Introduction

Enzymes have been used directly and indirectly by mankind for thousands of years. Their initial discovery and use was lead to several observations, adoption and continued adaptation. In general the enzymes themselves were expressed through the use of live microorganisms. Today, enzymes have many applications in a wide variety of areas of which the general consumer is unaware.

The world market for enzymes is over 1.5 billion and anticipated to double by the year 2008. There has been 12% increase in the volumes of enzymes manufactured in last 10 years. Approximately 400 companies are currently involved in the manufacture of enzymes. Over last 5 years several new companies have emerged with interesting new technologies for enzyme isolation, 60 % of enzyme production oceans in Europe, with 15% in the US and 15% in Japan rest 10 percent from other countries production of enzymes has greatly expanded since 1960s due to widespread introduction of fermentation technology.

In terms of dollar usage, the US & Europe each consume 30% of world output. Approximately 75% of industrial enzymes are for hydrolysis and de-polymerization of complex natural substances with proteases dominating due to their use in detergent and dairy industries. Food application microorganisms are now becoming the dominant source for enzymes for a wide variety of types. This trend will increase in future due to ease in genetic manipulation and also wide variety of enzymes available from microorganisms found in diverse and extreme environments. Microbial enzymes have been found and developed to replace existing enzymes of animal and plant origin (Lowe, 2002).

Among lipases of plant, animal and microbial origin, it is the microbial lipases that find immense application. This is because of microbes can be early cultivated and their lipases can catalyze a wide variety of hydrolytic and synthetic reactions. Currently enzymes are produced from a wide range of biological sources, an approximate break down of the sources for bulk enzyme sources mainly from filamentous fungi 60%; bacteria 24%; animal 6%; yeast 4%; streptomycin 2% its in the last decade that lipases have gained importance to a certain extent over proteases and amylases (Saxena, *et al.*, 1999).

Current Status of Enzyme Applications

The practical application of enzyme catalysis is big business. The total world market for enzymes in 1981 was estimated at 65000 tonnes with a value of 400 x 10^6 dollars; by 1985 this was expected to grow to 75000 tonnes (600 x 10^6) Enzymes are used in four distinct fields as: therapeutic agents; Manipulative tools, for e.g. in gene manipulation; analytical reagents; and "industrial catalysts". The largest of this market area is the use of enzymes as industrial catalysts (Stanbury and Whittaker, 1987).

Twenty five companies engaged in enzyme production in the western world, only a small handful is involved in bulk production. Novo Industries of Denmark produces 50% of the enzymes sold on the 'Western market place' GIST Brocades of Holland 20% while the total output of USA accounts for only 12% of the total. Among Enzymes produced annually 80% are hydrolytic enzymes out of these 59% are proteases, 28% are carbohydrases, others 10% where as lipases produces annually only 3% (Godfrey and Reichert, 1983).

Enzymes are among the most important products obtained for human needs through the microbial sources. Enzymes have been employed in food processing operations since the dawn of Civilization although their characterization was carried out in last century (Bhotmange and Shastri, 1994).

Enzymes, the catalysts of biological system, are remarkable molecular devices; the catalytic properties are quite specific. They also mediate transformation of different forms of energy. Some enzymes consist of protein only but most enzymes contain an additional non-protein component such as carbohydrates, lipids, metal ions and phosphates and some other organic moiety. The biochemical diversity of microorganisms makes them logical sources of wide variety of enzymes for use in food and other biotechnological system (Toylor and Richardson, 1979).

The use of enzymes and microorganism in processing of raw materials from plants animals has been practiced from long time. In the traditional processes, such as production of alcoholic beverages and yeast fermented dough in baking bread all these activities were practiced by predetermining use of living microorganisms (Word, 1989) further examples are the processes for preserving food, such as vegetable conversion by

fermentation with lactobacilli (sauerkraut) or processing milk by making cheese (Uhlig, 1998).

Importance of Lipases in Present Scenario

Enzymes have captured an important place in contemporary organic synthesis the present trend is evident by the fact about 10% of all papers published in the area of organic synthesis relate steps of biotransformation and use of one (lipase or the other enzyme (Qazi, 1998) more than 60% of these enzymes are hydrolases that constitute a group of lipases, esterases and proteases. A large number of this class of enzymes have in fact been in use for a long time and some of these enzymes are commercially available as ready use as catalysts (Qazi, 1998).

In large scale applications of over 2500 Known enzymes only 250 have been commercially exploited and only about 25 enzymes account for more than and only about 25 enzymes account for more than 80% all applications. Therefore, such as limited exploitation of the enzyme pool so far, a tremendous potential of enzymes for future is evident (Qazi, 1998). Lipases (Triacyl glycerol acyl hydrolases) occupy a place of prominence among biocatalysts owing to their novel and multifold applications in oleo chemistry (Mackre and Hammand, 1985) organic synthesis, detergent formulation (Bjorkling *et al.*, 1991) lipases are unique in catalyzing the hydrolysis of fats into fatty acids and glycerol at the maximum lipid interface and reversing the reaction in a non aqueous media. A change in conformation of the enzyme occurs when there is a contact with water insoluble substrates (Ghosh *et al.*, 1996 and Saxena *et al.*, 1999).

