J. Agric. Sci. Mansoura Univ., 30 (12): 8053 - 8073, 2005

BIOCONVERSION OF BARLEY STRAW INTO YEAST BIOMASS AND ALCOHOL

Olfat, S. Barakat*; S. A. El Gizawy*; E.M.A. Araud and M.K. Zahra***

* Agricultural Microbiology Dept., Fac. Agric., Cairo Univ., Giza.

** Food Technology Dept., Fac. Of The Scince Of Technol. And Engin., Sabbha Univ., Libya.

ABSTRACT

Barley straw was examined as a substrate for yeast biomass or ethanol production. Physical and chemical procedures were applied in order to convert the cellulosic fraction of the straw into soluble sugars, mainly glucose, which in turn could be used by a yeast strain as a carbon source. Pulverization and heat treatment (steaming at 121°C for 1 hour) were carried out in addition to acid or alkali pretreatment using either H_2SO_4 or NaOH at different concentrations. In this regard, 4%H_2SO_4 solution was the most effective in hydrolyzing the straw which was indicated by the release of the highest amount of soluble sugars. The supernatant resulted after acid pretreatment was detoxified and neutralized using overliming with different alkali, $Ca(OH)_2$, NaOH, $Ba(OH)_2$, KOH. Among the examined alkaline solutions; $Ca(OH)_2$ was the best. The supernatant was not the suitable substrate in batch fermentation for yeast biomass production using *Sacch. cerevisiae* 2030, therefore, it was examined for ethanol production using *Sacch. cerevisiae* O-14. The highest yield of alcohol (75-78.3 % of the theoretical) did not greatly affected by the yeast (*Sacch. cerevisiae* O-14) inocula size; nevertheless, the fermentation period was shortened when batches were inoculated with 10%. The solid cellulosic residues separated after pretreatment were subjected to chemical hydrolysis using different concentrations of NaOH or H_2SO_4 and autoclaved at different temperatures (100, 121, 136°C) for 1 hour. The maximum amount of glucose was recorded with 2% H_2SO_4 solution at 136°C for 1 hour. Production of yeast biomass from glucose, molasses and acid hydrolyzate in 9-hour fed batch fermentations was investigated. The highest yield coefficient (210%) was obtained with glucose while the yield coefficient was decreased to 105% and 80% when yeast was cultivated on molasses or acid hydrolyzate , respectively.

Keywords: Barley straw, yeast biomass, ethanol production, sugar-can molasses.

INTRODUCTION

Yeasts are very important not only for bakery production, but also as sources of some vitamins, proteins, enzymes, fermented foods, yeast extract, and ethanol either in beverages or as fuel and disinfectant (Hay, 1993). Recently, *S. cerevisiae* has been involved in the production of recombinant proteins, e.g. insulin, vaccine against Hepatitis B virus (Fredriksson, 2001). The possible application of cheap carbon sources for large scale yeast biomass production have been investigated (Kostitsina and Mysyakova, 1968;El Nawawy, 1969;Tahalasso *et al.* 1999;Ferrari *et al* 2001;Ryohei *et al.* 2003).

Barley straw is one of agricultural wastes consists mainly of cellulose, hemicellulose and lignin (Givens *et al.*, 1993). Hydrolysis of the cellulosic

fraction into fermentable sugars is difficult due to the rigid nature of the lignin cellulose complex (Dekker, 1983). Physical and chemical pretreatments have been suggested for improving saccharification of other lignocellulosic materials (Zaaky, 2002). In this regard, pretreatments have been used by Fan *et al.* (1982) and Sinitsyn *et al.* (1991) to reduce the particle size, lignin content, hemicellulose content and cellulose crystallinity. Cellulose was expected to be the major component of the residual solid fraction separated from the solution of acid or alkali pretreatment of the straw. So, the residual solid could be hydrolyzed into glucose. Saccharification of lignocelluloses by concentrated acid hydrolysis is a well known process however, dilute-acid solution has been recommended by Taherzadeh *et al.* (1997) to facilitate conversion of celluloses into sugars. The resultant acid hydrolyzate contained a number of chemical compounds which inhibit microbial growth, even at very low concentrations (Taherzadeh, 1999). Among these compounds, furfural, hydroxymethyl furfural, levulinic acid, formic acid, methanol, acetic acid, aromatic, polyaromatic and phenolic compounds have been reported (Sjöström, 1993; Taherzadeh *et al.*,1997). A suitable method of detoxification should be followed to reduce the toxicity of these compounds (Larsson *et al.*, 1999).

The present work was planned to study the possibility of using barley straw for production of Bakers` yeast biomass. Physical and chemical methods were carried out for hydrolyzing barley straw into glucose which could be used as carbon source for Bakers` yeast propagation.

MATERIALS AND METHODS

Yeast strains

Saccharomyces cerevisiae O-14 was obtained from Institute of Bakery Moscow, Russia and used only for ethanol production while *Sacch. cerevisiae* 2034 obtained from El-Hawamdia Factory, Egyptian Co. For Sugar and Integrated Indust., Giza, was used for yeast-biomass production .

Raw materials and chemicals

Cane molasses of 50% sugar, 80.5% total solids, and pH 5.2, obtained from El-Hawamdia Factory, Egyptian Co. For Sugar and Integrated Indust., Giza was used in yeast production, after being clarified, neutralized, and sterilized (Amin, 1978). Barley straw was obtained from "Zentain city area, Libya", pulverized and sieved to pass through 4 mesh screen before use. Glucose and other chemicals used throughout this work were obtained from El-Gomhoreia Co. for Chemicals and Medical Apparatuses.

The bioreactor

The experimental fermentor was a glass cylindrical vessel of 70 cm height and 8 cm diameter. A sterilized air stream was supplied to the vessel through an air distributing system fitted to its bottom, after being passed through a series of sterilized flasks containing 30% H_2SO_4 solution, 10% Na OH solution, sterilized distilled water, and a solution-adding flask respectively.

The dissolved oxygen was measured by an oxygen meter (LT Lutron DO-5510), and the pH was measured by a Jenway 3020 pH-meter.

Chemical determinations

Moisture and ash contents of the straw were determined according to the methods described in A.O.A.C.(1990). Crude protein was determined using micro-Kjeldahl method adapted by Cotteni *et al.*, (1982). The methods described by Goering and Van Soest (1970) were applied for determination of neutral-detergent fiber (NDF), acid–detergent fiber (ADF) and acid detergent lignin (ADL). The cellulose, hemicellulose and lignin fractions were calculated. Hemicellulose was calculated by difference between neutral-detergent fiber and acid-detergent fiber.

Total sugars were determined according to the method described by Smith *et al.* (1956) and DNS (dinitrosalisylic acid) was used for determining reducing sugars (Miller, 1959). The enzymatic colorimetric method (Trinder, 1969) was followed to determine glucose concentration using GOD-PAP kits (Diamond Diagnostics CO., USA). Ethanol was determined according to Plevako and Bakoshinskaya (1964).

Alkaline and acid Pretreatments of barley straw

Different weights *i.e.* 1, 2, 4, 6, 8, and 10 g of straw were added to 100 ml solution aliquots of increasing NaOH concentrations from 1 to 10, 15 and 20 %, or to 100 ml H_2SO_4 aliquots of the same concentrations for acid pretreatment. The mixtures were autoclaved at 121 °C for 1 hr. The overliming technique described by Larsson *et al.*, (1999) was followed in order to detoxify the supernatant produced after acid pretreatment using NaOH, $Ca(OH)_2$, Ba $(OH)_2$ and KOH.

