CHAPTER 5

CONCLUSIONS

The scope of research showed and achieved a mixed culture from the upflow anaerobic sludge blanket (UASB) wastewater treatment plantthat produced and accumulated polyhydroxybutyrate (PHB) in cells using glycerol as the sole carbon source to yield pure PHB.Although the results were less than 50% PHB content and then we tried to find many strategies for increasing PHB accumulated in cells such as nitrogen limiting and adding co-substrate.

Initially, the research found that the PHB productivities depended on the cultural broth in the 1-L shaken flask. The cultivation was accomplished an agitation rate of 200 rpm at 30°C, pH 7.A suitable medium was supplemented with 10% v v^{-1} glycerol concentration as primary carbon source and then the percentage of PHB contents was 49.63 ± 1.11% at 5 days (Table 3.1).Subsequently, cultivation in the 1-L shaken flasks was scaled up into a 10-L reactor under similarly condition. The PHB content and PHB productivity were 51.13 ± 1.70 % and 0.009 g PHB L^{-1} h^{-1} at 120 h in the 10-L reactor. In addition, microbial community dynamics during cultivations were examined and applied to tracking the changing bacterial populations pattern with a denaturing gradient gel electrophoresis (DGGE) subsequently the dominant bands were sequencing 16S rRNA for identifying bacterial strains.The 16S rDNA was illustrated the main bacteria in sludge belong to genus of *Bacillus*, *Bacteroides*, *Citrobacter*, *Clostridium*, *Dysgonomonas* and *Klebsiella* on the other hand, *Dysgonomonas* strain could not find in published papers. Furthermore, stability of the community cultures was investigated to explain bacterial communitystructure in each interval time corresponded to the PHB accumulated in cells.

Afterwards, the study tried to induce the percentage of PHB contents by adding glucose as a co-substrate at various concentrations and/or deducting a nitrogen source contained the medium. The results carried out the percentage of PHB content of 69.50 ± 2.29% in medium supplemented with 10 % v v^{-1} glycerol and 3 g L^{-1} ammonium sulphate as carbon and nitrogen source, respectively at 120 h. The nitrogen limiting process in medium could reduce substrate costs and raise the PHB production. Additionally, the research continuously studied the effects of various glucose concentrations at these

conditions. The results evaluated significantly between glycerol and glucose. The results showed the low PHB productivity of bacterial cultures when cultivated in medium without glycerol. The combination of 10% v v^{-1} glycerol and 1 % w v^{-1} glucose was the optimum condition that gave the PHB content as 89.01 ± 0.13 % during 3 days under the batch system on 1-L shaken flasks. Moreover, adding glucose as co-substrate was not only supported the productivities but also saved time. Consequently, the optimum condition was applied to scaling up into a 10-L simplify reactor using the similar control.

The study in the 10-L reactor was 83.01 ± 1.20 % after 3 days that it was less production than 1-L around 0.933 fold time. Nonetheless, a little difference was the optimal feed rates of the carbon and nitrogen substrates, maintaining dissolved oxygen throughout studied, affected by disturbances foaming on surface from turbines even if an optimum dispersion of medium in reactor were not considered which it could be interrupt cell growth and/or PHB accumulated in cells. The reactor could show possibility of scaling up from flask into 10-L bioreactor that was not complicated and more effective if concerning other parameter were considered. Interestingly, the capability of PHB accumulation in mixed culture reached approximately 85% of cell dried weight for 3 days using the batch process that there have never been reported previously. It can use a reference for further research into the manufacture of PHB productions using scale up from shaken flask to 10-L reactor. Furthermore, the results will also support possibility of using mixed culture for expansion into industrial scale.

Finally, additional research was done in microbial communities, carbon sources and fermentation systems from other sources by a ribosomal intergenic spacer analysis (RISA) technique. The RISA was applied to analyse dynamic of microbial communities in activated sludge that were capable of converting waste glycerol to PHA under cultivation on 5% v v^{-1} crude glycerol as a carbon source. 16S rRNA sequences of dominant RISA bands illustrated bacterial communities cultivated in waste glycerol were closely related to *Azoarcus* sp., *Bacillus cereus*, *Bacillus pseudofirmus*, *Flavobacterium columnare* and *Thauera* sp.