CHAPTER ONE

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INTRODUCTION

1.1 General introduction

Poly(3-hydroxyalkanoates) (PHA) is a polymer of biological origin which has similar characteristics as the petrochemical derived plastics (Hocking and Marchessault 1994). It has a huge potential of being an alternative to the synthetic plastics in numerous applications which is further made attractive by the fact that the PHA is readily biodegradable and naturally assimilated in the environment. However, the PHA is yet to make a significant impact as a viable choice in the envisaged clean technology world. This is due to the fact that it is very expensive to produce this polymer in bulk amount even for material testing purposes i.e. 18 times more expensive than polypropylene (USD16 per kg PHA to less than USD1 per kg polypropylene) (Lee 1996, Reddy *et al.* 2003). Although the environmental friendly factor is evident, it is not economically-viable in the long term for the consumers to pay a premium for its usage. One way to offset this price difference is to have high PHA yield and productivity such that its production is cost-effective and sustainable in the long run. It is imperative that the current approach on developing the PHA production process emphasizes on these parameters if the PHA were to find its niche in the lucrative consumers market.

PHA is produced employing fermentation process where the biosynthetic capability of microorganisms is exploited to achieve bioconversion of the substrate to the product. The microorganisms accumulate PHA as an intracellular reserved material in response to the imbalance in the growth environment; where a suitable carbon source is present in excess and one or more nutrients are limiting e.g. nitrogen, oxygen, phosphorus etc (Braunegg *et al.* 1998).

PHA can be divided into two classes: (a) the short-chain-length PHA (scl-PHA) where the length of the monomers' carbon atom is four or five. Typical examples of the scl-PHA are poly(β-hydroxybutyrate) (PHB) and poly(β-hydroxybutyrate-*co*-valerate) (PHBV). A well known producer of scl-PHA is *Alcaligenes eutrophus*; (b) the medium-chain-length PHA (mcl-PHA) contains monomers with the carbon atom length ranging from 6 to 18. This class of PHA is primarily produced by the fluorescent pseudomonads (Huisman *et al.* 1989).

The scl-PHA has been produced at the industrial scale as early as in 1982 by Imperial Chemical Industries (ICI) using *A. eutrophus* (Reddy *et al.* 2003). The mcl-PHA, on the other hand, failed to penetrate the consumer market as it has not been produced at a sustainable rate in a large-scale fermentation. This is attributed primarily to the low yield and productivity coupled with expensive carbon feedstock.

There are a wide range of carbon substrates that can be used for the production of PHA. Some examples are shown in Table 1.1.

Table 1.1 Production of PHA using different carbon sources

	Substrate	Organism	References
scl-PHA	Gluconate Propionate Octanoate	*A. eutrophus*	Reddy *et al.* (2003)
	Methanol	*Methylobacterium extorquens*	
mcl-PHA	*n*-Octane	*Pseudomonas oleovorans*	Preusting *et al.* (1993)
	Palm kernel oil	*P.putida*	Tan *et al.* (1997)
	Tallow	*P.resinovorans*	Ashby and Foglia (1998)

For the production of mcl-PHA, one of the most preferred feedstock is the highly reduced and long carbon number molecules such as the animal or vegetable oils or their

free fatty acids. These substrates have high energy content which is excellent for good cell growth and energy metabolism. Furthermore, the structural similarity of the fatty acids with the mcl-PHA made it an even better choice.

It is suggested that these oils in the semi-purified form can be a cheaper substrate for mcl-PHA fermentation as compared to the purified, single-type fatty acids. However, most of the studies on the PHA fermentation employed the latter as the carbon source which gives more defined fermentation components. Relatively high yield and productivity have been reported on the use of pure, single type fatty acids as the fermentation feedstock (Durner *et al.* 2001). The usage of mixed type free fatty acids or their oils, however, have not been a popular choice. As a result, the published literature on this subject is quite scarce.

As the PHA accumulation by bacteria is a response to the imbalance in growth environment, where carbon source is in excess and other nutrient e.g. nitrogen is limiting, this physiological condition is to be exploited in the fermentation process to achieve high yields and productivity. Ammonium ion can be chosen as the limiting nutrient as it is relatively easier to make a bacterial culture ammonium-limited than to make it other element-limited because the growth of microorganisms is more dependent on nitrogen than on other mineral ions and the nitrogen source is assimilated more rapidly than are other mineral ions (Suzuki *et al.* 1986a). However, very limited information is available in the literature on the effect of residual ammonium ion concentration on the production of mcl-PHA using saponified palm kernel oil (SPKO). Also no information is available on the nature of nitrogen source on the yield, monomer composition and molecular weight of the polymer produced. The main objective of the research was to understand the role of ammonium nitrogen on mcl-PHA production.

The specific objectives are:

In shake-flask studies,

(i) to determine the optimum carbon-to-nitrogen ratio (C/N) for PHA production by *P.putida* PGA1; and to find out the effect of increasing SPKO concentrations on organism's growth and PHA production;

(ii) to determine the effect of ammonium ion concentrations on the specific growth rate, μ of the organism;

(iii) to investigate the kinetics of ammonium uptake and relate it to the growth and PHA accumulation processes;

(iv) to investigate the effect of using different alternative nitrogen sources of ammonium on the PHA production, its monomer composition and molecular weight;

(v) to determine the optimum inoculum preparation time for fermenter experiments.

In fermentation experiments,

(i) to study the effect of SPKO on the oxygen transfer capability of a fermenter;

(ii) to investigate the fermentation profiles of batch and fed-batch cultivation modes with respect to the biomass growth, substrates utilization (SPKO, ammonium, oxygen) and PHA production trajectories;

(iii) to study the effect of limitation or exhaustion of substrates such as ammonium and oxygen on the yield and productivity and to compare the batch and fed-batch fermentations in terms of their biomass growth, PHA yield and productivities, the kinetics of PHA biosynthesis and internal degradation along with the kinetics of SPKO and ammonium uptakes;

(iv) to investigate the consistency of the monomer composition of the PHA produced;

(v) to investigate the intracellular metabolites evolution during the PHA biosynthesis and internal biodegradation by monitoring the intracellular metabolites such as ATP, ADP, acetylCoA, CoA, NADH and NAD^+ during the fermentation process as they are central to the metabolism of fatty acids and PHA, to help in the understanding of the more fundamental aspect of the medium-chain-length PHA production process;

(vi) to develop a mathematical model based on the fermentation results, which adequately describes the PHA production process with ammonium as the single limiting nutrient during the fermentation and to use the experimental data in a process design simulation to theoretically evaluate the feasibility of using chemostat culture for PHA production using SPKO.