CHAPTER FIVE

CONCLUSIONS

5.1 Conclusion

The industrial success of PHA is limited by the high cost of production. Capability of producing PHA from renewable resources, such as palm oil, would help in reducing the cost. Another way to improve productivity is by employing an efficient cultivation technique such as fed-batch cultivation. The technique has been a popular method to achieve high cell density, productivity and yield of desired product. Yamane *et al.*, (1988) discussed theoretically, the economic significance of increasing cell mass concentration for metabolite production. It mentioned that when metabolite accumulate intracellularly, the direct production cost is minimised by obtaining the greatest amount of cells from a fixed amount of nutrient consumed in the shortest operating time.

Comparison of the batch and fed-batch culture indicated that there was a significant increase in cell density and PHA accumulation in *P. oleovorans* when OA was used as carbon substrate (Table 5). There was no significant difference in cell yield when SPO was used as the carbon source (Table 5) during fed-batch cultivation although there was higher accumulation of PHA. Addition of SPO during fed-batch cultivation resulted in appearance of solid suspension which sometimes clumped together. This uneven distribution of SPO could have resulted in less efficient mass transfer and thus affected the final cell yield and PHA accumulation.

Thus, future studies should include investigation of the effect of substrate concentrations and cultivation conditions on growth. Another area to consider is to investigate the effect of supplementation of other nutrients (e.g. ammonium, mineral solution) during fed-batch cultivation.

This study showed that during batch cultivation OA supported growth of *P. oelovorans* better than SPO. This was shown by higher specific growth rate achieved when OA (0.3491 per hour) was used compared to SPO (0.2164 per hour). During batch cultivation, the PHA yield ($Y_{p/s}$) obtained when OA and SPO were used as carbon substrates were 8.05 mg/g and 10.47 mg/g, respectively. This indicated that SPO had supported higher conversion to PHA.

In SCL_{PHA} producing bacteria, PHA accumulation is triggered by limitation of elements such as nitrogen or phosphate (Kim *et al.*, 1996) which affects the intracellular metabolites such as acetyl-coA (Oeding and Schlegel, 1973). β-ketothiolase is the key enzyme in SCL_{PHA} synthesis in *Alcaligenes eutrophus*, but the enzyme may play little or no role in the synthesis of MCL_{PHA} in *P. oleovorans*. Therefore, nutrient limitation imposed during the cultivation might have been unnecessary and might have impaired the active growth. Ramsay *et al.*, (1991) reported that MCL_{PHA} synthesis by *P. oleovorans* grown on octane was not significantly stimulated by nitrogen limitation in a chemostat culture.

Preusting *et al.*, (1992) suggested that for efficient PHA production, it is important that *P. oleovorans* continues growing during the PHA accumulation. It means that complete deficiency of nitrogen source is undesirable. This was contrary to what was reported by Lageeven, *et al.*, (1988). Future studies should consider investigating the effect of introduction of ammonium during cultivation on the PHA production.

Even though *P. oleovorans* is known to accumulate MCL_{PHA}, this study has shown that the PHA monomers ranged from C_4 to C_{14} when either SPO or OA was used as carbon source. The incorporation of SCL_{PHA} had previously been reported (Gross *et al.*,1989 and Preusting *et al.*,1990). Even though a simple explanation such as cometabolism could have resulted in the incorporation of SCL_{PHA}, Tan *et al.*, (1998) however reported that this was not the case in their set of experiments. In relation to the use of palm oil and fatty acids as the carbon source, a detailed study on the specificity of the PHA synthase in *P. oleovorans* could shed light on the incorporation of C_4 monomer in a predominantly MCL_{PHA}. However, one should not eliminate the possibility of contamination by low molecular weight PHB found in the plasma membranes of bacteria. As it is always found associated with other macromolecules, it is called complexed PHB (c-PHB) (Reusch, 1995). At this point therefore, the appearance of C_4 in the MCL_{PHA} was not conclusively attributed to the ability of *P. oleovorans* to accumulate a wide range of 3-hydroxyalkanoic acids from OA and SPO.

POME is a suitable source to obtain bacterial isolates which are able to utilise palm oil as carbon source. This study resulted in the isolation of bacterial isolates capable of utilising PO directly for growth and PHA accumulation. Of these isolates, FLP1 was viewed as a superior isolate in term of high biomass and PHA accumulation. The isolate was identified as *Pseudomonas* sp. and the PHA that it synthesised was characterised as PHB, a SCL_{PHA}.

The successful isolation of PHA-producing bacteria capable of utilising palm oil, e.g. FLP1, provides chances of obtaining novel PHA with unusual combination of hydroxyalkanoic acids. Therefore, the search for new PHA-producing bacteria should be a continuous process.

While this study has isolated a few bacterial strains capable of utilising palm oil for growth and PHA accumulation, the strategies employed could be improved in terms of rapidity and reliability of screening for PHA producers, selection for a wider range of bacteria, and growth maintenance. Recently, Spiekermann *et al.*, (1999) has reported a simple and sensitive method of screening for PHA producers. The study demonstrated the use of Nile Red and Nile Blue A directly to medium and agar plate for staining without negatively affecting the growth of the cells. In terms of bacterial maintenance, extensive subculturing on nutrient agar, a rich medium, could result in loss of desired ability such as PHA synthesis or lipase excretion. Perhaps, synthetic mineral medium could be formulated that could conserve

the needed capability. This medium could be limiting in some nutrients and containing palm oil as the carbon source.

This study has established that *P. oleovorans* could utilise OA and SPO, two readily available renewable resources in Malaysia, as carbon source to synthesise MCL_{PHA}. The yield of PHA from OA and SPO was enhanced in a fed-batch culture compared to a batch culture. A bacterial strain, *Pseudomonas* sp., isolated from POME, could utilise palm oil directly to synthesise SCL homopolymer, PHB. Thus, palm oil appears to be a suitable carbon source which could be converted to MCL_{PHA} as well as SCL_{PHA} depending on the bacteria metabolising the oil.