ABSTRAK

PENINGKATAN KESTABILAN ENZIM LIPASE DARI BAKTERI *Klebsiella* sp. LPG172 DENGAN IMOBILISASI METODE ADSORPSI PADA MATRIKS HIDROKSIAPATIT

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Penelitian ini dilakukan untuk mempelajari enzim lipase dari bakteri *Klebsiella* sp. LPG172 terimobil dengan hidroksiapatit mengenai kemampuannya sebagai katalis pada reaksi hidrolisis dan transesterifikasi. Pada penelitian ini dilakukan peremajaan dan produksi lipase dari bakteri *Klebsiella* sp. LPG172 yang memiliki kondisi optimum pertumbuhan pada pH 7 dan waktu inkubasi 66 jam pada media NA/NB mengandung minyak zaitun, serta serangkaian pemurnian enzim lipase seperti fraksinasi ammonium sulfat, dialisis dan kromatografi kolom filtrasi gel sephadex G-75 untuk memperoleh enzim lipase murni. Kemudian dilakukan imobilisasi pada matriks hidroksiapatit, ditentukan kondisi optimum enzim imobil dan dilakukan pemakaian berulang.

Ekstrak kasar enzim memiliki kadar protein sebesar 3,34 mg/mL dan aktivitas unit enzim sebesar 34,6 U/mL. Setelah pemurnian kromatografi kolom filtrasi gel sephadex G-75 enzim lipase memiliki kadar protein sebesar 0,70 mg/mL dan aktivitas unit sebesar 1.401,67 U/mL pada uji hidrolisis, sedangkan uji transesterifikasi sebesar 1.723,6 U/mL. Imobilisasi lipase pada matriks hidroksiapatit menunjukkan aktivitas unit sebesar 962,67 U/mL pada uji hidrolisis dan 1.334,8 U/mL pada uji transesterifikasi.

Imobilisasi lipase *Klebsiella* sp. LPG172 pada matriks hidroksiapatit mengubah kondisi optimum aktivitas enzim. Pada reaksi hidrolisis kondisi optimum lipase imobil memiliki pH 8, suhu 50˚C dan inkubasi 15 menit dibandingkan enzim bebasnya yang menunjukkan aktivitas optimum pada pH 7, suhu 80˚C, dan inkubasi 25 menit. Pada reaksi transesterifikasi memiliki kondisi optimum lipase imobil pH 8, suhu 60, dan inkubasi 10 menit dibandingkan enzim bebasnya yang optimum dengan pH 7, suhu 70˚C, dan inkubasi 25 menit. Kondisi optimum enzim imobil tersebut digunakan untuk pemakaian berulang yang stabil pada reaksi hidrolisis sebanyak 2 kali pemakaian dengan aktivitas sisa sebesar 30% dari enzim bebasnya dan aktivitas enzim imobil sebesar 37% dari enzim bebasnya dipemakaian ke 4 pada reaksi transesterifikasi.

Kata kunci : *Klebsiella sp.* LPG172, imobilisasi, hidroksiapatit, hidrolisis, transesterifikasi.

ABSTRACT

INCREASING THE STABILITY OF LIPASE FROM THE BACTERIA *Klebsiella* sp. LPG172 BY IMMOBILIZING THE ADSORPTION METHOD ON THE HYDROXYAPATITE MATRIX

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This study was conducted to study lipase from *Klebsiella* sp. LPG172 bacteria immobilized with hydroxyapatite regarding its ability as a catalyst in hydrolysis and transesterification reactions. In this study, the rejuvenation and production of lipase from *Klebsiella* sp. LPG172 were carried out which had optimum growth conditions at pH 7 and an incubation time of 66 hours in NA/NB media containing olive oil, as well as a series of lipase purifications such as ammonium sulfate fractionation, dialysis and sephadex G-75 gel filtration column chromatography to obtain pure lipase. Then immobilization was carried out on the hydroxyapatite matrix, the optimum conditions of the immobilized enzyme were determined and repeated use was carried out.

The crude enzyme extract has a protein content of 3.34 mg/mL and an enzyme unit activity of 34.6 U/mL. After purification by sephadex G-75 gel filtration column chromatography, the lipase has a protein content of 0.70 mg/mL and a unit activity of 1,401.67 U/mL in the hydrolysis test, while the transesterification test is 1,723.6 U/mL. Immobilization of lipase on hydroxyapatite matrix shows a unit activity of 962.67 U/mL in the hydrolysis test and 1,334.8 U/mL in the transesterification test.

Immobilization of *Klebsiella* sp. LPG172 lipase on hydroxyapatite matrix changes the optimum conditions of enzyme activity. In the hydrolysis reaction, the optimum conditions of immobilized lipase have a pH 8, a temperature 50˚C and an incubation 15 minutes compared to the free enzyme which shows optimum activity at pH 7, a temperature 80˚C, and an incubation 25 minutes. In the transesterification reaction, the optimum conditions of immobilized lipase are pH 8, a temperature 60˚C, and an incubation 10 minutes compared to the free enzyme which is optimum at pH 7, a temperature 70˚C, and an incubation 25 minutes. The optimum conditions of the immobilized enzyme are used for stable repeated use in the hydrolysis reaction as many as 2 uses with a residual activity of 30% of the free enzyme and the activity of the immobilized enzyme is 37% of the free enzyme in the 4th use in the transesterification reaction.

Keywords : *Klebsiella* sp. LPG172, immobilization, hydroxyapatite, hydrolysis, transesterification.