradable and preferred substrates for hydrogen production. However, pure carbohydrate sources are expensive raw materials for hydrogen production. Major waste materials which can be used for biohydrogen gas production may be are biodegradable carbohydrate containing and non-toxic industrial effluents such as dairy industry, olive mill, baker's yeast and brewery wastewaters can be used as raw material for biohydrogen production. Carbohydrate rich food industry effluents may be further processed to convert the carbohydrate content to organic acids and then to hydrogen gas by using proper bio-processing technologies (Kargi *et al*., 2006) [3]. Lactose-rich wastewater can be found in the cheese and dairy industry wastewater. Cheese whey contains about 5% lactose, which can be a substrate for fermentation purposes (Hassana *et al*., 2009) [2], conducted experiment to study the possibility of hydrogen production from crude cheese whey by Clostridium saccharoperbutylacetonicum. Hydrogen production rate was affected by pH with the optimum at mild acidic range. The waste sludge generated in wastewater treatment plants contains large quantities of carbohydrate and proteins which can be used for energy production such as methane or hydrogen gas. Anaerobic digestion of excess sludge can be realized in two steps. Organic matter will be converted to organic acids in the acidogenic phase and the organic acids will be used for hydrogen gas production by using photo-heterotrophic bacteria. Many agricultural and food industry wastes contain starch or cellulose which are rich in terms of carbohydrate contents (Kargi *et al*., 2006) [3]. Starch wastewater has high organic content and suspended solids and nutrients (Tawfik *et al*., 2013) [7].

2. Material and methods

For conducting the experimental work Dairy wastewater collected from Ujjain Dugdh Sangh Sahakari maryadit (Sanchi dairy), Maxi Road Ujjain Madhya Pradesh, India was used as substrate. The dairy wastewater was the mixed effluent from the different activities regarding the washing of equipment and making of products. The characteristics of the dairy wastewater above was considered the mixtures of the effluent from utensil cleaning plus other acuity related to the production site. From the range of the data obtained the Sanchi dairy wastewater sample had slightly different characteristics compared to the reported typical quality of dairy wastewater. The wastewater can be considered as complex in nature due to the presence of proteins, carbohydrates, and lipids content.

Table 1: Characteristics of untreated wastewater sample

S. No.	Properties	Values
01	pH	7.5
02	Total Dissolved Solid (TDS)	2053 mg/l
03	Total Solid (TS)	7235 mg/l
04	Total suspended solid (TSS)	5182 mg/l
05	Chemical Oxygen Demand (COD)	4550 mg/l
06	Biological Oxygen Demand (COD)	2210 mg/l

(Malik V., 2015).

2.1 Sample Preparation Method

After collection, the dairy wastewater was transferred immediately to the laboratory and stored at 4 ^{0}C. A known volume 1000 ml of dairy Wastewater was mixed with 100 ml sewage wastewater, 10 gm. Yeast culture and fungus added to the requisite organic loading rate (OLR) prior to feeding into the reactor and pH adjustment. The whole experiment work was performed at room temperature. The experiment work conducted at the laboratory of the Chemical Engineering Department, Ujjain Engineering College, Ujjain (M.P.) India and sample test were carried out in the laboratory of Pollution Control Board, Ujjain (M.P.).

2.2 Batch mode anaerobic studies:

The production of biohydrogen gas and removal of pollution load were studied by batch technique. These indicate that the dairy wastewater participated as primary carbon source in metabolic reactions involving molecular H_2 generation. The substrate in borosil glass reactor placed on magnetic stirrer for known period of time. The substrate was checked daily for pH, temperature, colour and volume of gas obtain. The balloon test was applied for the analysis of biohydrogen gas. The pH value was obtained using pH meter.

3. Results and Discussion

This Section presents the results obtained from the batch studies of biohydrogen gas production and removal of pollution load by the dairy wastewater as substrates. Industrial effluents and wastewater are one of the significant environmental problems due to their highly toxic nature, so it is necessary to have treatment of wastewater and simultaneously producing valuable biohydrogen gas. The parameters studied include pH, atmospheric temperature and colour of the substrate.

3.1 Effect of time, pH and Avg. Temperature on production of biohydrogen gas by using dairy wastewater as substrate

Effect of various parameters like time, Avg. Temperature, pH and colour changes were observed during reactor operation for production of biohydrogen from Dairy wastewater as substrate (Table No. 2)

Table 2: Various parameters changes during reactor operation for production of bio hydrogen from Dairy wastewater as substrate.

