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PRODUCTION OF GLUCOSE AND ETHANOL FROM SOME CELLULOSIC RESIDUES USING THE PRODUCED CELLULASE ENZYME FROM *T. reesei*, *T. harzianum* AND *A. niger*.

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ABSTRACT

The lignocellulosic materials, faba bean straw and green pea pods were chemically pretreated by sodium hydroxide or glacial acetic acid. The amount of cellulose residues from faba bean straw and green pea pods decreased gradually by increasing NaOH concentration under conditions of 3 h, 100°C and 48h, 25°C. While, pretreatment of faba bean straw with glacial acetic acid showed higher yield of cellulose (80% and 70%) under the same above conditions. The chemical composition of faba straw and green pea pods were determined. Cellulose, hemicellulose and lignin contents were 32.3, 23.0 and 13.1% for crude faba bean straw and 29.2, 20.4 and 10.6% for crude green pea pods. After pretreatment, the extracted cellulose, hemicellulose and lignin contents were found to be (72.5, 10.5 and 7.2%) in faba bean straw and (80.0, 8.1 and 4.8%) in pea pods, respectively. The rate of saccharification process were 62.2% by *T. reesei*, 59.9% by *T. harzianum* and 56.2% by *A. niger*, for extracted cellulose of faba bean straw at concentration of 8% and the period of reaction was 6h. While, the saccharification rates were to 66.3, 58.8 and 65.0% by using the same above-mentioned fungi for extracted cellulose of green pea pods residue at concentration of 10% after 6 h.

Production of ethanol by fermentation of the resultant glucose which produced from saccharification processes amounted to 89.5, 84.7 and 85.7% by using hydrolyzed carboxymethyl cellulose of *T. reesei*, *T. harzianum* and *A. niger*, respectively. While, the ethanol produced from saccharification processes of faba bean straw and green pea pods estimated to (89.5, 89.5 and 88.5%) and (83.6, 89.5 and 88.0%) respectively by using produced cellulase from the above-mentioned fungi.

INTRODUCTION

Lignocellulosic waste materials are considered as a most important renewable reservoir of carbon. Wood and forestry waste and agricultural residues are abundant feedstocks for saccharification and subsequent

conversion to food; fuels and chemicals, (Deschainps, *et al.*, (1985). The application of cellulytic enzymes for the bioconversion of lignocellulosic residues into fermentable sugars can be used as chemical feedstocks for the production of industrial chemicals including fuel alcohol's.

Hoda, *et al.*, (1990) used the enzyme culture filtrate of *P. funiwlosum* for saccharification of the cellulosic wastes (pea hulls, rice straw, carrot leaves and guava seeds). The maximum amount of sacchaification process was 30.29, 39.99, 15.87 and 8.44 g/L, respectively after 65 h with 5 % substrate concentration.

Gabr, *et al.*, (1991, b) treated wheat straw and cotton stalks with peracetic acid. They noticed that peracetic acid is highly effective on rice straw. Also, they observed that the rate of saccharification of all cellulosic materials under their studies was directly proportional to the concentration of peracetic acid.

Abd El-Malak (1995) studied the enzymatic hydrolysis of some agricultural by-products, pretreated by NaOH and NH_4OH (1.0M, 24h at room temperature). The highest value of saccharification process was 89.8% from the extracted cellulose of soybean straw at concentration of (2.0%) after 48h.

Okeke and Obi (1995) studied the enzymatic hydrolysis of some agrowastes under the effect of two fungi isolates i.e. *Sporotichum pruimosum* and *Arthrographis sp.* They mentioned that the highest degrees of hydrolysis 15.1 % for *Sporotrichum pruinosum* and 7.5 % for *Athroqraphis sp.* were observed with alkali pretreated seed shells as substrate.

Szczodrak (1988) used the pretreated wheat straw for production of ethanol at 43 °C in the simultaneous saccharifiction and fermentation (SSF) process by *T. reesei* cellulase and kyveromyes fragils. They obtained 2.4 % (w/v) ethanol from 10% (w/v) of chemically treated straw after 48 h.

Gunasekaran and Kamini (1991) found that ethanol production by *Kluyveromyces fraqilis* was 65 g/L using 150 g/L lactose while, *Zymininas mobiles* produced 72 g ethanol /L under the same conditions.