Saccharification with sulfuric acid

Five g portions of pretreated barley straw were thoroughly mixed with either 100 ml aliquots of diluted sulfuric acid solutions (1, 10, 15, and 20%) and autoclaved at 121°C for 1 hour, or mixed with 20 ml of concentrated sulfuric acid solutions (40, 50, 60 and 70%) and kept at room temperature for 30 min, then completed to 100 ml with tap water and finally autoclaved at 121°C for 1 hr.

To study the effect of steaming temperature and duration on sacchrification of the pretreated straw, 5g samples of pretreated barley straw were added to 100 ml solutions of different H_2SO_4 concentrations (1.0 , 2.0, 3.0 and 4.0%) and subjected to different temperatures *i.e.*, 100, 121, and 136°C for 1 hr. Another 5g portions of pretreated straw were mixed with 100 ml of 4% H_2SO_4 and autoclaved at 121°C for 3 different time intervals *i.e.*, 1, 2 and 3 hrs. Reducing sugars in the saccharified solutions were determined after each heat treatment. The acid hydrolyzates were detoxified, neutralized then used for yeast propagation.

Saccharification with Na OH

Different concentrations of Na OH (1 to 10, 15, and 20%) were examined for saccharifying 5 g portions of pretreated barley straw at 121°C for 1 hr. To study the effect of steaming period on sacchrification of the pretreated straw, 100ml aliquots of 4% Na OH solution were added to 5g samples of pretreated barley straw, and autoclaved at 121°C for 3 different

time intervals (1, 2, and 3 hr). Reducing sugar contents of the saccharified solutions were determined after each heat treatment.

Fed-batch fermentation by using mineral glucose medium

Sugar amounts required for feeding 10 g of seed yeast (75% moisture) at each hour of a fed-batch fermentation process for yeast biomass production were calculated as described by White (1954). The fermentor was inoculated with 10 g of seed yeast (75% moisture) and suitable volumes of a solution containing ($g.L^{-1}$): glucose 50; yeast extract 4, and the mineral solution recommended by Berry (1989) were added to the fermentor hourly with increased exponentially feeding rate lasting 7 hrs after which the culture was left 2 hrs for cells maturing and stabilization. The pH values were controlled between 4 and 5 using 0.1N Na OH or 0.1N HCl throughout the fermentation period. Dissolved oxygen (DO), budding ratio, residual sugar content, yield coefficient, yeast concentration and development yield and specific growth rate were measured at the end of each feeding hour. Yeast moisture and percentage of productivity were measured at the end of fermentation process.

Fed-batch fermentation using molasses medium

Molasses supplemented with nitrogen (urea), phosphorus (H_3PO_4), sulphur and magnesium ($MgSO_4$) according to Rosen (1987) was used for feeding a fed-batch fermentation for yeast biomass production. Based on molasses sugar content the amounts of clarified molasses were calculated and added hourly. The conditions and parameters were applied and measured similarly as in the previous experiment.

Fed-batch fermentation using supernatant produced from acid pretreatment

The suitable amounts of sterilized supernatant containing the required amount of sugars and other nutrients (as mineral glucose medium) were added to the fermentor in a fed-batch fermentation lasted 7 hrs. The conditions and parameters were followed as in previous process.

Ethanol production from supernatant produced after acid pretreatment

Fifty grams of straw were added to 1000 ml of 4% H_2SO_4 (v/v) solution and autoclaved at 121°C for 60 min. The mixture was kept at 40°C for 48 hrs and filtered. After detoxification and neutralization, 300 ml aliquots were added to 100 ml of sterilized mineral yeast growth medium. The mixture was inoculated with 0.1, 1 or 10% of *Sacch. cerevisiae* O -14 biomass and incubated at 30°C for 72 hrs. Ethanol and total sugars were determined every 24 hrs.

Fed-batch fermentation by acid hydrolyzate

Fifty grams of residual pretreated barley straw were added to 1000 ml of 4% H_2SO_4 (v/v) and autoclaved at 121°C for 60 min. The mixture was kept 48 hrs at 40°C. The hydrolyzate was filtered, detoxified and neutralized as abovementioned. The fermentor was inoculated with 10 g of seed yeast (75% moisture), suitable amounts of sterilized acid hydrolyzate containing the required amount of sugars and other nutrients (as mineral glucose medium)

were added to fermentor for 7 hrs. The same conditions and parameters applied in the previous fed-batch fermentation experiments were followed.

RESULTS AND DISCUSSION

Chemical composition of barley straw

The raw barley straw under investigation contained 92%, 3.1% and 3.04% total organic matter, total ash and crude protein, respectively (Table,1). These results are slightly different from those obtained by Stritzler *et al.* (1992). However, Mathison *et al.* (1999) recorded that barley straw contains higher amounts of crude protein ranged between 3.4-4.8%. The percentages of fiber fractions NDF (76.38%) obtained from chemical analysis of barley straw from Zentain, Libya, was almost similar to that recorded by Stritzler *et al.* (1992); Mathison *et al.* (1999) and Caneque *et al.* (1998). Meanwhile, the ADF fraction percentage (52.39%) was higher than that recorded by Caneque *et al.* (1998) and Mathison *et al.* (1999) who reported that the ADF fraction ranged between (41.11 and 46.2%). Also the ADL fraction content (9.37%) was higher than that stated by Caneque *et al.* (1998).

Table (1): Chemical composition of barley straw.

Components	g/100 g of dry weight
Total organic matter	92.0
Ash	3.10
Crude protein	3.04
Fiber fractions: NDF	76.38
ADF	52.39
ADL	9.37
Cellulose	43.02
Hemicelluloses	23.99
Lignin	6.27

Cellulose and hemicelluloses content determined in straw under investigation were 43% and 23.99 %, respectively. Meanwhile, Belkacemi *et al.* (1998) as well as Mathison *et al.* (1999) found that cellulose content of barely straw ranged between 37.7% and 39.8%.

Straw pretreatment with alkaline

From data given in Table (2), it could be noticed that at low substrate concentrations, *i.e.*, 1 and 2%, the amount of sugars was ranged between 0.06 and 0.68 g/100ml even at high concentrations of NaOH. The best results were obtained with straw concentrations (4–6%) and the suitable NaOH concentration for pretreatment of the straw was 4%. Data in the table also suggested the use of 4% NaOH solution for pretreating 6% barley straw. These results are in conformity with those obtained by Zaaky

(2002).However, the best polysaccharide recovery from barely straw was obtained using 2% NaOH at 121°C (Attia, *et al.*, 1988; Kaullas *et al.*, 1991; Durand *et al.*, 1992).

Table (2): Total sugars released from barley straw pretreated with different concentrations of sodium hydroxide.

