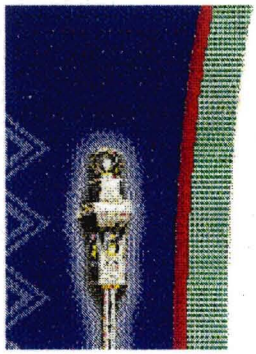

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DEPARTMENT OF APPLIED BIOLOGY AND BIOCHEMISTRY

PROJECT TITLE:

OPTIMISATION OF LIPASE PRODUCTION IN *AUREOBASIDIUM PULLULANS* ISOLATES IN ZIMBABWE

UNDERTAKEN BY

FAITH MANDIVENGA (N990109B)

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A dissertation presented in the partial fulfillment of the requirements for a Bachelor of Sciences Honours Degree in Applied Biology and Biochemistry from NUST, Bulawayo, Zimbabwe.

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ABSTRACT

Local isolates of *Aureobasidium pullulans* have shown the ability to produce appreciable levels of lipases when grown on appropriate carbon sources. The present study aimed at optimizing the production of these lipases. Three isolates (Ap25, Ap20 and Ap38) previously screened for lipase activity were used in this study.

Optimization of enzyme production was achieved by altering, one at a time, the nitrogen and carbon sources, the initial pH of the culture medium, and the growth period. Temperature and pH stability of the enzyme was also assessed for a period of one hundred and fifty minutes. Lipase activity was determined from culture filtrates by measuring the increase in the concentration of ρ- nitrophenol by the method of Mackness *et al*, (1983). Para- nitrophenol acetate was used as the substrate. Production of extracellular lipases was substantially enhanced when the carbon source, nitrogen source, the initial pH of the culture medium and the growth period were consecutively optimized.

The optimum growth period for Ap38 and Ap25 isolates was five days while that of isolate Ap20 was six days. The initial pH of the culture medium that was optimal for the three isolates was pH 7.0. At the optimum pH, a combination of 0.5% olive oil and 0.5% Tween 80 was the best carbon source for the production of lipases while the maximum specific activity was obtained when diammonium hydrogen phosphate was used as the nitrogen source. The enzyme was fairly stable between pH 4 and 7 retaining more than 80% of its activity after an incubating period of ninety minutes. The stability at pH 5 and 6 was equal at the end of incubation although the enzyme retained 97% of its activity at pH6 after 90 minutes compared to 84% at pH 5 for the same incubation period. In alkaline condition that is at pH 8 the enzyme was very unstable losing 15% of its activity in the first 30 minutes of incubation.

It can therefore be concluded that for maximum enzyme production, the isolates of *A. pullulans* should be incubated in minimal salts medium with a combination of

polyoxethylenesorbitan monooleate (Tween 80) and olive oil as the carbon source, diammonium hydrogen phosphate as nitrogen source. The initial pH of 7.0 should be used and the isolates incubated at 25°C. Harvesting time should be five days for the isolate Ap38 and Ap25 and six days for the isolate Ap20.