Use of *Candida rugosa* lipase as a biocatalyst for L-lactide ring-opening polymerization and polylactic acid production

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ARTICLE INFO

ABSTRACT

Candida rugosa lipase (CRL, EC Number: 232–619-9) is a stable enzyme, and its use is relatively widespread in the field of biotechnology, especially in hydrolysis reactions, esterification, transesterification and enantioselective biotransformation. This study examined the role of CRL as a biocatalyst for L-lactide ring-opening polymerization in the production of metal-free polylactic acid (PLA). The ring-opening polymerization reaction was conducted at various temperatures (70, 90, 110 and 130 °C) and CRL concentrations (1, 2, 3 (%w/w)). The results revealed a strong relationship between PLA formation and CRL activity. The highest CRL activity was obtained in ring-opening polymerization of L-lactide at a temperature of 90 °C and concentration of 2% (w/w). Under these conditions, the weight-average molecular weight (Mw) and number-average molecular weight (Mn) of PLA analyzed by gel permeation chromatography was highest at 5428 and 2854 g/mol respectively, with a yield of 93% and the enzyme activity 0.39 U. The polymerization of lactide at 90 °C occurred only in the presence of the catalyst. The crystallinity and melting point of PLA were 31% and 120 °C, respectively. Scanning electron microscopy (SEM) analysis of the morphology of PLA revealed smooth and uniform pores in each region, with mass percentage of the elements carbon (C) and oxygen (O) of 52% and 48%, respectively.

1. Introduction

Candida rugosa lipase (CRL) is a biocatalyst, which has extensive applications in the fields of biotechnology, as well as in hydrolysis, transesterification, esterification and enantioselective biotransformation reactions (Chang et al., 2014; Bezbradica et al., 2006). CRL is stable and tolerant of reaction media (Guncheva et al., 2014). Moreover, it is relatively less expensive than lipases derived from other sources (Yang et al., 2011; Bezbradica et al., 2006). CRL is commercially available and does not require a cofactor in its application (Herbst et al., 2014). Several studies showed that CRL was effective as a catalyst in the polymerization of monomers, including lactones (propyl malolactonate), at 45–60 °C (Varma et al., 2005) and 1,4-Dioxane-2-one (poly-dioxane) at 100 °C for 48 h (Albertsson and Srivastava, 2008). In PLA polymerization applications, there have been no studies of the use of CRL as a catalyst in the polymerization of crude lactide (i.e. lactide produced without purification prior to polymerization). Therefore, the objective of this study was to determine the effect of various temperatures and CRL concentrations in the polymerization stage on the yield and molecular weight of PLA produced.

Polylactic acid (PLA), a biodegradable polymer derived from renewable resources, has potential as a replacement for conventional plastics (Tsukegi et al., 2007; Huang et al., 2014). PLA is widely used for various applications, especially in the fields of medicine, packaging and textiles (Gozan et al., 2018). In the medical field, PLA is used in sutures for operating purposes (surgical implants) and as a shell material for capsules used as drug delivery systems. PLA is also used in the repair of body tissue (as a scaffold for cell growth) (Saeidlou, Huneault, Li, and Park, 2012). In the field of packaging, PLA is employed in the manufacture of plastic bags (retail industry), containers and thin film

coatings or rigid thermoform (Düşkünkorur et al., 2015). In the field of textiles, PLA is used in the manufacture of shirts (Nampoothiri et al., 2010; Kamel et al., 2011).

PLA is manufactured from lactic acid by via ring-opening polymerization of L-lactide through a three-stage process: polycondensation, depolymerization and polymerization. The catalysts used in ring-opening polymerization are generally metal complexes of Al, Mg, Zn, Ca, Sn, Fe, Y, Sm, Lu, Ti and Zr (Kimura and Yoshiharu, 2014). Stannous octoate/Sn (II) 2-ethylhexanoate (Sn(Oct)$_2$) is the most used catalyst to synthesis high molecular weight PLA (Purnama et al., 2012; Kimura and Yoshiharu, 2014). A drawback with the use of metal catalysts in the manufacture of PLA is contamination of the product by the metals used. Thus, a purification process is required, especially when the polymer is targeted for biomedical applications. In addition, to obtain a high-purity monomer, process conditions of high temperature (200–250 °C) and vacuum pressure are required. The U.S. Food and Drug Administration agency has regulated the maximum tolerance of tin in commercial and biomedical product for as much as 20 ppm (Gao et al., 2011). In the ring-opening reaction, tin catalytic reduction is not possible because tin functions as the initiator of the reaction (Stjerndahl et al., 2008).

The trend to use of lipases are an alternative to metal catalysts is growing rapidly. Lipases/esterases belong to a group of biocatalysts with the ability to synthesize organic compounds, especially polyesters (Yang et al., 2011). Lipases are enzymes that catalyst the hydrolysis of lipids produced by various organisms. Although lipases exhibit various substrate specificities, they share structural and functional similarities. The active site of lipase is composed of three amino acid residues (i.e. serine, histidine and aspartate or glutamate) known as a 'catalytic triad' (Guncheva et al., 2014). The use of lipase catalysts in the manufacturing of PLA has several advantages, such as a reduction in the processing temperature from 200° to 250 °C to 60–110 °C, thereby reducing energy consumption (Idris and Bukhari, 2012). Further, lipase is derived from a renewable resource, and lipases can be produced in an environmentally friendly manner from various microorganisms, ranging from *meso*philes to thermophiles (Kim, Song, and Kim, 2013).