Ahuja *et al.*, (1992) used the enzymatically hydrolyzate of rice straw to produce ethanol with *Saccharomyces cerevisia.* The maximum yield of ethanol was (4.12 % v/v) after 36 h incubation.

Lisbethoisson and Bärbel, (1996) found that ethanol produced from lignocellulosic hydrolyzates in an economically feasible process requires micro-organisms that produce ethanol with a high yield from all sugars present, and have a high ethanol productivity from lignocellulosic hydrolysate.

Moritz and Duff (1996) found that ethanol productivity was increased up to 65% over conventional (nonextra-reactive) fed-bach simultaneous saccharification of cellulosic substrates. In batch simultaneous saccharification

and extractive fermentation (SSEF) reactors with 2.5% aqueous phase, 50% conversion of 25% (aqueous phase concentration) Solka Floc could be achieved in 48 h using 2 FPU/g cellulose.

The aim of the present work is to study the conversion of some locally-available agro-waste materials (faba bean straw and green pea pods) by using different fungi cellulases (*T. reesei*, *T. harzianum* and *A. niger*) to soluble sugars and ethanol.

MATERIALS AND METHODS

1. Sources of cellulosic substances:

Faba bean straw (*Vicia faba*) and pea pods (*pisum sativum*) were utilized in this study. Faba bean straw was obtained from the experimental station at Nobaria, Agricultural Research Centre. However, green pea pods were obtained from Montana Co., for Food Industry, Qalama, Qaliub, Qaliubia Governorate, Egypt.

2. Chemical analysis of Faba bean straw and green pea pods:

Moisture content, ash, total lipids total nitrogen and crude protein Values were determined according to the methods described by A.O.A.C (1980). Lignin content was determined gravimetrically by the method reported by Adams (1965). Cellulose and hemicellulose contents were achieved according to the method described by Chen and Anderson (1980). Total carbohydrates were estimated by the method reported by Montgomery (1961). Total reducing sugars were determined accoreding to Somogyi (1952) by using the chromogenic reagent of Nelson (1944).

3. Pretreatment of lignocellulosic materials :

The milled agricultural wastes (Faba bean straw and green pea pods) were subjected to various chemical concentrations of NaOH and glacial acetic acid. The concentrations of NaOH solutions were 1, 2, 3, 4, and 5 % (w/w) while, the concentrations of glacial acetic acid solutions were 4, 8,12, 16, 18 % (v/w).

The pretreatment of lignocellulosic materials were accomplished according to the method described by Chen and Anderson (1980) under different conditions 3 h at 100°C and 48 h at room temperature.

4. Enzyme assays:

Enzyme assays were carried out on culture filtrates at pH 4.8, (0.1 M citrate buffer) and 50°C. The activity was expressed as micromoles of reducing sugars. Carboxymethyl cellulase (CMC-ase) and Filter paper-ase (FP-ase) activities were measured according to the method described by Tanaka, *et al.*, (1985).

5. Saccharification process :-

The saccharification process was carried out by the method described by Szczodrak (1988). The percentage of saccharifiaction (%) was calculated using the following equation as reported by Okeke and Obi (1995).

Saccharifi cation % = $\frac{\text{amount of reducing sugar (mg) x 0.9 x 100}}{\text{amount of substrate (mg)}}$

6. Alcohol production from the lignocellulosic materials

The method of Ban and Han (1990) was used to produce the alcohols from CMC, Faba bean straw and green pea pods. The resultant alcohol was determined by gas chromotography (GC) HP 5890 F. Spectrophotometer with refractive index detector and amines HP5 columm (cross linked 5 % pH Mesilion 30m × 0.32mm × 0.25mm) Flm.

RESULTS AND DISCUSSION

1. Chemical Pretreatment of lignocellulosic Materials:

Pretreatment of lignocellulose can enhance its hydrolysis by increasing the accessibility of its components cellulolytic enzyme.

Sodium hydroxide (NaOH) and glacial acetic acid were used to increase the susceptibility of lignocellulosic crop residues. The pretreatment with (NaOH) remove a large amounts of hemicellulose and lignin, which increase the accessibility to the cellulase, and improve the fiber to be more permeable to cellulase.

