Synthesis polylactic acid by lipase catalyzed polymerization biology essay

Polylactic acid (PLA), the biodegradable polymer, has received increasing attention as alternative materials in packaging and biomedical applications. The general method for synthesis of PLA using chemical-catalyzed polymerization produces the catalysts residues which are toxicity. Therefore, the enzymatic polymerization is a green alternative method to decrease this problem. Several researches attempt to improve the optimal condition for synthesis of PLA by using lipase as enzymatic-catalyzed.

For an example, Lassalle et al. (2008) reported the synthesis of PLA

by using lipase as biocatalyst and focused on the procedure. The results found that immobilized CAL-B was the most effective biocatalyst with 60% LA conversion

and 55% recovered solid polymer in the reaction working at 60 °C for

96 h. Furthermore, Hans et al. (2009) researched to confirm the mild reactions conditions for the ring-opening polymerization of lactides by using Novozyme 435 (immobilized CAL-B) 12% wt. concentration in toluene to synthesize the polymer at 70 °C, D-lactide was catalyzed and 33% of monomer was converted and could be isolated a polymer with 25% yield for a number-average molecular weight of 3, 300 g mol-1. Finally, Garcia-Arrazola et al. (2009) reported the synthesis of poly-L-lactide by used immobilized

CAL-B (Novozyme 435) as biocatalyst for the ring-opening polymerization of L-lactide at 65 °C could be achieved using supercritical carbon dioxide (scCO2). The L-lactide monomer could be converted as the PLA with a

molecular weight 12, 900 g mol-1 under the condition at a biphasic scCO2/organic liquid system media and the optimum of temperature for the lipase activity. All of these present studies are the novel route to produce the polylactic acid and relate improvement of the new biomaterials.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

9 Number-average molecular weight as a function of temperature

for the ring opening polymerization of DD-lactide at different

monomer conversion with 15 wt.-% of Novozyme 435 and

a monomer to toluene ratio 1: 3 12

SYNTHESIS OF POLYLACTIC ACID BY LIPASE-CATALYZED POLYMERIZATION

INTRODUCTION

Lipase

Lipases or triacylglycerol acylhydrolases EC 3. 1. 1. 3 are hydrolase which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids under aqueous conditions. In addition, lipases catalyze the tranesterification of other esters under micro-aqueous conditions. The ability of lipases has received increasing attention for used as catalyze in a wide array of biotechnology industry, such as food technology, detergent, chemical industry, cosmetic, organic synthesis, biomedical sciences and pharmaceutical applications (Gupta et al., 2004; Treichel et al., 2010).

Lipases are produced by various plants, animals and microorganisms. Many microorganisms which are known as producers of extracellular lipases, including bacteria, yeast, and fungi. Especially, bacterial lipases and fungal lipases are most widely used as a class of commercial enzymes in many applications. The important commercial microbial lipases are Achromobacter sp., Alcaligenes sp., Arthrobactersp., Bacillus sp., Burkholderia sp., Chromobacterium sp., and Pseudomonas sp. from bacteria which are used

successfully in the market with several products names, such as Lumafast, Lipomax, Combizyme and Greasex (Gupta et al., 2004). Moreover, fungi produces the important commercial lipases are Rhizopus sp., Aspergillus sp., Penicillium sp., Geotrichum sp., Mucor sp., and Rhizomucor sp. (Treichel et al., 2010) which are used in the market with many products names, such as Lecitase, Lipozyme, and Novozym 435 (CAL-B).

Of these, the lipases from microbial have a stability, selectivity, and broad substrate specificity for cultivation such as an applications by used substances form oil mill wastewater, slaughterhouse wastewater, agroindustrial waste and corn steep liquor

(Gupta et al., 2004; Treichel et al., 2010). Therefore, the recent microbial lipases have gained special industrial attention for used as biocatalyst in rapidly growing biotechnology.

Polylactic acid

Polylactic acid or the short name is PLA is a thermoplastic aliphatic polyester which a synthetic polymer based on lactic acid (LA) and have a helical structure was shown in Figure 1. PLA derived from the fermentation of renewable resources such as corn starch, tapioca products and sugarcanes.

