

**B. V. BHOOMARADDI COLLEGE OF ENGINEERING & TECHNOLOGY,
VIDYANAGAR, HUBLI- 580031**

(AFFILIATED TO VISVESVARAYA TECHNOLOGICAL UNIVERSITY, BELGAUM)



DEPARTMENT OF BIOTECHNOLOGY

A Project on

**PURIFICATION AND CHARACTERIZATION OF LIPASE FROM
ASPERGILLUS SPECIES AND ITS POTENTIAL APPLICATION IN
BIODIESEL PRODUCTION AND DETERGENT INDUSTRIES**

SPONSORED BY

KARNATAKA STATE COUNCIL FOR SCIENCE AND TECHNOLOGY

(KSCST)

Under The Guidance Of

Prof. Mrs. ZABIN K BAGEWADI

By

Preeti Sarashetti

2BV08BT030

Rameshwari Rayaji

2BV08BT032

Abstract:

To date, a large number of lipases from filamentous fungi have been extensively studied both from the biochemical and from the genetic point of view because of their high production, good stability, recognize a wide variety of substrates and catalyze many different reactions. The lipase-catalyzed trans-esterification reaction is increasingly becoming more attractive for use in the production of biodiesel fuels and the latest trend in detergent industry is lipase preparation usage for stain removal, which are compatible with the detergent formulations. Hence the present study is initiated. The potential fungal isolates were screened using Rhodamine B and Trybutyrin plate Assay method. Further production of lipase was carried out by optimizing physico-chemical parameters like pH and temperature, and optimized values were pH 7, 10 and 30⁰C, 50⁰C. The lipase activity was measured using p-nitro phenyl Palmitate as substrate by spectrophotometric method. Purification of lipase was carried out by ammonium sulphate precipitation followed by dialysis and ion exchange chromatography for G2, G1 24hr sample and G2 72 hr sample which showed fold purification of 5.1, 7.3 and 9.6 respectively. To improve strains physical mutation (UV irradiation) was carried out and 6 different mutants were obtained and kill curve was analyzed. Characterization of partially purified lipase showed that the effect of temperature on relative activity for both G1, G2 i.e 24th hr and 72nd hr samples showed increase in activity upto 80⁰C, whereas for B1 (72 hr) and B4 (24 hrs) showed increase in activity upto 70⁰C. The effect of pH on partially purified lipase confirmed that B1, G1, G2, were acidic lipase producers and B4 was basic lipase producer. The effect of various metal ions (1mM) confirmed that Ca²⁺, Mg²⁺ stimulated lipase activity when compared to control sample. Whereas other metal ions like Zn²⁺, Cu²⁺, Hg²⁺, Co²⁺, K⁺, Na⁺, EDTA showed relative inhibition. In present study the lipase activity was not totally inhibited by any of the metal ions. The effect of surfactants like Tween 20 and Triton-X 100 on lipase activity was higher compared to partially purified lipase, hence surfactants showed stimulatory effect on lipase activity. This partially purified lipase was used in the production of biodiesel from pongamia pinnata oil using various organic solvents by enzymatic tranesterification method and methanol was found to be the best solvent for production of biodiesel. Various properties of biodiesel measured were found to be in line with standard diesel values. In detergent industry wash performance for combination of lipase with detergents and tap water was higher than the normal tap water and detergent. Wash performance analysis of the commercial detergent for removal of fatty stains improved upon addition of this lipase.

Keywords: *Aspergillus niger* (B1, B4), *Aspergillus flavus* (G1, G2), Lipase, Purification, Mutation, *Pongamia pinnata*, Viscosity, Acid value, Biodiesel, Detergents.