5. SUMMARY

In the present work, the lignocellulosic materials of wheat straw, corn cobs, corn stalks and sugarcane bagasse have been investigated and more information were obtained about its pretreatments and chemical features of these agricultural wastes. Besides, cellulase complex enzymes, β-glucosidase, hemicellulase/xylanase enzymes mixture and immobilized glucose isomerase enzyme have been evaluated to obtain optimum conditions. Saccharification, monomerization and isomerization were accomplished using these enzyme to obtain the most suitable conditions for the conversion of the abovementioned lignocellulosic materials to high fructose syrup. In addition, attempts were carried out to immobilized β-glucosidase enzyme and the kinetic behaviour of immobilized forms were determined.

The obtained results indicated that wheat straw, corn cobs, corn stalks and sugarcane bagasse contained a high percentage of cellulose and hemicelluose polysaccharides i.e. (35.9, 32.9%); (33.1, 25.8%); (36.5, 27.2%) and (38.5, 31.5%).

Pretreatment of the abovementioned lignocellulosic materials with 10% NaOH at 80 ^{o}C for h was achieved to convert the crystallinity structure of these polymers to amorphous state to enhance the enzymatic attack toward these polysaccharides.

Evaluation of different enzymes activities were accomplished, the results are summarized in the following points

A) The effect of substrate concontration:

The effect of substrate concentration using carboxymethyl cellulose (CMC) as standard, and extracted cellulose from different lignocellulosic materials on cellulase enzyme complex (celluclast 1.5L), indicated that Vmax with CMC (252.3 mM/L) was higher than that other materials i.e. wheat straw (201.4 mm/L), corn cobs (208.3 mM/L), corn stalks (204.2 mM/L) and sugarcane bagasse (229.2 mM/L). On the other hand, Km values of cellulase for the abovementioned substrates were 4.4 g/L; 3.09 g/L; 3.0 g/L, 2.6 g/L and 2.5 g/L for CMC.

The effect of substrate concentration (cellobiose) on the reaction velocity of β-glucosidase (Novozym 188) enzyme showed that Vmax and Km of 55.83 μmol glucose/min/0.1 ml enzyme and 0.45 mM, respectively. While the effect of this parameter on reaction activity of hemicellulase/xylanase enzymes mixture using xylan as substrate indicate that Vmax and Km were 93.3 mM/L and 12.3 g/L at 60 min incubation period. On the other hand, the effect of glucose concentration on the reaction activity of sweetzyme type-T illustrated that the Vmax and Km were 750 μmol fructose and 2.3 mM at incubation time 60 min.

B). Effect of pH.

The effect of pH on the reaction activity of the four different enzymes were studied. The results indicated that the optimum pH value for cellulase and β-glucosidase enzymes was 4.8. While that for hemicellulase/xylanase (viscozyme 120 L) was 5.0 for both xylan and isolated hemicellulose from agricultural residues under the study.

The optimum pH value of immobilized glucose isomerase (sweetzyme type T) equalled 7.5.

C). Effect of temperature:-

The effect of temperature on the enzyme activity were reported. The optimum temperature of cellulase, β-glucosidase and viscozyme 120 L equal to 50 ^{o}C. The fourth enzyme (Sweetzyme type T) had optimum temperature equals to 60 ^{o}C.

D). Effect of enzyme concentration

The effect of cellulase enzyme concentration on the reaction activity indicated that the reaction activity of this enzyme reached its

maximum (4.31 mM/L/min) at the enzyme concentration 200 μ L 100ml buffer with CMC. While, the enzyme had a maximum activity equalled to 3.61, 2.92, 3.01 and 3.01 mM/L min at enzyme concentration of 350, 350, 300 and 250 μ L/100ml buffer for wheat straw, corn cobs, cornstalks and sugarcane bagasse, respectively.

The highest activity of β-glucosidase enzyme i.e. 83.0 μmol glucose/min reached at the enzyme concentration of 200 μL/100ml buffer. On the other hand, immobilized glucose isomerase enzyme (Sweetzyme type-T) revealed its maximum activity (60.74 μmol fructose/min) at the enzyme concentration of 500 mg/10 ml reaction mixture.

Immobilization of β-glucosidase enzyme:

Immobilization of β-glucosidase enzyme on solids supports is an important advantage, since it enables the continuous reutilization and recyclation of the enzyme support system.

The β-glucosidase enzyme from Asperiglius niger has been immobilized on 6 different support materials i.e. Concanavalin A-Separose (con A-Sepharose), cyanogen bromide activated sepharose (CNBr-sepharose), calcium-Alginate gel, polyacrylamide gel, bovine serum albumin and sand as an inorganic support.

Kinetic studies of the immobilized enzyme with different supported were characterized. The obtained results indicated that the rate of most enzyme reactions increased up to a certain point with increasing concentration of substrate. The native enzyme showed Km value of 0.45 mM which was decreased after immobilization to 0.40 and 0.35 mM for con A-sepharose and polyacrylamide gel supports, respectively. On the contrary, the Km values for the two other supports calcium alginate and CNBr-sepharose were increased (1.8 and 0.55 mM), respectively.

The stabilities of different preparations of immobilized enzyme were assayed at 50°C and pH 4.8. The obtained results indicated that CNBr-sepharose-enzyme complex was the most stable one, its relative activity was 96.7% compared with the native enzyme (90.6%) after 120 h. Also, sand as a solid and very cheap support showed an important result, since it retained about 86.3% of the total relative activity after 120 h.

The obtained data indicated that CNBr-sepharose and sand accomplished a very reasonable results. Since the first showed a retention activity after the binding of about 88.0% and ca 80.0% for the second, respectively, compared with the activity of the native enzyme.

Therefore, different preparations of β-glucosidase immobilized by CNBr-sepharose and sand were studied. The optimum factors influence the immobilized complexes reaction and stability were estimated for both supports. The obtained resultes indicated that the maximum activity (55.1, 46.1) was at pH 4.5 for both native and β-glucosidase-CNBr-sepharose complex form.

While immobililzed β-glucosidase on sand reached its maximum activity (45.7) at pH 4.8. Also these results revealed that the optimum temperature of the native and immobilized enzymes on CNBr-sepharose was 50°C. On the other hand, the maximum activity of β-glucosidase-sand complex occurred at 55 °C.

Enzymatic saccharification of different pretreatment cellulosic materials.

The acquired results of saccharification processes for different treated lignocellulosic materials indicated that the maximum values of saccharification processes were 79, 89, 81 and 79%, respectiviely for extracted cellulose of wheat straw, corn cobs, corn stalks and sugarcane bagasse at concentrations 25, 50, 50 and 50 g/L with incubation periods 96,96, 36 and 72h. The maximum saccharification value of extracted cellulosic material from corn cobs was higher than that attained by other substances.

Monomerization and isomerization of saccharified lignocellulosic materials:-

The obtained results indicated that the maximum values of conversion process by using CNBr - sepharose - β-glucosidase complex for monomerization, followed by isomerization process were higher than that the obtained values using sand β-glucosidase complex. In addition, it has to be mentioned that the highest values of the isomerization process (78.7%) was acquired with saccharified cellulosic material of corn cobs. This result may be due to its highest percentage of saccharification process (89.0%).