Lipases have become a subject of intense research during the last decade, especially due to their potential applications. Particularly, Microbial lipases exhibit very different enzymatic properties, stability selectivity and substrate specificity making lipases suitable for many industrial processes (Taipa *et al.*, 1995).

LIPASES: Meaning and Definition

Enzymes are capable of catalyzing the hydrolysis of triglycerides including lipases and esterases which differ principally in their relative activities on substrates in solution form and in emulsion form. Lipolysis is defined as the enzyme catalysed hydrolytic cleavage of triglycerides resulting in release of free fatty acids (Arnold *et al.*, 1974).

Lipases are serine hydrolases and contain consenses sequence G-X1-S-X2-G as the catalytic moiety where G= Glycine, S= Serine, X1-histidine and X2= glutamic or Aspertic acid (Svedson, 1994)

It has been well established and definition of lipases since the mid 1980's that lipases (E.C. 3.1.1.3) catalyze both the hydrolysis and synthesis of esters of widely different structures and molecular back bones. These transformations are highly chemo-region, and enentioselective (Poppe and Novak, 1992)

Lipases are defined as corborxyl esterases catalyzing the hydrolysis and synthesis of long chain acyl glycerols (Ferrato *et al.*, 1997). There is no definition available for the term "Long chain", but esters of glycerol with an acyl chain length to 10 carbon atoms can be regarded as lipase substrates, with trioleoyl glycerol being the standard substrate. The hydrolysis of glycerol esters with esters acyl chain length of 10 carbon atoms eg, tributyryl glycerol (tributyrin) as a standard substrate usually indicates the presence of esterase (Jenson, 1983) from microbiologists point of view. The majority lipases are perfectly capable of hydrolyzing these esterase substrates (Willis, 1960 and Hayes, 1963). These lipases are specific for lysis of triacylglycerols of small molecular mass (Petrovic, *et al.*, 1990).

In biological system lipases (acylglycerol acyl hydrolases (E.C. 3.1.1.3) initiate the catabolism of fats ester bonds of acyl glycerols. Work from several laboratories has established that the activities of lipases are not limited to this reaction. They also catalyse the synthesis and transesterification of glyceride (Posorske *et al.*, 1988 and Bloomer *et al.*, 1990) and phosphoglyceride (Svedson *et al.*, 1990 and Yagi *et al.*, 1990) ester bonds, and the synthesis and hydrolysis of a variety of non-glyceride esters (Kalartis *et al.*, 1990 and Miller *et al.*, 1988). In addition, lipases are active in both aqueous and non-aqueous solvent systems (Dorolick *et al.*, 1989 and Wong *et al.*, 1989). Therefore those lipases have considerable biotechnological potential for the general synthesis and hydrolysis of esters (Slatger and Zbioinska, 1988).

Screening for Lipase Producing Microorganisms

In isolation of industrially important microorganism according to Buckland (1992) the advances of Recombinant DNA have resulted in extremely valuable new commercial

processes and have improved many other fields with all the recombinant technique, that sales of new microbial secondary metabolites produced from natural isolates were introduced in 1980's was greater than the sales of all recombinant products added together (Stanbury and Whittaker, 1995). The diversity of microorganism may be exploited still by searching for strains from the natural environment able to produce products of commercial value.

Screening of microorganism for potential industrial application, the isolation involves obtaining either pure or mixed cultures followed by their assessment to determine which carryout the desired reaction or to produce the desired product (Demian and Davis, 1999). In some cases of possible screening procedures it is possible to design the isolation procedure in such a way that the growth of producers is encouraged or the organism recognized at Isolation stage (Bull, 1979).

Isolating the microorganisms from environment is the microbiologists First step in Screening for natural products, such as secondary metabolites and Enzymes. Unfortunately for industry, no single isolation method will reveal the total member and variety of microorganisms present in a sample (Hungate, 1962 and Slatger *et al.*, 1983). The ideal isolation procedure commences with an environmental source (Frequently Soil) which is highly probable to be rich in desired types is so designed as to favour the growth of those organism possessing industrially Important characteristic i.e., industrially useful characteristic is used as selective factor and also that should be simple test to distinguish the most desirable types for particular industrial application.

The sources of microorganisms are mainly and varied the choice of natural sources is often based on the reason that the samples from widely different locations are most likely to yield novel isolates which intern might produce novel metabolites and also enzymes (Williams and Willington, 1982).

