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Microbial Production of Hydrogen from Sugarcane Bagasse using *Bacillus Sp.*

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Abstract : Production of Hydrogen by batch culture of *Bacillus Sp.,* strain was investigated using Bagasse Extract as a carbon source. The objective of the study was to optimize the cost of production by using Bagasse Extract which is unfit for consumption. In this study, the effect of Time, initial pH, Temperature and Bagasse Extract concentration on Hydrogen production were investigated. The optimum values were found to be: Time- 48 h, initial pH - 7.0, Temperature - 32°C, and Bagasse Extract - 1.0% (v/v). At these optimized condition the maximum Hydrogen production was found to be 0.23 mol H_2 / mol substrate respectively. From these results, it can be concluded that the use of Bagasse Extract for Hydrogen production increases the yield.

Keywords: Biohydrogen, Bacillus Sp., Bagasse optimization

Introduction

Today environmental pollution is a great concern to the world, mainly due to rapid industrialization and utilization. Since large amount of CO_2 are discharged by the combustion of fossil fuel, development of alternative energy resources that have minimal environmental impact is desired. Hydrogen is considered to be an ideal source of energy for the future because it is easily converted to electricity by fuel cells, does not evolve the green house gas carbon-di-oxide in combustion and is cleanly combustible. Among the many process of hydrogen production, microbial hydrogen synthesis is gaining momentum because it is an energy saving process [1], since; the gases produced by biological process mostly contain hydrogen (60-90% v/v). However, different impurities like CO_2 and O_2 are present in the gas mixtures[2]. Microorganisms are capable of producing hydrogen via either fermentation[3] or photosynthesis[4,5]. The former is generally preferred, because it does not rely on the availability of light sources and the transparency of the mixed liquor[6]. Pure cultures known to produce hydrogen from carbohydrates include the species like *Enterobacter*, *Bacillus* and *clostridium*, etc. Studies in the laboratory have concentrated on pure substrates including glucose, starch and cellulose, often in batch processes[7]. Hydrogen production using anaerobic strains from different effluents like orange-processing, milk industry, sugar refinery, paper mill effluents and cow dung have been reported[8]. Recently the development of economical process to produce hydrogen using microorganism from biomass such as waste-water and organic wastes discharged from food industry have been demonstrated[9,10]. Since the sugars such as glucose and starch are especially good substrate for hydrogen producing microorganisms. Most of the work reported for hydrogen production was pertaining to anaerobic fermentation and using photo fermentations[4,5]. In commercial fermentation, maintaining anaerobic condition is somewhat difficult. In the present study an aerobic fermentation was attempted, for hydrogen production. Sugarcane bagasse hydrolysate was used as a sole carbon source for hydrogen fermentation. The conditions were optimized for the *Bacillus Sp.,* strain for the economical production of hydrogen.

Materials and Methods

Acid Treatment

Hydrolysis of bagasse was carried out by treating of powdered bagasse with different acids. Five gram of each sample along with 100 mL of different dilute sulfuric acid concentrations such as 1, 2, 3, 4 and 5% (v/v) were placed in an autoclave (Hitech equipment, India) and hydrolyzed for 1 h (121 °C and 15 Psi). The sample was cooled in a tap water and filtered through filter paper. The filtrate was collected separately for all the runs and the sugar content was assayed as given below. The acid hydrolysates of all the substrates were neutralized using 1 N NaOH.

Bacterial strain and growth condition

The strain which gave maximum hydrogen yield and better substrate consumption was selected and used for all hydrogen fermentation studies. *Bacillus Sp.,* MTCC, 297 used in this work was obtained from MTCC, Chandigarh. The organism was maintained on agar slants by keeping it in refrigerator at 4°C and was sub cultured at fortnight time interval. The culture was maintained on agar slant [0.1 %(w/v) Beef extract, 0.2 %(w/v) Yeast Extract, 0.5 %(w/v) Peptone, 0.5 %(w/v) NaCl, 51.2 %(v/v) Bagasse Extract (equivalent to 1.0g% (w/v) Glucose) and 1.5 %(w/v) Agar]. About 100ml of the Bacillus medium containing 51.2 %(v/v) Bagasse Extract as carbon source with pH 7 was sterilized in an auto clave and then cooled to room temperature. A loop full of the test organism taken from the sub-cultured slant was inoculated. The cultures were incubated in shake condition at 30°c in a rotary shaker incubator (Remi equipments, India) at 200 rpm for one day. A one day pre-grown culture of 2.0 %(v/v) was used as an innoculum for the hydrogen fermentation medium.

