Summary

Enzymes are considered as natures catalysts. Most enzymes today are produced by the fermentation lipids constitute a large part of the earth's biomass. Lipolytic enzymes play an important role in turn-over of these water-insoluble compounds (lipids). The lipolytic enzymes are involved in breakdown and mobilization of lipids within cells of individual organism. Microorganisms have been found to produce emulsifying agents or biosurfactants to solubalise lipids. These Microbial enzymes are often more useful than enzymes from plants or animals because of the great variety of catalytic activity available, the high yields possible, case in genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of Microorganism on in expensive media. Microorganism are also more stable than their corresponding plant and animal enzymes and their production is more stable than their corresponding plant and animal enzymes and their production is more convenient and safer.

Lipolytic enzymes are currently attracting an enormous attention because of their biotechnological potential. They constitute the most important group of biocatalysts for biotechnological applications. The novel biotechnological application has successfully established using lipases for the synthesis of the industrially important chemicals manufactured from fats and oils by chemical processes could be produced by lipases with greater rapidity and specificity under mild conditions. The chemo-regio and enentio specific behavior of these enzymes has caused tremendous interest. As a result the Microbial lipases used for the synthesis of biopolymers and biodiesel, the production of enentiopure pharmaceuticals, agrochemicals and flavor compounds.

Only about 2% of the world's microorganisms have been tested as enzyme sources. A relatively small number of bacterial lipases have been studied; fungal lipases have been studied since 1950S. Fungal lipases have been exploited due to low cost of extraction, temperature and pH stability.

In an optimization of production parameters of lipase depends on various environmental factors such as pH, Nitrogen, carbon and lipid sources lipase production is generally stimulated by lipids. The lipase activity steadily increases to a peak and declines. Lipase production is usually coordinated with and dependent the availability of

triglycerides, besides these free fatty acids, hydrolysable esters, bile salts and glycerol also stimulate lipase production.

Most of the commercially important enzymes have been produced from a limited range of microbial cultures, which are generally recognized as safe (GRAS) and are non pathogenic producing no known toxins and having a well established record of safety.

The first step in enzyme manufacture is to identify microbial strains that produce an enzyme with appropriate catalytic specificity and the desired physical properties. Modification of the producer microbial strain is generally required in order to increase greatly the amount of the enzyme produced per unit cell mass. Composition of the culture medium and the fermentation conditions are important determinants of the yield and cost of an enzyme preparation.

In view of the overwhelming microbial diversity present in nature, the selection/ isolation of new sources is still worth while. To screen large number of organisms, cheap, simple, rapid and selective detection methods should be available. Selective strain selection methods for colonies on plates are commonly used for this purpose. When a prospective producer microorganism has been identified several steps are required to convert it into a strain that is suitable for commercial use. A good industrial strain produces a high yield of enzyme and in high concentration. One major impediment to high levels of enzyme and in high concentration.

Subsequent operations include optimization of cell cultivation media, bioprocess engineering analysis and design for large scale enzyme production, followed by a strategy for recovery and purification of the desired enzymes.

In recent research and development efforts in microbial enzyme technology hinge on the following aspects:

- Screening rare or little investigated genera and species of microorganisms from the natural environment and exploring their potential as sources for enzymes with novel properties.

- Strain improvement for hyperproduction of the desired enzyme to make the process economically attractive. Classical mutation coupled with advanced molecular techniques is employed to achieve this objective.

- Optimizing the bioengineering parameters to facilitate process scale-up and achieve high production yields of the desired enzyme.

- Heterologous gene expression in prote in hypersecreting strains of filamentous fungi like *Aspergillus niger*. *Trichoderma reesei* or other suitable hosts. Gene expression to achieve production of thermostable enzymes from hyperthermophiles in mesophilic hosts is a very promising approach for extermozymes manufacture on commercial scale.

- Some of the lead laboratories abroad have successfully isolated DNA from the hyperthermophilic environment such as deep sea smoke chimney vents and after successfully cloning in bacterial hosts, developed screening techniques with high speed robotics to identify clones which can secrete high temperature stable enzymes. This technique overcomes the difficulties associated with the 'in vitro' culture and conservation of the hyperthermophiles isolated from these harsh environments.

The summary of the results obtained and the conclusions arrived at on the basis of these results presented in the present thesis are briefly outlined as below;

1. The Chapter I includes a brief introduction to the research undertaken, where in the necessity of undertaking the work is justified and the aims and objectives of the study are specified.