The molecular weight and yield of PLA vary, according to the type of lipase, temperature, reaction time, reaction media and amount of water (Ma et al., 2009). In general, the enzymatic polymerization reaction rate increases with temperature (Herbst et al., 2014). In esterase-catalyzed ε-caprolactone polymerization, a higher reaction temperature led to higher monomer conversion. At a temperature of 45 °C, ε-caprolactone conversion was 84%, whereas it was 100% at 80 °C (Ma et al., 2009). The increased monomer conversions and molecular weights at higher temperatures were partly caused by the decreased diffusion constraints in the reaction mixture, resulting from high viscosity of the product (Ma et al., 2009). However, in the case of the ring-opening reaction of L-lactide, with catalyst immobilization of *Candida antarctica* lipase, monomer conversion decreased with temperature, probably due to the heat-labile nature of the lipase. At temperatures of 50, 70 and 90 °C, monomer conversion was 80%, 60% and 25% respectively (Hans et al., 2009).

In the enzymatic ring-opening polymerization reaction, the concentration of the enzyme greatly affects the level of monomer conversion. Polymerization of ε-caprolactone at 80 °C for 72 h, increasing the concentration of the enzyme increased the conversion of the monomer (Ma et al., 2009). When the enzyme concentration exceeded 25 mg/ml, monomer conversion was more than 99%. The enzyme concentration range is 5–75 mg/ml (Ma et al., 2009). However, the relationship between the enzyme concentration and value of number-average molecular weight (*Mn*) of PLA remains unclear. A previous study reported that the *Mn* value did not depend on the enzyme concentration (Ma et al., 2009), whereas other research found a decrease in *Mn* with increasing concentrations of the enzyme in the polymerization of ε-caprolactone (Deng and Gross, 1999).

2. Material and Methods

2.1. Materials

Commercially available L-lactic acid (liquid, 90%) and chloroform were obtained Merck, Jakarta, Indonesia. *Candida rugosa* lipase (CRL, EC Number: 232–619–9) was purchased from Sigma-Aldrich, Singapore). Nitrogen gas was procured from Windu Prasetya Manunggal Co., Cilegon, Indonesia. Methanol was obtained from Brataco Co., Jakarta, Indonesia.

2.2. Methods

2.2.1. L-lactide synthesis

L-lactide synthesis was achieved via polycondensation of L-lactic acid and depolymerization, as described by Rahmayetty et al. (2017). The L-lactic acid was placed in a four-necked flask, which was equipped with a magnetic stirrer, temperature controller and condenser, which was connected to a distillation column. The reaction was carried out at 120 °C for 1 h, with continuous nitrogen gas flow to push evaporative water into the condenser.

Next, polycondensation was carried out at 150 °C for 2 h and 180 °C for 2 h, without nitrogen gas flow. The product of the polycondensation process was an oligomer (OLLA). The prepared OLLA was reheated at 210 °C for 3 h, with vacuum pressure of 76 Torr. The flask was equipped with a magnetic stirrer, temperature controller and distillation column, which was connected to a vacuum pump. The reaction was carried out at 210 °C, with weight of SnCl$_2$ 0.1 (w/w) and 76 Torr until distillate production ceased. After completion of the depolymerization process, the vacuum valve was closed slowly. The L-lactide was then collected in the sample container.

2.2.2. Ring-opening polymerization of L-lactide

L-lactide was weighed and placed in a four-neck flask, which was equipped with a magnetic stirrer and thermometer. L-lactide was heated until it melted. The melting temperature (Tm) of L-lactide is 93 °C. Powder CRL was then added at concentrations of 1%, 2%, 3%, 5% and 10% (w/w). Nitrogen gas was allowed to flow for some time to remove oxygen in the reactor. The mixture was heated to 70, 90, 110 and 130 °C and stirred with a magnetic stirrer for 72 h. Upon completion of the polymerization step, the polymerization product mixture was cooled to room temperature, and chloroform (5 ml) was added to the mixture to dissolve PLA in the mix. The resulting solution was centrifuged to separate lipase from PLA dissolved in chloroform, prior to filtration. The filtrate was transferred dropwise into excess methanol. White solid PLA gradually formed. The PLA was filtered and dried in an oven at 45 °C for 4 h.

2.3. Characterization

2.3.1. FTIR Characterization

The functional groups of the PLA product were characterized using a Fourier transform infrared spectrometer (FT-IR, Agilent Technologies type Cary 630, USA).

2.3.2. NMR Characterization

The molecular structure was observed using a nuclear magnetic resonance (NMR spectra recorded on JEOL spectrometer operating at 500 MHz, USA). Proton NMR was obtained using chloroform as a solvent, and tetramethylsilane was used as the internal standard.

