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Polylactic acid Synthesis via Direct Polycondensation Method Using *Candida rugosa* Lipase Catalyst

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ABSTRACT

Polylactic acid (PLA) is a biodegradable, biocompatible polymer and is produced from renewable natural resources. PLA synthesis through a polycondensation mechanism generally uses a metal catalyst, such as Zn and Sn oxides. The disadvantages of using metal catalysts are the contamination of products produced by the metals used so that unsafe products are used for biomedical applications and food packaging. The purpose of this research is to synthesize the safe PLA used for food packaging and biomedical applications. Polycondensation is done by pretreatment of lactic acid at 120°C for 1 hour. Diluted lactic acid is mixed with *Candida rugosa* lipise catalyst with a certain concentration (1, 2, 3 and 4% w / w). The mixture was heated at certain temperature (60; 80; 100 and 120°C) and vacuum pressure of 0.1 bar for 72 hours. The highest viscosity and density of PLA in this study was produced at 100°C and using 3% *Candida rugosa* lipase. The highest viscosity and density of PLA were 2443,9 CSt and 1231,9 mg / l respectively. *Candida rugosa* lipase concentration does not affect PLA yield at constant polycondensation temperature. PLA yield is affected by temperature. PLA yields at 60, 80, 100 and 120 ° C are 97.98; 97,65; 96.78; 96.13% respectively. The molecular weight of PLA at temperature 100°C for 1436-1482. Lipase concentration does not affect the molecular weight of the PLA.

Keywords: *lactic acid, Candida rugosa lipase, polylactic acid (PLA), polycondensation*

1. INTRODUCTION

Polylactic acid (PLA) has great potential to be developed as a substitute for conventional plastics. PLA is a biocompatible, biodegradable polymer and derived from renewable resources (Lopes & Jardini, 2012). PLA can be obtained from lactic acid derived from sugars, starches, cellulose and glycerin from waste biodiesel (Lasprilla et al., 2012). PLA is non-toxic and noncarsinogenic polymer for the human body so it is very well used for biomedical applications such as sewing thread for surgery, clips, and drug delivery systems. In the early 1970s, The US Food and Drug Administration (US-FDA) agency has set maximum limits of metals that are allowed in commercial products for medical applications is 20 ppm (Xiao, et al., 2011). The tendency to utilize lipase enzymes as an alternative to metal catalysts is growing rapidly because it is environmentally friendly and non-toxic to the body. The advantage of enzymatic polycondensation particularly by using a lipase catalyst is a high catalytic activity, low reaction conditions (60-110°C) and byproducts less. Enzymatic polycondensation is a process of synthesis of environmentally friendly polymeric materials (Kobayasi s, et al., 2009; Heise A, et al., 2010).PLA is safe in direct contact with fluids present in the body or used in the human body (Rasal, 2010). To produce a safe PLA used for biomedical applications, it is highly dependent on the mechanism of making the PLA. PLA is generally made by direct polycondensation and ring opening polymerization method. Compared with ring-opening polymerization, direct condensation polymerization has simpler and cheaper stages, and is easier to manipulate and commercialize.

PLA synthesis through polycondensation mechanism generally uses a metal catalyst, such as Zn and Sn oxides. The disadvantages of using metal catalysts in producing PLA are the contamination of products by the metals catalyst so that they are not safe for biomedical and food packaging applications. The FDA has set a maximum tin limit that is allowed to be in a commercial product and for medical needs is 20 ppm (Xiao, et al., 2011). The tendency to utilize lipase enzymes as an alternative to metal catalysts is growing rapidly because it is environmentally friendly and non-toxic to the body. The advantage of enzymatic polycondensation particularly by using a lipase catalyst is a high catalytic activity, low reaction conditions (60-110 ° C) and byproducts produced less. Enzymatic polycondensation is a process of synthesis of environmentally friendly polymeric materials (Kobayasi s, et al., 2009; Heise A, et al., 2010).

The synthesis of PLA by using enzyme catalyst in research scale and industrial implementation is an attempt to find a substitute of metal catalyst. In enzyme polymerization, several lipases from different sources have been used to form polyester. Example lipase from *Candida antarctica, Candida rugosa, Pseudomonas fluorescens, Pseudomonas cepacia and porcine pancreas* (Yang et al., 2011).