Figure 1 Chemical structure of Polylactic acid: PLA.

PLA has received increasing attention as alternative materials in packaging and biomedical applications due to PLA is a biodegradable polymer, it easily degrades by simple hydrolysis of microorganisms under the appropriate conditions (Garlotta, 2001; Avinc and Khoddami, 2009). PLA has a high-

strength, high-modulus, brightness, barrier properties and good moisture management as a result of its interesting for used in packaging and composite materials for clothing applications (Garlotta, 2001). Furthermore, PLA has a biocompatible and bioabsorbable properties which can be used for wide range applications in biomedical and pharmaceutical technology, such as surgical sutures, tissue engineering scaffolds, absorbable bone plates, artificial skin, and controlled drug-release systems (Lassalle and Ferreira, 2008; Avinc and Khoddami, 2009; Hans et al., 2009).

Because of its compost based on a natural substance which make

a biodegradability, PLA is to be a more environmentally-friendly polymer than poly ethylene terepthalate (PET) which is derived from a synthetic petrochemical-based materials due to PLA is lower greenhouse gas emission and significant energy savings, PLA avoids the problems related to plastic waste accumulation. The result of comparison between PLA and PET polymer was shown in Table 1.

Table 1 Comparison of raw material type and possibility of recycling and biodegradation between PLA and PET polymer.

Indexes

PLA

PET

Initial raw material base

Renewable plant stock

Petroleum products

Non-renewable resources

Recycling of polymer wastes

Total recycling possible

Total recycling possible

Biodegradation of polymer wastes

Total

Does not degrade

Source: Avinc and Khoddami (2009)

PLA products are easily composted or recycled under appropriate conditions at the end of the product life. The Figure 2 show the life cycle of PLA material degrades first by microbial hydrolysis, then the carbon dioxide and water which obtained from reaction became the basic necessities for a new growth and leading to produced lactic acid (LA) for re-used as a monomer in the production of a new PLA (Avinc and Khoddami, 2009).

Figure 2 Life cycle of PLA.

Synthesis of polylactic acid: PLA

The synthesis of PLA starts with the extraction of sugars (e. g., glucose and dextrose) from natural substances which used as a substrate in fermentation of lactic acid by microorganisms. Lactic acid (LA) is the starting material for the PLA production process, through polymerization. There are two major routes to synthesize PLA from LA monomer which are showed in Figure 3 (Avinc and Khoddami, 2009).

Figure 3 Polymerization routes to PLA.

From the Figure 3, polymerization routes to PLA are distributed as two processes, the first route is a polycondensation polymerization and the second route is a ring opening polymerization.

The conventional process for synthesis of PLA

The production process to synthesize PLA by polycondensation of LA is the conventional process for making PLA. This process need to carry out under high vacuum and high temperature, solvent is used to extract the water through the condensation reaction (Avinc and Khoddami, 2009).

However, PLA polymer products obtained tends to have low molecular weight. Therefore, the second route is improved by ring opening polymerization of LA which is condensed of water and then converted into cyclic dimer of LA or lactide for used as a monomer in ring opening polymerization. PLA polymer products obtained higher molecular weight than the first route and used milder conditions.

Polymerization of PLA need to use a catalyst for supporting the conversion of LA to PLA. The catalysts are divided into two types, the first is the chemo-process which is the polymerization by used a metal as a catalyst and the second is the bio-process which is the polymerization by used a LA-polymerizing enzyme as a catalyst.

The chemo-process made the residues of heavy metals based catalysts, such as oxides of Zinc (Zn) and Stannum or Tin (Sn) which are toxicity. Furthermore, the process need high purity monomers, high temperature and high vacuum for serving conditions reactions. On the other hand, the bio-process used an enzyme based catalysts such as lipases which are non-toxic. Also, PLA polymer products can be used for biomedical and pharmaceutical applications. Moreover, polymerization reaction can be run under mild and environmentally-friendly conditions (Taguchi et al., 2008; Lassalle and Ferreira, 2008; Hans et al., 2009).