2.3.3. GPC Characterization

The molecular weight distribution was obtained using gel permeation chromatography (GPC-8052, Italy). Tetrahydrofuran was used as the mobile phase, at a flow rate of 1.0 ml/min, with a column temperature of 40 °C. Calibration was performed using standard
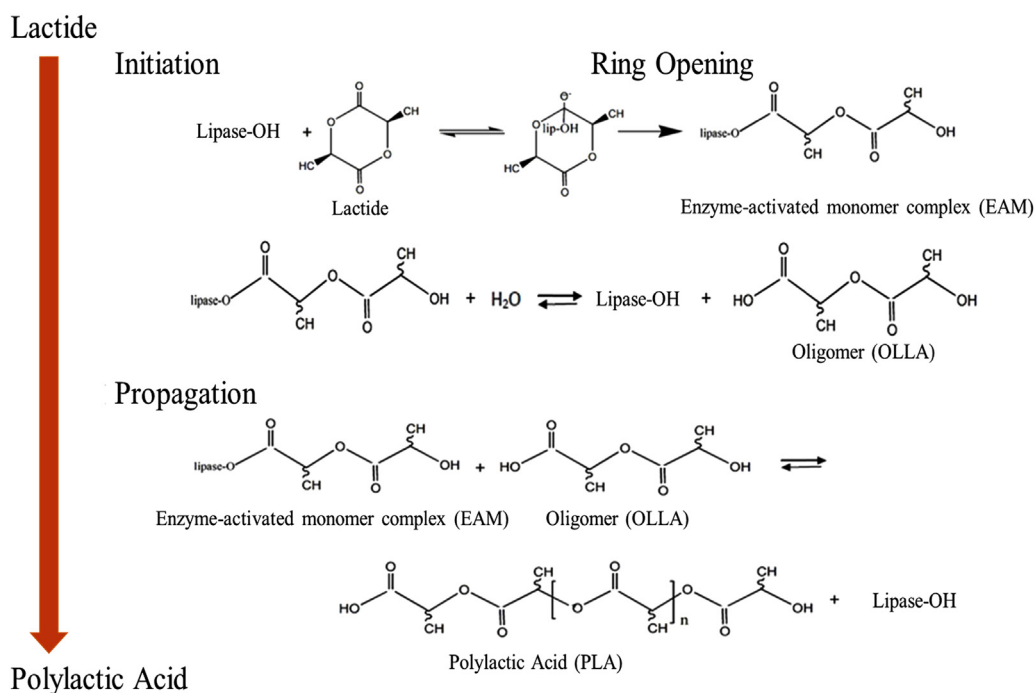
Fig. 1. Synthesis of polylactic acid through ring opening polymerization of l-lactide.
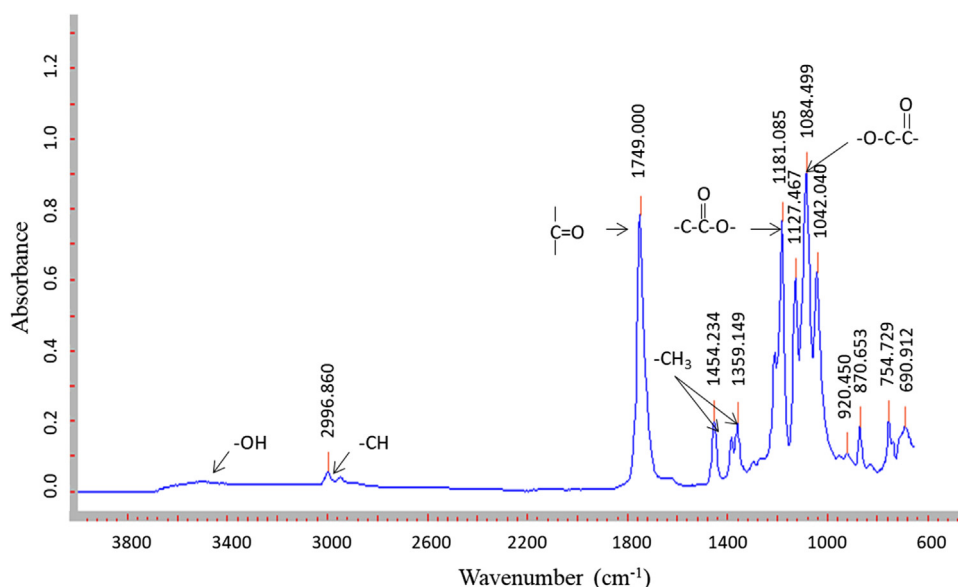

Fig. 2. The FTIR Spectrum of Poly Lactid Acid (PLA). The functional groups of PLA assayed at the stage of ring-opening polymerization of the lactide at 90 °C and CRL concentration 2% (w/w) were indicated by the present of -C=O (1749 cm^{-1}), -CH$_3$ (1359 – 1454 cm^{-1}), and -OH (3550 cm^{-1}).

polystyrene with a similar molecular weight distribution.

2.3.4. XRD Characterization

The degree of crystallinity was measured by X-ray diffraction (XRD, Empyrean, Malvern PANalytical, United Kingdom).

2.3.5. SEM Characterization

The morphology was characterized by scanning electron microscopy (SEM-EDX, JSM 6510LA-JEOL, USA).

2.3.6. DSC Characterization

The melting temperature (T_m) was estimated using differential scanning calorimetry (DSC, PerkinElmer, type JADE DSC, USA) at temperatures of 30–500 °C.

3. Results and discussion

The synthesis of L-lactide from L-lactic acid through a two-stage process, namely polycondensation and depolymerization, was carried out as described previously (Rahmayetty et al., 2017). L-lactide was then used for the synthesis of PLA via ring-opening polymerization. Fig. 1 shows the proposed synthesis reaction from lactide to PLA through ring-opening polymerization. The weight-average molecular weight (*Mw*) of the oligomers (OLLA) obtained in the polycondensation stage was 2820, and the *Mn* was 2389, with PDI of 1.18 (Rahmayetty et al., 2017). The crude lactide yield obtained from the depolymerization stage was 79%, in which the L-lactide purity was 81% (Rahmayetty et al., 2015). Ring-opening polymerization of L-lactide using the CRL catalyst produced PLA powder with the same functional groups as those

of the oligomer (OLLA). The characteristics of PLA produced from ring-opening polymerization of L-lactide with CRL are discussed at Section 3.1.

3.1. Characterization of functional groups of PLA

3.1.1. FTIR Characterization

The functional groups of PLA and the OLLA were the same. The PLA-produced functional groups at the stage of ring-opening polymerization of the lactide is shown in Fig. 2. One of the identical FTIR spectra shows the characteristic absorption band s of PLA. The peaks at 1749 and 1181 assigned to the -C = O carbonyl stretch and -C = O carbonyl bending, respectively. Two -C-H bonds were observed at 2996 cm^{-1} (asymmetric) and 2940 cm^{-1} (symmetric). The -OH bond stretching was observed at wave number 3550 cm^{-1}, which is characteristic of a carboxylic acid. Due to very strong hydrogen bonds in carboxylic acids, OH bond stretching from the carboxylic acid was lower than that of alcohol (3300 cm^{-1}). An -C = O and OH bond was discovered at 1220 cm^{-1} and 1064 cm^{-1}, respectively. However, bonding at lower wavelengths tended to show considerable overlap, causing difficulty in characterization. The infrared spectroscopy wavenumbers (cm^{-1}) for the bonds and PLA functional groups were as follows: -OH stretch (free): 3100; -CH-stretch: 2997 (asymmetric), 2946 (symmetric), 2877; -C = O carbonyl stretch: 1748; -CH3 bend: 1456; -CH- symmetric and asymmetric: 1382 and 1365; -C = O bend: 1225; -C-O- stretch: 1194, 1130, 1093; -OH bend: 1047; -CH3 rocking modes: 956, 921; and -C-C- stretch: 926, 868 (Auras et al., 2004).

