CHAPTER V
CONCLUSIONS AND SUGGESTION

This chapter contains conclusions of the results obtained from this study. Finally, the last section of suggestion is addressed for the further work.

5.1 Conclusions

5.1.1 The characterization of sugarcane juice

Total sugar concentration in sugarcane juice was approximately 200 gL^{-1}. Sugar in sugarcane juice was mainly composted of 130 gL^{-1} sucrose, 9.25 gL^{-1} glucose and 6.20 gL^{-1} fructose. Total soluble solid was approximately 20°Bx while weakly acid showed at about 4.9-5.4. Sugarcane juice was appeared clearly and darkness color and total sugar concentration including sucrose and glucose were increased after treating by heat treatment.

5.1.2 Isolation and screening of PHAs producing microorganisms

There are 36 bacterial strains that were screened from the soil and sugarcane samples. All of them can be grown on sucrose yeast extract medium. Since, 17 isolated strains gave a positive result after staining with Sudan black B. However, only 4 isolated strains showed capability to accumulate of PHAs in short time and gave dark color in their cells. These are coded as SV13, SV19 and SC114. Three of them are gram-positive and only the isolate SC126 is gram-negative. Among 4 isolated strains, SV13, SV19 were chosen to further study. However, the efficient strain of PHAs production was SV13. It was reached to maximum of PHAs content of 0.1770 gL^{-1} at 20 hr. Then the strain SV13 was identified using 16S rDNA gene sequence technique and was found closely to *Bacillus cereus* with 99% identity.

5.1.3 Production of PHAs by pure bacterial strain of *Alcaligenes latus* TISTR 1403

The pure bacteria of *Alcaligenes latus* TISTR 1403 was used to produce PHAs via batch fermentation of sugarcane juice in flask scale. The maximum PHAs content was found about at 0.13873 gL^{-1} after 24 hr cultivation. Compared to the isolated

strain SV13, it gave much lower than for the PHAs production. Therefore, only the strain SV13 was used to produce PHAs using statistical optimization.

5.4.1 Statistical process optimization of PHAs production by isolated strain SV13

5.4.1.1 There were 4 factors of initial sugar, agitation rate, nitrogen and pH were affected on PHAs production especially for DCW at 95, 90, 85 and 70% confidence levels, respectively after screening by Plackett and Burman design. Two factors as pH and nitrogen were affected to PHAs content at 70 and 65% confidence levels.

DCW was increased when of initial total sugar and agitation rate were increased. Increasing of nitrogen was affected as PHAs content and DCW were increased while PHAs content and DCW were decreased when pH was increased.

5.1.4.2 Three factors of agitation rate (100-300 rpm), pH (6-9) and nitrogen (0.0-2.0 gL^{-1}) were obtained when response surface methodology (RSM) by using central composite design (CCD) was applied. The coefficient of determination of R-Squared (R^2) was 0.9464 but the "Pred R-Squared" of 0.6152 was not close to the "Adj R-Squared" of 0.8849. It was indicated that there was a large block effect or a possible problem to the model.

5.1.4.3 Increasing and decreasing productivity were existed as the factors were varied. Increasing of agitation rate, PHAs production was increased while decreasing of pH and nitrogen, PHAs production was also increased.

5.1.4.4 RSM by using CCD was desired to optimize PHAs production by 2 factors as pH (5.0-6.5) and 0.5-2.75 gL^{-1} nitrogen revealed that the coefficient of determination of R-Squared (R^2) at 0.9818 (R^2 = 0.9805 in replication 2) and final equation factors were obtained;

$$Y\ (PHAs) = +696.80 +90.49*pH -2.37*Nitrogen -181.01*(pH)^2$$
$$-63.50*(Nitrogen)^2 +13.95*(pH)*(Nitrogen)$$

In term of pH, pH^2 and $nitrogen^2$ were significant (Prob > *F* less than 0.0500).

5.1.4.5 Validation of response surface model under PHAs production in flask scale was revealed the actual value of PHAs content obtained nearly the prediction value. The validation of PHAs production using the optimize condition in flask was obtained 0.6-0.79 gL^{-1} PHAs, 0.03 $gL^{-1}h^{-1}$ productivity and 0.009 g g^{-1} h^{-1} specific productivity, respectively.

5.1.5 Production of PHAs in shake flask and fermentor using optimized condition

5.1.5.1 The production of PHAs in shake by strain SV13 flask was obtained at 1.546 gL^{-1} PHAs, 38.31% (w/w) PHAs content and 0.0773 g L^{-1} h^{-1} productivity by using optimize condition (pH 5.9 and 1.63 gL^{-1} nitrogen).

5.1.5.2 The maximum PHAs production in 5 L fermentor under optimize condition (pH 5.9 and 1.63 gL^{-1} nitrogen) was obtained at 0.034 gL^{-1} while PHAs content and productivity reached at 2.98% (w/w) and 0.0014 g L^{-1} h^{-1}, respectively.

5.2 Suggestion

Here, these are all suggestions have been made based on the results obtained from this study that would be useful and improve the understanding for the future work.

5.2.1 Since, sugarcane juice mainly contains of sucrose. Primary treatments of the juice perhaps need to consider such as acid or base hydrolysis and enzyme conversion, to convert sucrose into monosaccharide such as glucose.

5.2.2 Highly sugar content in sugarcane should be avoided treatment by heat. The juice should heat at low temperature to prevent the inhibitors such as hydroxyfurfural occurring leads to inhibit growth of bacteria. Moreover, reducing sugar such as glucose or fructose are binding with amino acid group occurring complex compound as glucose-amine compound that made the juice has dark color and leads to low efficient of metabolisms.

5.2.3 The period time to harvest the cells is one of important factors. Suitable time period to harvest cells leads to obtain maximum PHAs production and to prevent the degradation of PHAs inclusion. Therefore, it should be considered.

5.2.4 As pH was strongly affected to PHAs production, therefore pH in range of optimal condition is need to control by adding acid or/and base solution or preparation of cultivation medium in the buffer condition.

5.2.5 The intracellular product of PHAs desires the appropriate method to recovery such as bead beating, high speed extraction, microwave extraction and etc. Especially, in the step of cell disruption, to break cell wall however it should not degrade or destroy biopolymer product. Therefore, chemical reagents or lytic enzymes perhaps are alternative options to investigate for product recovery.