NaOH Concentration (%)	Weight of straw (g/ 100 ml)					
	1	2	4	6	8	10
	Total sugars (g/100 ml)					
1	0.06	0.27	0.63	0.67	0.74	0.61
2	0.07	0.36	0.65	1.16	0.84	0.63
3	0.11	0.43	0.65	1.23	0.86	0.74
4	0.11	0.50	0.66	1.52	0.91	0.80
5	0.10	0.62	0.67	1.40	0.91	0.80
6	0.10	0.55	0.64	1.40	0.90	0.80
7	0.10	0.55	0.64	1.40	0.90	0.80
8	0.10	0.55	0.64	1.40	0.90	0.80
9	0.10	0.55	0.64	1.40	0.90	0.80
10	0.10	0.55	0.64	1.40	0.90	0.80
15	0.10	0.68	0.64	0.90	0.90	0.80
20	0.10	0.68	0.64	0.87	0.90	0.80

Pretreatment of straw using sulphuric acid

Data in Table (3) show the amounts of total sugars produced as a result of acid pretreatment of barley straw at 121°C. Generally, the amount of total sugars increased as straw concentration increased up to 6% then the amount of total sugars gradually decreased as straw concentration increased up to 10%. Regardless of acid concentration, saccharification of 1% barley straw resulted in low percentages of sugars ranged from 0.04 to 0.19%.

Table (3): Total sugars released from barley straw pretreated with different concentrations of sulfuric acid.

H_2SO_4 Concentration (%)	Weight of straw (g/ 100 ml)					
	1	2	4	6	8	10
	Total sugars (g/100 ml)					
1	0.04	0.13	0.60	1.44	1.11	0.90
2	0.12	0.19	0.60	1.52	1.11	0.93
3	0.18	0.20	0.60	1.56	1.19	0.96
4	0.19	0.21	0.70	1.66	1.19	1.10
5	0.19	0.20	0.70	1.56	1.19	1.10
6	0.19	0.20	0.70	1.47	1.19	1.10
7	0.19	0.20	0.70	1.36	1.19	1.10
8	0.14	0.20	0.70	1.35	1.19	1.10
9	0.14	0.20	0.70	1.26	1.19	1.10
10	0.14	0.20	0.70	1.61	1.19	1.10
15	0.13	0.20	0.70	1.31	1.02	0.96
20	0.04	0.20	0.55	0.64	0.90	0.82

The highest amount of sugars (1.66%) obtained at 6% straw concentration treated with 4% H_2SO_4 and the lowest amounts were obtained at the highest H_2SO_4 concentration (20%). Accordingly, in order to obtain a high sugar-containing-solution from barley straw, 4% sulfuric acid solution would be recommended for pretreatment of 6% of straw suspension. Many workers used dilute acid solutions 0.2 and 0.5% H_2SO_4 combined with high temperatures (up to 200°C) for pretreatment of lignocellulosic materials to remove hemicelluloses and amorphous cellulose (Chung and Lee, 1985; Qureshi and Manderson, 1995 and Taherzadeh *et al.*, 1997). In the present experiment, it was planned to use higher acid concentration (4% H_2SO_4) at lower temperature (121°C) for achieving a sufficient removal of hemicellulose (Lee *et al.*, 1997).

From data it could be recommended that acid pretreatment of 6% straw suspension is more efficient than alkali pretreatment because of the higher resulted sugars (1.66 %) compared to that produced with alkali (1.52 %).In addition to that, acid treatment is more practical and cheaper than alkali treatment.

After acid pretreatment of straw, the supernatant which was obtained by filtration was neutralized and used for cultivation of yeast to produce biomass or alcohol. Disposal of such aqueous acidic solution would be environmentally unsafe and lead to loss in the carbon fraction from the raw material. However, the supernatant is known to contain some inhibitors originated from lignin and liberated during the hydrolysis (Clark and Mackie, 1984; Chung and Lee, 1985; Palmqvist, 1998). Therefore, overliming with alkalines ($Ca(OH)_2$, $Ba(OH)_2$, NaOH and KOH) was carried out to overcome the toxicity problems. The alkali was added to the supernatant in order to adjust the pH to 9 or 10, followed by acidification to pH 5, which is suitable for the fermentation process. In order to verify the toxicity of the supernatant, the supernatant was inoculated with 0.5 % yeast inoculum and grown in shake culture for 24 hr at 30°C and 150 r.p.m.

Values of biomass concentration, total new yeast, yield and specific yield of the cultivated yeast are presented in Table (4). Calcium hydroxide proved to be the most active detoxifying agent as all parameters which indicate yeast survival and activity were recorded when yeast was cultivated in a medium treated with $Ca(OH)_2$.

Table (4): Effect of using different alkali with acedic supernatant on yeast propagation.

detoxifyingagent	Biomass g/l	Total new yeast (g/l)	Yield (g/g)	Specific yield (g/g)
$Ca(OH)_2$	6.7	1.7	0.34	0.1
$Ba(OH)_2$	5.9	0.9	0.18	0.05
NaOH	5.2	0.2	0.04	0.01
KOH	5.0	0.0	0.0	0.0

Working volume = 100 ml. **Sugar = 2 %.**
Fermentation period: 24 hr. **Inoculum size = 1%.**
Specific yield = g product/g sugar. **Yield = g product/ g biomass.**
Initial pH, 5.5. **Incubation temperature , 30 °C.**

Unexpectedly the yeast strain failed to grow on a supernatant pretreated with KOH. These results are in agreement with those reported by Larsson *et al.* (1999). Calcium hydroxide was shown to catalyze the condensation reaction of formaldehyde (Niitsu *et al.*, 1992) as well as other kinds of aldehydes in the hydrolyzates, e.g., furfural and HMF (Taherzadeh, 1999).

Saccharification of pretreated straw

Acid hydrolysis

Data illustrated by Fig (1) reveal that by increasing acid concentration of the hydrolysis mixture from 1 to 4% enhanced the amount of glucose released from 6.04 to 7.87g/l. However, no genuine increases in the amount of glucose produced when the concentration of H_2SO_4 was increased from 5 to 20%. By the contrary, using 40% of H_2SO_4 acid solution sharply increased the hydrolysis of the cellulosic residue and the hydrolyzate contained 24.4g/l glucose. The maximum hydrolysis was reached using 70% H_2SO_4 solution with a conversion efficiency 52.6%.

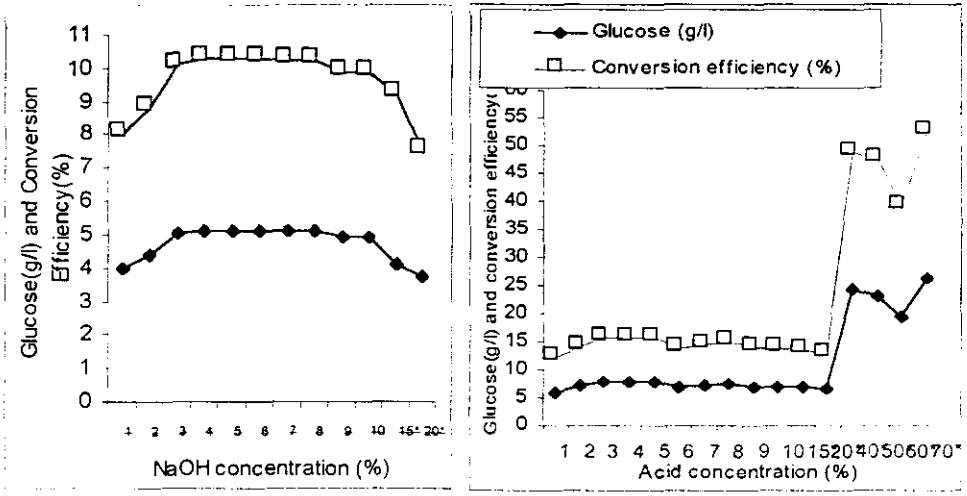


Figure (1): Effect of H_2SO_4 or NaOH concentration used in hydrolysis of residue cellulose at 121°C for one hour on the amount of glucose produced (g/l) and the conversion efficiency (%)

The use of 40% H_2SO_4 solution could, practically and economically, be recommended for the hydrolysis of such cellulosic material at 121°C for one hr. Further increases in acid concentration to 50 or 60% did not improve both the amount of glucose produced and the conversion efficiency. These results agree with those reported by Katzen and Monceaux (1995) and Qureshi and Manderson (1995) for the conversion of cellulose in wood into glucose.

