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Effect of Different Substrate Concentrations in Starch Hydrolysis Reaction by Free α -Amylase Enzyme

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Structured Abstract

Background: Starch is a complex semi-crystalline structure comprised of amylose and amylopectin. Amylose is the linear chain while amylopectin is the branched chain connected by glycosidic bond. It is widely acknowledged as the primary energy source in human diet due to its high carbohydrate content that could be digested into glucose as the stored energy. The study holds a significant application in industries of food and beverage, pharmaceuticals and biofuel production. Therefore, the study aims to determine the effect of different substrate concentrations ranging from 0.2%, 2.0%, and 4.0% on reducing sugar production by free α -Amylase enzyme using starch as substrate.

Methods: The α -Amylase enzyme was extracted from *Aspergillus oryzae*, substrate with 0.2%, 2.0%, and 4.0%, DNS reagent and Sodium Phosphate buffer were prepared. The study was conducted under controlled conditions of pH 7, temperature 40°C, and under reaction time of 4 hours during the starch hydrolysis reaction before quantification by DNS assay. The Michaelis-Menten and Lineweaver-Burk model were used in determining the enzyme's kinetic parameters (V_{max} and K_m).

Results: The experimental results showed that the reducing sugar or glucose production rate increased from 0.06, 0.47, and 1.10 (g/L) with increasing substrate concentration. The relationship obtained from experimental data has proved the Michaelis-Menten theory where the higher the substrate concentrations, the higher the rate of starch hydrolysis. Meanwhile, the Lineweaver-Burk plot method was used to calculate the enzyme kinetic parameters of V_{max} (0.01 M/min) and K_m (97.29 mol/L) to describe the α -Amylase's catalytic efficiency and its affinity to the substrate for catalysis.

Conclusion: In conclusion, the findings of this study indicated that the higher the substrate concentration, the higher the rate of starch hydrolysis at once increasing the amount of glucose produced. However, the values of V_{max} and K_m obtained were far from the expected values, suggesting in increasing the range of substrate concentrations.

Keywords: Substrate concentration, starch hydrolysis, enzyme activity, alpha amylase, DNS reagent

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