CHAPTER ONE

INTRODUCTION

Enzymatic processes have been used for centuries. The fermentations for making beer, wine, bread and cheese are older than recorded history (Underkoefler, 1976). All fermentations are enzymatic conversions mediated through the metabolism of living organisms. When a system of several enzymes is necessary to produce desired changes, this is most conveniently done by intact cells in fermentation process. Where a single enzyme or a simple system of only two or three enzymes is involved in a desired reaction, cell-free enzyme preparations are usually preferred to intact cells.

The practical use of enzymes to accomplish reactions apart from the cells goes back into antiquity, long before the existence, the nature, or the functions of enzymes were understood. Malt for starch conversion to sugar for brewing, stomach mucous for clotting milk in cheese making and papaya juice for tenderising meat are examples of ancient uses of enzymes. Later crude enzyme preparations extracted from animal and plant tissues such as pancreas, stomach mucosa, malt and papaya fruit found application in textile, leather, food, beverage and other industries. The malting of barley and use of the

extract for starch liquifaction is another such application. In 1883, Payen and Persoz showed that the agent responsible for starch solubilization, which they called diastase, was destroyed by boiling, acted catalytically and could be concentrated and purified by precipitation with alcohol (Wang et al., 1979). Buchner extracted active enzymes from living cells. He initiated efforts to isolate and purify individual enzymes (Bailey and Ollis, 1986). In 1878, the word enzyme was introduced by Kuhne. In 1885, Bluementhal described one of the first large scale extraction and purification processes for an enzyme, renning (Wang et al., 1979). After the biocatalytic enzymes responsible for the action of the crude preparations became recognized and understood, a search began for better, less expensive and more readily available sources of similar enzymes. The development and continual improvement of methods for large scale production of enzymes, along with better understanding and means of controlling enzymatic processes and applications have resulted in a sizable number of commercial enzyme products and industrial uses of them. As early as 1902, Emmerich, Low and Korshun suggested that bacterial enzymes could be used clinically (Wang et al., 1979). In 1908, Wallenstein described the stabilization of malt diastase by calcium sulphate and in 1915 Rohm found that the laundry clothes could be cleaned more easily and at low temperatures when pretreated with lipases and proteases (Bailey and Ollis, 1986). The first successful isolation of pure and crystalline enzyme, urease, was achieved in 1926 by Sumner. Studies of the isolated enzyme showed it to be a protein, a

property now well established for enzymes in general (Bailey and Ollis, 1986). Now, large number of enzymes are known and many are being commercially produced.

Since all living cells produce enzymes, it is possible to obtain useful enzyme products from animal tissues, plant tissues or microorganisms. However, microbial enzymes have the enormous advantage of being produced in large quantities by established fermentation techniques. It is also easier to improve the productivity of a microbial system compared to a plant or animal one. Microbial cells have the capacity of producing numerous different enzymes. In order to grow and remain alive, microorganisms have to carry out a number of enzyme-catalysed reactions. Hundreds of enzymes have to be formed and used in an integrated manner in a microbial cell. Enzyme production is closely controlled in microorganisms and in order to improve productivity these controls may have to be exploited or modified. Such control systems as induction may be exploited by including induceres in the medium, whereas feedback repression may be removed by mutation and selection techniques (Stanbury and Whitaker, 1984). Productive capacity for microbial enzymes may be expanded without limit to meet all demands (Underkoefler, 1976). Developments in the production of microbial enzymes have assured potentially unlimited supplies and also have made available

enzyme systems which cannot be readily obtained from plant and animal sources.

1.1 CHARACTERISTICS OF ENZYMES

A knowledge of the properties or kinetics of enzymes is essential to a basic understanding of enzymic conversions, whether they are occurring inside whole cells as in fermentations or in enzyme reactors.

An enzyme is usually defined as a protein biocatalyst produced by a living cell. The molecular weight of enzymes range from 15,000 to over a million. Like all catalysts, enzymes affect the velocities of chemical reactions. Enzymes only catalyze reactions which are thermodynamically possible. An enzyme lowers the amounts of activation energy required by the reaction. Hence, enzymes are able to catalyze reactions under very mild conditions at normal temperature and pressure reducing the possibility of damage to heat-sensitive substrates and also reducing the energy requirements of the process.

Enzymes differ from other catalysts in several respects, mainly because of their proteinous nature. Being proteins, the enzymes are denatured or

inactivated when subjected to pH change, heat or strong chemicals. Hence, two distinctive properties of enzymes are their thermal liability and their sensibility towards acids and bases.

The enzymes produced by living cells are of course, for the purpose of accomplishing their specific metabolic needs. Since enzymes can be separated readily from the cells that produce them and can perform their catalytic activities entirely apart from the cells, they are available to mankind for useful practical applications. The use of enzymes has now been extended into a variety of fields such as brewing, food production, textile industry and health care. The recent developments in biochemistry has resulted in the understanding of mechanisms of enzyme reactions and the development of new enzyme sources. Together with progress in applied microbiology these developments extended the range of enzyme applications (Chibita, 1979).

