Kinerja enzim kolesterol oksidase streptomyces sp. terimobilisasi magnetit modifikasi dan rhodococcus erythropolis Bl21(DE3) untuk reaksi oksidasi kolesterol = Performance of the streptomyces sp. cholesterol oxidase immobilized modified magnetite and rhodococcus erythropolis BL21(DE3) for cholesterol oxidation reactions

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Abstrak

Penelitian ini mencakup rangkaian oksidasi kolesterol berupa studi oksidasi kolesterol, oksidasi dengan menggunakan substrat hewani, oksidasi dengan enzim terimobilisasi material magnetit silikon dioksida (M-SiO2)/magnetit kitosan (M-Chit) dan oksidasi dengan protein rekombinan Rhodococcus erythropolis BL21(DE3) (RhoChoA). Enzim kolesterol oksidase yang diproduksi dengan metode submerged fermentation dari Streptomyces sp memiliki nilai aktivitas sebesar 5,12 U/mL dan aktivitas RhoChoA sebesar 17,9 U/mL. Studi kinetika dilakukan dengan menggunakan orde satu dengan reaksi irreversible. Optimasi produksi enzim dilakukan dengan memperhatikan faktor suhu dan jenis substrat. Untuk meningkatkan karakteristik enzim, imobilisasi dilakukan pada enzim kolesterol oksidase Streptomyces sp. Material magnetit disintesis dengan metode sol-gel dengan modifikasi menggunakan magnetit silikon dioksida dan magnetit kitosan yang diberi kode M-SiO2 dan M-Chit secara berurutan. Enzim hasil produksi diimobilisasi dengan menggunakan teknik cross-linking. Hasil karakterisasi FTIR dari material magnetit menunjukkan gugus fungsi M-O di bilangan gelombang 559,88; 598,91 dan 680,1 cm-1 , gugus Si-O di bilangan gelombang 1615,78 dan 1761,65 cm-1.
Uji oksidasi dilakukan dengan beberapa variabel bebas yaitu konsentrasi enzim (0,5; 1; 2 mg/mL), konsentrasi substrat (0,75; 1,25; 2,5 mg/mL), waktu oksidasi (5, 30, 60, 120, 180 menit), serta bentuk enzim (ekstrak kasar enzim kolesterol oksidase dan enzim kolesterol oksidase terimobilisasi). Hasil uji oksidasi dikuantifikasi dengan menggunakan HPLC untuk menganalisis konsentrasi substrat dan konsentrasi enzim yang optimum dalam oksidasi yang dijadikan sebagai referensi dalam penentuan uji biosensor kolesterol. Oksidasi kolesterol dengan menggunakan substrat hewani dilakukan dengan ekstraksi dengan pelarut lemak. Kuantifikasi kadar kolesterol dalam sampel menunjukkan susbtrat dari lemak hewani memiliki konsentrasi kolesterol tertinggi dari kuning telur dengan konsentrasi 1,94 mg/mL, hati ayam (0,93 mg/mL), daging sapi (0,25 mg/mL) dan daging ayam (0,23 mg/mL). Enzim kolesterol oksidase dengan konsentrasi 2 mg/mL dapat mengoksidasi ekstrak kasar kolesterol dari kuning telur, hati ayam dan daging ayam hingga teroksidasi 20%, sedangkan ekstrak kasar kolesterol dari daging sapi teroksidasi sebesar 10%. Hasil uji oksidasi dengan menggunakan HPLC diperoleh konsentrasi substrat secara optimal dioksidasi oleh enzim terimobilisasi M-SiO2 dengan konsentrasi 20 mg/mL serta konsentrasi kolesterol 1,94 mM sebesar 90%, sedangkan enzim kolesterol oksidase bebas mengoksidasi kolesterol sebesar 80%. Uji oksidasi kolesterol menggunakan enzim kolesterol oksidase terimobilisasi magnetit kitosan (M-Chit) ditemukan konsentrasi substrat yang optimum adalah 2,5 mg/mL dan konsentrasi enzim yang paling efektif adalah 2 mg/mL. Reaksi oksidasi kolesterol dengan kondisi optimum dan menggunakan enzim terimobilisasi M-Chit dapat mengoksidasi kolesterol sampai 10%. Uji penggunaan kembali material M-Chit dalam proses imobilisasi dapat digunakan sebanyak 2 kali.

.....This research includes a series of cholesterol oxidation in the form of cholesterol oxidation studies, oxidation using animal substrates, oxidation with immobilized enzymes of magnetite silicon dioxide (M-SiO2) / magnetite chitosan (M-Chit) and oxidation with recombinant protein Rhodococcus erythropolis BL21 (DE3) (RhoChoA). Cholesterol oxidase enzyme produced by the submerged fermentation method from Streptomyces sp has an activity value of 5.12 U / mL and a RhoChoA activity of 17.9 U / mL. The kinetic study was carried out using first order with an irreversible reaction. Optimization of enzyme production is carried out by controlling the temperatur and type of substrate. To improve the characteristics of the enzyme, immobilization was carried out on the cholesterol oxidase enzyme Streptomyces sp. The magnetite material was synthesized by the sol-gel method with modification using magnetite silicon dioxide and magnetite chitosan which were coded M-SiO2 and M-Chit, respectively. The immobilized enzymes are produced using a cross-linking technique. The FTIR characterization results of the magnetite material showed the M-O functional group at wave number 559.88; 598.91 and 680.1 cm-1, the Si-O group at wave numbers 1615.78 and 1761.65 cm-1.

The oxidation test was carried out with several independent variables, namely enzyme concentration (0.5; 1; 2 mg / mL), substrate concentration (0.75; 1.25; 2.5 mg / mL), oxidation time (5, 30, 60, 120, 180 minutes), as well as the form of the enzyme (crude extract of the cholesterol oxidase enzyme and the immobilized cholesterol oxidase enzyme). The results of the oxidation test were quantified using HPLC to analyze the optimum substrate concentration and enzyme concentration in oxidation which were used as references in determining the cholesterol biosensor test. Cholesterol oxidation using animal substrates was carried out by extraction with fat solvents. The quantification of cholesterol levels in the sample showed that the animal fat substrate had the highest cholesterol concentration from egg yolks with a concentration of 1.94 mg / mL, chicken liver (0.93 mg / mL), beef (0.25 mg / mL) and chicken meat. (0.23 mg / mL). Cholesterol oxidase enzyme with a concentration of 2 mg / mL can oxidize the crude extract of cholesterol from egg yolk, chicken liver and chicken meat up to 20%, while the crude extract of cholesterol from beef is oxidized only 10%. The results of the oxidation test using HPLC showed that the optimal substrate concentration was oxidized by the immobilized enzyme M-SiO2 with a concentration of 20 mg / mL and a cholesterol concentration of 1.94 mM of 90%, while the free cholesterol oxidase enzyme was 80% oxidized. Cholesterol oxidation test using immobilized cholesterol oxidase enzyme magnetite chitosan (M-Chit) found that the optimum substrate concentration was 2.5 mg / mL and the most effective enzyme concentration was 2 mg / mL. Cholesterol oxidation reaction under optimum conditions and using the immobilized enzyme M-Chit can oxidize cholesterol up to 10%. The M-Chit reuse test in the immobilization process can be used 2 times