The effect of sodium hydroxide (1.0-6.0 w/w) and glacial acetic acid (4.0-20.0 v/w) on the extraction of cellulose and hemicellulose in Faba bean straw and green pea pods at different time and temperature are tabulated in Tables (1) and (2).

The obtained results showed that the amount of cellulose residues from Faba bean straw and Pea pods decreased gradually by increasing (NaOH) concentration under conditions of 3h, 100°C and 48 h, 25°C.

Also, pretreatment of Faba bean straw with glacial acetic acid in Table (2) showed higher yield of cellulose (80 %, 70 %) at 3h, 100 °C and 48h, 25°C respectively with 4 % glacial acetic acid, compared with green pea pods which yielded 63.3 % and 68.0 % under the above-mentioned conditions. The obtained results are in agreement with those obtained by Gabr, *et al.*, (1991, b).

The results in Table (1) showed that the highest obtained value i.e. (80 %) of extracted cellulose from pea pods was higher than that of Faba bean straw which contained 72.5 % at the same conditions (3h, 100°C). On the other hand, the amount of isolated hemicellulose from pea pods reached its highest content (28.1 %) at 5 % NaOH while, Faba bean straw contained less amount (27.5%) under the same conditions. This observation may be due to

the loss of significant amount of hemicellulose in the washing stage under high NaOH concentration, (Fox, *et al.*, 1989).

Table (1): Effect of NaOH concentration, temperature and time on the extraction of cellulose and hemicellulose from lignocellulosic materials.

NaOH Conc. w/w %	Faba bean straw				Green pea pods			
	Cellulose %		Hemicellulose %		Cellulose %		Hemicellulose %	
	3h, 100 °C	48h, 25 °C	3h, 100 °C	48h, 25 °C	3h, 100 °C	48h, 25 °C	3h, 100 °C	48h, 25 °C
1	72.5	65.0	10.5	5.6	80.0	60.0	8.1	9.2
2	70.0	60.0	12.5	14.0	70.0	49.6	15.0	17.0
3	67.5	57.5	17.5	16.5	58.0	38.7	18.0	19.4
4	65.0	55.0	21.0	20.5	49.6	29.8	23.0	22.2
5	60.0	50.0	27.5	25.6	40.3	24.5	28.1	27.5
6	54.4	44.6	25.9	26.8	34.2	20.2	27.2	25.0

Table (2): Effect of glacial acetic acid, temperature and time on the extraction of cellulose and hemicellulose from lignocellulosic materials.

Glacial acetic acid (%) v/w %	Faba bean straw				Green pea pods			
	Cellulose %		Hemicellulose %		Cellulose %		Hemicellulose %	
	3h, 100 °C	48h, 25 °C	3h, 100 °C	48h, 25 °C	3h, 100 °C	48h, 25 °C	3h, 100 °C	48h, 25 °C
4	80.0	70.0	5.7	6.4	63.3	68.0	6.7	8.2
8	73.3	60.0	11.2	13.6	56.6	64.0	14.2	12.6
12	66.7	50.0	17.0	16.4	50.0	50.0	17.3	15.8
16	60.0	43.0	22.7	20.6	43.3	42.0	20.9	18.9
18	50.0	38.0	22.3	23.2	33.3	35.0	26.5	25.0
20	46.8	30.0	21.9	23.8	29.4	30.0	25.6	23.8

On the other hand, cellulose content in this study was decreased by increasing the NaOH concentration. The acquired results are in a good harmony with those found by Diwamy *et al.*, (1986). The obtained results may be due to that NaOH led to breakdown of intermolecular hydrogen bond of lignocellulosic fibers which promote the swelling of fibers beyond water-swollen dimension and thereby increasing enzymatic and microbiological penetration into the cell wall fine structure (Tarkaw and Feist, 1969). Also, alkali treatment disrupts or breaks the bonds of lignin moieties and reduces lignin content (Blanch and Wilke, 1982).