Because of the resultant hydrolyzate contained high amount of H_2SO_4 and was not suitable for microbial growth without neutralization, a dilute acid hydrolysis process was studied at three different temperatures in order to

reach the maximal amount of glucose as shown in Fig (2). Regardless of acid concentration, heating the cellulosic materials at 100 °C for 1 hr resulted in lower amounts of glucose (1.21-1.97 g/l) compared with those obtained at temperatures higher than 100°C. On the other hand, 2% H_2SO_4 solution exerted the highest hydrolytic capability of celluloses heated at 136°C. Similar results were recorded by Chung and Lee (1985) and Taherzadeh *et al.* (1997).

In the present study, higher temperature than 136°C was not applicable under laboratory conditions. So, a separate experiment was carried out to examine the effect of different exposure times at 121°C on cellulose hydrolysis in the residue by 4% H_2SO_4. Data on the amount of glucose produced (g/l) and the calculated conversion efficiency (Table 5) indicate that autoclaving time had no effect on the hydrolysis efficiency of the cellulosic residues.

Steaming has been known as one of the most effective methods for pretreatment of lignocellulosic materials (Gregg and Saddler, 1996). Temperature could be used alone for autohydrolysis of cellulosic materials at 180°C (Schmitt and Murphy, 1980), within a range between 190-200°C (Wright, 1988 ; Walch *et al.*, 1992), or within a range of 220-270°C (Gregg and Saddler, 1995). Alkali hydrolysis

Fig (1) presents the amount of glucose determined after alkali hydrolysis of solid residues using NaOH at concentrations ranged between 1 and 20% at 121°C for 1 hr. Glucose production was increased from 4.00 to 5.13 g/l as the NaOH concentration was increased from 1 to 4%. Moreover, the amount of glucose was almost constant (5.13-4.93 g/l) even when the concentration was increased up to 10%. The conversion efficiency didn't exceed 10.3% even when NaOH solutions reached up to 20%.

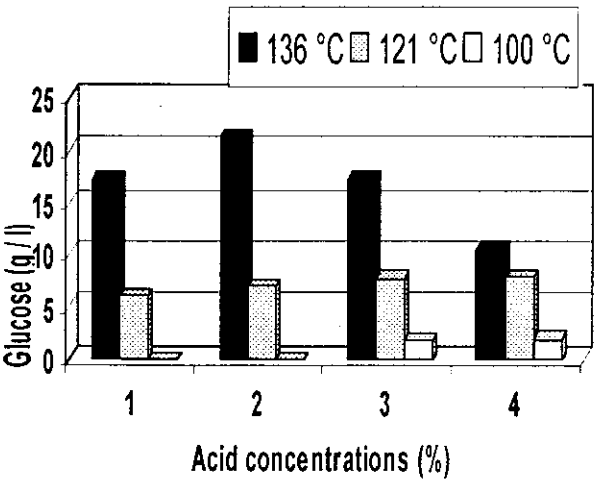


Fig (2): The effect of temperature and acid concentration on the amount of glucose released from the hydrolysis of residual cellulose.

In an attempt to improve the alkali hydrolysis of cellulosic residue, residual cellulose was treated for different time intervals with 4% NaOH at 121°C. The amount of glucose in the hydrolyzate was determined as well as the conversion efficiency (Table, 5). It could be observed that the time of heat treatment had almost no effect on either the amount of glucose produced or the conversion efficiency. In other words, production of glucose from the cellulosic residue autoclaved for 1 hr was not higher than those heated for 2 or 3 hr.For an economic reason, the use of alkaline as ahydrolysing agent for cellulosic residues is preferable. However, it could only be used for pretreatment of the raw barley straw. Alkaline pretreatment of lignocellulosic materials has been known to enhance the enzymatic hydrolysis of the residual cellulose (Goel and Ramachandran, 1983; Attia *et al.*, 1988; Kaullas *et al.*, 1991; Zaaky, 2002).

Data in this study suggests the possible use of chemical hydrolysis of the cellulosic residues. Hydrolysis process would rather be fruitful with acid than alkali. Hydrolysis of the cellulosic residue in 2 % H_2SO_4 solution at 136 °C for 1 hr is recommended in order to obtain a hydrolyzate containing 21.3 g/l glucose (Fig.1). Acidity in the resultant hydrolyzate should be neutralized with Ca $(OH)_2$ in for its use in bakers` yeast biomass production.

Table (5): Effect of heat treatment exposure time on the amount of glucoserecovered from cellulosic residues.

Heat treatment period (hr)	Hydrolysis with 4% H_2SO_4 at 121°C		Hydrolysis with 4% NaOH at 121°C	
	Glucose (g/l)	Conversion efficiency %	Glucose (g/l)	Conversion efficiency %
1	7.87	15.74	5.13	10.26
2	7.91	15.82	4.94	9.88
3	7.98	15.96	4.94	9.88

Production of Bakers` yeast using glucose as a carbon source

Results illustrated by Fig (3) reveal that yield coefficient of yeast increased with increasing cultivation time from one hour to another reaching a maximum value of 223.3% by the end of the fourth hour. Thereafter, the yield coefficient decreased gradually through the subsequent two hours to reach 195.7% by the end of the seventh hour. This might be attributed to the accumulation of the sugars added into the cultivation broth. Unbalanced conditions may also resulting in a switch from respiratory to fermentative activity of the yeast cells which in turn would lower the yield (Hospodka, 1966; Amin, 1978).

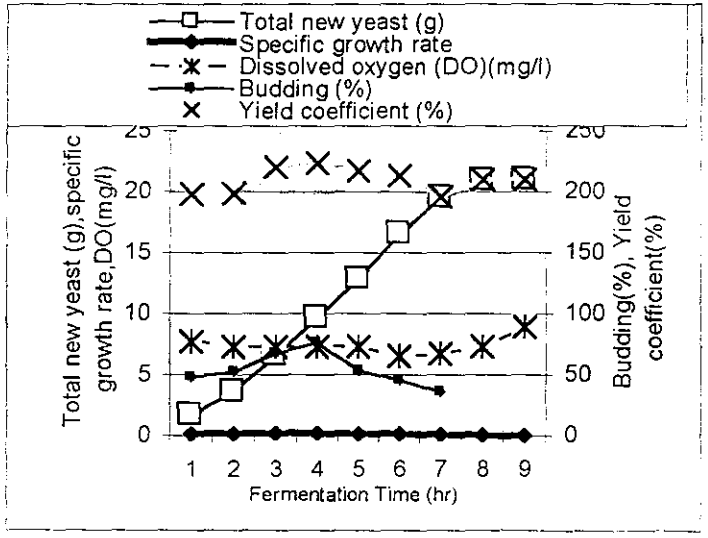
Production of Bakers` yeast using glucose as a carbon source

During the last two hours, there was no additional feeding. The growing yeast cells had to be matured and stabilized, so the yield coefficient was increased to 210.2%. This might be attributed to assimilation of the residual sugars and other compounds formed during the metabolic activity of the yeast cells (Zahra *et al.*, 1974; Mostafa, 2000).

