CHAPTER VII
CONCLUSIONS AND RECOMMENDATION

7.1 Conclusions

The research was success in producing multibiofuels (biohydrogen, methane and ethanol) from sugarcane by using *C. butyricum* and mixed cultures. Hydrogen was optimally produced from sugarcane juice at an initial pH of 6.5 and initial sucrose concentration of 25 g-COD/L by free and immobilized *C. butyricum*. The maximum hydrogen production at the optimum condition for free and immobilized cell experiments were 2.96 L H_2/L substrate and 2.84 L H_2/L substrate, respectively, and the maximum HY were 2.66 and 2.67 mol H_2/ mol sucrose consumed, respectively. The hydrogen content in the biogas was 44-45% and there was no methane produced. The hydrogen production by *C. butyricum* was essentially butyrate-type fermentation. Main by-product was HBu in which accounted for 45-66% of VFAs. Solvents i.e. ethanol and butanol were detected in this study. Immobilized *C. butyricum* in batch fermentation can be reused up to 5 times without a reduction in HY and HPR. In repeated-batch fermentation, if the HY was considered as the most important parameter for hydrogen production, the optimum the end-of-the-batch harvested volume would be 50% with the maximum HY of 3.07 mol H_2/mol sucrose. However, if the HPR was considered as the most important parameter, the end-of-the-batch harvested volume of 75% with the maximum HPR of 3.50 L H_2/L substrate.day would be the most suitable for repeated-batch hydrogen production. The biohydrogen produced from repeated batch was purified and connected to a fuel cell system in which its ability to generate the maximum and constant electrical power of 49 mW during 8–20 hours of the fermentation could be achieved. In order to supply the electricity for 100 households, the 15,000 KW-h/month (648 GJ/year) was needed (PEA, 2009). Thus, it is necessary to cultivate 22 ha/year of sugarcane to sufficiently supply the substrate i.e., sugarcane juice for the hydrogen-electricity generation system.

The sugarcane juice was also used to produce bio-hydrogen and ethanol by *C. butyricum* under non sterile condition in two stages fermentation. Bio-augmentation

of *C. butyricum* for continuous hydrogen production from sugarcane juice in the CSTR at various HRT was examined. The maximum hydrogen production rate and yield of 3.38 mmol H_2/L substrate.h and 1.00 mol H_2/mol hexose consumed could be achieved at the optimum HRT of 4 h. Results indicated that hydrogen production under non-sterile condition by *C. butyricum* could be conducted. However, the hydrogen content obtained was observed to be low (less than 25%). The relationship of the augmented microorganism i.e. *C. butyricum* and normal flora in the fermentation system under non-sterile condition was analyzed by the Denatured Gradient Gel Electrophoresis (DGGE) method at every HRT. The DGGE results revealed that augmented microorganism, *C. butyricum*, was dominant and played an important role on hydrogen production with the support of normal flora, *K. pneumoniae*, under non-sterile condition. *L. harbinensis*, one of dominant normal flora, was observed to be able to grow in the hydrogen fermentation system but played a role in reducing hydrogen production efficiency resulting in a low hydrogen content obtained.

SCB was used to produce hydrogen by *C. butyricum* in order to implement the total use of sugarcane plant. We demonstrated that acid hydrolysate of SCB was suitable for producing hydrogen by *C. butyricum* due to its high sugar concentration (glucose, xylose, arabinose) and low growth inhibitors concentrations (HAc and furfural). The optimal condition for the hydrolysis of SCB was the use of 0.5% H_2SO_4 under 121 °C and 1.5 Kg/cm^2 in autoclave for 60 min. The highest HPR and HY of 1,611 mL H_2/L.day and 1.73 mol H_2/mol total sugar, respectively, were obtained at the optimal fermentation condition of initial pH 5.5, 37°C, and an initial total sugar in SCB hydrolysate of 20 g-COD/L. The hydrogen performance were compared favorably to those reported in the literature ensuring that SCB hydrolysate could be used as a fermentation media for hydrogen production by *C. butyricum.*

