1. INTRODUCTION

Enzymes are as protein biocatalyst for biochemical reactions that occur in living organisms. In other words, the utilization of enzymes have gradually expanded into various fields, such as brewing, food production, textiles, tanning, and medicine. On the other hand, enzymes are generally expensive, have limited stability, and cannot be used in organic solvents or at elevated temperatures. Consequently, chemists have developed various techniques for decreasing enzyme expense, reusability, and increasing enzyme stability. The best technique is a process known as immobilization of enzymes. Enzyme immobilization can be defined as the attachment of an enzyme to an insoluble polymeric matrix or support, inert or reactive to form support-enzyme complex **(Mosbach, 1988 and Weetall, 1993)**.

Enzymes immobilization on insoluble supports has been extensively developed in the industrial applications of enzymes. Advantages are the possibility of using continuous processes with insoluble enzymes, facilitation of enzyme recovery, and in some cases immobilization of an enzyme will result in an enhanced stability against the denaturing effect of heat and pH changes and organic solvents. This enhanced resistance to generally unfavourable conditions is of particular importance for immobilized catalysts used in continuous processes, which must be adapted for long-term operation with the minimum need for renewal of the catalyst **(Woodward and Capps, 1992 and Lamb and Stuckey, 1999)**.

Immobilization of enzymes can be achieved in many ways. But, various types of techniques based on the structure of enzymes and the nature of support. The adsorption of an enzyme on to an insoluble support is the simplest method of enzyme immobilization and often has little effect on catalytic activity. Many different supports are used under physical adsorption method, such as ion exchange resins polystyrene, controlled-porous glass, alumina, silica gel, chitin, calcium carbonate, hydroxyapatite, activated carbon, and granular chicken bone as in the case of industrial applications **(Schafhauser and Storey, 1992; Sardar *et al.*, 1997 and Riccio *et al.*, 1999)**.

The entrapment method of immobilization is based on the localization of an enzyme within the lattice of a polymer matrix or membrane in such a way as to prevent the released protein whilst allowing penetration of substrate **(Kierstan and Coughlan, 1985)**.

Many different synthetic supports have been used such as polyacrylamide, polyurethane and natural polymers as collagen, agar, cellulose, Ca-alginate and K-carrageenan **(Conlon and Walt, 1986; Martinsen *et al.*, 1989 and Prabhune and Sivaraman, 1991)**.

Covalent coupling for the immobilization of enzymes is based upon the formation of a covalent bond between the enzyme molecules and support material.

In general, covalent attachment procedures involve the formation of an activated solid support, followed by reaction of the activated support with an enzyme to form a immobilized

enzyme. A single reaction may be involved, or several steps may be required to prepare an activated carrier and the coupling.

The immobilization of an enzyme by covalent attachment to a support matrix must involve only functional groups of the enzyme that are not essential for catalytic action, and therefore no reagents must be used which could affect the binding and active sites of the enzyme **(Mosbach, 1976)**.

The majority of used support materials do not possess these reactive groups, but have hydroxyl, amino, amide, or carboxyl groups which require activation before their application for immobilization. However, only a few supports contain these reactive groups for direct coupling of enzymes, including maleic anhydride-based copolymers, methacrylic acid anhydride-based copolymers and idoalkyl-methacrylates. Many different supports have been used under this technique such as agarose, cellulose, silicon, porous glass, dextran-modified silica, polystyrene particles and polyvinylalcohol **(Garcia *et al.*, 1989; Plant *et al.*, 1991; Bahar and Celebi, 1998 and Subramanian *et al.*, 1998)**.

Microencapsulated enzymes are formed by enclosing enzymes within spherical semipermeable polymer membrane and still have activity on substrates which differ into the capsule. During the microencapsulation, the enzyme in the aqueous microsphere comes into contact with organic solvents and chemical monomer which can readily denature it to protect the enzyme from deactiviation, mixed with a polymer as bovine serum albumin, haemoglobin, or polyethyeneimine prior to microencapsulation **(Klei *et al.*, 1985 and Kurokawa *et al.*, 1998)**.

The aim of this investigation was to study the attempts for immobilization of alpha-amylase and amyloglucosidase enzymes which are considered as the most popular enzymes used for industrial purposes in the starch solubilization process. Different supports were used in techniques of immobilization i.e. Ca-alginate gel beads, sand, chitin, cyanogen bromide activated-Sepharose (CNBr-Sepharose) and concanavalin A-Sepharose (Con A-Sepharose). Also, the optimal conditions and kinetic behaviour of free and immobilized enzymes were examined. Besides that, the liquefication and saccharification processes were thoroughly studied to obtain the most suitable conditions for glucose syrup production from starch.