2. Chemical composition of lignocellulosic materials:-

The chemical composition of lignocellulosic materials i.e. Faba bean straw and green pea pods are tabulated in Table (3). The obtained results

showed that cellulose, hemicellulose and lignin are the main constituents while ash and protein occur in lesser amounts in crude and extracted lignocellulosic materials under investigation. The results in Table (3) show that crude Faba bean straw contained the highest amount of cellulose (32.3%) while, crude pea pods contained lesser amount (29.2 %). The lignin amounted to 13.1% in Faba bean and 10.6% in pea pods.

On the other hand, pretreatment of lignocellulosic materials with NaOH under the above-mentioned conditions increased the amount of isolated cellulose and decreased the amount of isolated hemicellulose and lignin content. The extracted cellulose, hemicellulose and lignin contents after this pretreatment were found to be (72.5 %, 10.5 % and 7.3 %) in Faba bean straw. Also, the extracted cellulose, hemicellulose and lignin were found to be (80.0 %, 8.1% and 4.8%) in pea pods.

Table (3): Chemical composition of crude and extracted faba bean straw and pea pods.

Contents	Faba bean straw		Pea pods	
	Crude	Extracted	Crude	Extracted
Moisture	2.6	1.8	2.4	1.1
Cellulose	32.3	72.5	29.2	80.0
Hemicellulose	23.0	10.5	20.4	8.1
Lignin	13.1	7.3	10.6	4.8
Ash	2.5	1.2	2.2	0.9
Protein	3.8	2.3	3.4	2.1
Total Lipids	4.2	-	3.2	-
Total hydrolysable carbohydrate	13.9	2.9	23.1	3.2
Total soluble sugar	2.6	-	3.6	-

Percentage on dry weight basis under experimental conditions

From the obtained results, it could be concluded that the pretreatment of lignocellulosic materials with NaOH decreased the lignin to a large extent. Consequently, cellulase enzyme became more effective towards the extracted Faba bean straw and pea pods, Fox *et al.*, (1989).

3. Effect of different treatments of cellulosic materials on enzymatic saccharification:-

Saccharification processes were applied on different extracted cellulose from ligoncellulosic material i.e. Faba bean straw and green pea pods at substrate concentration of (2.0, 4.0, 6.0, 8.0 and 10.0% w/v) using (0.1M) acetate buffer solution pH 5.0. These processes were carried out by utilizing cellulase enzyme produced by *T. reesei*, *T. harzianum* and *A. niger* at enzyme concentration of 54.5, 40.6, 51.3 units protein enzyme. The experiments were achieved in a shaking incubator at 200 r.p.m and 50 °C.

The rate of enzymatic saccharification was accomplished for different periods ranged between 3h and 6h. Saccharification of different

treated ligoncellulosic materials are recorded in Tables, (4 , 5) and Figs (1,2). The results in (Table 4) indicated that, the highest value of saccharification process were 46.9% by *T. reesei*, 43.7 % by *T. harzianum* and 46.4% by *A. niger*, for the extracted cellulose of Faba bean straw at concentration of 8 % and the period of reaction 3 hrs. On the other hand, at the same concentration of extracted cellulose from Faba bean straw, the saccharification percentage amounted to (62.2, 56.4 and 56.0 and 56.0% %) by using *T. reesei*, *T. harzianum* and *A. niger*, respectively for a period of 6hrs. These results are in agreement with those previously obtained by Vega *et al.*, (1991) and Okeke and Obi, (1995).

Table (4): Enzymatic saccharification of treated faba bean straw.

Source of Enzyme	3 hrs					6 hrs				
	Substrate concentration (% w/v)					Substrate concentration (% w/v)				
	2	4	6	8	10	2	4	6	8	10
T. reesei	36.7	38.6	40.2	46.9	44.4	38.5	45.0	53.5	62.2	61.9
T. harzianun	25.8	34.3	39.0	43.7	42.0	32.4	40.0	50.7	56.4	56.0
A. niger	23.4	35.0	39.9	46.4	43.7	34.5	38.9	47.0	56.0	53.2

Table (5): Enzymatic saccharification of treated pea pods.