Analytical Methods

Estimation of total gas

The gas produced during the fermentation was collected in a graduated aspirator bottle by water displacement method. The total gas evolved was noted as the volume of water replaced by the gas. The percentage of hydrogen present in the gas was assayed by the following procedure. The hydrogen produced is expressed in terms of yield and is calculated as mentioned below.

Yield of Hydrogen = Amount of H_2 produced (mol) /Amount of substrate consumed (mol)

Estimation of Hydrogen

The amount of gas produced in the batch reactor was recorded during regular time intervals using water displacement method at regular time intervals. The amount of hydrogen constituted in the total gas was determined with a Gas chromatograph (NUCON 5765,) equipped with a Thermal Conductivity Detector and a 2.0 m (1/4 in. inside diameter) steel column filled with Porapak Q (50/80 mesh) using nitrogen as the carrier gas at a flow rate of 30 ml/ min. Injector, Oven and Column temperature was set at 150°c, 80°c and 200°c respectively. The gas samples for GC analysis were collected through a hypodermic needle fixed in the stopper. A 5ml gas sample was injected in to the GC prior to the analysis. Pure Hydrogen (99.8% purity) was injected to obtain the necessary standard. Other gases (CO_2 and CH_4) that are evolved during the process were not detected in this column. Hence they can be collectively called as other gases amounting for the remaining consumption for the total value.

Results and Discussion

Effect of acid concentration on the hydrolysis of substrate

In order to get an optimum conversion of substrate, the experiments were carried out for various acid concentrations. Different concentration of sulfuric acid and 1N HCL such as 1, 2, 3, 4 and 5% (v/v) were used to hydrolyse the substrate of sugar cane bagasse. All hydrolysis studies were carried out in an autoclave for 1 h at constant temperature (121°C). 1% (v/v) sulfuric acid concentration gave the highest glucose yield of 0.25 g g~' for the substrate of sugar cane bagasse (Fig. 1). At higher sulfuric acid concentrations say 2, 3, 4 and 5% (v/v), the yield dropped rapidly. The yield decreased as acid concentration increased. Higher glucose yield was obtained at a lower temperature, indicating that glucose decomposition rate was slow at the lower temperature (not shown in this text). Overall, the results of acid hydrolysis of different substrate indicate that the effect of acid concentration on the hydrolysis of substrates to glucose is more critical at higher concentrations due to the decomposition of monomeric sugars. For lower acid concentration levels, increased conversions could be achieved by extending the hydrolysis time[11].

Effect of pH

The effect of initial pH on production of hydrogen using newly *Bacillus Sp.,* strain was investigated by conducting the fermentation for 48 h at different pH ranging from 5.0 to 8.0. Initial pH of the medium influences the rate of hydrogen production**.** It was found that the hydrogen production increases with increase in pH up to 7 and then decreases. At lower pH there could be increased formation of acidic metabolites, which destabilize the cell's ability to maintain internal pH, resulting in lowering of intracellular level of Adenosine triphosphate (ATP) and inhibiting glucose uptake[12] . An optimum pH was found to be 7.0 which results the higher yield of hydrogen (0.12 mol H_2/mol substrate) (Fig 2). Similarly, the total gas production is also follows the same trend as the hydrogen profile. Fall in total gas production was observed at two extreme pH levels when compared to neutral pH. At the pH range from 5.5 to 7.0, a phenomenal increase in the activity of the strain was observed. A difference in the projection of hydrogen was observed between the pH ranges from 5.5 to 7.0. This may be due to the lower metabolic activity of the organism in turn it lowers the enzyme secretion which involved in hydrogen production Pathway.