Taguchi et al (2008), synthesized polyesters using Escherichia coli recombinant strains by introducing polyhydroxyalkanoate (PHA) synthesis gene. Copolymers of 6 mol% lactate and 94 mol% of 3-HB unit with a molecular weight of 1.9 x 10^5 were achieved by one-stage fermentation (Taguchi et al., 2008). One-stage fermentation method is very difficult to produce PLA homopolymer. Production of copolymer PLA with carbon source in the form of glucose and using recombination of E. coli JLXF5 strain in fed batch culture yields P(3HB-co-39.4 mol% LA) with molecular weight of 141,000 Da and polymer content of 43% in medium. Cultivation was done at temperature 30°C and pH 7 for 72 hours (Jung & Lee, 2011). Matsumura et al (Matsumura et al., 1997a) also reported lipase *Porcine pancrease lipase* (PPL) and *Pseudomonas. sp* as a cyclic diester polymerization catalyst at a temperature of 80-130 °C to produce polylactic acid with a molecular weight up to 12,600 Da.

Arrazola et al (2009) synthesized PLA with a lipase catalyst from *Candida antartica*, the reaction took place at a pressure of 300 ± 5 bar and a temperature of [illegible] ± 2 °C. The yield of the polymer obtained was 11.03% with a molecular weight of 12,000 g/mol (García-Arrazola et al., 2009). Lagunes, F.G, et al (2012) synthesize the PLA using a lipase catalyst from *Bulkholderia cepacia*. The reaction takes place at a pressure of 50 bar and a temperature of 105°C. The yield of the polymer was > 50% (Guzmán-Lagunes et al., 2012).

The focus of the research to be carried out is based on the literature study of the use of lipase catalysts derived from various sources of microorganisms that have been successful in synthesizing the PLA. Most monomers are polymerized using lipases from a PPL source, *Candida antarctica* (Novozym 435), *Pseudomonas. sp, Burkholderia cepacia* (PS) and *Pseudomonas cepacea* (PCL). In this research will be polymerization of lactic acid using the source of Candida rugosa lipase. The operating conditions of polymerization at 60-120 °C are the temperature for the activity of thermophilic bacteria. Polymerization takes place on a bulk medium without solvent.

2. LITERATURE REVIEW

PLA (Polylactic acid) was discovered in 1932 by Brothers (DuPont). The PLA produced at that time has a low molecular weight by heating lactic acid under vacuum. In 1954, Du Pont produced polymers of greater molecular weight. In 2002, Cargill Dow established a factory to produce polylactic acid from sweet corn starch with a capacity of 300 million pounds per year (under the trade name NatureWorks). The resulting lactic polyacids have low molecular weight. (Kiran & Divakar, 2003).

PLA can be made from lactic acid by various processes ie polycondensation, ring opening polymerization, and by direct methods such as dehydration of azeotropes and enzyme polymerization. Currently, the widely used method for producing PLA is direct polycondensation and ring opening polymerization. The presence of hydroxyl groups (-OH) and carboxyl (-COOH) in lactic acid allows lactic acid to be converted directly to the polyester through a polycondensation reaction.

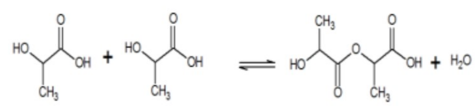

Figure 1 Reaction polycondensation of lactic acid

In the lactic acid polycondensation reaction to produce the PLA, the presence of water molecules must always be minimum or even absent, so that the equilibrium reaction is always shifted to the right or the product (lactic acid), so the water in the feed needs to be removed by evaporation at temperatures above the boiling point of water and atmospheric pressure. The use of solid catalysts such as SnO2 and SnCl2 can increase the rate of reaction towards the formation of PLA while eliminating the water formed (Södergård, A. and M. Stolt, 2010).

Mitsui Toatsu Chemical Company uses direct polycondensation of the solution by mixing lactic acid, tin catalyst and high boiling organic solvent and producing 300,000 molecular weight (Mw) poly-DL-lactic acid (Lopes & Jardini, 2012). Tin will be incorporated into the product so that when used for medical and food application the tin reduction process should be done first. The US Food and Drug Administration (FDA) has set a maximum tin limit that is allowed to be in commercial products and for medical needs of 20 ppm (Xiao et al., 2011). In polycondensation reactions, tin catalyst reduction is not possible because the tin function is the initiator of the reaction (Gao, Ma, & Xu, 2011). The way to avoid tin residues is to use an initiator containing atoms other than lead, for example, zinc. The use of zinc catalyst in the polymerization process results in too low molecular weight of the polymer for use in industrial applications (Gao et al., 2011).

3. RESEARCH METHODOLOGY

This research was conducted at Chemical Engineering Operations Laboratory of Universitas Sultan Ageng Tirtayasa. The materials and procedures are described in this section.

3.1 Materials and Tools

L-lactic acid with a purity of 90% and Chloroform (Merck, Indonesia); *Candida rugosa* lipase (Sigma-Aldrich, Indonesia); methanol (Barataco Co., Indonesia) and nitrogen gas (Windu Prasetya Manunggal Co., Cilegon, Indonesia); were used in this study.

