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Studies on optimization of fermentative hydrogen production by an isolated strain

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ABSTRACT

The facultative strain of bacteria capable of producing hydrogen was isolated from soil sample rich in decomposed biomass. The isolated strain was characterized and the process parameters, namely pH, temperature and fermentation time were optimized for the maximum hydrogen production. The optimum conditions were found to be: pH-7, temperature-34ºC and fermentation time-42hrs. Under these optimum conditions, the isolated strain achieved the highest hydrogen production rate of 2.84 ml H_2/L.h.

Keywords— *Biohydrogen, Facultative bacteria, pH, Temperature, Fermentation time*

1. INTRODUCTION

From food to fuel, hydrogen is considered as a vital component of society. Biohydrogen production is considered as an attractive way for the production of hydrogen compared to other available methods, including hydrocarbon reformation, coal gasification and partial oxidation of heavier hydrocarbons. Unlike other methods, biohydrogen production is catalyzed by microorganisms under ambient temperature and pressure (Lee et al., 2011). The microorganism can utilize renewable organic materials for the production of hydrogen, which can provide 2.75 times higher energy yields than hydrocarbons (Han and Shin, 2004; Mudhoo A et al., 2011). The culture conditions of the organism like pH and temperature are known to influence the metabolite levels, including hydrogen (Ferchichi M et al., 2005; Kotay SM and Das D, 2007). The present work focuses on the isolation of hydrogen-producing bacterial strain and the optimization of its process parameters.

2. MATERIALS AND METHODS

2.1 Isolation of microorganism

Facultative bacterial strains capable of hydrogen production were isolated from soil samples enriched with decomposed biomass. Single colonies were obtained by serially diluting the soil samples and plating on nutrient agar. Pure cultures were obtained by plating repeatedly. The isolates were screened based on their higher hydrogen yield. The screened isolates were maintained on agar slants at 30°C and stored at 4°C.

2.2 Batch fermentation studies

Batch fermentation studies were carried out in 250ml Erlenmeyer flasks with 100ml of fermentation medium. The fermentation medium consists of glucose, 10g/L; malt extract 1g/L; yeast extract, 2 g/L; peptone, 5 g/L and NaCl, 0.5 g/L. After adjusting the initial pH to the desired level, the media was autoclaved. The sterilized media after cooling was inoculated with 10ml of 24 hr grown culture. The temperature was maintained by keeping the reactor setup in a thermostat.

2.3 Gas chromatography

The gas produced during fermentation was collected by water displacement method in an inverted conical flask with a sampling septum. Gas sample collected in the conical flask was taken out using a gas-tight syringe and analyzed with a gas chromatograph (AIMIL- NUCON 5765, Mumbai, India) equipped with a thermal conductivity detector and a 2 meter long steel column packed with Porapak Q. Nitrogen gas at a flow rate of 30 ml/min was used as the carrier gas. The injector, oven and column temperatures were 150°C, 80°C and 200°C respectively.

3. RESULTS AND DISCUSSION

3.1 Isolation of hydrogen producing strain

Serial dilution and subsequent plating of soil samples resulted in 10 morphologically different colonies [S1, S2, S3… S10]. Pure cultures were obtained by repeated plating and each isolate was analyzed for their hydrogen production efficiency at a temperature and initial pH of 30°C and 7 respectively. Among the ten isolates, three isolates, S3, S5 and S8 were able to produce hydrogen. S5 being the highest hydrogen producer with hydrogen productivity of 98 ml H_2/L followed by S8 (73.13 ml H_2/L) and S3 (42.15 ml H_2/L) were selected for further studies.

3.2 Effect of pH

Though controlled pH is reported to be effective for the enhancement of fermentative hydrogen production (Liu IC et al., 2011), this study is focused only on the effect of initial pH. The effect of initial pH on the production of hydrogen by the isolated strain was studied by varying the pH between 4 and 8, with a constant temperature of 30°C. Fig 1 shows the effect of initial pH on the hydrogen production capacity of the isolated strain. The hydrogen production rate and productivity (98.09 ml H_2/L) were increasing with increasing pH from 5 to 7. Increase in pH after 7 resulted in a decrease in the hydrogen production rate. At lower pH, there could be an increased formation of acid metabolites, which inhibits the glucose intake of the organism and thereby the hydrogen production. Optimum initial pH for hydrogen production was found to be 7 where a maximum hydrogen production rate (2.044 ml H_2/L.hr) and hydrogen productivity (98.09 ml H_2/L) were obtained. Irrespective of the initial pH, the final pH of the fermentation medium was in the range of 4 to 4.3. Many other studies have reported a lower pH as optimum pH for the production of hydrogen (Ren et al., 2008; Saripan AF and Reungsang A, 2012), but there are reports that lower pH of 4 and 4.5 can cause longer lag periods (Lee YJ et al., 2002).