Time in Days	Avg. Temperature in ^0C	pH	Gas obtained in mL	Colour
1	28.2	6	Nil	Antique white
2	29.0	5.9	Nil	
3	28.0	5.8	15	
4	29.2	5.6	20	
5	29.6	5.5	30	Almond
6	28.2	5.4	24	
7	28.0	5.4	18	
8	28.2	5.2	13	
9	28.4	5.2	10	
10	28.9	5.0	8	Blanched Almond
11	29.2	4.9	5	
12	29.6	4.9	5	

3.1.1 Effect of time
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 (2 days) of initiation period the production of biohydrogen gas was found to be around 20ml of the gas produced. Then the biohydrogen gas production was gradually increased and it reaches the maximum of 30ml of the gas collected in the 5th-6th day of reactor operation (Figure 1).

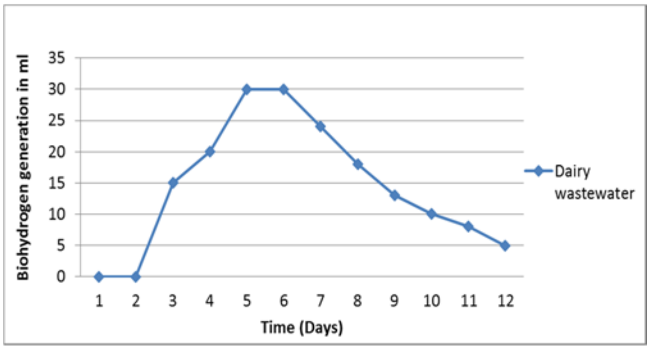

Fig 1: Effect of Time on production of biohydrogen from dairy wastewater.

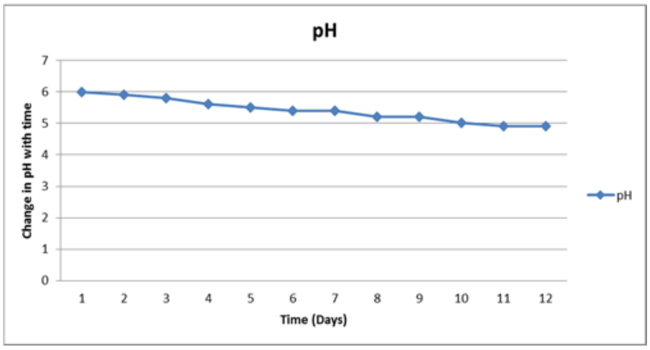

Fig 2: Change in pH with respect to time by dairy wastewater as substrate.

The result obtained from this study are effective for production of biohydrogen gas from Dairy wastewater and the Change in pH (decreases) with respect to time due to acid formation into the reactor show in figure 2.

3.1.2 Effect of PH
Bacteria respond to change in pH by adjusting their activity and synthesis of proteins. This is especially important for fermentative H_2 production where activity of acidogenic group of bacteria is considered to be crucial and rate limiting. The result obtained from this study at pH range 5.4-5.6 is ideal to avoid methanogenesis and produce maximum biohydrogen gas.

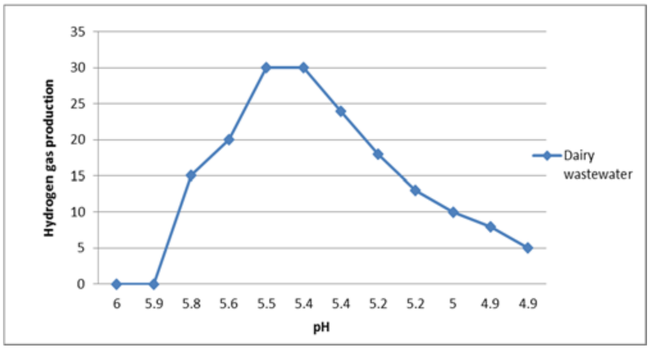

Fig 3: production of biohydrogen gas with respect to pH from dairy wastewater as substrate.

4. Conclusion
The study indicated that the feasibility of hydrogen generation from dairy wastewater by anaerobic fermentation in a batch reactor with magnetic stir. Production of biohydrogen gas and removal of pollution load maximum in Dairy wastewater due to high organic matter (lipid, protein and carbohydrate). The selected reactor operating conditions (acidophilic pH 6) were found to be favourable for effective bio hydrogen gas. The experiment results showed that maximum H_2 production (about 92 ml) at day 4-7 and pH value is 5.4-5.6, substrate colour show Almond and temperature showed 28±2 ^0C. The described process has a dual benefit of biohydrogen gas production with simultaneous reduction of pollution load of dairy wastewater in an economical, effective, and sustainable way.

5. Acknowledgement
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