The reaction was performed using the reactor in the form of a four-neck flask with a capacity of 500 ml and equipped with a magnetic stirrer and thermometer. The reactor condenser is connected with a screw and condensate receiver. The vacuum pump is connected via the top of the condenser to the water trap.

3.2 Research Flow Diagram

The research flow diagram is shown figure 2.

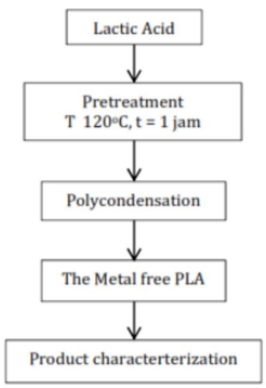

Figure 2 The research flow diagram

4. Results and Discussion

In this research we will discuss the influence of temperature and lipase concentration on viscosity, density and molecular weight of PLA.

4.1 Effect of Temperature and Lipase Concentration on the viscosity of PLA

Differences in temperature also cause differences in the viscosity of PLA. The viscosity of PLA increased with the raising of temperature polycondensation . The viscosity of PLA produced in polycondensation is shown in Table 1.

Table 1. Effect temperature and lipase concentration to viscosity of PLA

Sample	Lipase conc (%w)	Temp (°C)	Time (s)	Viscosity (CSt)
Lactic acid		28	2,93	25,8
Polycondensation (60°C)	1	28	30,68	238,6
	2	28	39,83	348,9
	3	28	51,26	440,2
	4	28	52,12	451,8
Polycondensation (80°C)	1	28	82,71	764,4
	2	28	90,66	828,9
	3	28	93,64	845,8
	4	28	94,83	887,5
Polycondensation (100°C)	1	28	268,47	2186,6
	2	28	290,46	2308,2
	3	28	292,98	2443,9
	4	28	262,64	2098,7
Polycondensation (120°C)	1	28	228,35	1856,0
	2	28	241,47	1984,7
	3	28	252,64	2010,4
	4	28	238,86	1966,4

In Table 1, it is seen that viscosity has increased with increasing of polycondensation temperature. At the beginning of the raw material in the form of lactic acid has a viscosity of 25.8 Cs. After polycondensation using *Candida rugosa* lipase catalyst ,viscosity of polymer solution increase. Increased viscosity indicates that there is a weight increase of molecular weight in PLA products. The PLA produced at a polycondensation temperature of 120°C has a lower viscosity compared to temperature of 100 °C.

Table 1 shows also the effect of lipase concentration on the viscosity of the PLA solution. Addition of lipase concentrations at 60 and 80°C led to increased viscosity of the PLA solution product. At the polycondensation temperature of 100 and 120 °C, the addition of 4% lipase catalyst leads to a decrease in the viscosity of the PLA when compared with 3% lipase concentration. The lipase can act as a catalyst in the hydrolysis reaction at certain temperatures and concentrations, so that structure molecule of PLA is broken and obtained water as byproducts. The breakup of the PLA bond and the formation of water causes the PLA density to decrease.

4.2 Effect of temperature and Lipase Concentration to the density of PLA

Analyze the density of PLA is done using piknometer. The density of PLA solution that obtained in this study can be seen in Table 2.

Tabel 2. Density lactic acid and PLA solution

Sample	Lipase Conc. (%b)	Density (mg/l)
Aquadest (27°C)	-	996,5 Volume = 9,9965 ml
Lactic acid	-	1195,7
Polycondensation (60°C)	1	1198,2
	2	1199,8
	3	1200,6
	4	1205,8
Polycondensation (80°C)	1	1209,7
	2	1210,4
	3	1214,8
	4	1215,0
Polycondensation (100°C)	1	1228,6
	2	1230,5
	3	1231,9
	4	1224,7
Polycondensation (120°C)	1	1220,5
	2	1225,6
	3	1226,8
	4	1223,5

The density analysis showed that density increased from lactic acid as raw material compared to polycondensation product at 60, 80, 100 and 120°C. The increasing of polycondensation temperature causes the density of the solution to raising. The density of the PLA solution at 120°C is decrease compared to the density of the PLA produced at 100 °C. The decrease in the density of the PLA solution is due to the lower PLA viscosity.

4.3 Effect of Temperature and Lipase Concentration on the PLA Yield

The polycondensation process is the stage of incorporation of lactic acid molecules into larger molecules in the presence of heat and catalyst. Temperature greatly affects the yield of PLA produced. The resulting PLA yield is calculated using the equation:

$$Yield\ PLA = \frac{weight\ of\ PLA\ solution}{weight\ of\ lactic\ acid\ teoritis} \times 100\%$$

The PLA yield at each polycondensation temperature is shown in Figure 3.