Process for synthesis of PLA by lipase-catalyzed polymerization

From the advantages of the bio-process or the enzymatic-catalyst polymerization, there are several researches attempts to synthesize PLA by used enzyme as catalyst such as lipase-catalyzed in the ring opening polymerization. The reaction of polymerization can be set up follow with the Figure 4. In the reactor compounded with LA, lipase, solvent and purge gas which is used for protection to occur of the regeneration of PLA. Furthermore, the total reactions need to control the optimal temperature and reaction time.

Figure 4 Polymerization reactions to synthesize PLA.

The measurements which used to represent the properties of PLA polymer products are considered in several parameters. The important of evaluations are the conversion of LA, the molecular weight of PLA polymer products, the recovery of PLA and the recovery of lipases at the end of reactions.

Influence of several factors for the polymerization

Production of a good PLA, must be use a good set up reaction of polymerization. Otherwise, the influence of the several factors such as a kind of lipases, enzyme concentration, monomer concentration and temperature needs to be considered together.

Influence of the kind of lipase

Lassalle et al. (2008) researched the influence of the kind of lipase for the synthesis of polylactic acid (PLA) by using the three kind of lipases as biocatalysts. Porcine pancreatic lipase (PPL) from mammalian, Candida antarctica lipase B (Immobilized CAL-B) from fungal, and Pseudomonas cepacia (PCL) from bacterial origin were used in the experiment. The reaction was carried out by operating of LA, lipase, and solvent at 60 oC for 96 h. The performance of the three lipases was evaluated in a term of the conversion of LA to PLA and expressed as percentage (%) conversion.

Table 2 Conversion (%) of LA, isolated enzyme after reaction, recovered PLA, and molecular weight (Mn) (Da) as a function of the kind of the different lipase.

Enzyme

% Conversion

% recovered PLA

% recovered lipase

Mn

(Da)

Imm. CAL-B

58

55

85

446

PCL

88

12

34

400

PPL

96

2

90

768

Source: Lassalle and Ferreira (2008)

The result was presented in the Table 2, using the immobilized CAL-B as catalyst obtained 58% conversion of LA, 55% recovered PLA, 85% recovered lipase, and 446 Da of Molecular weight. For using PCL as catalyst obtained 88% conversion of LA, 12% recovered PLA, 34% recovered lipase, and 400 Da of Molecular weight. For using PPL as catalyst obtained 96% conversion of LA, 2% recovered PLA, 90% recovered lipase, and 768 Da of Molecular weight.

From the result found that higher conversion levels were measured in the case of soluble enzymes, but only traces of solid polyesters were recovered in this cases. In contrast, amounts of solid PLA were recovered using immobilized CAL-B, and the conversion was lower than soluble lipases. For the conclusion of the experiment, the immobilized CAL-B was the most effective biocatalyst with 60% conversion of LA and 55% recovered solid polymer in the reaction working at 60 oC for 96 h.

Influence of the enzyme concentration

There are several researches used the immobilized CAL-B lipase for esterification reaction due to its high catalytic activity but it does not propagate in polymerization reaction. So, Hans et al. (2009) researched to

confirm the synthesis of PLA by immobilized CAL-B (Novozyme 435) catalyst in ring opening polymerization of lactide. The reaction was improved by adding nitrogen gas into the reactor for protected regeneration of PLA to LA and used toluene as a solvent for enzymatic polymerization. The objective of this study is find an optimal reaction condition such as enzyme concentration, monomer concentration and optimal temperature.

Figure 5 Lactide conversion as a function of reaction time for the ring opening polymerization of DD-lactide at 70 oC with a monomer to toluene ratio of 1: 2 (g: mL) and use different concentration of Novozyme 435.

The first factor is influence of the enzyme concentration. The result was presented in Figure 5, the overall monomer conversion increases when increasing amounts of enzyme. The reaction catalyzed with 25 wt.-% of enzyme up to 100% monomer conversion after 2 days, while the reaction catalyzed with 10 wt.-% of enzyme up to only 25% monomer conversion.