3.1.2. NMR Characterization

The molecular structure of the PLA produced from the polymerization stage was evaluated using ^{1}H NMR and ^{13}C NMR, as shown in Fig. 3(a) and (b), respectively. Fig. 3(a) shows the ^{1}H NMR spectrum of PLA powder obtained at 90 °C using CRL 1%. The spectrum shows the H-doublet signal for methyl proton (^{d}H) resonance at 1.59 ppm and H-multiplet signal for methine (^{a}H) resonance in the main chain of PLA at 5.18 ppm. The spectrum at 1.57; 3.19 and 4.37 ppm was assigned to the methyl proton and methine proton next to the terminal hydroxyl group and carboxyl group, respectively. The ^{1}H NMR spectrum of PLA was similar to that reported by others (Choubisa et al., 2013; Ding et al., 2011; Kadota et al., 2010; Umare et al., 2007).

Fig. 3(b) shows the ^{13}C NMR spectrum of PLA powder. In the ^{13}C NMR spectrum of PLA, peak signals were visible at 16.73, 69.17 and 169.78 ppm, indicating the molecular structure of methyl carbon, carbon methane and carbon ester, respectively. The ^{13}C NMR spectrum of PLA in the present study was similar to that observed by Ding et al. (2011) obtained by a reaction using an Nickel (II) complex, Nickel (II) -Lanthanide (III), as catalysts.

Table 1 shows the chemical shifts of lactic acid (raw material), OLLA (polycondensation product), lactide (depolymerization product) and PLA (main product) from ring-opening polymerization of lactide, which clearly demonstrates the change of chemical structures.

The spectrum of ^{1}H- and ^{13}C NMR structural assignments of the synthesized PLA were recorded in chloroform at room temperature, as shown in Table 2. The structural assignments of the synthesized PLA were similar to the PLA structure reported by Sobczak and Kolodziejski (2009).

3.2. Analysis of molecular weight of PLA

Initially, the ring-opening polymerization of lactide was carried out at 70, 90, 110 and 130 °C with CRL at a concentration of 1%. The influence of temperature on the molecular weight of PLA is depicted in Fig. 4(a). As shown, polymerization at 90 °C produced PLA with an *Mw* of 2833 g/mol and *Mn* of 1451 g/mol. Increasing temperature cause decreasing of the Candida rugosa lipase activity which can effect decreasing the value of Mw and Mn. At a polymerization temperature

above 90 °C, the lipase undergoes discoloration from yellowish white to dark brown. High temperatures cause denaturation of proteins of enzymes. Fig. 5 shows the lipase diffractogram spectrum before and after polymerization at 130 °C. This proves the change of lipase structure due to denaturation after the polymerization process.

To examine the effect of CRL on the polymerization stage, a polymerization reaction was performed at 90 °C, with or without the CRL catalyst at concentrations of 1%, 2%, 3%, 5% and 10%. In the polymerization of lactide without the catalyst, no polymer was produced. The liquid reactants without the catalyst did not change the viscosity and did not harden at room temperature. These results proved that polymerization of lactide at 90 °C occurred only in the presence of the catalyst. The effect of the CRL concentration on the polymerization process is shown in Fig. 4(b).

Fig. 4(b) shows that a 2% concentration of CRL yielded PLA with an *Mw* of 5428 g/mol, *Mn* of 2854 g/mol and PDI of 1.9. At CRL concentrations lower than 2% (w/w), the molecular weight of PLA decreased. Similar results were reported by Deng and Gross (1999) and He et al. (2008). Deng and Gross (1999) reported that the addition of catalysts decreased the *Mn* value. He et al. (2008) stated that the addition of porcine pancreatic lipase at concentrations of 0.1–0.5% (w/w) caused a decrease in the *Mn* of poly (5-allyloxy-1,3-Dioxans-2-one) from 48,700 to 14,300 g/mol.

Based on the results above, the optimum conditions for the lactide ring-opening polymerization reaction with CRL was at a temperature of 90 °C and concentration of 2%. Under these conditions, *Mw* and *Mn* reached the highest values of 5428 g/mol and 2854 g/mol.

3.3. Yield of PLA

The weight of PLA produced in the polymerization process was measured after the deposition process with methanol. The yield of PLA was calculated using the following equation:

$$\text{PLA yield} = (\text{weight of PLA that formed})/(\text{weight of lactide used}) \times 100\%. \quad (1)$$

PLA yields at the various CRL concentrations and temperatures were calculated by the Eq. (1) (Fig. 6). PLA yields using 1% CRL at temperatures of 70, 90, 110 and 130 °C were 9%, 88%, 64% and 60%, respectively. Increasing the concentration of CRL to 2% slightly improved the yield of PLA to 11%, 93%, 73% and 71% at temperatures of 70, 90, 110 and 130 °C, respectively. At 3% CRL, an increased yield of 15.24% was obtained at 70 °C. However, PLA yields at 3% CRL decreased to 72%, 34% and 21% at 90, 110 and 130 °C respectively. High PLA yields were obtained at CRL concentrations of 1%, 2% and 3% at a temperature of 90 °C. The highest PLA yield (93%) was obtained by CRL at 2% concentration with the enzyme activity 0.39 U. The yield and purity at each step of the PLA synthesis are shown in Table 3.

