5. SUMMARY

The various procedures, different supports and reactions were carried out in an attempt for the immobilization of some industrial enzymes, i.e. α-amylase and amyloglucosidase on different supports. These enzymes have been immobilized on five different supports materials, Ca-alginate gel beads, sand, chitin, concanvalin A-sepharose (Con A-sepharose) and cyanogen bromide-activated sepharose (CNBr-sepharose).

The activities of native α-amylase and amyloglucosidase enzymes were determined before immobilization processes. The achieved data indicated that the activity of free α-amylase was found to be 96.0 μmM glucose/L/min. While, amyloglucosidase enzyme appeared activity equalled to 93.8 μmM glucose/L/min.

The effect of Na-alginate concentrations on preparations of immobilized alpha-amylase and amyloglucosidase were studied. The accomplished results illustrated that the retention activities were 67.2% and 78.4% at 2% and 4% Na-alginate for α-amylase. On the other hand, the retention activity for amyloglucosidase forms was found to be 70% and 76% at a concentration 2% and 4% (w/v) Na-alginate, respectively.

Alpha-amylase and amyloglucosidase were bounded with above-mentioned different supports. From these results the highest retention activity was found by cyanogen bromide-activated sepharose and the retention activity amounted to 91.7% for alpha-amylase, but the retention activity with Con A-sepharose was 90.4%. On the other hand, sand and chitin gave

the lowest bounded material which accompanied with the lowest retention activity, 71.2 and 72.8%, respectively.

While, the retention activities of amyloglucosidase with the same above-mentioned supports were 90.4 and 89.0% for CNBr-sepharose and Con A-sepharose, respectively. On the other hand, the retention activities were 70 and 66% for sand and chitin as another supports, respectively.

The optimum factors influence the free and immobilized enzyme preparations and stability were determined. The effect of pH on the reaction activity of free and immobilized alpha-amylase and amyloglucosidase with Ca-alginate gel beads showed that the optimum pH was 6.0 for free and pH 6.2 for immobilized α-amylase forms. The free amyloglucosidase showed its maximum activity at pH 4.8, while, the immobilized form gave a maximum activity at pH 5.0.

The effect of temperature on the reaction activity of free and immobilized α-amylase and amyloglucosidase with Ca-alginate beads were evaluated. Maximum activity for free and immobilized forms were obtained at the same temperature, 50°C. On the other hand, the maximum activity for free and immobilized amyloglucosidase forms were obtained at 50 and 55°C, respectively.

The effect of substrate concentration on the reaction velocity of free and immobilized α-amylase and amyloglucosidase within Ca-alginate gel beads were estimated. The maximum reaction velocity V_{max} of free α-amylase was 2029.4 μmM/L and K_m was found to be 0.55 g/100 ml buffer for free amylase form. However, the V_{max} value equalled to

1579.4 μmM/L and K_m constant equalled 0.75 g/100 ml buffer in the case of immobilized form. On the other hand, the maximum reaction velocity (V_{max}) was found to be 2224 μmM/L and K_m equalled 0.60 g/100 ml buffer for free glucoamylase enzyme. However, these values were 2140.6 μmM/L and 0.80 g/100 ml in the case of immobilized form.

The stability of immobilized α-amylase with different supports was determined for each enzyme forms under optimum conditions. The relative activities of bounded α-amylase were 76.55, 74.15, 66.59, 92.14 and 93.62% for sand, chitin, Ca-alginate, Con A-sepharose and CNBr-activated sepharose after incubation period 96 days, respectively. However, the stability of immobilized amyloglucosidase forms was measured and the obtained results indicated that the preparations of immobilized glucoamylase almost more stable and yielded the highest retention activity.

Reusability of immobilized α-amylase enzyme forms was assayed after 7 times use. The highest relative activity (92.96%) however it lost 7.04% of its activity after 7 cycles with immobilized enzyme by CNBr-activated sepharose. But, Con A-sepharose enzyme complex lost about 9.84% of its activity after 6 times. However, the Ca-alginate enzyme gel beads decreased approximately 30.0% of its original activity after 5 times use.

On the other hand, the immobilized amyloglucosidase with sand and chitin lost 19.05% and 21.51% of its original levels after 4 times, respectively. But, the relative activities of Con A-sepharose and CNBr-activated sepharose complexs were

85.34% and 90.41% after 7 times cycles. While, gel beads of Ca-alginate complex lost 34.29% of initial activity.

The effect of incubation periods on the enzymatic hydrolysis of soluble starch by using free, immobilized α-amylase, free and immobilized amyloglucosidase enzymes forms were estimated. The maximum reducing sugars were found to be 2256.5, 1670.50, 1621.51, 1172.28 and 2262.18 μ mM glucose/L for free and immobilized α-amylase on sand, chitin, Ca-alginate and CNBr-sepharose, respectively after 120 min, while, Con A-sepharose yielded 2210.05 μ mM glucose/L after 150 min.

On the other hand, the maximum reducing sugars obtained with immobilized amyloglucosidase on the above-mentioned supports were 2650.27, 1765.23 and 1422.65 μ mM glucose/L for free and immobilized forms on sand and Ca-alginate gel beads after 150 min. While, the chitin enzyme complex and CNBr-activated sepharose complex yielded of 1696.22 and 2394.28 μ mM glucose/L after 2 h.