Figure 6 Molecular weight as a function of conversion plots for the ring opening polymerization of DD-lactide at 70 oC with a monomer to toluene ratio of 1: 2 (g: mL) and use different concentration of Novozyme 435.

In contrast, the relation of molecular weight and conversion are represented in Figure 6. The result found that 25 wt.-% of enzyme obtained the molecular weight of PLA lower than 15 wt.-% of enzyme and 10 wt.-% of enzyme at the same conversion due to higher enzyme concentrations have more water which is introduced into the reaction and leads to a decrease of the molecular weight.

Amounts of water within the reaction have an influence for the molecular weight PLA polymer products (Hans et al., 2009). The normal of reaction for synthesis PLA by lipase-catalyst distribute into 3 step, the first step is the monomer activation which is the combination of lipases and lactic acid (LA), then the lipase-LA combine with water for extension of pre-polymer and release the component of lipase-OH in the initiation step, the last step is the chain propagation which increase the number of monomer within polymer chain. In any case, if there is a lot of water in the reaction, it will occur the conformation of the other component as free water and a linkage between lipase and water by loosely bound and tightly bound. The free water and lipase-water loosely bound can break the polymer chain in the initiation and affect to decrease a molecular weight of PLA polymer products.

Influence of the monomer concentration

Hans et al. (2009) studied influence of the monomer concentration by expected that increasing monomer concentration, the polymerization rate and the overall monomer conversion will increase.

Figure 7 Lactide conversion as a function of reaction time for the ring opening polymerization of DD-lactide at different monomer to toluene ratio (monomer concentration) at 70 oC with 15 wt.-% of Novozyme 435.

From the Figure 7 observed at the monomer to toluene ratio 1: 2 and 1: 3, the high conversion increase and then decrease when the monomer concentration decrease. Exclusion a monomer to toluene ratio 1: 1, the conversion is also lower which might result from a poor solubility of the substrate and the precipitation of PLA.

For the conclusion of the experiment, the immobilized CAL-B was the most effective biocatalyst with 33% of monomer was converted and could be isolated a polymer with 25% yield for a number-average molecular weight of 3, 300 g mol-1.

Influence of the temperature

Furthermore, Hans et al. (2009) expected that the temperature affect to PLA polymer products in ring opening polymerization as show in the Figure 8.

Figure 8 Lactide conversion as a function of reaction time for the ring opening polymerization of DD-lactide at different temperatures with 15 wt.-% of Novozyme 435 and a monomer to toluene ration 1: 3.

From the Figure 8 observed that increasing temperature, the monomer conversion decrease. At 80 oC and 90 oC, a monomer conversion does not exceed 25 % in 2 days while at 60 oC and 70 oC, a monomer conversion reaches about 60 % and at 50 oC, a monomer conversion reach to 80 %.

In the case of ring opening polymerization of lactide by lipase-catalyst at higher temperature might induce an enhanced deactivation of the enzyme which led to low monomer conversion.

Figure 9 Number-average molecular weight as a function of temperature for the ring opening polymerization of DD-lactide at different monomer conversion with 15 wt.-% of Novozyme 435 and a monomer to toluene ratio 1: 3.

The relative of molecular weights and temperatures at different conversions are presented in the figure 9, at 60 % and 50 % conversion obtained a highest molecular weights at 60 oC and drop off at higher temperatures. Explanation is an increase temperature release of free and loosely bound water which make denaturation of the enzyme. The other reason is a decrease in temperatures also induces a lower solubility of the polylactide and affect difficult to maintain a homogeneous solution.

The improvement of process for lipase-catalyzed synthesis of PLA

From the study about the influence of several factors for ring opening polymerization by lipase-catalyst observed that the enzymatic synthesis of PLA by use volatile organic compounds solvent do not encouraging due to a poor solubility of the substrates in polymerization reactions. In addition, the high temperature to reach the melting point of LA at 92 oC-95 oC might cause partial enzyme deactivation (Garcia-Arrazola et al., 2009).