Effect of Time

The Effect of Time study on hydrogen production was carried out for 60 h at room temperature 30°C. The culture was inoculated with 2.0% (v/v) in a pre-cultured broth. The optimum time was found to be 48 h, at which a maximum yield of 0.13 mol H_2 /mol substrate (Fig 3) was obtained.

Effect of Temperature

The Effect of temperature on hydrogen production were studied by conducting the experiments for 48 hrs at various temperatures ranging from 30 to 40°c. It was observed that hydrogen rate increased with operating temperature until 32°C but dropped significantly at 40°C (Fig 4). Drop in hydrogen

production rate at 40°C could be attributed to the protein deactivation. The optimum temperature for hydrogen production was found to be 32°c because it gives a maximum yield of 0.2 mol H_2 /mol substrate

Effect of substrate concentration

Initial bagasse concentration of 0.5% showed highest production rate, hydrogen production rate was maximum at 1.0% (Fig 5). The rate increased with increase in initial bagasse concentration from 0.5 – 3% but dropped at 1.5% indicating substrate inhibition. Several authors have reported similar profile between initial substrate concentration and H_2 production rate[12,13,14,15]. As the initial Bagasse Extract concentration increases, the production of total gas, hydrogen and ethanol increases and reaches a maximum of 0.21 mol H_2 /mol substrate of 1.0 %(v/v) bagasse extract concentration. Further increase in bagasse extract concentration drastically affects the growth rate of the organism that results with the fall in production of total gas and hydrogen. It is concluded that the carbon source level considered to be important parameter because the gas production is completely depends on the growth rate of the organism.

Effect of inoculum

Up to 4% inoculum volume, there was increase in hydrogen production rate with the increase in inoculum volume (Fig 6) but thereafter the rate reduced at 6% and 8%. At higher inoculum volume, more carbon source was devoted for biomass than hydrogen production. The optimum innoculum for hydrogen production was found to be 4 % (v/v) because it gives a maximum yield of hydrogen 0.23 mol H_2 /mol substrate.

Conclusion

Microbial production of hydrogen using *Bacillus Sp* strain was investigated and the factors affecting production were optimized. The initial pH of the culture medium significantly affects the total gas evolved and the hydrogen contribution content in it. A suitable bagasse extract concentration level is essential to get high hydrogen production; however, excessively high or excessively low concentrations of bagasse extract level affect the growth of organism resulting in reduced production. Temperature is another important factor and it also affects the growth of the microorganism. The optimum values were found to be: Time- 48 h, initial pH - 7.0, Temperature - 32°C, and Bagasse Extract - 1.0% (v/v). At these optimized condition the maximum hydrogen production was found to be 0.23 mol H_2 /mol substrate respectively. From these results, it can be concluded that the use of Bagasse Extract for Hydrogen production increases the yield.