The organism will grow on particular substrates in the presence of certain compounds or under some cultural conditions adverse to other types. In other words, the isolation procedure may be designed to exclude certain microbial 'weeds' and to encourage more novel types (Bull, 1992).

Isolation of Lipase Producing Microorganisms

Microbial biodiversity is far greater than those of plants and animals and they are present even under environments which are unfavorable for higher life forms. Lipases are synthesized by microorganisms, which grow on oils or fats. There are various microorganism known to produce lipases which include bacteria, fungi are potent producers are among fat producing organisms (Reed, 1999) how ever no relationship between fat production and lipase production (Moat and Faster, 1995). In the field of microbial enzyme technology current research and development efforts, put for the screening of rare or little investigated genera and Species of microorganism from natural environment and exporting their potential as sources for enzymes with novel properties (Glazer, 1996).

In the natural environment, the microorganisms co-exists but their number as well as nutritional requirements vary considerably, As a result, the more abundant and nutritionally non- fastidious groups of organism get easily isolated during routine plating procedures. In the soil, microbes exist on vegetative cells or spores with varying degree of survival potential under adverse environmental conditions. Further the media used for isolation of bacteria, fungi and yeasts vary widely in composition and innovative approaches are necessary to isolate the rarer and less distributed genera and species in pure culture, it is apparent that global interest in screening of natural isolates from the ecosystem requires a sound knowledge of microbial Ecology.

A relatively smaller number of bacterial lipases have been well studies compared to Plant and fungal lipases (Pondey, 1996).

Bacterial lipases are glycoproteins, but some extra cellular bacterial lipases are lipoproteins. Winkler (1979) reported that enzyme production in most of the bacteria is affected by certain polysaccharides. Most of the bacterial lipases reported so for are constitutive and are nonspecific in their substrate specificity and few bacterial lipases are thermostable (Macre *et al.*, 1985).

Among bacteria, *Achromobacter* sp. *Staphylcooccus* sp. *Alkaligenes* sp. *Chromobacteria, Pseudomonas* sp. have exploited for the production of lipases. *Staphylococcal* lipases are lipoprotein in nature (Brune and Gotz, 1992). Isolating

microorganisms from environmental niche is the microbiologists first step in screening for natural products such as secondary metabolites and enzymes (Hunter and Beld, 1999). Unfortunately for industry no single isolation method will reveal the total number and variety of organisms present in the sample (Feldmann and Idczak, 1992). Screening of lipases from microorganisms and their various environmental niches are wool scour effluent (Brahimi *et al.*, 1991), copra waste (Benjamin and Pondey, 1996), vegetable oil processing industry (Sangliyandi and Gunasekharan, 1996). Isolation of *Humicola* from compost in Japan (Arima, 1972) from decomposting-leaf litter (Venkateshwaralu and Reddy, 1993). Isolation of thermophilic *Bacillus* for alkaline lipase (Sharma, 2002) *Penicillium roqueforti* S-86 from cheese (Petrovic *et al*, 1990), soil near an oil extraction unit (Gupta, 2002).

Microbial Production of Lipases

Both, SMF and SSF are now being widely employed for the exploitation of cell-bound or secreted microbial metabolites. Lipases are one of the primary metabolites abundantly available in the microbial world. In the past, interest in microbial lipases resulted from investigation of food spoilage, especially of dairy products. In contrast, free fatty acids (FFA) in some dairy products, notably cheese, and contribute to desirable flavors. So that action of the lipases resulting from mold growth can contribute to the flavour. Many other food commodities utilize microbial lipases either *in situ* or *ex situ* to obtain desired flavors and textures. This has led the industrial applications of lipases in the modern technologies, especially in fats and oil industry (Rao *et al.*, 1990).

Therefore, in present study efforts are being made to isolate the organisms, (Bacteria, Moulds and Yeasts) from ground nut oil mill waste and nearer oil contaminated soil samples, identity and screen them for lipase activity. Such lipase producers were further subjected to study the various physical and nutritional factors with special reference to media formulation and optimization studies of solid state fermentation was studied. The findings were discussed in light of the present status and future prospects of the microbial derived lipases.

It has been felt that often beneficial to turn to natural habitats for fresh Microorganism isolates, The Microorganisms widely distributed in nature. The ultimate natural sources of organisms for industrial use are soil, water river mud, plant and animal.

The present study is proposed to carry out on the followings.

1. The field survey and sample collection from groundnut industrial wastes.
2. Screening for lipase catalyst microorganisms by using different plate assays.
3. Characterization and identification of lipase producing microorganisms.
4. Microbial lipase production by submerged fermentation in conical flasks.
5. Microbial lipase production by solid-state fermentation using different substrates.
6. Enzyme assays carried out with the crude enzyme preparations using the spectrophotometer.