3.4. Characterization of the degree of crystallinity, T_m and morphology of PLA

The degree of crystallinity of the PLA product was analyzed using X-ray diffraction. Polymers can contain crystalline regions that are randomly mixed with amorphous regions. The emergence of high intensity in a diffractogram denotes a polymer with a crystalline structure, whereas a polymer with an amorphous structure tends to produce ramps and wider bands. The diffractograms of PLA generated in this study are shown in Fig. 7.

The XRD characterization results in Fig. 7 show two 2θ diffraction peaks at around 16.84° and 19.10°. These peaks coincide well with those in previous reports (Carrasco et al., 2010; Gates et al., 2014; Guo, 2012; Tabi et al., 2010). Quantitative determination of the degree of crystallinity was performed using XRD data. The width of the diffraction peaks can provide information about the crystal structure of PLA, with an increase in the peak pointing to a more crystallized PLA. The

(a)

(b)
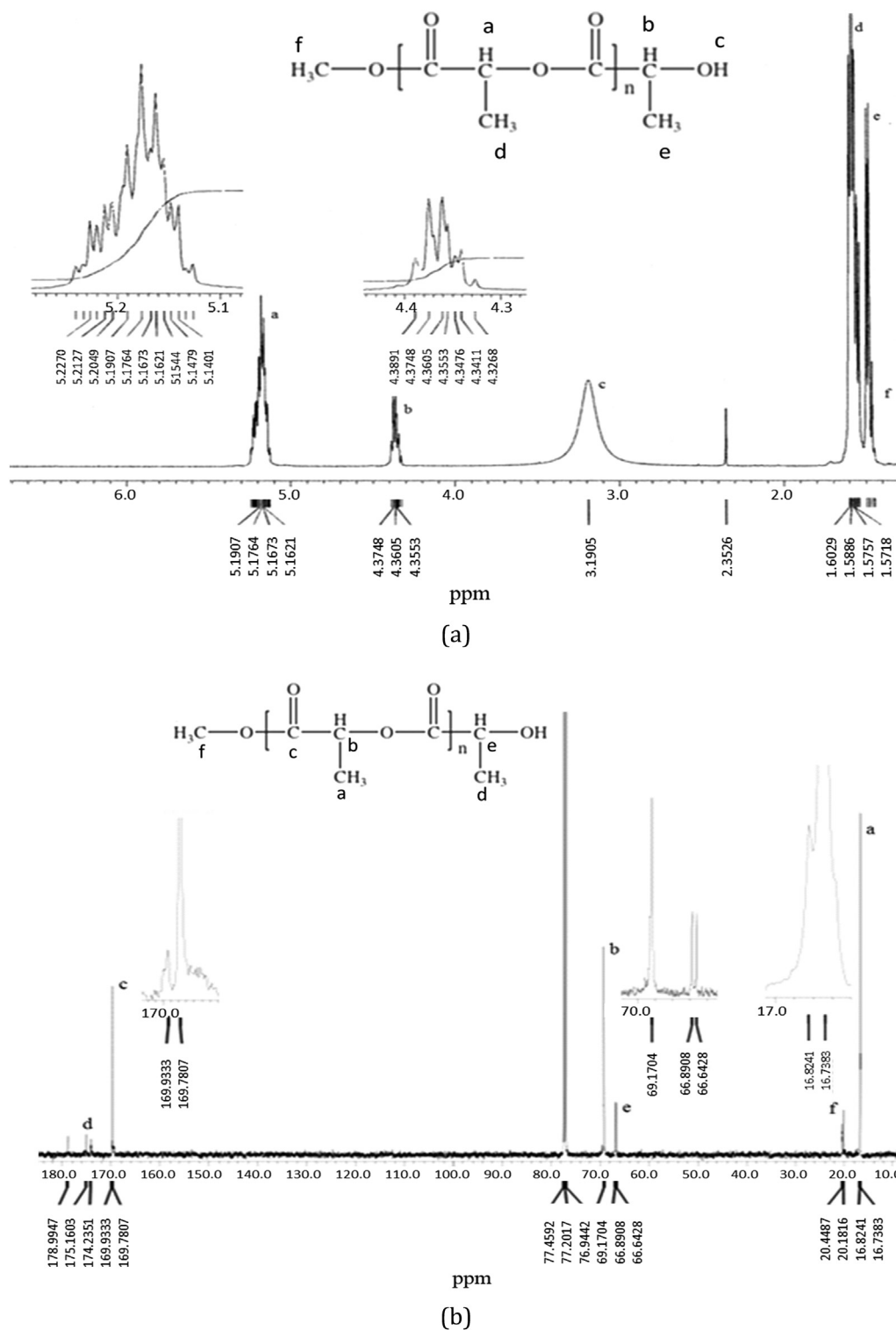
Fig. 3. The representative NMR Spectrum in chloroform of PLA (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum. PLA was synthesized by CRL 1% (w/w) at 90 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded on a spectrometer using tetramethylsilane (TMS) as an internal reference and chloroform-d (CDCl$_3$) as a solvent. The spectrum was recorded at room temperature (25 °C).
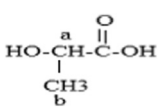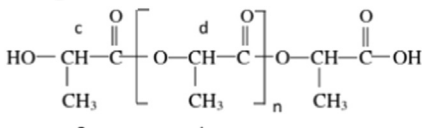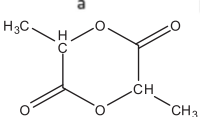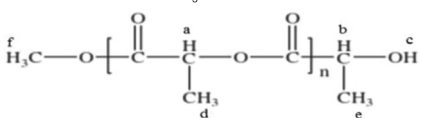
degree of crystallinity can be determined by XRD based on the assumption that crystalline and amorphous regions are substantially the same and have equivalent scattering power. The degree of crystallinity was determined by compared the area of crystalline regions and the total area. Total area was sum of crystalline and amorphous area. Eq. (2) with modification was used to calculate the degree of crystallinity (Kurniawan et al., 2017).

