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# Dark fermentative hydrogen production rate from glucose using facultative anaerobe bacteria E coli

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*Abstract*—**Hydrogen is a best and environmental- friendly energy source, its combustion results only in water as product. Hydrogen may be produced by a number of diverse processes including biological processes using dark fermentation, direct or indirect bio photolysis and photo fermentation. The higher hydrogen production rates were acquired using dark fermentation process on the contrary to other bioprocesses. Dark fermentation can be accomplished by facultative and obligate anaerobic microorganisms. In this study, the hydrogen production was investigated using facultative anaerobe bacteria E coli. The optimization of some key factors for biohydrogen production considerably elevates the biohydrogen production effectiveness.**

**Keywords**—***Biohydrogen; Dark fermentation; E coli; Hydrogen production rate.***

## I. Introduction

Hydrogen gas is a clean and high energy fuel which can be produced in fuel cells for electricity generation. Hydrogen is considered as the major energy carrier of the future. Unlike fossil fuels, hydrogen is not available in nature and requires expensive production methods [1]. Biological production of hydrogen is considered the most environment- friendly route of production of hydrogen, which is the most promising alternative to fossil fuels because it is clean, efficient, and renewable [2, 3].

Biological method mainly includes photosynthetic hydrogen production and fermentative hydrogen production. The efficiency of photosynthetic hydrogen production is low and it cannot be operated in the absence of light, while fermentative hydrogen production can produce hydrogen continuously without light. Moreover, compared with photosynthetic hydrogen production, fermentative hydrogen production has higher hydrogen production efficiency, higher hydrogen production stability, higher feasibility for industrialization, simpler control requirement and lower operating costs. Therefore, Dark fermentative hydrogen production is more feasible, widely used and it has been received increasing attention in recent years [4].

Escherichia coli is a facultative anaerobe organism, it is characteristic of mixed acid fermentation, it is the best-characterized bacterium [5]. E coli produce hydrogen via its formate hydrogen lyase (FHL) system from formate [6].

This Study has been carried out concerning the effects of temperature and pH on fermentative hydrogen production and to optimize the fermentation factors for improved hydrogen production rate using Escherichia coli ATCC 8739 strain and glucose as substrate.

## II. Materials and Methods

E. coli ATCC 8739 strain was cultured in modified M9 medium containing 2 mL of Pfennig and Lippert's trace element solution [7]. The culture bioreactors were sparged with argon (Ar) gas (99.9%) to ensure that they were completely devoid of $O_2$ [8]. Glucose was used as carbon source. Batch cultivations were carried out in bioreactors under varying temperatures (25– 40°C) and pHs (5.5- 7.0) at 150 rpm.

The produced gas is measured by water displacement method. It was collected in a graduate glass tube of 150 ml, containing 20% NaOH solution for selective absorption of carbon dioxide, sealed in its top by a septum for gas analysis. The extremity of the tube was directed through a babbler. The gas composition was confirmed by gas chromatography (SHIMADZU GC-14B) equipped with a thermal conductivity detector (TCD). The injector, the column and the detector temperatures were 100°C, 200°C and 100°C respectively. Argon was used as the carrier gas at a flow rate of 1 l/h [8].

## III. Results and Discussion

### A. *Effect of initial pH*

The effect of pH on hydrogen production rate was studied by varying the initial pH of the M9 medium between 5.5 and 7.5. It can be seen from Fig. 1 that the hydrogen production rate varied considerably with pH, it increased with increasing the pH up to pH 6.5 and then the hydrogen production rate was reduced. Experimental data revealed also that the maximum hydrogen production rate was obtained at pH 6.5.

The lower values of hydrogen production rate, were obtained at pHs extremes, higher than 7 and lower than 6, it is may be due to the effect of this pH range on the transport across the cell membrane, on the activity of some cell

biocatalysts, and on the redirection of their metabolic pathways which are competing for pyruvate, in consequence the pH is able to change the composition of fermentation end products that comprise a mixture of fatty volatiles acids, alcohols and hydrogen. Bowles et al [9] and Chittibabu et al [10] reported that, at lower pH, the increased formation of acidic or alcoholic metabolites, which destroys the cell's ability to maintain internal pH [9], it might have resulted in poor hydrogen production and lowering of intracellular level of ATP, thereby inhibiting glucose uptake [10]. So, the pH gradient across the cytoplasmic membrane determines the glucose transport rate. Additionally, Rossmann et al [11] reported that, at alkaline pH the formate excrete out cells through a symport mechanism Therefore, the intracellular formate concentration reduced and we can conclude that the FHL enzyme complex involved in formate conversion into H2 and CO2 could become less active and thus the conversion rate decreased.