3.3 Effect of temperature

The isolated strain of bacteria produces hydrogen in the mesophilic range of temperature (20-40°C) and the effect of temperature on the hydrogen production were studied by varying the incubation temperature from 30°C to 40°C. Fig 2 explains the effect of temperature on the production of hydrogen. Maximum hydrogen productivity of 119.97 ml H_2/L was obtained at the temperature of 34°C, above and below which a decreasing trend was observed for hydrogen production rate and productivity. Wang J and Wan W (2008) have reported the highest hydrogen production rate at temperature 34.2°C, which is close to the result obtained here. They also showed that a higher temperature of 42.8°C can reduce the lag phase, but with a decreased hydrogen production rate.

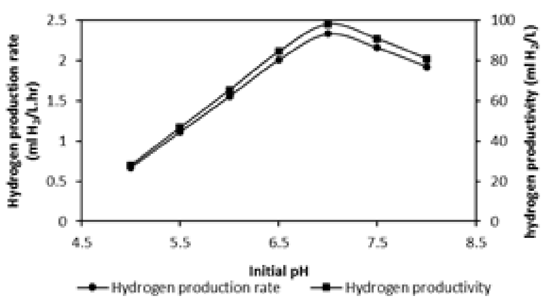

Fig. 1: Effect of initial pH on hydrogen production

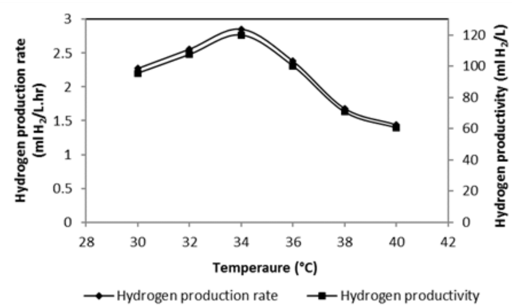

Fig. 2: Effect of temperature on hydrogen production

Effect of fermentation time: The time profile of hydrogen productivity and hydrogen production rate by the isolated strain is shown in Fig 3. Maximum hydrogen production rate and hydrogen productivity of 2.84 ml H_2/L.hr and 119.14 ml H_2/L respectively, were obtained at fermentation time 42 hrs. A decrease in hydrogen productivity was observed after 42 hrs of fermentation. After 42 hrs, gas production was ceased and there was a reduction in the percentage of hydrogen in the collected gas. Dissolution of hydrogen in water might have caused a decrease in percentage hydrogen in the collection flask after 42 hrs.

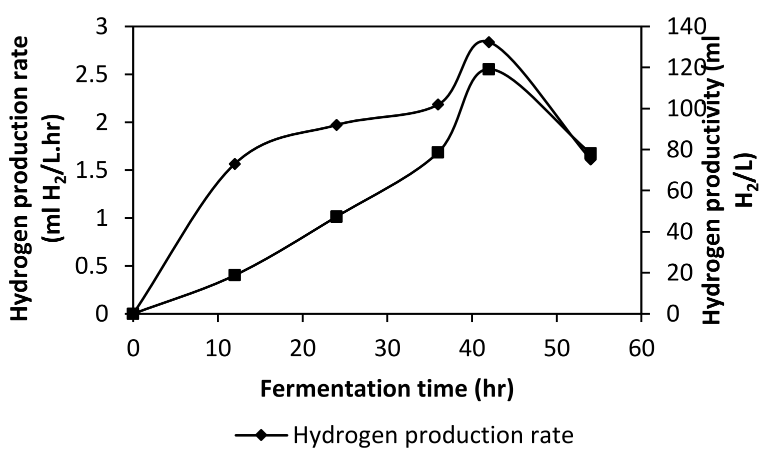

Fig. 3: Time profile of hydrogen production by the isolated strain

4. CONCLUSION

A facultative strain capable of hydrogen production was isolated from soil. The initial pH, temperature and fermentation time were optimized for maximizing the hydrogen production. Utilizing glucose as the substrate, maximum hydrogen productivity of 119.14 ml H_2/L was obtained under the optimum conditions.

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