The budding cells ratio was increased by time to reach the highest level after the first 4 hours from the beginning of the cultivation period. Then, it gradually decreased till the end of cultivation time. A directly proportional relationship was observed between the budding ratio and yield coefficient, with higher values recorded by the end of the fourth hour. However, this finding disagrees with the results obtained by Wiemken *et al.* (1969) and Amin (1978) who stated that the budding ratio reached its maximal value one hour earlier before the maximal yield coefficient.

The calculated values of specific growth rate were increased gradually reaching their highest peak (0.22) at the 3rd hour then decreased thereafter (Table, 6). The high specific growth rate might result in high biomass yield in a short time (Yuan *et al.*, 1993; Mostafa, 2000). However, a relationship between budding ratio and the specific growth rate could be observed. Higher values of specific growth rate obtained as the budding ratio increased.

The concentration of dissolved oxygen in the fermentation broth at the commencement of the fermentation process was lowered from 10 to 7.7 mg/l after one hour of cultivation and further decreased to 7.3 mg / l at the second hour, then remained constant at this level till the end of the fifth hour. These reductions in DO concentration were accompanied by an increase in yeast growth rate. However, the available amount of DO had a great effect on the yeast yield coefficient. In other words, the lowest yield coefficient (195.7%) was accompanied by the lowest amount of DO at the seventh hour of cultivation. In this regard, Mostafa (2000) attributed the reduction in yeast yield to the air supply depletion and to the increase in alcohol production as well.



pH:4.5-5 seed culture:10g Initial dissolved oxygen(DO):10mg/l
incubation temperature :30°C

Fig (3): Yeast growth parameters during its cultivation in glucose mineral medium.

Table (6): Specific growth rate and total residual sugar obtained during yeast cultivation in different growth media.

Aerobic Cultivation Time (hr)	Glucose Medium		Molasses medium		Supernatant produced after acid pretreatment		Acid hydrolyzate	
	Specific growth rate	Total residual glucose (g)	Specific growth rate	Total residual glucose (g)	Specific growth rate	Total residual glucose (g)	Specific growth rate	Total residual glucose (g)
1	0.17	-	0.13	0.037	0.03	0.564	0.10	0.267
2	0..17	-	0.16	0.051	0.04	1.132	0.10	0.939
3	0.22	-	0.21	0.071	0.03	1.338	0.10	0.820
4	0.19	-	0.19	0.084	0.03	1.665	0.09	1.275
5	0.16	0.071	0.16	0.089	0.04	1.975	0.09	2.975
6	0.16	0.089	0.16	0.094	0.06	2.305	0.10	3.146
7	0.11	0.144	0.13	0.091	0.07	2.635	0.11	3.52
8	0.05	0.090	0.03	0.091	0.01	ND	0.01	ND
9	0.0003	0.090	0.0001	-	0.005	ND	0.006	3.422

ND: Not determined **Values are means of three replicates**

Production of bakers' yeast using molasses as a carbon source:

The yeast yield coefficient was calculated on the basis of the amount of sugars contained in molasses fed during the yeast cultivation. It could be observed that, the smallest yeast yield coefficient (151.2%) was obtained during the first hour of cultivation and the greatest one (223.3%) was recorded during the fourth hour of cultivation (Fig. 4).

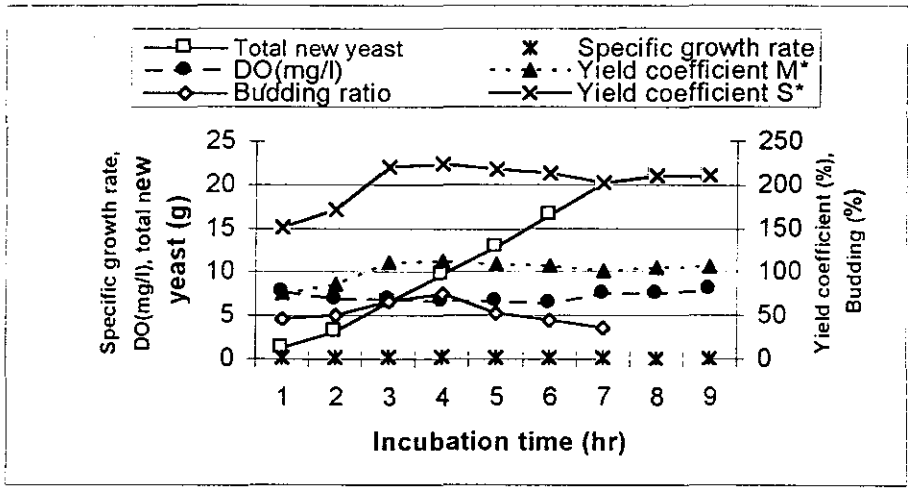
In spite of the reduction in the yeast yield coefficient by the seventh hour, it was increased again to reach 210.2% at the last hour of the cultivation period. This might be explained by the presence of furfural and other components in molasses which may result in delaying the yeast growth during the first two hours of cultivation (Sjostrom, 1993; Taherzadeh *et al.*, 2000).

With regard to the yeast yield coefficient on the basis of the weight of molasses fed as a raw material, its value reduced by one-half since molasses contained 50% (w/w) sugars. The lowest value was recorded (75.6 %) during the first hour of cultivation and increased to the maximum value 111.7% during the fourth hour of cultivation. However, the yield coefficient at the end of the fermentation period was 105.1%. In agreement with these results, Amin (1978) obtained a yield coefficient ranged between 79.7 and 115.4% when yeast was grown under aerobic conditions in molasses medium during 15 transferring stages (10 hours each). However, in this experiment the yield coefficient was (105.1%) by the end of the experiment after 9 hours of cultivation in one stage. Also it could be noticed that the yield coefficient is almost equal during the last 2 hours of cultivation, which might suggest that the fermentation period could be reduced to 8 hours only.

The budding ratio was increased by increasing the time to reach a maximum value of (74.6%) during the fourth hour of cultivation. It was gradually decreased by time to reach its lowest value (35.5%) by the end of the seventh hour of cultivation. In comparison, there were no big differences

in budding ratio between yeast grown in glucose medium and that cultivated on molasses (ranged between 0.5 and 2%).

Regarding the specific growth rate of yeast grown in molasses (Table, 6), a similar trend was observed as that noticed when the yeast was cultivated in glucose medium. It is worthy mentioned that, the growth rate during the first 2 hours (0.13 and 0.16) was lower when yeast was grown in molasses than that obtained with glucose medium, although, the specific growth rate was higher at the third and the seventh hours of cultivation when yeast was grown in molasses medium than in glucose. This might be due to the presence of some growth factors and nutrients needed for yeast growth, e.g., biotin, Ca-D-pantothenate and Inositol (White, 1954; Reed and Reppler, 1973; Reed and Nagodawithana ,1991; Ringbom *et al.* 1996).