The optimum pH of 6.5 observed in this study to maximize hydrogen production rate is in good agreement with the reported values of similar studies using glucose and a pure culture of E coli strain DJT135 [12]. However, Chittibabu et al. [11] reported that pH 6.0 was most suitable for the hydrogen production by E coli strain BL-21. This disagreement in performance of bioprocess with respect to the initial medium pH for enhancing the rate of hydrogen production is dependent on the mechanism of fermentative hydrogen production, on the type of microorganisms and on the experimental conditions.

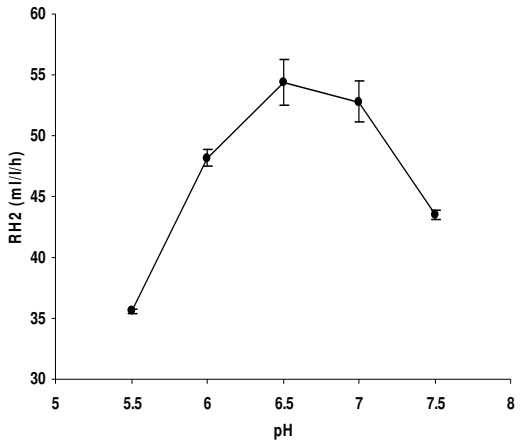


Fig. 1. Effect of pH on hydrogen production rate

*B. Effect of Temperature*

In order to determine the optimal temperature for elevating hydrogen production rate by E coli ATCC 8739 from glucose, the effect of temperature ranging from 25°C to 40°C on fermentative hydrogen production was investigated in batch tests as presented in Fig. 2.

The results showed that the hydrogen production rate increased with increasing temperature from 25 °C to 35°C, however, it decreased with further increasing temperature from 35°C to 40°C. The possible reason for the development in bioprocess efficiency with increasing temperature from 25°C to 35°C was that the ability of hydrogen-producing bacteria to degrade substrate and produce hydrogen increased with increasing temperatures. Moreover, literature shows that lower hydrogen production rate was observed at the temperatures higher than 45°C [13] because some essential enzymes and proteins associated with cell growth or hydrogen production, such as hydrogenase, may be inactivated by an increase in denaturation rate of the enzymes when the temperature gets too high [14, 15].

Maximum hydrogen production rate was obtained with temperature of 35°C, using E coli ATCC 8739, which was also shown using Escherichia coli DJT135 [12].

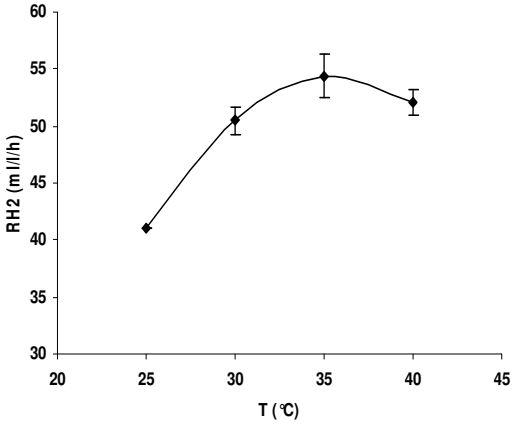


Fig. 2. Effect of temperature on hydrogen production rate

### III.3. Comparative Hydrogen Production Rates of Different Strains of E coli

In the present study, the hydrogen production rate of E. coli ATCC 8739 strain was compared with those of various strains of E.coli in batch process (Table I).

TABLE I. HYDROGEN PRODUCTION RATE OF VARIOUS E COLI STRAINS

| Strain | Culture and Substrate | Hydrogen Production Rate | References |
|---|---|---|---|
| *E coli ATCC 8739* | Batch Glucose | 56.84 ml/l/h | This work |
| *E coli HD 701* | Batch Glucose | 52 ml/l/h | [16] |
| *E coli W3110 (K-12)* | Batch Glucose and formate | 0.3 ± 0.1 mmol/h/g dry cell | [17] |
| *E coli K-12* | Batch Glucose and formate | 45.8 ± 4.1 mmol/h/g dry cell | [18] |

## IV. Conclusions

This work shows that pH and temperature had an significant influence on the hydrogen production rate. The most suitable pH and temperature for optimal performance of the hydrogen production by E coli ATCC 8739 strain were pH 6.5 and 35°C, respectively. Different E coli strains were compared for their capability in hydrogen production under anaerobic conditions. The experimental data revealed that the

E coli ATCC 8739 strain is a promising hydrogen producer bacterium.

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