Throughout the successful process of hydrogen production from sugarcane juice, large amounts of organic wastewater were generated. Wastewater contained residual organic matter such as butyric and acetic acids and residual sugar, which were valuable as substrates for methane and ethanol productions. Therefore, the possibility of using hydrogenogenic effluent to produce methane and ethanol was explored in this study. RSM with CCD was used to optimize the factors affecting the

methane production. Results demonstrated significant effect of the model ($p < 0.05$) on the improvement of methane production yield. However, only substrate concentration had significant individual effect on SMP. The interactive effects for all of these factors were found to be insignificant ($p > 0.05$). The optimum conditions for methane production obtained in this study were substrate concentration of 10.10 g-COD/L, ratio of $NaHCO_3$ to substrate of 5.53 and initial pH of substrate of 7.46 which gave the maximum response value for SMP of 1,994.44 mL CH_4/g-VS_{added}. The model validation experiment confirmed that the SMP from experimental data was close to the predicted data by using CCD and RSM.

The possibility of using hydrogenogenic effluent from the CSTR to produce ethanol by carried over microorganism consortia from previous hydrogen fermentation was further explored at various initial pH and sucrose concentration. The highest ethanol yield of 0.31 mol EtOH/mol hexose consumed and ethanol production of 1,418 mg COD/L, respectively, were achieved at the optimum initial sugar concentration of 25 g-COD/L and pH of 7. A simultaneous hydrogen and ethanol production could be found in both hydrogen production using the CSTR and ethanol production in batch system. In addition, the metabolic pathway was shifted from alcohol production to butyrate-type hydrogen production which was confirmed by the HP values that increased from 68 to 166 mL H_2/L substrate when the sugar concentration was increased from 25 to 100 g-COD/L. *C. butyricum* was the dominant species during ethanol fermentation of hydrogenogenic effluent at every pH values and sugar concentrations. The performances of sugarcane as a feed stock to produce multibiofuels are summarized in Table 44.

Table 44 The performance of using sugarcane as a feed stock to produce multibiofuels

Bio-fuel	Substrate	Fermentation mode	Inoculum	Optimum condition				Yield
				Substrate Conc.	pH	Temp. (°C)	HRT (hr)	
H_2	Sugarcane juice	Batch	*C. butyricum* /Free cell	Sucrose 25 g-COD/L	6.5	37	-	2.67 mol H_2/mol sucrose
H_2	Sugarcane juice	Batch	*C. butyricum*/ Immobilized cell	Sucrose 25 g-COD/L	6.5	37	-	2.66 mol H_2/mol sucrose
H_2	Sugarcane juice	Repeated batch	*C. butyricum*/ Immobilized cell	Sucrose 25 g-COD/L	6.5	37	-	3.04 mol H_2/mol sucrose
H_2	Sugarcane juice	CSTR	*C. butyricum*/ Free cell plus indigenous microorganisms	Sugar 25 g-COD/L	6.0	37	4	1.0 mol H_2/mol hexose
H_2	SCB Hydrolysate	Batch	*C. butyricum*/Free cell	Sugar 20 g-COD/L	5.5	37	-	1.73 mol H_2/mol sugar
CH_4	Hydrogenogenic effluent	Batch	Anaerobic sludge/Free cell	VFAs 10.10g-COD/L	7.46	30	-	1994.44 mL CH_4/g-VS $_{added}$
Ethanol	Hydrogenogenic effluent	Batch	Carried over microorganisms consortium	Sugar 25 g-COD/L	7	37	-	0.31 mol EtOH/mol hexose

7.2 Recommendation

Since *C. butyricum* can convert sugar to hydrogen in a low content under non-sterile condition, therefore a genetic modification of *C. butyricum* to enhance its capability to efficiently use sugar should be conducted to eliminate the limitation.