Source of Enzyme	3 hrs					6 hrs				
	Substrate concentration (% w/v)					Substrate concentration (% w/v)				
	2	4	6	8	10	2	4	6	8	10
T. reesei	38.2	54.0	60.1	59.1	62.7	39.4	59.0	65.3	64.2	66.3
T. harzianun	30.8	43.0	49.9	51.7	55.8	45.0	54.5	56.7	55.4	58.8
A. niger	27.6	35.9	46.2	48.8	56.0	38.4	44.7	56.7	58.2	65.0

However, the maximal improvement in the saccharification rate of any cellulosic material under this investigation is still relatively low. This observation may be attributed to that the surface area of cellulose is not major limiting factor but the crystallinity of cellulosic materials might play a bigger limiting rate as illustrated by Fan, *et al.*, (1980). In addition the poor improvement of physical and chemical pretreatments might be due to its incapability to remove the lignin which accounts for the most hinderance towards saccharification process. Also, the end -product inhibition and to

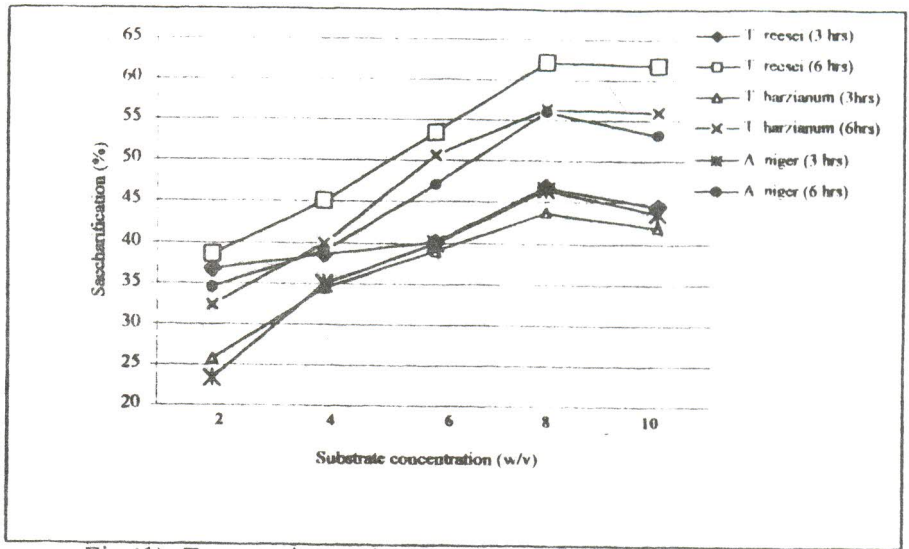

Fig (1): Enzymatic saccharification of treated faba bean straw

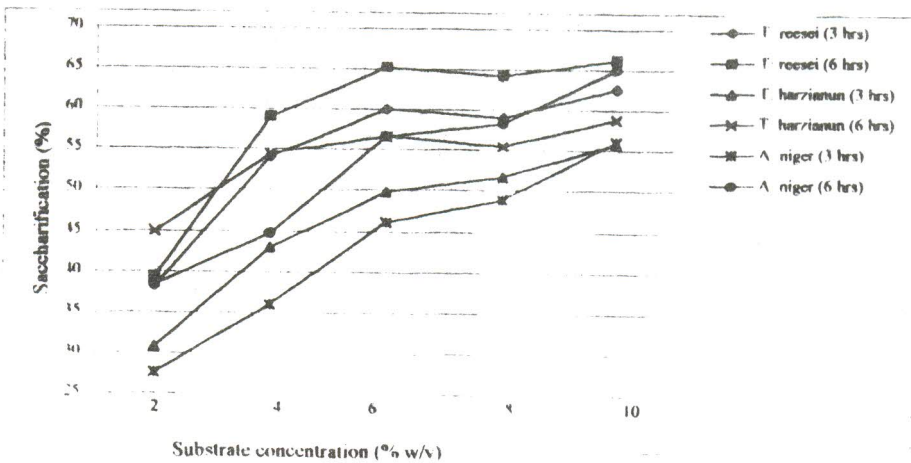

Fig (2): Enzymatic saccharification of treated pea pods.

some extent thermal instability during the long reaction period. Okeke and Obi (1995). Also, from the obtained data which are shown in table (5) and fig (2), it might be concluded that the maximum values of saccharification process were 62.7, 55.8 and 56.0% by using *T. reesei*, *T. harzianum* and *A. niger* respectively, at concentration 10 % and period of 3h. While at the same concentration of the extracted cellulose from pea Pods, the saccharification percentage equalled to 66.3, 58.8 and 65.0 % respectively, by the same fungi after 6 h. The obtained data is coincident with that reported by Vega *et al.*, (1991).