Garcia-Arrazola et al. (2009) improved the polymerization reaction to obtain PLA by used supercritical carbon dioxide (scCO2) as a solvent replacement of the volatile organic compound (VOCs). The advantage of scCO2 is non-expensive, non-flammable, non-toxic, low melting point, low viscosity, high diffusion coefficient, and friendly in synthetic processes.

Table 3 Results obtained for the ring opening polymerization of L-LA in scCO2 with

20 % (w/v) of L-LA and initial water content (aw) < 0. 16.

Entry

Biocatalyst (wt%)

Time (days)

Polymer yield (%)

1

10

1

5. 70

2

10

2

9. 77

3

10

3

11. 03

4

10

4

1. 64

5

15

1

3. 2

6

15

3

5. 16

7

15

5

5. 35

8

15

7

<1

Source: Garcia-Arrazola et al. (2009)

The result from this improvement of process by use scCO2 as a solvent was presented in the Table 3, the ring opening polymerization of LA at 10 wt% of enzyme obtained highest yield of PLA as 9. 77 % and 11. 03 % in 2 days and 3 days respectively. When increase amounts of enzyme to 15 wt%, polymer yield was lower than 10 wt% of enzyme with obtained highest yield of PLA only 5. 16 % and 5. 35 % in 3 days and 5 days respectively.

The experiment pointed out that 10 wt% of the immobilized CAL-B in the ring opening polymerization for synthesis of PLA by use scCO2 at 300 bar and 65 oC is the most appropriate condition to obtain PLA with a molecular weight as 12, 900 g mol-1.

CONCLUSION

Polylactic acid (PLA) is a biodegradable which derived from renewable resources. The synthesis of PLA from ring opening polymerization of lactic acid (LA) start at polycondensation of LA for obtain lactide formation, then lipase catalyze the reaction for obtain PLA. Using lipase-catalyst in ring opening polymerization is the environmentally-friendly reaction due to lipase-catalyst is non-toxic and can be run under a mild condition. Furthermore, PLA polymer product can be used in biomedical and pharmaceutical applications.

The appropriate of synthesis of PLA from LA by use lipase as biocatalyst must consider in several factors such as the kind of lipase, enzyme concentration, monomer concentration, reaction temperature and the kind of solvent. Performance and properties of PLA need to consider in the molecular weight at the first, due to the applications of PLA depends on the molecular weight which related with the properties of PLA products applications.

LITERATURE CITED

Avinc, O. and A. Khoddami. 2009. Overview of poly(lactic acid) (PLA) fibre; Part I: production, properties, performance, environmental impact, and end-use application of poly(lactic acid) fibres. Fibre Chemistry. 41(6): 391-401.

Garcia-Arrazola, R., D. A. Lopez-Guerrero, M. Gimeno and E. Barzana. 2009.

Lipase-catalyzed synthesis of poly-L-lactide using supercritical carbon dioxide.

J. Supercritical Fluids 51: 197-201.

Garlotta, D. 2001. A literature review of poly(lactic acid). J. Polymers and the Environment. 9(2): 63-84.

Gupta, R., N. Gupta and P. Rathi. 2004. Bacterial lipases: an overview of production, purification and biochemical properties. Appl. Microbiol. Biotechnol. 64: 763-781.

Hans, M., H. Keul and M. Moeller. 2009. Ring-opening polymerization of D-lactide catalyzed by Novozyme 435. Macromolecular Biosci. 9: 239-247.

Lassalle, V. L. and M. L. Ferreira. 2008. Lipase-catalyzed synthesis of polylactic acid: an overview of the experimental aspects. J. Chem. Technol. Biotechnol. 83: 1493-1502.

Taguchi, S., M. Yamada, K. Matsumoto, K. Tajima, Y. Satoh, M. Munekata, K. Ohno,

K. Kohda, T. Shimamura, H. Kambe and S. Obata. 2008. A microbial factory for lactate-based polyesters using a lactate-polymerizing enzyme. PNAS. 105(45): 17323-17327.

Treichel, H., D. Oliveira, M. A. Mazutti, M. D. Luccio and J. V. Oliveira. 2010. A review on microbial lipases production. Food Bioprocess Technol. 3: 182-196.