$$\%\text{Crystallinity} = \frac{\text{Crystalline area}(5-80°)}{\text{Total area}(5-80°)} \quad (2)$$

Based on Fig. 7 and Eq. (2), it can be seen that the degree of crystallinity of PLA was 31%. The crystallinity value shows that PLA resulting from polymerization with CRL resulted in semi-crystalline polymers that formed stereoisomers, such as PLA. Auras et al. (2005)

Table 1
^{1}HNMR Chemical shift of lactic acid as raw material, OLLA as polycondensation product at gradually temperature of 150 °C for 2 h and 180 °C for 2 h, Lactide as depolymerization product at 210 °C with weight of SnCl2 0.1% (w/w) and 76 Torr, and PLA as main product from main product from ring opening polymerization of lactide at 90 °C using CRL 1% (wt/wt).

| Compound	Structure	H-doublet (ppm)	H-quartet (ppm)
L-lactic acid		b = 1.49 − 1.48	a = 4.38–4.36
Oligomer (OLLA)		b = 1.59 − 1.56 a = 1.48 − 1.47	d = 5.14–5.18 c = 4.35
Lactide		b = 1.65–1.68	a = 5.07–5.02
PLA		d,e,f = 1.57–1.59	a = 5.17–5.19 b = 4.37–4.36 c = 3.19

Table 2
^{1}H dan ^{13}CNMR chemical shift and structural assignments of PLA.

Chemical shift in ppm		Structural assignments
This study	Sobczak & Kolodziejski	
5.17–5.19	5.17	(1H, q, -**CH**(CH3))
4.36–4.37	4.36	(1H, q, -**CH**(CH3)OH, end grup)
1.57–1.59	1.58	(3H,d,-**CH₃**)
3.19		(H, **OH** pada hydroksil)
169.8–169.9	169.80	(-**C**(O)O-)
69.17	69.2	(-**CH**(CH3)-)
16.82	16.8	(-**CH3**)

reported a similar degree of crystallinity of PLA (i.e. 29 ± 0.5%). Gupta et al. (2007) also found that PLA had a degree of crystallinity of 0–37%.

DSC was used to measures the glass temperature (T_g), crystalline temperature (T_c) and T_m of the product. The DSC results in Fig. 8 showed that the resulting PLA had a T_g of 65 °C and that its T_c and T_m were 103 °C and 119 °C, respectively. According to Dechy-Cabaret et al. (2004), PLA with sequential amorphous and crystalline structures had melting temperatures of about 130 °C and 180 °C, respectively. Based on these findings, PLA that formed had an amorphous structure. These results are in accordance with those published by others (Auras et al., 2005; Guo, 2012). Guo (2012) reported that PLA had a T_g value of 60 °C, T_c value of 110 °C and T_m value of 165 °C, whereas Auras et al. (2005) reported that PLA had a T_g value of 62 °C and T_m value of 150 ± 0.5 °C. The molecular weight of PLA determines its T_m and T_g. Both Tm and Tg increased with increasing molecular weight. As reported earlier, the T_g value was determined by the degree of crystallinity (Gupta et al., 2007).

Scanning Electron Microscopy (SEM) was used to observe the morphology of PLA. SEM images of PLA at a magnification × 1000 are shown in Fig. 9. As apparent in the figure, the morphology of PLA was smooth, with uniform pores in each region. Energy-dispersive X-ray spectroscopy (EDX) was used to determine the elemental composition of PLA. X-ray energy detected by the EDX detector revealed the PLA contained elements C and O, with C and O accounting for 52% and 48% mass and 59% and 41% atoms, respectively. The elemental content in PLA was equivalent to the molecular structure obtained from the analysis of the ^{13}C-NMR structure. The mass percentage of PLA prepared in
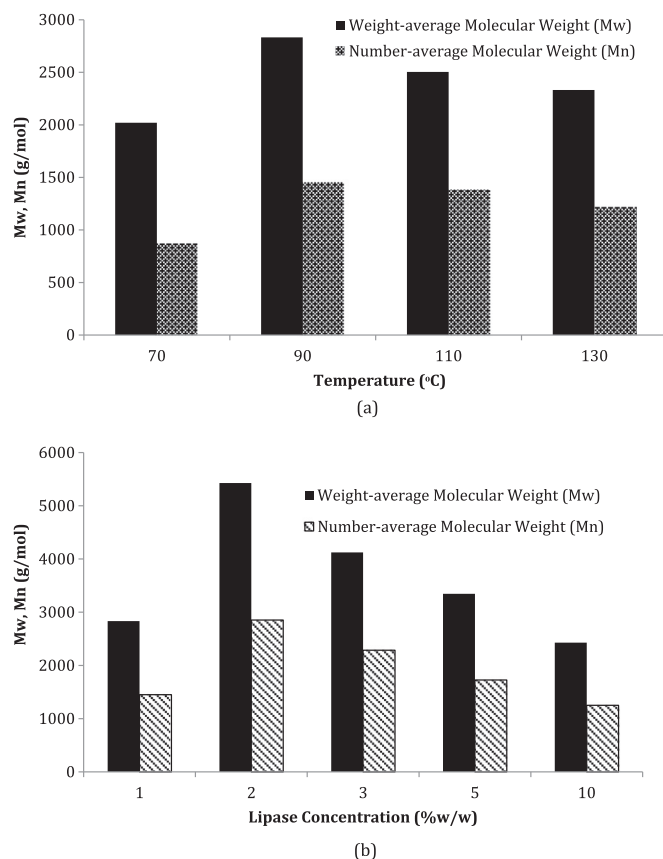