However, the results pointed that, the extracted cellulosic materials from green pea pods achieved maximum saccharification values higher than Faba bean straw. This observation may be attributed to that the extracted cellulose from pea pods contained a less amount of hemicellulosic polysaccharides which act as impurities in the sacchasification process. And hence, these polymers led to decrease the saccharification values of treated Faba bean straw.

4. Production of ethanol by fermentation of lignocellulosic materials:-

Fermentation of the lignocellulosic hydrolyzate to ethanol was accomplished as an ultimate step in the present bioconversion scheme. The starting materials used here were the hydrolyztates attained from the enzymatic degradation under the optimal saccharification condition. The unusual yeast strain *Saccharomvces cerevisia* (V-21) was chosen to ferment these hydrolyed products, since it is capable of producing ethanol from CMC, treated Faba bean straw and treated pea pods, Delgenes, *et al.*, (1996). To allow a clear evaluation of the ethanol productivity of this yeast, it was grown on variable carbon sources including CMC, Faba bean straw and pea pods by 5.0 g/100 glucose. The results are shown in Table (6) indicated that the high efficiency of the present yeast for conversion of CMC and treated faba bean straw into ethanol by *T. reesei*, *T. harzianum A. niger* were 89.5 %; 84.7 %; and 85.7 % respectively. These results are in agreement with those previously reported by Roca, *et al.*, (1996).

Table (6): Fermentation of the lignocellulosic hydrolyzates to ethanol.

Source of straw	Ethanol (%)		
	T. reesei	*T. harzianun*	*A. niger*
CMC	89.5	84.7	85.7
Faba bean	89.5	89.5	88.5
Pea pods	83.6	89.5	88.0

REFERENCES

Abd El-Malak, G. (1995): "The effect of extraction processes on the activity of some cellulosic hydrolyzing enzymes of agricultural by-products". M.Sc. thesis Fac. of Agric., Moshtohor, Zagazig univ.

Adams, G.A. (1965): "Methods in carbohydrate chemistry" Edited by R. L. Whistler, J. N. BeMiller and M. L. Wolform, Academic press, New York and London, 5, 185-187.

Ahuja, V.; Singh, A. and Punj, V. (1992): "Effect of supplementation of cellobiase from T.reesei on saccharification of rice straw and simultaneous fermentation to alcohol". J. of Res. Punjab Agric. Univ., 28, (2), 234 - 242.

A.O.A.C., (1980): "Official Methods of Analysis of the Association of official Agriculture Chemists", 13th ed. published by the Association official Agriculture Chemists Washington, D.C., U.S.A.

Ban, L.K. and Han; Y.W. (1990): "Alcohol production from pineapple waste". World J. of Microbiol. and Biotech., 6, 281 - 284.

Blanch, H.W. and Wilke, C.K. (1982): "Sugars and chemicals from cellulose". Rev. Chem. Eng., 1,71- 119-924.

Chen, W.P. and Anderson A.W. (1980): "Extraction of hemicellulose from grass straw for the production of glucose isomerase and use of resulting straw residue for animal feed".Biotech. and Boieng. 22, 519-531.

Delgenes, J.P., Moletta, R. and Navarro, J.M. (1996): "Effect of lignocellulose degradation products on ethanol fermentations of glucose and xylose by *Saccharomyces cerevisiae*, *Zymomonas mobilispichia* stipitis, and *Candida shehatae*". Enzyme and Microbiol. Tech. 19:220-225.

Deschainps, F.; Giuliano, C.; Asther. M.; Huet, M. C., and Roussost, S. (1985): "Cellulase production by *Trichoderma harziamim* in static and Mixed solid-state Fermentation Reactors under Nonaseptic Conditions". Biotech. and Boeng., 27 (1), 1385-1388.

Diwany, A.I., Shaker, H.M., Farid, M. A., and El -Abd, H. A. (1986): "Effect of chemical treatments on saccharification of rice hulls and yeast growth". Agric. Wastes, 18, 1-7.

