PURIFICATION AND CHARACTERIZATION OF EXTRACELLULAR LIPASE PRODUCED ON SOLID STATE FERMENTATION BY *RHIZOPUS ARRHIZUS*

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Abstract

Lipases are enzymes of great industrial importance that are widely used in various fields of industry including food industry. They catalyze two types of reactions - hydrolysis and transesterification. The hydrolysis reaction of water-insoluble triglycerides takes place on the substrate-water interface as the ultimate acceptor of fatty acids is the water molecule. Transesterification reactions usually proceed in an organic medium with low water content. Due to the numerous applications of lipases in industry, there is a need to study their characteristics. The aim of the present work is isolation and purification of lipase obtained by solid-state fermentation of *Rhizopus arrhizus*.

The proposed scheme for isolation and purification of lipase, involved precipitation with 65% i-propanol and ion exchange chromatography with diethylaminoethanol covalently linked to sepharose (DEAE-Sepharose). Chromatographic isolation was performed on fast protein liquid chromatography (FPLC). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to monitor the purification process and to determine the molecular weight of the enzymes.

As a result of the applied procedure for isolation and purification of lipase, multiple forms of the enzyme, designated as Lipase 1 and Lipase 2, were isolated to a homogeneous type. The two enzymes differed in their biochemical characteristics. Lipase 1 had a molecular weight of 55,000 Da and exhibited maximum catalytic activity at pH 8.0 and 40 ^{0}C. Isolated Lipase 2 was characterized by a molecular weight of 28,000 Da and showed maximum catalytic activity at pH 9.0 and a temperature of 45 ^{0}C. Results describing the thermal stability of the two enzymes were presented. Lipase 2 was characterized by a lower value of Km = 0.54 mM and a higher value of Vmax = 0.22 μmol/(min.mL) compared to Lipase 1 with Km = 0.98 mM and Vmax = 0.09 μmol/(min.mL). The activation energy of the reactions catalyzed by the two lipases was also determined. The reaction catalyzed by Lipase1 was characterized by Ea = 123.22 kJ/mol, and the reaction catalyzed by Lipase 2 by Ea = 97.51 kJ/mol.

Two lipases isolated differed in their biochemical characteristics. Lip 2 exhibited higher activity at higher temperature and pH in comparison to Lip 1 and was characterized by a lower Km value, which indicated a higher affinity of the enzyme to the substrate. Both enzymes showed high activity at alkaline pH of 8.0-9.0 and at relatively high temperatures of 40 - 45 ^{0}C, which allows them to be used in the production of detergents.

Key words: *Lipase, Rhizopus arrhizus, Purification, Properties.*