Fig. 4. The molecular weight of PLA (a) with variation of temperature using 1% (w/w) CRL, (b) with variation of CRL concentration at 90 °C. Molecular weight of PLA was calculated as a result of ring opening polymerization. Molecular weight was assayed using gel permeation chromatography (GPC) with tetrahydrofuran (THF) as the solvent with column temperature of 40 °C at 1.0 ml/min. The calibration was performed using polystyrene as the standard polymer molecular weight distribution.

this study was close to the values reported by Park and Xanthos (2009), who found values of 55% and 44% for elements C and O in PLA, respectively.
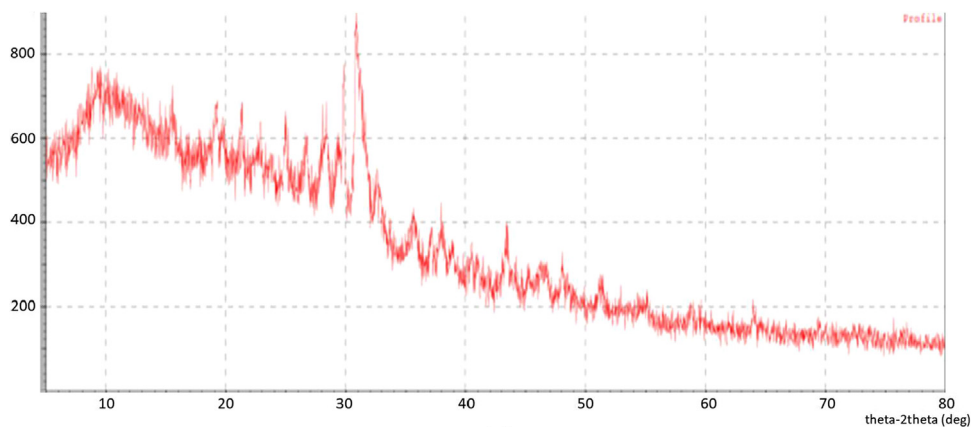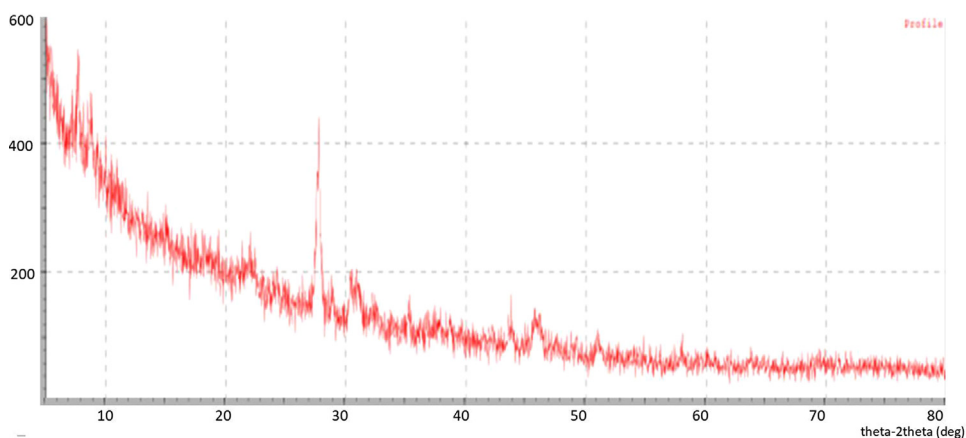
Fig. 5. Lipase diffractogram spectrum (a) before use; (b) after polymerization at 130 °C.
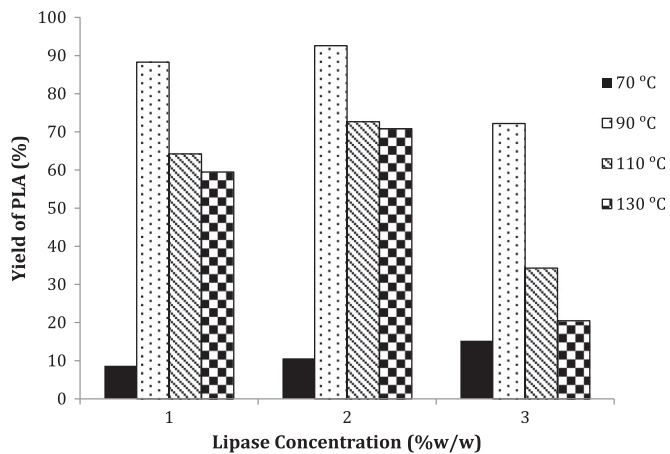

Fig. 6. The effect of temperature and CRL concentration on PLA yield. The PLA yield was calculated at various CRL concentration and temperatures. Temperatures and CRL concentration were varied at 70, 90, 110, 130 °C and 1, 2, 3% (%w/w), respectively.
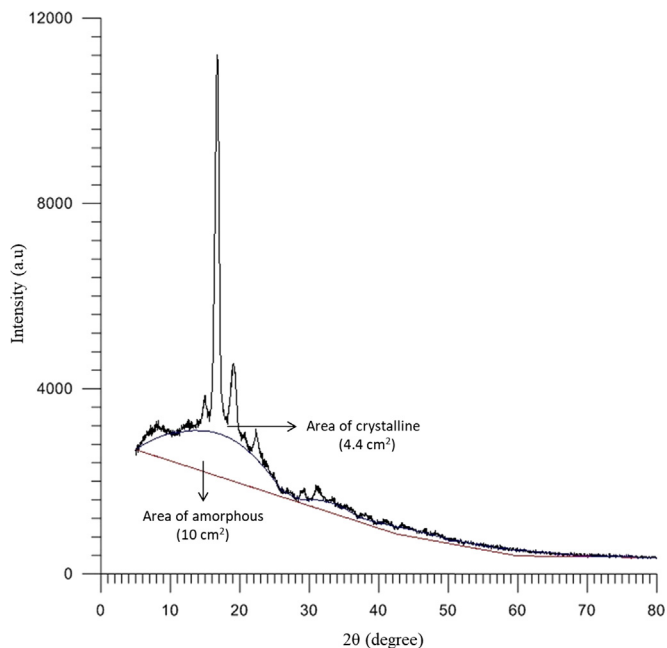