*M Yield coefficient based on total molasses fed pH:4.5-5 seed culture:10g
*S Yield coefficient based on total sugars fed Initial dissolved oxygen(DO):10mg/l
incubation temperature :30°C

Fig (4): Yeast growth parameters during its cultivation in molasses medium

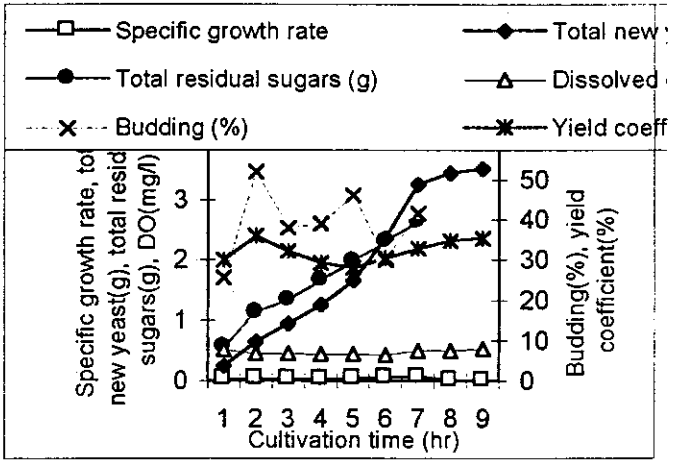
The amount of DO (10 mg/l) at the beginning of the experiment was reduced to 7.7 mg/l during the first hour of yeast growth and to 6.4 mg/l at the 6th hour of cultivation. Thereafter, DO was then increased reaching (8.0 mg/l) by the end of the experiment. High concentration of soluble substances in molasses might increase the viscosity and decrease oxygen solubility in the fermentation mixture.

Production of barkers' yeast using straw hydrolyzate as a carbon source

The supernatant produced after acid pretreatment of raw barley straw or hydrolyzate resulted from acid hydrolysis of the cellulosic residues were neutralized using Ca $(OH)_2$ before feeding into the fermentation batches.

Data illustrated by Figs (5 and 6) show that, the yield coefficient of yeast biomass produced from the straw supernatant was found very low and ranged between 28 and 35.9%. Meanwhile, the yield coefficient was rather high (77.3- 84%) and increased almost twice as a result of yeast cultivation using glucose solution obtained from acid hydrolysis of residual cellulose).

Acid hydrolyzate of lignocelluloses has been used by many investigators as a substrate for bakers' yeast, single-cell protein, and alcohol production (Nemirovskii and Kostenko, 1991 ;Taherzadeh, 1999). Low yield coefficient of yeast grown on a supernatant resulted from pretreatment of raw barley straw could be explained by the fact that such supernatant contained pentoses, hexoses, carboxylic acids and phenolic compounds (Clark and Mackie, 1984; Stewart *et al.*, 1995 ;Palmqvist, 1998). Yeast cells can grow and assimilate hexoses but it can't grow on pentoses (Berry, 1989 ;Walker, 1998). The presence of hydroxymethyl furfural (HMF) which is a by-product formed during acid hydrolysis of lignocellulosic materials (Taherzadeh *et al.*, 2000) might inhibit yeast growth during the fermentation of dilute acid hydrolyzates (Azhar *et al.*, 1982; Sjostrom, 1993 ; Taherzadeh *et al.*, 1997).



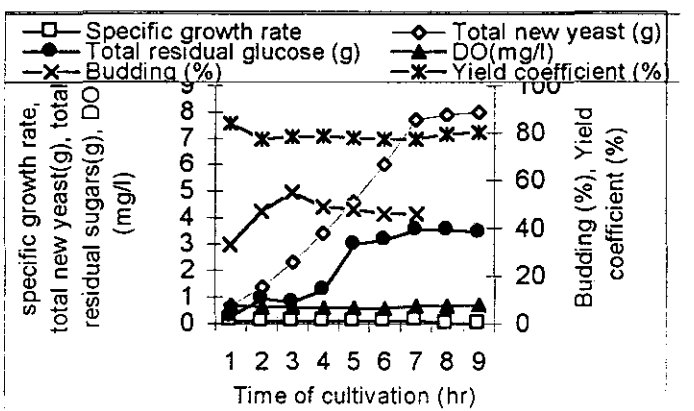
pH:4.5-5 seed culture:10g Initial dissolved oxygen(DO):10mg/l incubation temperature :30°C

Fig (5): Changes in yeast growth parameters during yeast cultivation in supernatant from pretreatment of barley straw.

The maximal values of budding ratio obtained after different cultivation periods depending upon the type of hydrolyzate. A maximal budding ratio (52%) was recorded after 2 hours of yeast cultivation in the supernatant. Meanwhile the maximal value of budding ratio (55%) was achieved after 3 hours of yeast cultivation in a hydrolyzate resulted from acid hydrolysis of residual cellulose. Microscopic examination of yeast samples obtained from the different batches along the cultivation period, revealed a very small size of cells grown in batches fed with the supernatant produced after acid pretreatment of raw barley straw as compared to those cultivated in the hydrolyzate obtained from acid hydrolysis of residual cellulose.

The specific growth rate (Table, 6) was almost constant and ranged between 0.03 and 0.1 with the maximal value recorded at the seventh hour of cultivating yeast on glucose solution obtained by acid hydrolysis of residual cellulose. It could be observed that values of yeast specific growth rate in acid or in acid hydrolyzate characterized lower those of yeast grown in either glucose or molasses media.

The residual sugars were accumulated by time collapsing to reach the maximal values at the seventh hour of cultivation. Thereafter; the amount of residual sugars was decreased during the last two hours of cultivation. Although, no further feedings were carried out during the last two hours and the yield coefficient of yeast was increased probably due to the consumption of the accumulated sugars by the growing yeast cells. However, the amount of total residual sugars recorded when yeast cultivated in supernatant produced after acid pretreatment was lower than that obtained when yeast was cultivated on glucose solution obtained either by acid hydrolysis or enzymatic hydrolysis of residual celluloses. This might be attributed to the assimilation of sugars by the cultivated yeast cells to produce metabolites rather than biomass.



PH: 4.5-5 seed culture: 10g Initial dissolved oxygen (DO):10mg/l; incubation temperature :30°C

Fig (6): Changes in yeast growth parameters during yeast cultivation in acid hydrolyzate as a carbon source.

The results reveal that, the kind of substrate did not affect the amount of (DO) in the fermentation brews. In all cases the amount of DO reduced from 10 to 7.7 mg/l during the first hour of cultivation and to 7.4 mg/l during the 6th hour of cultivation. After that, the amount of DO increased to (8 mg/l) at the end of the cultivation time.

Alcohol production by yeast grown in supernatant obtained from acid pretreatment of raw barley straw using different inocula sizes.