Unlike ordinary chemical catalysts enzymes are very specific in action. With enzymes, substrate specificity and reaction specificity are distinguished. Examples of substrate specificity are the hydrolysis of sucrose by sucrase and the oxidation of glucose by glucose oxidase. Examples of reaction specificity are the action of proteinases which are capable of splitting particular peptide bonds of proteins. These peptide bonds are amide linkages between carboxyl and amino groups of amino acids. Individual proteinases have narrow bonds

they can split. For example, hydrolysis of only those bonds linking the carboxyl groups of the two basic amino acids, lysine and arginine, to other amino acids. Trypsin has little or no effect on peptide bonds formed by other amino acids (Underkoefler, 1976).

The speed of an enzyme reaction is greatly influenced by parameters such as substrate concentration, enzyme concentration, pH, temperature, time, presence or absence of activators or inhibitors which affect the activity of the enzyme. It is well established that the enzyme takes an actual part in the reaction by combining with the substrate; when the reaction products are formed, the enzyme reappears in the original form.

There are six major classes of reactions, namely, oxidation-reduction, transfer of functional groups, hydrolysis reactions, addition to double bonds, isomerization reactions and formation of bonds with ATP cleavage, which enzymes catalyse. These reactions form the basis of enzyme classification as oxidoreducatases, or transferases, lyases, isomerases, hydrolases and ligages by Enzyme commission (EC) system assigning index numbers to all enzymes.

1.2 IMMOBILIZATION OF ENZYMES

Some of the advantages of free enzymes may be disadvantageous in practical use. Enzymes are generally unstable and cannot be used in organic solvents or at elevated temperatures. It is also very difficult to recover active enzyme from the reaction mixture. In order to obtain superior catalysts for practical applications, two approaches have been investigated (Chibita, 1979). One is the synthetic approach, using recently developed techniques of organic synthesis and polymer chemistry to synthesize catalysts having enzyme-like activities. The second approach is the modification of enzymes by immobilization. One of the most interesting developments in the last three decades has been the increase in our knowledge of methods for immobilizing enzymes successfully and utilizing them in industrial applications. The definition of immobilized enzyme is: "enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously" (Chibita, 1979). The choice between the free and immobilized forms of an enzyme for any particular use depends on the nature of the conversion process, the relative stabilities of the two forms and the cost. Various immobilization methods like carrier-binding, cross-linking and entrapment methods were studied. In carrier-binding, enzymes are bound to water-insoluble carriers with physical,

ionic or covalent bonds. In the second method, inter molecular cross-linking of enzymes by means of bi-functional or multi-functional reactions such as glutaraldehyde is carried out. In entrapment method, enzymes are incorporated into the lattice of gel matrix or enclosed in a semipermeable polymer membrane. In the rapidly developing field of enzyme technology, immobilized enzymes are playing a very important role.

For effective immobilization of an enzyme, carrier selection and immobilization techniques are important when considering selection of a support and adjustment of its surface properties, interaction between support surface and reaction mixture should be considered. The coupling procedure should be simple and amount of enzyme activity which can be recovered in the immobilized system should be high (Sastry, 1994). Even though reaction systems may permit repeated use of immobilized enzyme, loss of enzyme in the coupling procedure can very often make the process uneconomical.

1.3 OBJECTIVES OF THE PRESENT STUDY

Covalent bonding, crosslinking and adsorption of enzyme urease on different supports were investigated in the present study. The supports or carriers chosen should be stable in reaction medium and not deteriorate under

operational conditions. They should be mechanically rigid and have little compaction at high flow rate in continuous operations using packed bed reactors. The choice of the support was made by the conditions under which the enzyme was immobilized and the method of coupling involved. In the present study, vermiculite, Nylon 6/6 tubes and Amberlite MB-1 were used as supports. They are chemically and thermally stable and resistant to microbial attack.

The aim of the present study is to develop an economical and immobilized urease system using vermiculite, a cheap mica type mineral, Nylon 6/6 tubes or Amberlite MB-1 as carriers. The objectives of the present study are:

a). to study the effect of pH, urease concentration and time on immobilization of urease using vermiculite + EDTA and vermiculite + EDTA + glutaraldehyde.

b). to determine the thermal stability, optimum temperature, optimum pH, storage stability, optimum substrate concentration, amount of protein bound and reusability of urease immobilized on vermiculite using EDTA and EDTA + glutaraldehyde.

c). to study adsorption of urease on vermiculite saturated with cations like potassium, calcium and aluminum under various conditions and evaluate the properties of adsorbed urease.

d). to study the coupling of urease on a cheap non-biodegradable, commercial grade partially hydrolyzed Nylon tubes using glutaraldehyde as coupling agent and determine properties of the immobilized enzyme like optimum pH, temperature, storage stability and reusability.

e). to study immobilization of urease on Amberlite MB-1 with glutaraldehyde as bifunctional agent under various conditions such as pH, enzyme concentration and enzyme coupling time and determine the properties of immobilized urease such as optimum pH, temperature, thermal stability, storage stability and reusability .