DETERMINATION OF ENZYME KINETIC PARAMETERS ON SAGO STARCH HYDROLYSIS BY LINEARIZED GRAPHICAL METHODS

(Penentuan Enzim Parameter Kinetik pada Hidrolisis Kanji Sagu dengan Kaedah Grafik Lelurus)

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Abstract

Amyloglucosidase (E.C. 3.2.1.3) from Aspergillus niger was used to hydrolyze the sago (Metroxylon sagu) starch into reducing sugars. The experiment was conducted at constant temperature, 55 °C; pH, 4.5 and enzyme amount, 0.2 U/ml, respectively. In this investigation, the substrate concentration was varied ranging from 1.0 – 7.0 g/L. The obtained data were then fixed into linearized plots namely Lineweaver-Burk and Langmuir models to calculate enzyme kinetic parameters, $K_m$ and $V_{max}$. Both of the $K_m$ and $V_{max}$ (mM, mol/min) values from each plot were: Lineweaver-Burk (26.53, 3.31) and Langmuir (13.52, 2.35). Among the linearized models, $K_m$ and $V_{max}$ values acquired from Langmuir plot was chosen.

Keywords: enzyme kinetic parameters, $K_m$ and $V_{max}$, Langmuir, Lineweaver-Burk, sago starch hydrolysis

Abstrak

Amyloglucosidase (EC 3.2.1.3) dari Apergillus niger digunakan untuk menghidrolisis kanji sagu (Metroxylon sagu) kepada gula penurun. Eksperimen ini dijalankan pada suhu 55°C; pH, 4.5 dan enzim 0.2U/ml. Kecepatan substrak yang diuji dalam kes ini adalah dalam lingkungan 1.0 – 7.0 g/L. Data yang diperolehi akan digunakan untuk terus ke dalam plot linear Lineweaver-Burk serta Langmuir bagi tujuan mengira enzim parameter kinetic: $K_m$ dan $V_{max}$. Dua-dua nilai $K_m$ dan $V_{max}$ (mM, mol/min) dari Lineweaver-Burk dan Langmuir masing – masing adalah 26.53, 3.31; 13.52, 2.35. Nilai $K_m$ dan $V_{max}$ dari Langmuir model dipilih dalam kajian ini.

Kata kunci: enzim parameter kinetik, $K_m$ dan $V_{max}$, Langmuir, Lineweaver-Burk, hidrolisis kanji sagu

Introduction

Enzyme kinetic is the branch of enzymology that deals with the factors affecting the rate of enzyme-catalyzed reactions. In general, the most important factors are those of enzyme concentration, ligand concentrations (substrates, products, inhibitors and activators), pH, ionic strength and temperature. When all these factors are analyzed properly, it is possible to learn a great deal about the nature of the enzyme-catalyzed reaction. In addition, a kinetic analysis can lead to a model for an enzyme-catalyzed reaction and conversely, the principles of enzyme kinetic can be used to write the velocity equation for a model which can then be tested experimentally [1]. In general, the Michaelis and Menten [2] framework has proven to be simple yet powerful approach to describe the kinetic of most enzyme reactions (Eq. 1.0):