Abstract

Hydrogen (H₂) is an environmentally clean energy-carrier that may be a valuable alternative to the limited fossil fuel resources of today. Biological hydrogen production processes are carried out at ambient temperature and atmospheric pressure and hence are less energy intensive compared to chemical or electrochemical processes. Among various processes of biological hydrogen production, fermentation, in particular, has several advantages to its credit. It enables higher rate of production and does not rely on the availability of light energy. Moreover fermentative organisms are able to use a large variety of substrates, both pure as well as waste materials without much pretreatment of feed stocks. However, the major bottleneck of dark fermentation process stems from lower achievable yield of hydrogen per mol of substrate and thus the process is not economically viable in its present form. The pathways and experimental evidences cited in literature reveal that at most 4 mol of hydrogen could be obtained from each mol of glucose as substrate during acetate fermentation. Therefore, amelioration of hydrogen productivity by increasing its yield has become a challenging R & D task in hydrogen biotechnology. Two-stage fermentation process, described in the present work, is an approach to that end. The process described in the present work is a combination of dark and photofermentation in a sequential batch mode. In the first stage glucose is fermented to acetate, CO₂ and H₂ in an anaerobic dark fermentation by Enterobacter cloacae DM11. This is followed by a successive second stage where acetate is converted to H₂ and CO₂ in a photobioreactor by photosynthetic bacteria, Rhodobacter sphaeroides O.U. 001.

Preliminary experiments were carried out to study the effect of initial substrate concentration, initial medium pH, temperature and iron concentration on fermentative hydrogen production in a batch process using E. cloacae DM11. The cumulative hydrogen production was found to increase with increasing initial glucose concentration in the MYG medium. However, there was noticeable decrease in hydrogen production beyond 1% (w/v) of initial glucose concentration. At 1% (w/v) glucose concentration, the molar yield of hydrogen was 3.31 mol H₂ (glucose)⁻¹. The pH of 6.5±0.2 at a temperature of ~37°C was found most suitable with respect to maximum rate of production of hydrogen in batch
fermentation. Results indicated that at higher initial pH there was a greater drop in final pH with a shorter duration of hydrogen production. The value of activation enthalpy of hydrogen production was 47.34 kJ mol⁻¹, which was slightly higher than those obtained in other fermentation processes. However, the value activation enthalpy of thermal deactivation, 118.67 kJ mol⁻¹K⁻¹, agrees well with those found in similar processes. The studies on the effect of inoculum size revealed that maximum cumulative volume of hydrogen production was obtained with inoculum of 10% (v/v). Studies on effect of various concentration of Fe²⁺ on hydrogen production and biomass growth revealed that the presence of iron (Fe³⁺) in the media up to the concentration of 20 mg l⁻¹ had a marginal enhancing effect on total hydrogen production. Energy analysis on the basis of gross heating value shows that about 31.89% energy could be recovered as gaseous hydrogen using glucose as substrate and this is higher than the values reported in literature for other biohydrogen production processes.

Spent media from dark fermentation by Enterobacter cloacae DM11 was found to have the ability to photoproduce hydrogen by Rhodobacter sphaeroides O.U. 001 in a two-stage batch fermentation process. The yield of hydrogen in the second stage was about 1.5 - 1.72 mol H₂ (mol acetic acid)⁻¹. This indicates that the overall yield of hydrogen obtained in the combined process was higher than that of an uncombined single process. Increase in acetic acid concentration from 0.035 to 0.25 % (w/v) (5.8 to 41.6 mM) resulted in subsequent increase in the amount of hydrogen production by 4.2 times. Increased light intensity resulted in an increase in cumulative hydrogen production and also rate of hydrogen production. However, light conversion efficiency decreased by increasing light intensity. A four-fold increase in light intensity resulted in three-fold decrease in light conversion efficiency although the cumulative volume of hydrogen gas production increased. It was observed that only a maximum of 0.51% light conversion efficiency could be achieved but at the expense of very low light intensity of 2500 lux (3.75 W m⁻²). Effect of supplementation of various nitrogen sources to the spent media revealed that except for L-glutamic acid no significant improvement in hydrogen production took place.

Various waste water such as distillery waste, casein whey, starch manufacturing waste and
black strap molasses, a byproduct of sugar industry were used as substrate for biohydrogen production. Among these feedstock, casein whey responded well in the production of hydrogen by dark fermentation using *E. cloacae* DM 11. It was observed that maximum 742 ml of hydrogen could be produced from whey sample with 20 times dilution. However, the rate of hydrogen production was slightly higher with 50 fold dilution. Starch manufacturing waste, which showed a high value of COD at the beginning, also responded to dark fermentation by *E. cloacae* DM11. However, unlike casein whey, the increasing dilution of starch waste water resulted in progressively lower cumulative hydrogen production. Hydrogen production was carried out with molasses both with and without the addition of yeast extract. However, molasses supplemented with yeast extract gave better production of hydrogen. Several models were used to predict the kinetic behavior of the biomass growth, substrate utilization and hydrogen production in the two-stage process. Monod model, with incorporation of substrate inhibition term, has been used to determine the growth kinetic parameters for the first stage. The values of maximum specific growth rate ($\mu_{\text{max}}$) and $K_s$ (saturation constant) were 0.398 h$^{-1}$ and 5.509 g l$^{-1}$ respectively using glucose as substrate. The experimental substrate and biomass concentration profiles have good resemblance with those obtained by kinetic model predictions. Testing of variance methods was applied to find out the statistical significance of the experimental data points and those predicted by the model. Substrate inhibition model following Andrew’s equation was found suitable for hydrogen production using glucose as substrate in the first stage of fermentation compared to the classical Monod model. For initial acetic acid concentration of 2.5 g l$^{-1}$ in the spent medium the maximum specific growth rate was found to be 0.099 h$^{-1}$ using Logistic model in the second stage. Both the curve fitting and statistical analysis showed that the modified Gompertz equation was suitable to describe the progress of cumulative hydrogen production.