The low yield coefficient of yeast cultivated in supernatant obtained after acid pretreatment of raw barley straw suggests the examination of such substrate in a further experiment for alcohol production. Therefore, an efficient alcohol–producing-strain of *Sacch. cerevisiae* O-14 was used as

inocula at different sizes between 0.1 and 10.0% (w/v). The inoculated supernatants were incubated in batches at 30°C for 72 hours. Samples were taken each 24 hours to determine the amount of alcohol as well as the amount of residual sugars in the fermentation brew (Table, 7).

Generally, the ability of *Sacch. cerevisiae* to produce alcohol was affected by the inoculum's size where the amounts of alcohol ranged between 9.2 and 18.0 g/l. The amount of alcohol produced using high-density inoculum was higher than those obtained using low-size-inoculum at different fermentation periods. A shorter fermentation period was observed when the batches received high inoculum size (10.0%) with maximal ethanol concentration (18.0 g/l) and fermentation efficiency (78.3 % of the theoretical) achieved within 24 hours. These results are in agreement with those obtained by Chung and Lee (1985) and Belkacemi, *et al.* (1998).

Table (7): Effect of yeast inocula size and fermentation time on the amount of alcohol produced by *Sacch. cerevisiae* O-14 cultivated in supernatant after acid pre-treatment of raw barley straw using batch culture.

Inoculum size (w/v)	Incubation time (hr)	Alcohol concentration (g/l)	Theoretical yield (%)	Yield (g/g)	Productivity (g/lhr)	Residual sugars (%)
Control	0	0	0	0	0	4.5
0.1	24	9.22	40.1	0.2	0.38	2.69
	48	13.32	57.9	0.3	0.28	1.88
	72	ND	ND	ND	ND	ND
1.0	24	10.5	45.7	0.23	0.44	2.44
	48	16.97	73.8	0.38	0.35	1.18
	72	17.3	75.0	0.38	0.24	1.13
10.0	24	18.0	78.3	0.4	0.75	0.81
	48	18.0	78.3	0.4	0.38	0.8
	72	18.0	78.3	0.4	0.25	0.8

ND: Not determined, Incubation temperature: 30°C, Yield: g ethanol/g substrate. Initial pH value: 5, Productivity: g ethanol /hr, Working volume: 400 ml, Initial sugars concentration : 4.5%.

As expected, the two parameters which indicate the alcohol production (ethanol yield and ethanol productivity), were higher using 1.0% inoculum than those obtained using 0.1%, during all the fermentation periods. High ethanol yield was recorded (0.38) at 1.0% inoculum's size after 48 hours. Prolongation of the fermentation period to 72 hr didn't affect this value. Unlike the ethanol yield, ethanol productivity was decreased by increasing the fermentation time. The maximum value of both ethanol yield and productivity (0.40 and 0.75 g/l/h, respectively) were recorded at 10.0% yeast inoculum. These results are higher than those obtained by Belkacemi *et al.*(1998), where they obtained productivity of 0.19 and 0.13 g/l.h after 24 hours and 48 hours of cultivation, respectively.

Although, the high ethanol yield (78.3% of the theoretical), the amount of residual sugars was found relatively high (0.8%) compared with the initial sugar concentration. This could be attributed to incomplete assimilation of reducing sugars, since yeasts are known to be unable to convert pentoses,

e.g., xylose, resulted from acid hydrolysis of hemicelluloses into ethanol (Belkacemi *et al.*, 1998).

Finally, the supernatant resulted from pretreatment of barley straw is more suitable for producing alcohol than producing biomass. Previous studies confirmed this finding as some of toxic substances, as furfural and HMF, are taken up by yeast cells during ethanol fermentation (Chung and Lee, 1985;Taherzadeh *et al.*, 2000). Besides the enzymes involved in cell growth are affected by the presence of some inhibitors while those involved in the production of alcohol are not (Safi *et al.*, 1986).

REFERENCES

A.O.A.C. (1990). Association of Official Analytical Chemists, Official Methods of Analysis. Fifteenth Edition Published by the Association of Official Analytical Chemists INC. 2200 Wislon Boulevard, Suite 400. Arlington, Virginia, 22201.

Amin, G. A (1978). Further Studies on Bakers` Yeast .M. Sc. Thesis. Fac. Agric., Cairo Univ.

Attia, P M.; Mahrous, T.S.; Aliind, F.A. and Nasr, A.A. (1988). Biochemical conversion of some agricultural wastes by cellulases isolated from some microorganisms (Part I). J. Agric, Sci. Mansoura Univ., 13 (4): 1877-1885

Azhar, A.F.; Bery, M.K.; Colcord, A.R. and Roberts, R.S. (1982). Development of a yeast strain for the efficient ethanol fermentation of wood acid hydrolysate. Dev. Ind. Microbiol., 23:351-360.

Belkacemi, K.; Turcotte, G.; Halleux D.; Savoie, L.; De-Hall-Eux, D. Finkelstin, M. and Darison, B.H. (1998). Ethanol production from AFEX-treated forages and agricultural residues .Enzy. Engin. and Biotech., 70 (72): 441 – 462.

Berry, D. R. (1989). Growth of yeast. In: Fermentation Process Development of Industrial Organisms. Justino, O. Neway (Ed). Marcel Dekker, INC. New York. pp, 277-301.

Caneque, V.; Velasco, S.; Sancha, J.L.; Manzanares, C. and Souza, O. (1998). Effect of moisture and temperature on the degradation of fiber and on nitrogen fractions in barley straw treated with urea. Anim. Feed Sci. and Technol., 74:241-258.

Chung, I. S. and Lee, Y.Y. (1985). Ethanol fermentation of crude acid hydrolyzate of cellulose using high-level yeast inocula. Biotechnol. and Bioeng., 27:308-315.

Clark,T.A. and Mackie, K.L. (1984). Fermentation inhibitors in wood hydrolysates derived from the softwood *Pinus radiate*. J. Chem. Tech. Biotechnol., 34B: 101-110

Cotteni, A.: Verloo, M.: Kiekens, L.; Velghe, G. and Camerlynck, R. (1982). Chemical analysis of plants and soils. Institute of Analytical Biochemistry, Gent Univ., Belgium.

Dekker, R. F. (1983).Biocoversion of hemicellulose; Aspects of hemicellulase production by *T. reesei*; QM9414 and enzymatic Saccharification of hemicetlulose. Biotechnol. and Bioeng., 25: 1 127-1146.

Durand, J. S.; ph Beaumatin, R.Z. and Hannequart, G. (1992). Effects of sulphate supplementation of untreated and alkali-treated wheat straws on ruminal fermentation and microbial protein synthesis in a semi-continuous fermentor. Anim. Feed. Sci. Technol., 36:287-301.

El-Nawawy, A.S. 1969. Utilization of bagasse pith for the production of fodder yeast by a new isolate of *C. pelliculosa.* Antonie van Leeuwenhoek J. Microbiol., 35: 46.

Fan, L. T.; Lee Y. H. and Gharpuray, M. M. (1982).The nature of lignocellulosics and their pretreatments for enzymatic Hydrolysis. Adv. Biochem. Eng. Biotechnol., 23: 158-183.

Ferrari, M. D.; Dianco, R.; Froche, C. and Loberena, M.L.(2001). Baker's yeast production from molasses /cheese whey mixtures. Biotechnol. Letters, 23(1):1-4.

