Introduction
1. INTRODUCTION

Enzymes are considered as nature’s catalysts that accelerate reactions by lowering the activation energy barrier. These may be intracellular or extracellular. These have attracted researchers the world over due to their wide range of applications in food, dairy, pharmaceutical, textile, paper and pulp, leather, and beverage industry. The particular benefits offered by enzymes are their specificity and mild reaction conditions. Increasingly, enzyme based strategies are replacing traditional chemical methods in both laboratories and industries. The major industrial enzymes include alkaline protease, α-amylase, xylanase, lipase, cellulase, penicillin acylase, laccase, glucose isomerase, tannase, pectinase, phytase etc. Among the currently used industrial enzymes, hydrolases such as lipases and proteases are dominant and extensively used in detergent, food, dairy, and chemical industries. Various carbohydrases, primarily amylases and cellulases, represent the second largest group of industrial enzymes (Kirk et al., 2002). India imports about 70% of the total enzyme consumption. The major enzyme manufacturing Indian industries are Novozyme, Biocon, Advanced Biochemicals, Rossari Biotech, Zytex, Jay Biozyme Technologies, etc. (Chandel et al., 2007).

The blooming industrial enzyme market is one of the major revenue generators in the life sciences industry sector. The world market for enzymes is estimated to grow 8% per year to eight billion dollar in 2013 (www. Reportlinker.com). According to Global Industry Analysts Inc., the global market for enzymes is expected to reach 4.3 US billion dollar by the year 2015. The factors driving market growth include ever increasing requirements of the pharmaceutical industry and robust expansion of the biocatalyst sector. In addition, advances in enzyme engineering using biotechnological methods and processes, are likely to fuel the market growth and the major product segments include carbohydrases, proteases and lipases (www.reportlinker.com/World-Industrial-Enzymes-Market). The use of industrial enzymes allows the technologists to develop processes that more closely approach the gentle, efficient processes in nature. Current commercial use of industrial enzymes, together with new applications, results in significant savings in resources such as raw materials and water consumption, as well as the improvement of
energy efficacy for the benefit of both the industry in question and the environment. This will continue to play an important role in maintaining and enhancing the quality of life we enjoy today and the sustainability for generations to come.

Lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) belongs to the group of hydrolases whose main biological function is to catalyze the hydrolysis of insoluble triacylglycerols to generate free fatty acids, mono- and diacylglycerols and glycerol. These enzymes catalyze the hydrolysis of long chain acylglycerol at an oil-water interface. Lipases are highly diversified in enzymatic properties and substrate specificity. In addition to their natural function of hydrolyzing carboxylic ester bonds, lipases can catalyze esterification, interesterification, and transesterification reactions in non-aqueous media to synthesize a growing range of products of potential industrial interest (Schmidt-Dannert, 1999; Zhang et al., 2011). These reactions usually proceed with high chemo-, regio- and/or enantioselectivity, making lipases an important group of biocatalysts (Reetz, 2002; Snellman et al., 2002). Among the lipases distributed in microorganisms, animals and plants, the microbial lipases are commercially important (Houde et al., 2004; Jaeger and Eggert, 2002). This is because of the availability of a wide range of hydrolytic and synthetic activities, the high yields possible, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations, and easy cultivation of microbes on inexpensive media (Hasan et al., 2006; Saxena et al., 1999; Vakhlu and Kour, 2006; Wiseman, 1995). Many microorganisms are known as potential producers of extracellular lipase, including bacteria, yeast and fungi (Abada, 2008). Bacterial lipases are generally more stable than animal or plant lipases. Some important lipase-producing bacterial genera include Bacillus, Pseudomonas and Burkholderia. Bacterial lipases from Bacillus possess interesting properties that make them potential candidates for biotechnological applications.