Fig. 7. The XRD spectrum of PLA produced at 90 °C and 2% (w/w) CRL. The polymer crystal structure was analyzed by XRD of Panalitycal type Empyrean. The degree of crystallinity of PLA is 30.56%. The value of crystallinity shows semi-crystalline polymers which formed stereoisomers.
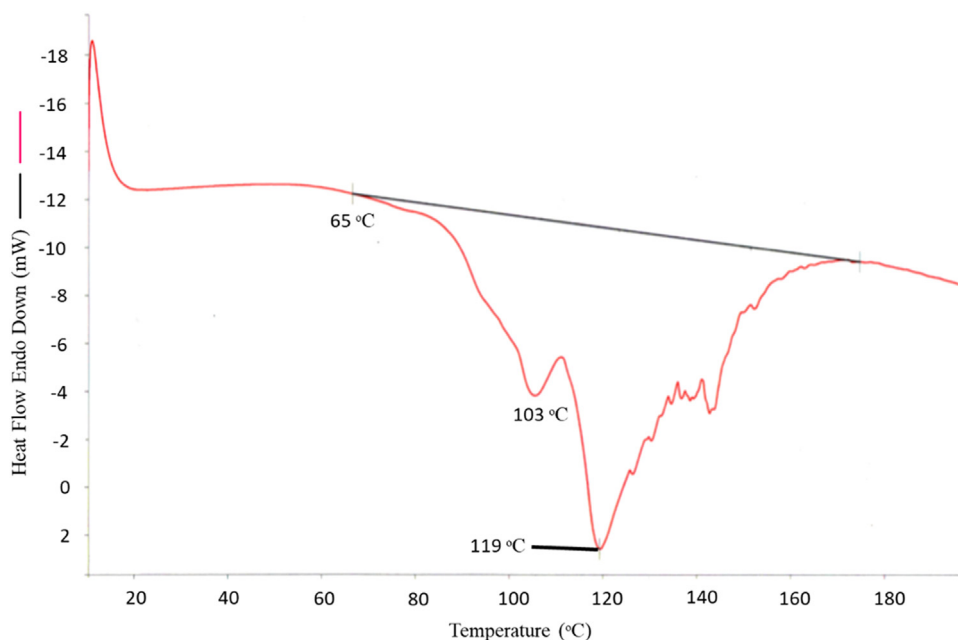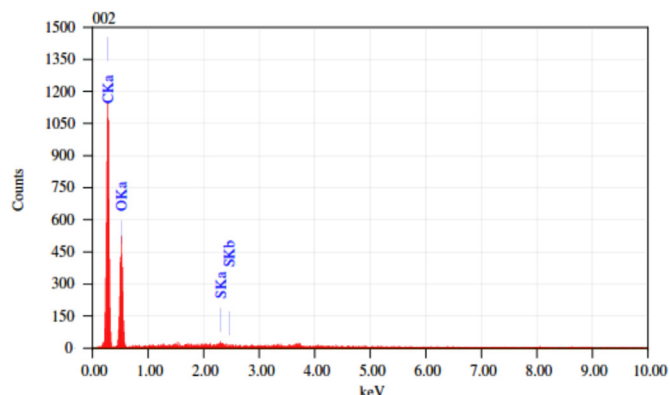
Table 3
Yield and purity at each step of PLA synthesis.

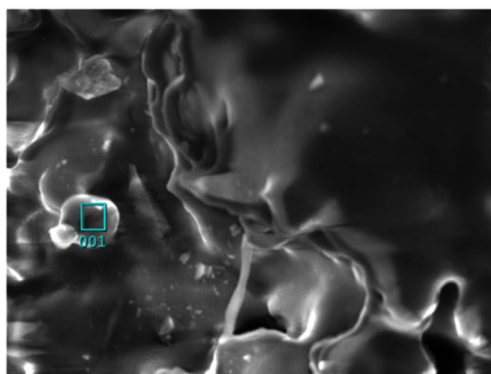| Compound	Step of process	Purity (%)	Yield (%)
L-lactic acid	Raw material	98	
Oligomer	Polycondensation	n.a	88
Lactide	depolymerization	81	79
PLA	Ring opening polimerization	n.a	92

Fig. 8. DSC Graph of resulting PLA. PLA had a Tg of 65 °C and that its Tc and Tm were 103 °C and 119 °C, respectively.

Fig. 9. The SEM images of PLA with magnifications of 1000 × . The PLA was synthesized at 90 °C and 2% (w/w) CRL. PLA morphology was analyzed using SEM *JEOL JSM 6510LA*. The results of energy-dispersive X-ray spectroscopy (EDX) show that the mass percentages of the elements C and O are 52.15 and 47.64 for percent mass and 59.26 and 40.65 for percent atom, respectively.

4. Conclusion

CRL was used as a catalyst in ring-opening polymerization of L-lactide. The catalyst showed the highest activity at a temperature of 90 °C and concentration of 2% (w/w). These experiments also proved that the polymerization of lactide at 90 °C occurred only in the presence of the catalyst. The resulting PLA had an *Mw* of 5428 g/mol and *Mn* of 2854 g/mol at a yield of 93% with the enzyme activity 0.39 U. Thus, this product can be recommended for biomedical applications. The crystallinity of PLA was 31%, and the mass percentages of elements C and O contained in the PLA, as determined by SEM-EDX analysis, were 52% and 48%, respectively.

Acknowledgements

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