Fan, L.T.; Lee, Y.H. and Beardmore, D.H. (1980): "Major Chemical and Physical features of Cellulosic materials as substrates for enzymatic hydrolysis". Adv. Biochem. Eng., 14:101- 116.

Fox, D.J.; Dunn, N.W.; Cray, P.P.; and Marsden W.L.,(1989): "Comparison of alkali and steam (acid) pretreatments of lignocellulosic materials to increase enzymatic Susceptibility: Evaluation under optimised pretreatment Conditions."J. Chem. Tech. Biotechnol., 44:135-146.

Gabr; S.A.; Sitohy, M.Z; Labib. S.M., and Ghozlan, M.S. (1991 b): "Enzymatic hydrolysis of some physically and chemically pretreated agricultural cellulosic residues". Zagazig, J. Agric. Res. 18, (2), 375- 391.

Gunasekaran, P. and Kamini, N.R. (1991): "High ethanol productive from lactose by immobilized cells of kluyveromyces fragilis and zymomonas mobilis". World J. of Microbiol. and Biotech., 7, 551 - 556.

Hoda, G.E.M.; Alian, A.M.; Nagwa, M.E.S. and Fadel, M.A. (1990): "Enzymatic hydrolysis of some cellulosic wastes for fodder yeast production. Transformation of wastes to fermentable sugars". Annual of Agriculture Science, Cairo, 35(1), 143-155.

Lisbethoisson, R. and Bärbel, H. (1996): "Fermentation of lignocellulosic hydrolysates for ethanol production". Enzyme and Microbiol Tech., 18, 312 - 331.

Montgomery, R., (1961): "Further studies of the phenol salphuric acid reagent for carbohydrates". Biochem. Biophys. Acta., 48, 591 - 593.

Moritz, J.W. and Duff., S.J.B. (1996): Simultaneous saccharification and extractive fermentation of cellulosic substrates. Biotech. and Bioeng., 49, 504-511.

Nelson, N., (1944): A photometeric adaptation of the Somogyi determination of glucose. J. Biol. Chem., 153, 375-380.

Okeke, B.C. and Obi, S. K. C. (1995): "Sacchrification of Agro-waste materials by fungal cellulases and Hemicellulases". Bioresource Tech., 51, 23 - 27.

Ooshima, H.; Kurakake, M.; Kato, J.; and Harano, Y. (1991): "Enzymatic activity of cellulase adsorbed on cellulose and its change during hydrolsis". Appl. Biochem. Biotech., 31, 253 - 266.

Roca, E.; Flores, J.; Nunez, M.J.; and Lema, JM. (1996): "Ethanolic fermentation by immobilized Saccharo-myces cerevisiae in a semipilat pulsing packed-bed bioreactor". Enzyme and Microbiol Tech 19, 132 - 139.

Somogyi, M.J. (1952): Notes on sugar determination. J. Biol. Chem., 195,19-23.

Szczodrak, J. (1988): "The enzymatic hydrolysis and fermentation of pretreated wheat straw to ethanol". Biotech. and Bioeng., (32):771 - 776.

Tanaka, M.; Robinson, C.W. and Moo-Young, M. (1985): "Chemical and enzymatic pretreatment of corn stover to produced soluble for mentation substrates".Biotech. and Bioeng., 27: 362-368.

Tarkaw, H. and Feist, W. C. (1969): "Mechanism for improving the digestibility of lignocellulosic `materials with dilute alkali and liquid ammonia". Adv. Chem. Ser., 95, 197 - 218.

Vega, J.L.; Klasson, T.; Clausen, E.C.and Gaddy, J.Z. (1991): "The saccharification of corn stover by cellulase from *Penicillium funiculosum*". Bioresource Tech. 35: 37 - 80.

