Abstract

Fuel is an inseparable part of our daily activity. The industrial revolution, on which the economic growth of modern society is based, is due to versatility and efficient utilization of the fossil fuel. However, the reckless use of fossil fuel has given adverse environmental impact, which lead to extinction of various flora and fauna from the earth and other human related health problems. Under such a grim situation, H_2 provides the best solution. It is considered as dream fuel for the future due to its environmental compatibility, renewable nature and versatility in use. The biological process for its production has an edge over its chemical counterpart due to its environmentally compatible process and renewable nature of the raw materials used. Facultative anaerobes of *Enterobacteraceae* family are the most potent biological material, so far as commercial exploitation of biological H_2 production is concerned. Very little information is available on H_2 production using *E. cloacae*. The present study deals with the enhancement of H_2 production using locally isolated facultative anaerobe *E. cloacae* IIT-BT 08. The bacterium produces H_2 at pH 5-6, temperature 32°-40°C using media comprising 1 % malt extract, 0.4 % yeast extract and 1 % glucose or sucrose. The strain also produces H_2 from other carbon sources including complex ones such as cellulose and starch which indicate the potentiality of this organism to use cheaper carbon sources for H_2 production. The maximum H_2 yield (mol / mol) are 6.0 and 5.4 from sucrose and cellobiose respectively. The maximum specific H_2 production rate is 29.6 mmol / (g dry cell h) at about 7th h of fermentation at 36°C with sucrose as substrate. The average H_2 content in the evolved gas is 92 % (v / v).

Enterobacter cloacae IIT-BT 08 also produces both ∝-amylase (130 U/ml) and H_2 (7.6 mmol / g starch) in a batch system using soluble starch as substrate. The optimum temperature and pH for the crude ∝-amylase activity are 60°C and 4.0 respectively.

H_2 is produced continuously in a packed bed reactor using immobilized of *E. cloacae* IIT-BT 08 on lignocellulosic solid matrices which are environmentally friendly. Among the three lignocellulosic carriers used, coir (SM-C) is the best in terms of cell retention (0.44 g dry cell / g dry carrier), packing density (100 g / l reactor volume), cell loading (44 g dry cell / l reactor volume) and hydrogen production rate (62 mmol / l h).

Gas hold-up is a major problem in tubular bioreactor with immobilized cells. It is reduced by 67 % using the rhomboid bioreactor as compared to tubular one. The maximum hydrogen production rate achieved is 75.6 mmol / l h at a dilution rate of 0.93 h^{-1} and recirculation ratio of 6.4. The maximum rate of hydrogen production is 3.4 times higher than that of batch system considering glucose as substrate.

The Cu and Zn content of the anaerobic cells are higher as compared to the aerobic cells. These metal ions may play an important role in the H_2 generation. The experimental results and electron microscopic studies show that coir adsorbed highest number of cells as compared to other solid matrices. The immobilized cells show surface associated growth and binary fission under immobilized and anaerobic conditions.

Improvement in H_2 production is achieved through redirection of metabolic pathways by blocking formation of alcohols and some of the organic acids in *Enterobacter cloacae* IIT-BT 08. The wild type strain is more susceptible to allyl alcohol (7 mmol) and combined effect of NaBr and $NaBrO_3$ (40 mmol each at pH 5.5). On the other hand, the double mutants of *E. cloacae* IIT-BT 08 with defects in both alcohols and organic acids formation pathways can tolerate the lethal concentration of these reagents and has better H_2 yield (3.4 mol / mol glucose) than wild type strain (2.1 mol / mol glucose).

Cell growth and substrate degradation kinetics in batch process of the newly isolated *Enterobacter cloacae* IIT-BT 08 are investigated with the help of Monod model. The values of μ_{max}, K_S and $Y_{x/s}$ of the cell are 0.568 h^{-1}, 3.658 g / l and 0.0837 respectively using glucose as a substrate. The simulated profiles of the substrate concentration and biomass concentration have significant variance with respect to the experimental values. One modified Andrew's model for substrate inhibition has been proposed and found good resemblance with the experimental results. In another study, hydrogen production is found to be growth associated.

Kinetic modeling of continuous H_2 production shows that both plug flow as well as diffusion models are equally valid for the system. The effect of diffusion along the axial direction is negligible on k_p which may be due to small size of the column. The experimental k_p values are plotted against $1/G^n$ for different values of n (between 0.1 to 1.0) and k values are obtained from them. For the present system, it is observed that only when $n \geq 0.91$ the k is positive. The following simulation model is proposed by fitting a straight line for different n values from 0.91 to 1.0:

$$(1 / k_p) = 0.012 + 0.563 / G^{0.99}$$

Both external film diffusion and intrinsic reaction rates are the limiting factors for the observed reaction rate. The effect of external film diffusion on the observed rate decreases with increasing flow rate. A correlation of the form $J_D = K\ N_{Re}^{-0.01}$ is proposed. This implies that J_D is nearly independent of the increase in flow rate.