Lipases have emerged as key enzymes which find usage in food, paper, textile, and detergent industries, wastewater treatment, production of fine chemicals, pharmaceuticals, cosmetics, etc. (Athawale et al., 2003; Charoenpanicha et al., 2011; Gupta et al., 2004; Haki and Rakshit, 2003; Jaeger et al., 1999; Pandey et al., 1999; Sharma et al., 2001). The commercial use of lipases is a billion dollar business that comprises a wide variety of different applications (Bornscheuer, 2000; Jaeger and Reetz, 1998). Following proteases and carbohydrases, lipases are considered to be the third largest group based on total sales
volume (Jaeger et al., 1999). The chemo-, regio- and enantiospecific behavior of these enzymes has caused tremendous interest among scientists and industrialists. Lipases can be used as biocatalyst in esterification and transesterification reactions. The biosynthesis of esters is currently of much commercial interest because of the increasing popularity and demand for natural products amongst consumer. Lipases can be used to resolve the racemic mixtures and to synthesize the desired chiral building blocks for pharmaceuticals, agrochemicals and pesticides (Andualema et al., 2012). To improve detergency, modern types of heavy duty powder detergents and automatic dishwasher detergents usually contain one or more enzymes, viz protease, amylase, cellulase and lipase (Ito et al., 1998). It is estimated that every year, about 1000 tons of lipases are added to approximately 13 billion tons of detergents (http://www.aukbc.org/beta/bioproj2/introduction.html). In textile industry, lipase is used to assist in the removal of size lubricants, in order to provide a fabric with greater absorbency for improved levelness in dyeing. Its use also reduces the frequency of streaks and cracks in the denim abrasion systems. In paper and pulp industry, lipase is used to remove the ‘pitch’ or the hydrophobic components of wood that interfere during the elaboration of paper pulp mainly triglycerides and waxes.

In view of the remarkable importance of lipases, it seems worthwhile for the industrial enzymologists to isolate microorganisms capable of producing novel biocatalysts with desirable properties. Microbial diversity is a major source for biotechnological products and processes as the biosphere is dominated by microorganisms. Although there are several reports on lipolytic microorganisms yet with the advancement in the biotechnology, there has been growing demand for the enzymes with novel properties.

Microbial lipases can be produced both by submerged (SmF) and solid state fermentation (SSF). Fungi are preferably cultivated in SSF whereas bacteria and yeasts are cultivated in SmF (Dutra et al., 2008). Each method has its own advantages and limitations. SmF is an attractive method as the process variables can be easily controlled while in SSF, the lower mass transfer processes, related to gases and nutrients diffusion and temperature may take place. In any fermentation process, medium composition is of crucial importance because it significantly affects product concentration, yield and productivity. In addition, enzyme production depends on several nutritional and physico-chemical factors.
Thus, to obtain maximum enzyme yield it becomes necessary to optimize fermentation conditions. It is therefore, worthwhile to optimize the growth parameters.

Parametric optimization can be done by using either one variable approach or statistical methods such as response surface methodology (RSM). Using statistical optimization, interaction among the factors can be easily studied which is not possible by one variable approach. A certain degree of purification of enzymes is needed for their application in industries like food, pharmaceuticals, chemical, cosmetics etc. Besides, purified enzyme is needed for investigation of its characteristics.

The industrial applications of soluble enzymes may be limited by their low storage, high costs of production and instability in adverse environments. These limitations may be overcome to a great extent by using immobilized lipase. The advantages of lipase immobilization include its reusability (and hence economical), improved stability, ease of product separation, greater efficiency and control of its catalytic activity, and termination of the reaction by removing the enzyme from the reaction mixture (Bhushan et al., 2008). Moreover, products are not contaminated with the enzyme, which is especially useful in the food and pharmaceutical industries. So, it would be pertinent to immobilize lipase and determine its potential use in industry.

Keeping the above facts in view, the present investigation was planned with the following objectives:

- Isolation & screening of lipase-producing microorganisms from natural sources
- Production of lipase in submerged fermentation by the isolated microorganism
- Optimization of parameters for enhanced lipase production
- Purification and characterization of lipase
- Immobilization of lipase
- Application of the isolated lipase