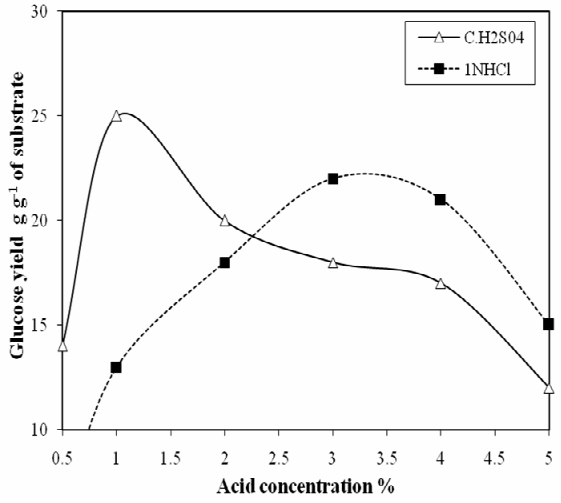

Figure 1 Effect of acid concentration on glucose yield in batch culture

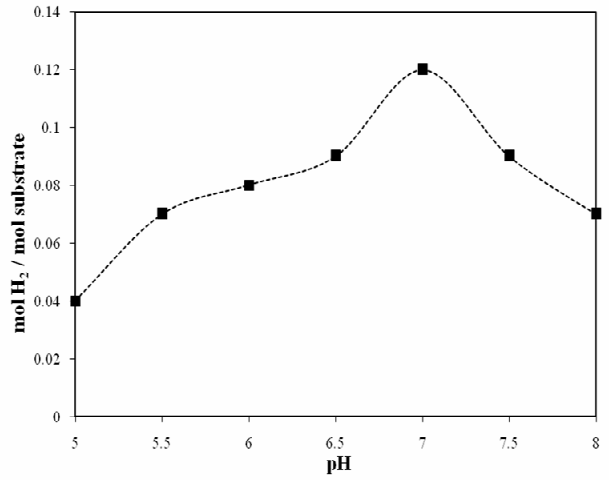

Figure 2 Effect of pH on hydrogen production in batch culture

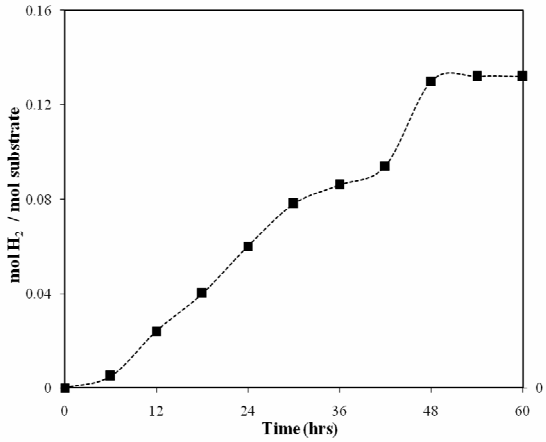

Figure 3 Effect of time on hydrogen production in batch culture

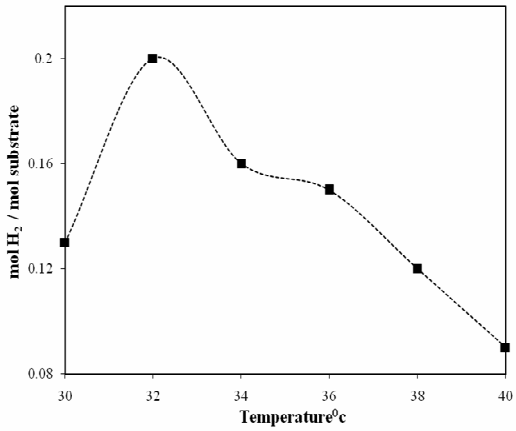

Figure 4 Effect of temperature on hydrogen production in batch culture

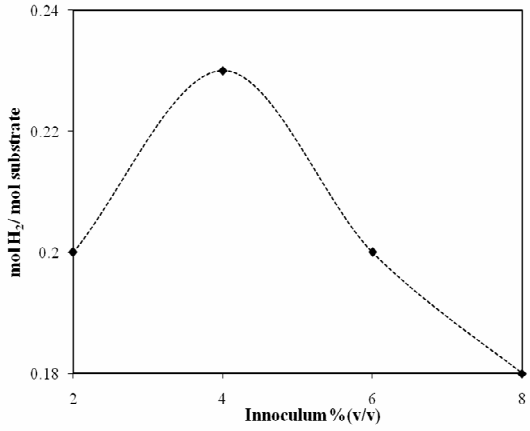

Figure 6 Effect of innoculum age on hydrogen production in batch culture

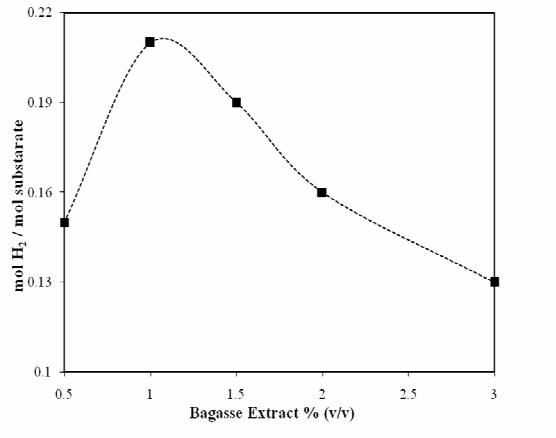

Figure 5 Effect of substrate concentration on hydrogen production in batch culture

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