Kinetic Analysis on Cell Growth and Biosynthesis of Poly (3-Hydroxybutyrate) (PHB) in Cupriavidus Necator H16

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***Abstract*—Poly (3-hydroxybutyrate) (PHB) is a biodegradable polymer that can be synthesized through bacterial fermentation. In this study, *Cupriavidus necator* H16 was used to synthesize PHB by using jatropha oil as its sole carbon source. Experiments using 12.5 g/L of jatropha oil with the highest PHB accumulation of 8.6 g/L at 61.5hr was used to assess its kinetic pattern. Logistic and Leudeking-Piret model was used to evaluate the cell growth and PHB accumulation respectively and the theoretical values obtained corresponded well with the experimental data with slight deviation in cell growth and PHB accumulation at the end of stationary phase.**

***Index Terms*—*Cupriavidus necator,* jatropha oil, kinetic model, poly (3-hydroxybutyrate).**

I. Introduction

The current concerns over the increasing usage of non-biodegradable plastics and its impact to the nature have pushed researchers to develop bioplastics that are biodegradable and environmental-friendly. These bioplastics are mainly polyesters of polyhydroxyalkanoate (PHA) polymers which are produced by various microbes under nutrient-limiting conditions (e.g.: limitation of sodium and phosphorus) but with an excess of carbon source [1].

PHAs are 100% biodegradable and biocompatible polymers with properties such as thermoplastic, elastomer, insoluble in water and also non-toxic in nature [2]. These polyesters have characteristics similar to those of polyethene and polypropylene, and can therefore be used instead of conventional plastics. One particular example of PHA is Poly (3-hydroxybutyrate) (PHB) which is a homopolymer that contains monomers of 3-hydroxybutyrate. It has crystalline properties with a melting point of around 170^{0}C [3]. PHA has garnered great interest due to its biodegradability and biocompatibility with properties such as non-toxic, optically-active, and insoluble in water [2]. PHAs non-toxic property also makes it suitable for use in medical, pharmaceutical and also food industries [4].

Nevertheless, usages of bioplastics from PHA are still limited mainly because its production cost is still very high when compared to petroleum-based polyesters. Previous researches have proven that using substrate from pure glucose or sucrose can be rather expensive for large scale production. At USD 16/kg, the production cost of bioplastics from glucose would be 18 times higher than the conventional polypropylene plastics [6]. Since approximately 50% of PHA net production cost comes from the cost of its raw material [7], utilizing plant oil as the carbon source for PHA synthesis might reduce the PHA production cost and thus making large-scale PHA production a more feasible approach.

Meanwhile, researchers have also focussed on the idea of using industrial and municipal wastes for the synthesis of PHA. Previous researches have reported the use of various industrial wastes such as activated sludge, dairy waste, cheese whey, molasses and so on. In a research done by Chua *et al.* [8], it was shown that PHA accumulation was higher with slight alteration of the sludge by adding acetate into the municipal wastewater to give a PHA yield of up to 30% of the sludge's dry weight. Studies done by Rogers and Wu [9] suggested the use of enhanced biological phosphorus removal (EBPR) in activated sludge to give a yield of 50% PHA content under aerobic and anaerobic condition Cheese whey is another type of solid waste that can be used for PHA synthesis. As a by-product of dairy industry, it is considered as a pollutant due to its high content of [10]. In a study done by Pandian *et al.* [11] a yield of up to 11.32 g/L of PHB dry weight was obtained by using this dairy waste as their main substrate.

Utilizing industrial wastes as carbon source for PHA production might prove to be valuable since this approach has an added advantage of reducing sludge handling cost. However, the low yield of PHA obtained from this approach may not be feasible for large-scale PHA production. Previous researches such as the ones done by Park DH [12] gave promising results with up to 83% of PHB content with soybean oil as their carbon source. Meanwhile, López-Cuellar *et al.* [13] achieved 92% PHA content from fed-batch fermentation of *Cupriavidus necator sp.* by using canola oil as their carbon source. Similarly, Ng *et al.* [14] reported a total of 87% of PHB accumulation from the fermentation of *Cupriavidus necator sp.* by using jatropha oil as their main feedstock. Jatropha oil is known to have a high saturated fatty acid content [14] which will facilitate the synthesis of PHB. Apart from that, *C. necator* has been proven to accumulate PHB up to 80% of its cell dry weight [15].

Nonetheless, to ensure the efficiency of PHB production, it is essential to have a clear-cut mathematical model that could express the kinetic parameters such as cell dry weight and PHB concentration. A well-defined kinetic model could facilitate in problem-solving during large-scale fermentation

Manuscript received March 29, 2013; revised May 30, 2013. This work was funded by Malaysian Ministry of Higher Education under Grant GRS110320.

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DOI: 10.7763/IJBBB.2013.V3.268