CHAPTER FIVE

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CONCLUSIONS

In this study, PHA was produced by *P.putida* PGA1 from free fatty acids mixture supplied by SPKO under ammonium-limited condition. SPKO also provided the carbon skeleton for the biosynthesis of PHA precursors which would then be polymerized into PHA. The study was carried out using shake-flasks and fermenter cultivations in both batch and fed-batch modes. The conclusions from the studies are:

(i) Shake-flasks

a) Optimum carbon-to-nitrogen (C/N) ratio, which was represented by SPKO-to-ammonium, for growth and PHA production is 6.9mole:1mole;

b) SPKO concentration of more than 10.0 g/L negatively affected the growth and PHA production;

c) Growth of *P.putida* PGA1 was optimal around 0.1 g/L ammonium ion. Higher concentrations resulted in substrate-inhibition;

d) The uptake of ammonium ion by the organism followed first-order kinetics indicating that the uptake rate directly is proportional to the initial concentration of ammonium ion. The uptake process was quite insensitive to inhibition by 20 mM sodium azide (which is a respiratory chain inhibitor), but severely affected by 78 mM potassium chloride (which caused dissipation of membrane potential normally involved in bacterial uptake processes);

e) Bactopeptone enhanced the PHA yield as compared to yeast extract, beef extract, ammonium and urea. However, a significant difference was observed on the PHA molecular weight when these different nitrogen sources were used. The highest molecular weight of PHA was obtained when its production was low (as in the ammonium cultivation) and the

lowest molecular weight PHA was observed at high production (as in bacto-peptone cultivation). This may indicate that when the PHA production is low, higher rates of chain propagation relative to the rates of chain termination and transfer in the polymerization reaction resulted in polymers with higher molecular weights;

f) Optimum inoculum preparation time for the transfer to fermenter was between 15-20 h.

(ii) Fermenter studies

a) SPKO concentration at 10.0 g/L reduced the oxygen mass transfer coefficient, K_La of the fermenter by 50%. The presence of biomass and fermentation byproducts further reduced the K_La of the system;

b) Fed-batch fermentation mode was superior to batch for PHA production in terms of product concentration (1.49 g/L to 0.09 g/L); PHA content (71.4% to 11.9%); volumetric productivity (0.07 g L^{-1} h^{-1} to 0.0028 g L^{-1} h^{-1}) and PHA yield on SPKO (0.11 g PHA g^{-1} SPKO to 0.02 g PHA g^{-1} SPKO);

c) Feeding with optimum C/N ratio (6.9 mole SPKO: 1 mole ammonium) solution improved the specific PHA production rate, qPHA of *P.putida* PGA1 in the fed-batch fermenter (0.6 g PHA g^{-1} residual biomass h^{-1}) as compared to batch (0.0062 g PHA g^{-1} residual biomass h^{-1}). The presence of small amount of ammonium in the fermentation culture was important to maintain the growth capacity of the organism. This is to maintain microbial metabolic activity as only viable residual biomass has the biochemical capability to synthesize and store PHA;

d) Maintaining ammonium concentration between 0.05-0.1 g/L in the fermentation media was important during the PHA biosynthesis phase. Exhaustion of ammonium caused biomass death and internal PHA

degradation. Thus, ammonium limitation stimulated PHA synthesis whereas its complete deficiency damaged the PHA biosynthetic activity;

e) The monomer composition of the PHA i.e. C_8, C_{10}, C_{12}, C_{14} and C_{16} -3-hydroxyacylesters remained relatively constant (although their relative mole percentages may vary) throughout the accumulation period in batch and fed-batch fermentations;

f) Internal PHA de-polymerization followed zero-order kinetic, when ammonium ions are exhausted from the medium;

g) Oxygen depletion is detrimental to the PHA accumulation with no net increase and often resulted in the de-polymerization of accumulated PHA.

h) Uptakes of SPKO and ammonium during the fermentations followed a zero-order and first order kinetics, respectively. The specific uptake rate constant of SPKO correlated well vis-à-vis to the specific uptake rate constant of ammonium.

i) It was observed that the ATP/ADP and acetylCoA/CoA ratios increased during growth and PHA accumulation phase. On the other hand, ATP/ADP ratio decreased whereas the acetylCoA/CoA ratio became constant during the internal PHA biodegradation phase. During both phases the $NADH/NAD^+$ remained relatively constant, indicating that a tight control of $NADH/NAD^+$ ratio is important in the regulation of *P.putida* PGA1 metabolism.

j) The growth and PHA production by *P.putida* PGA1 in the batch and fed-batch fermentations can be mathematically modeled and simulated using a relatively simple kinetics. However, the lack of rigorous theory underlying the biological mechanism with respect to the ammonium utilization and intracellular PHA metabolism dynamics affected the quantitative accuracy

of the mathematical model. Thus, the 'black-box' approach is not sufficient for the purpose of model construction of the studied process.

k) The simulation results also showed that a two-stage chemostat system is not productive for the PHA production process under investigation.