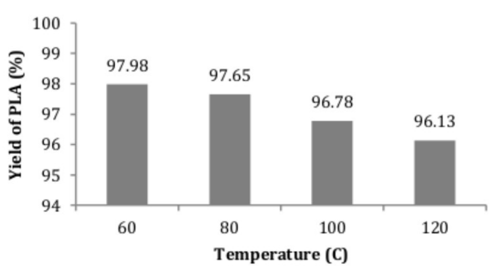

Figure 3 yield of PLA at variation of temperature

PLA yields obtained on catalyst concentration variations of 1, 2, 3 and 4% did not result in significant changes in PLA yield. This shows that in the polycondensation process, the addition of catalyst concentration does not increase the acquisition of PLA.

The PLA yield as shown in Figure 3 has decreased not too greatly in the rise in polycondensation temperature. The decreasing yield of PLA along with increasing polycondensation temperature is due to the presence of lactic acid, water and other components which are evaporated as well as intermolecular interaction during the condensation process. At high temperatures lactic acid will react quickly to extend the bond, but high temperatures cause lactic acid that will not evaporate. At a pressure of 760 Torr the boiling point of lactic acid at 217 ° C (Upare, P.P., et al., 2012).

Groot, et. al., (2010) reported that lactic acid molecules having hydroxyl functional groups (-OH) and carboxylic acids (-COOH) undergo esterification reactions due to inter- and intramolecular interactions. The intermolecular interactions of lactic acid molecules in the polycondensation reaction cause the formation of PLA dimer, trimer, and oligomers and produce water molecules (H_2O), whereas intramolecular interactions result in a change in the form of lactic acid dimer into lactide. Intramolecular interactions are caused by thermal degradation of PLA oligomers into short chain or lactide molecules (Achmad, F., et al., 2009). In this research, no lactide product was found. This shows that the polycondensation stage in this study does not occur intramolecular reaction.

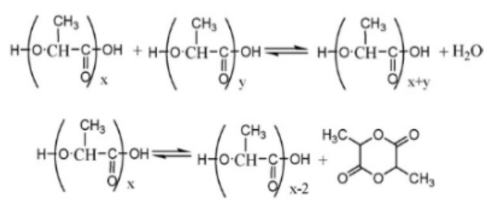

Figure 4 Two reactions involved in polycondensation (Maharana, et al., 2009)

The molecular weight of the polymerization solution by polycondensation is dissolved in chloroform and precipitated using methanol. The precipitation results do not produce white precipitate. Determination of the molecular weight of the PLA solution product of this study used liquid chromatography mass spectrometer (LCMS). The result of LCMS analysis is shown in Figure 5.

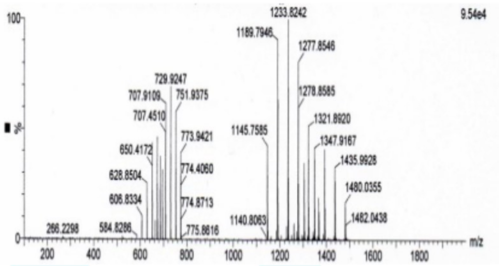

Figure 5 molecular weight of PLA with 3% lipase at 100°C

The molecular weight of PLA obtained from polymerization at 100°C with catalyst variations of 1,2,3 and 4% is seen in Table 4.3.

Table 3 Molecular weight of PLA solution

Lipase conc (%w/w lactic acid)	m/z
1	1436
2	1469
3	1482
4	1472

From the results of the LCMS analysis shown in Table 3 it is seen that at the polymerization temperature of 100oC, the lipase concentration does not affect the molecular weight of the PLA solution. The molecular weight (m/z) of the PLA solution is 1436-1482. This is similar to that reported by Namekawa et al. (1999), bulk polymerization using lipase at 60 °C to produce low molecular weight polymers (less than 2000).

5. Conclusion

Research activities that have been done successfully get PLA in the form of solution through polykondensasi process. Polycondensation with variations in temperature and lipase concentration obtained different viscosities, densities and yields. The conclusions for each stage of the process are as follows;

a. The rising of temperature cause the rising of viscosity and density of PLA solution. The highest viscosity and density of PLA in this study was produced at 100°C with 3% *Candida rugosa* lipase concentration. The highest viscosity and density of PLA were 2443,9 CSt and 1231,9 mg / l respectively.

b. *Candida rugosa* lipase concentration does not affect PLA yield at constant polycondensation temperature. PLA's yield is affected by temperature. PLA yields at 60, 80, 100 and 120 ° C were 97.98; 97,65; 96.78; 96.13% respectively.

c. The PLA molecular weight at 100°C is 1436-1482. Lipase concentration did not significantly affect the molecular weight of the PLA.

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