2. In Chapter II, an exhaustive review of the relevant aspects of application of lipases, lipases origin, chemistry and historical aspects, the characteristics of the and substrates, etc., are presented and also an attempt has been made to evaluate the different substrates used in fermentation for production of lipase.

3. The Chapter III deals with the materials used and methodology adopted in the present study. The aspects covered are mainly isolation, screening, the bacteria and fungi, SMF and SSF (solid state fermentation) procedure and methodology adopted to optimize the fermentation parameters, nutrient supplementation and comparison of lipase production.

4. In the chapter IV the results obtained during the present work are presented. The results arrived in brief indicated in following.
    i. For the isolation of microorganisms. Fungi are isolated from the groundnut industry samples collected during 2000 and 2004. these samples are brought to laboratory plating onto PDA media, only 28 isolates of fungi isolated by primary screening.

    ii. Screening for lipase producing ability from above 28 isolates, by plating onto olive oil and tween-80 supplemented modified CZA medium. 15 species of moulds and three species of yeasts are selected on the basis of utilization of olive oil.

    iii. Screening of lipase producing bacteria, primary screening of bacteria was done through serial dilution of the sample collected and plating onto NA medium and secondary screening for lipase producers tested on tributyrin supplemented medium, out of 23 isolates obtained from primary screening, the strain showing 0.8 cm zone of lipolysis of tributyrin plate assays was the chosen criteria to select the potent producer bacteria, further characterization of 8 bacterial isolates carried out.

5. Screening of various agricultural substrates were carried out. The results of suitability of substrate for solid state fermentation indicated that coconut cake; Groundout cake (1:1) was suitable substrate for lipase production.

    i. Submerged fermentation of five fungal isolates are subjected namely *Aspergillus niger* GC1, *Aspergillus niger* GCS 2, *Aspergillus terreus* GCN 8, *Aspergillus flavus* NCIM 650 and *Aspergillus niger* NCIM616. In comparison to all five isolates, *Aspergillus niger* NCIM 616 and

Aspergillus terreus GCN8 Identified as potential producer of lipase production revealed.

ii. Here in our investigation use of several substrates coconut oil cake, Ground nut oil cake GOC: GNC (1:1) were used to test the lipase yield from 04 Isolates of *Aspergillus* was compared.

iii. In *Candida cylendracea* activity 55 U/ml of broth and In *Candida rugosa* NCIM 3467 was found to be activity of 52 U/ml where as in starting from 24 hours to 144 hours of incubation all 3 isolates shown lipase activity of exponential yield.

iv. Amount the lipase producing eight species of bacteria are used, based on tributyrin hydrolysis and *Bacillus licheniformis* NCIM 2042. as an inoculum for fermentation. These bacterial strains cultured on medium composed of 30% peptone, 1% yeast extract, 0.5% Nacl and 1% olive oil at pH 7-7.2. The cultivation was done for 80 hours at 30-32°C.

6. The success and direction of fermentation depends on obtaining a proper balance between the substrate, the process itself and the fermenting organisms. Hence, optimization of solid state fermentation parameters like initial moisture content, Kept 70% Addition of 1% carbon source, Addition 3% Nitrogen source to coconut oil cake ground nut oil cake (1:1) lipase production was carried out. Once a parameter was optimized, the optimum level of the parameter was continued in the next step of experiment.

i. Clearly indicates for different incubation time from 24 hours to 96 hours. There is difference in yield of lipase in comparison with all 09 isolates. Two bacteria *Serratia marcesens* and *Bacillus licheniformis* are indicated as superior lipase producer.

ii. The results revealed that the all the carbon some employed under present study have enhanced the production of in 1% glucose when added with comparison to addition of other carbon sources except 1% glucose no

other carbon source have influenced the lipase production. Lipases catalyse the hydrolysis of triglycerids into diglycerides, monoglycerides.

iii. The results pertaining to the effect of addition 3% Nitrogen indicated in a chart that the production of lipase from *Aspergillus terreus* GCN 8. The highest production of lipase in 96 hours i.e. 12.2 U/ml broth recorded in Tryptone whereas in 48 hours and in 72 h, the use yeast extract + peptone (1:1) and tryptone yielded same U/ml. Addition of Y extract peptone casein and Beef extract has reduced yield noticed in 48 hours, 72 hours, and in 96 hours.

7. The bibliography referred in the present thesis is included in the references section at the end of the thesis.