**ABSTRACT**

Global energy requirements at present are mostly dependent on the fossil fuels, which eventually lead to foreseeable depletion of limited fossil energy resources. Among many alternatives, $H_2$ energy has been receiving special attention as a potential and sustainable replacement for fossil fuels as it has low emission and is environmentally benign and cleaner. $H_2$ can be produced by different routes and from various substrates. Biological production of $H_2$ is one of the alternative methods, which is being focused extensively by the research fraternity more recently. Biological $H_2$ production from a renewable biomass such as the organic fraction of waste or wastewater has considerable potential. In the present study extensive work was carried out on various pretreatment methods of the inoculum for enhancing the production of $H_2$ by anaerobic fermentation. Subsequently, investigations were undertaken to produce $H_2$ from composite chemical wastewater by anaerobic fermentative process in a bioreactor which was fabricated in the laboratory.

Optimisation studies were conducted initially to establish the best suitable operating conditions for effective $H_2$ production, and the conditions showing the best performance were applied for the reactor studies. Anaerobic mixed inoculum was subjected to various pretreatment methods inorder to arrive at the best suitable method for

$H_2$ production. Maximum $H_2$ production was observed for the experiments carried out with “PH” pretreated mixed anaerobic inoculum (0.2031 mmol/g COD) and minimum production was observed with the experiments carried out with “PHC” pretreatment method (0.0425 mmol/g COD). Among the individual pretreatment methods “C” pretreatment showed maximum efficiency for $H_2$ production (0.1854 mmol/g COD). Substrate removal efficiency was found to be highest for the experiments conducted with “PH” pretreated mixed anaerobic inoculum and the minimum was observed in the case of experiments with “HC” pretreated mixed anaerobic microflora.

Optimisation of carbon source was studied using various carbon sources like glucose, fructose, lactose, starch and sucrose to understand their influence on the $H_2$ production. Maximum $H_2$ production was observed in the case of glucose as carbon source (0.2106 mmol/g COD) and least $H_2$ production was observed (0.1256 mmol/g COD) with starch. Samples obtained from the experiments were analysed for the composition of the VFA in order to understand the possible metabolic pathway for the production of $H_2$. Acetic acid was the major acid in all the cases except for starch. In the case of glucose as carbon source maximum acetic acid (97.36%) production was observed when compared to others.

Optimisation studies of the nutrient source were carried out with DAP, combination of $NH_4Cl$ and $KH_2PO_4$ and combination of urea and

$KH_2PO_4$. Maximum $H_2$ production was observed in the case of combination of $NH_4Cl$ and $KH_2PO_4$ as nutrient source (0.2063 mmol/g COD) and minimum was observed in the case of urea and $KH_2PO_4$ (0.1098 mmol/g COD) as a combination.

Influence of fermentation pH and substrate composition (composite chemical wastewater as primary carbon source) on molecular $H_2$ production was studied in batch experiments. Effective $H_2$ production was evidenced at fermentation pH 6.0 (1.25 mmol $H_2$/g COD) compared to 5.0 (0.71 mmol $H_2$/g COD) and 7.0 (0.27 mmol $H_2$/g COD). Fermentation pH of 6.0 was found to be optimum for effective $H_2$ generation with pretreated inoculum. Addition of co-substrate (either glucose containing synthetic wastewater or sewage wastewater) along with chemical wastewater showed enhanced $H_2$ yield. At fermentation pH 6.0 VFA composition showed the presence of acetate, butyrate, and propionate with relatively lower concentration of ethanol. Acid-forming pathway with acetic acid as a major metabolite dominated the metabolic flow during the $H_2$ production.

Optimized conditions obtained in batch studies were used for the reactor studies. $H_2$ production with simultaneous wastewater treatment was studied in biofilm configured periodic discontinuous/sequencing batch reactor using chemical wastewater as fermentable substrate. Reactor was initially operated with designed synthetic wastewater (SW) at

OLR of 4.8 kg COD/$m^3$-day and subsequently operated using composite chemical wastewater (CW) at OLR of 5.6 kg COD/$m^3$-day by adjusting pH to 6.0 to inhibit the methanogenic activity. $H_2$ evolution rate differed significantly with the nature of wastewater used as substrate [SW-volumetric $H_2$ production rate-12.89 - mmol $H_2$/$m^3$-min and specific $H_2$ production rate-0.0084 mmol $H_2$/min-$gCOD_L$; CW-volumetric $H_2$ production rate-6.076mmol $H_2$/m3-min and specific $H_2$ production rate-0.0089mmol $H_2$/min-$gCOD_L$]. Substrate (COD) reduction of 32.4% (substrate degradation rate (SDR), 1.55 kgCOD/$m^3$-day) and 26.7% (SDR, 1.49 kgCOD/$m^3$-day) was observed with SW and CW, respectively.

The influence of substrate loading rate on fermentative $H_2$ production was evaluated in biofilm configured sequencing batch reactor using composite chemical wastewater as substrate. Reactor was operated with selectively enriched anaerobic mixed microflora at different organic loading rates (OLRs, 6.3, 7.1 and 7.9 kg COD/$m^3$ day) after adjusting the feed to a pH of 6.0. Variation in $H_2$ production rate was observed with change in OLR [specific $H_2$ yield – 13.44 mol $H_2$/kg $COD_R$ day (6.3 kg COD/$m^3$ day), 8.23 mol $H_2$/kg $COD_R$ day (7.1 kg COD/$m^3$ day) and 6.064 mol $H_2$/kg $COD_R$ day (7.9 kg COD/$m^3$ day)]. The $H_2$ generation efficiency was found to be dependent on the applied organic loading rate (OLR) of the wastewater. Increase in substrate loading rate showed

marked drop in $H_2$ production and yield. This phenomenon observed might be due to the increase in recalcitrant nature of the wastewater.

The study revealed that among the pretreatments methods of the inoculum, “PH” pretreatment was found to be the best. Among the carbon sources studied glucose was found to give maximum H2 production. A combination of $NH_4Cl$ and $KH_2PO_4$ was found to be best choice for nitrogen and phosphorous source. A pH of 6.0 was found to be optimum for $H_2$ production. Studies carried out with composite chemical wastewater revealed the feasibility of $H_2$ production with simultaneous treatment