CHAPTER IX
CONCLUSIONS

This research investigated the development of biohydrogen production from sweet sorghum syrup and a utilization of hydrogenogenic effluent for polyhydroxylalkanoates (PHAs) production. The experiment was divided into 6 parts. First, the environmental parameters for bio-hydrogen production from sweet sorghum syrup by mixed cultures in batch fermentation were optimized using statistical methods. Three significant variables affecting hydrogen production from sweet sorghum syrup by anaerobic mixed cultures i.e., total sugar, initial pH and $FeSO_4$ were selected by the Plackett- Burman design experiments. These variables were optimized by the Box-Benhken design to maximize hydrogen production. The maximum hydrogen yield (HY) of 2.22 mol H_2/mol hexose were achieved at the optimum conditions attained were 25 g/L total sugar, initial pH of 4.75 and 1.45 g/L $FeSO_4$. At the optimum condition, estimated hydrogen production was 6,864 mL H_2/L which was only 0.04% difference from the actual value, 6,897 mL H_2/L, obtained from the confirmation experiment which suggested that the optimal conditions obtained can be practically applied to produce hydrogen from sweet sorghum syrup with the least error. The optimum condition obtained was used to continuously produce hydrogen in anaerobic sequencing batch reactor (ASBR). The ASBR was conducted with different hydraulic retention time (HRT) of 96, 48, 24 and 12 hr and cycle periods consisting of filling (20 min), settling (20 min), and decanting (20 min) phases. Results indicated that HRT affected to hydrogen production i.e. content, yield and production rate. High hydrogen contents (43-44%) were obtained at longer HRT (48-96 hr) while maximum HY of 0.68 mol H_2/mol hexose obtained at 24 hr HRT. The hydrogen production rate (HPR) increased with a reduction in HRT i.e. from 0.60 L H_2/L-d (96 hr) to 1.12 L H_2/L-d (12 hr). Decrease in HRT resulted in a decrease of soluble metabolite production, especially acetone and butanol. HRT of 24 hr was the optimum condition for ASBR operation indicated by the maximum hydrogen yield obtained.

The effects of HRT on hydrogen production and microbial community analyzed by PCR-DGGE were further investigated. Results indicated that dominant hydrogen producers were *Clostridium butyricum*, *C. acetobutylicum* and *C. proteolyticum*. The present of lactic acid bacteria such as *Sporolactobacillus* sp., *Bacillus* sp., *Lactococcus* sp. and *Lactobacillus* sp. might be responsible for a low HY obtained. These microorganisms could excrete the bacteriocins and lactic acid causing the adverse effect on hydrogen producers.

The effects of organic loading rate (OLR) on continuously hydrogen production from sweet sorghum syrup by mixed cultures in ASBR operating with different OLRs i.e. 25, 30, 35 and 40 g/L-d were examined. Results indicated that OLRs affected hydrogen production, biomass concentration, substrate removal efficiency and microbial community. Biomass concentration increased with an increase in OLRs which is in contrast with substrate removal efficiency. The optimum OLR was found to be 30 g hexose/L-d in which a maximum HY and SHPR of 0.53 mol H_2/mol hexose and 32.52 mL/g MLVSS-d. Since the HY obtained at optimal HRT and OLR were very low, therefore experiment 5 has an attempted to enhance bio-hydrogen production from sweet sorghum syrup in ASBR by nutrient and vitamin supplementations. Results indicated that nutrient and vitamin supplementations could increase HPR (3.2 L H_2/L-d) and HY (1.6 mol H_2/mol hexose) up to 5 folds in comparison to the control (0.6 L H_2/L-d and 0.34 mol H_2/mol hexose respectively).

Throughout the successful process of hydrogen production, large amounts of organic wastewater were generated. It contained residual organic matter such as butyric and acetic acids and residual sugars. The effluent was used to produce PHAs by *Cupriavidus* sp. KKU38 in experiment 6. Results revealed that hydrogenogenic effluent was a potential substrate for PHAs production with the maximum PHAs concentration and content of 0.85 g/L and 65-68% (w/w), respectively. In addition, COD in the effluent was reduced up to 82.73%. Results from this study demonstrated that sweet sorghum syrup can be used to produce hydrogen and PHAs successfully.