إنتاج الجلوكوز والإيثانول من بعض المخلفات السليولوزية بإستخدام إنزيم السليوليز المنتج من تراى ريزيا، تراى هيرزينيم والأسبرجلاس نيجر

صلاح مصطفى محمود سعد*، فرحات فوده على فوده*،
عزت رزق جندى *، رأفت نظمى زكى**

* قسم الكيمياء الحيوية الزراعية - كلية الزراعة بمشتهر - جامعة الزقازيق - فرع بنها

** مركز البحوث الزراعية- قسم الميكروبيولوجى

يهدف هذا البحث إلى دراسة المواد اللجنوسليولوزية الخاصة بمخلفات قش الفول البلدى وبقايا قرون البسلة لإستخدامها فى إنتاج مواد لها قيمة غذائية مثل شراب الجلوكوز وإنتاج الإيثانول كمصدر للطاقة. وقد إستخدمت الصودا الكاوية وحامض الخليك الثلجى لمعالجة المخلفات السليولوزية بهدف التكسير البللورى لهذه الجزيئات لتحويلها إلى صورة غير بللوريه حتى يسهل لإنزيم السليوليز مهاجمتها وتحليلها.

وقد أظهرت النتائج أن معاملة هذه المخلفات بحامض الخليك الثلجى (٤% حجميه/وزنيه) أدى إلى إنتاج ٨٠%، ٧٠% من السليولوز المستخلص بالنسبة للفول البلدى وذلك على درجة الحرارة١٠٠°م لمدة ٣ ساعات، ٢٥°م لمدة ٤٨ ساعة على التوالى بينما بقايا قرون البسلة فكان السليلوز المستخلص ٨٠% ، ٦٠% عند معاملتها بالصودا الكاوية (١% وزنيه/وزنيه) على نفس الظروف السابقة.

وقد تم دراسة المكونات الأساسية لهذه المخلفات السليوليزية وأوضحت النتائج المتحصل عليها أن قش الفول البلدى الخام تحتوى على ٣ر٣٢% سليولوز، ٠ر٢٣% هيمسليولوز ، ١ر١٣% لجنين بينما نسبة هذه المكونات فى بقايا قرون البسلة الخام فكانت ٢ر٢٩%، ٤ر٢٠% ، ٦ر١٠% على التوالى. وبعد المعالجة الكيميائية لمخلفات قش الفول البلدى وبقايا قرون البسلة بواسطة الصودا الكاويه تحت الظروف السابقة فكانت نسبة السليولوز، الهيمسليلوز واللجنين (٥ر٧٢%، ٥ر١٠%، ٣ر٧%) فى حالة قش الفول البلدى المستخلص بينما كانت (٨٠%، ١ر٨%، ٨ر٤%) فى حالة بقايا قرون البسلة المستخلصة.

وقد تم إجراء عملية التسكر لإنتاج السكريات تحت الظروف المثلى للفول البلدى وبقايا قرون البسلة لمدة ٣، ٦ ساعة بتركيز ٢، ٤، ٦، ٨، ١٠% من المواد المتفاعلة.

وأظهرت النتائج المتحصل عليها أن أعلى معدل للتسكر فى ٦ ساعات وذلك فى وجود الإنزيم المنتج من فطر *A. niger ' T. harzianum ' T. reesei* كان (٢ر٦٢، ٤ر٥٦، ٠ ر٥٦%) بتركيز ٨% من السليولوز المستخلص من قش الفول البلدى بينما كان أعلى معدل للتسكر للسليولوز المستخلص من قرون البسلة٣ر٦٦، ٨ر٥٨، ٦٥% وذلك بإستخدام نفس الإنزيمات المنتجة من الفطريات السابقة فى ٦ ساعات بتركيز ١٠% من المادة المتفاعلة تحت نفس الظروف المثلى للتحليل الإنزيمى.

وقد تم دراسة إنتاج كحول الإثيلى من تخمر السكريات الناتجة من عملية التسكر بواسطة خميرة *Saccharomyces cervisia* وقد أوضحت النتائج أن نسبة التحويل كانت٥ر٨٩، ٧ر٨٤، ٧ر٨٥% من الجلوكوز الناتج من الكربوكسى ميثليل سليولوز للإنزيمات الناتجة من فطريات *A. niger ' T. harzianum ' T. reesei* على التوالى بينما كانت نسبة التحويل من الجلوكوز الناتج من قش الفول البلدى (٥ر٨٩، ٥ر٨٩، ٥ر٨٨%) وبقايا قرون البسلة (٦ر٨٣، ٥ر٨٩، ٨٨%) بإستخدام نفس الإنزيمات الناتجة من الفطريات السابقة الذكر.