Fredriksson, J. (2001). Probing control of glucose feeding in cultivation of *Saccharomyces cerevisiae*. M.Sc. Thesis, Lund Institute of Technology, Lund Univ., Sweeden.

Givens, D. I.; Clark, P.; Jacklin, D.; Moss, A.R. and Savery, C.R. (1993). Nutritional aspects of cereals, cereals grain by-products and cereal straw for ruminants. Home-Grown Cereals Authority Research Review No. 24. London, Uk.

Goel, S.C. and Ramachandran, K.B.(1983). Comparison of the rates of enzymatic hydrolysis of pretreated rice straw and baggase with celluloses. Enzyme Microb. Technol., 5: 281 – 284.

Goering, H.K. and Van Soest, P.J. (1970). Forage fiber analysis. Agriculture Handbook, 379, US Dept. Agric., Washington, DC., pp.387-598.

Gregg, D. and Saddler, J.N. (1995). Bioconversion of lignocellulosic residue to ethanol: Process flow sheet development.Biomass and Bioenergy, 9: 287-302.

Gregg, D.J. and Saddler, J.N. (1996). Review: Factor affecting cellulose hydrolysis and the potential of enzyme recycle to enhance the efficiency of an integrated wood to ethanol process. Biotechnol. Bioeng., 51: 375-381

Hay, J.D. (1993): Novel yeast products from fermentation processes. J. Chem. Technol., 58: 203-205.

Hospodka, J. (1966). Oxygen absorption rate-controlled feeding of substrate into aerobic microbial cultures. Biotechnol. Bioeng., 8:117.

Katzen, R. and Monceaux, D.A. (1995). Development of bioconversion of cellulosic wastes. Appl. Biochem. Biotechnol., 51/52: 585-592.

Kaullas, D.P.; Christakopoulos, P.; Kekos, D.; Macris, B.J. and Koukios, E.G. (1991). Correlating the effect of pretreatment on the enzymatic hydrolysis of straw. Biotechnol. and Bioengin., 39:113-116.

Kostitsina, V. P. and Mysyakova, E.A. (1968).Improvement in production technology. Gidroliz. Lesokhim. Prom. 21:22. Cited from Reed and Reppler (1973).

Larsson, S.; Reimann, A.; Nilvebrant, N.O. and Jönsson, L.J. (1999). Comparison of different methods for the detoxification of lignocellulose hydrolyzates of spruce., Appl. Biochem. Biotechnol., cited from Taherzadeh, (1999).

Lee, K. C. P.; Bulls, M.; Holmes, J. and Barrier, J.W. (1997). Hybrid process for the conversion of lignocellulosic materials. Appl. Biochem. Biotechnol., 66: 1-23

Mathison, G.W.; Soofi-Siawash, R.; Okine, E.K., Helm, J. and Juskiw, P. (1999). Factors influencing composition and ruminal degradability of barley straw. Can. J. Anim. Sci., 79:343-351.

Miller, G.L (1959). Use of D N S acid reagent for the determination of reducing sugar. Anal. chem., 31:426-428.

Mostafa, Enas M. (2000). Optimum production of Bakers' yeast. M. Sc. Thesis Fac. of Engineering, Cairo Univ.

Nemirovskii, V. D. and Kostenko, V.G. (1991). Transformation of yeast growth inhibitors Which occurs during biochemical processing of wood hydrolysates. Gidroliz Lesokhim Prom-st 1:16-17.

Niitsu, T. Ito, M. M. and Inoue, H. (1992). Analysis of the formose reaction system. J. Chem. Eng. Japan, 25: 480-485

Palmqvist, E. (1998). Fermentation of lignocellulosic hydrolysates: inhibition and detoxification. Ph.D. Thesis Lund Univ., Sweden

Plevako, E. A. and Bakoshinskaya, O.A. (1964). Microbiology, Chemistry and Technology Control of Yeast Production. Food Industry, Moscow. Cited from Amin (1978).

Qureshi, N. and Manderson, G.J. (1995). Bioconversion of renewable resources into ethanol: an economic evaluation of selected hydrolysis, fermentation, and membrane technologies. Energy Sources, 17: 241-265.

Reed ,G. and Nagodawithana, T.W.(1991).Yeast derived products. In: Yeast Technology ,PP.369-412. AVI Nostr and Reinhold, New York.

Reed, G. and Reppler, J.H. (1973). Yeast Technology. The AVI Publishing Company, INC. Westport, Connecticut.

Ringbom, K.;Rothberg, A. and Saxen, B. (1996). Model based automation of baker's yeast production . J. Biotechnol. , 51(1):73-82.

Rosen, K. (1987). Production of Bakers' yeast. In: Yeast Biotechnology D. R. Berry; I. Russell, and G. G. Stewart (Eds.). George Allen & Unwin, London, pp. 471-500.

Ryohei, U.; Naoko, H. and Naoto, U. (2003). Fermentation of molasses by several yeasts from hot spring drain and phylogeny of the unique isolate producing ethanol at 55 °C. J. of Tokyo Univ. of Fisheries, 90:23-30.

Safi, B. F.; Rouleau, D. and Mayer, R.C. (1986). Fermentation kinetics of spent sulfite liquor by *Saccharomyces cerevisiae.* Biotech. and Bioengin. 28:944-951.

Schmitt, R.C. and Murphy, V.G. (1980). Autohydrolysis process system for utilizing geothermal fluids in converting lignocellulose biomass to alcohol fuels. Trans. – Geotherm. Resour. Counc., 4(Geotherm.: Energy Eighties): 609-612

Sinitsyn, A.P.; Gusakov, A. V. and Valsenko, E.Y. (1991). Appl. Biochem. Biotechnol., 30-59.

Sjostrom, E. (1993). Wood Chemistry: Fundamentals and Applications, 2nd Ed. Academic Press. San Diego.

Smith, F.; Gilles, M.A.; Haithon, J.K. and Goees, P. A. (1956). Colorimetric method for determination sugar related substances. Annl. Chem., 28:350-356.

Stewart, D.; Wilson, H.M.; Hendra, P.J. and Morrison, I. M. (1995). Furrier-transform infrared and raman spectroscopic study of biochemical treatment of Oak wood (*Quercus rubra*) and barley straw (Hordeum vulgare)straw. J. Agric. Food Chem., 43:2219-2225.

Stritzler, N.P.;Wolstrup, J. ; Eggum, B.O. and Jensen, B. B. (1992). Factors affecting degradation of barley straw in sacco and microbial activity in the rumen of cows fed fiber-rich diet. Anim. Feed Sci. and Technol., 38: 263-280

Tahalasso,F.; Burgt, J. ;O`Flaherty, V. and Colleran, E. (1999). Large scale anaerobic degradation of betaine. J. of Chemical Technol. and Biotechnol., 74(12): 1176-1182.

Taherzadeh, M.J. (1999). Ethanol from Lignocellulose: Physiological Effects of Inhibitors and Fermentation Strategies. Ph.D. Thesis Chalmers Univ. Technol., Sweden.

Taherzadeh, M.J.; Gustafssonn, L.; Niklasson, C. and Liden, G. (2000). Physiological effects of 5-hydroxymethylfurfural on Saccharomyces cerevisiae. Appl. Micorbiol. Biotechnol. 53:701-708.

Taherzadeh, M. .J.; Niklasson, C. and Lidén, G. (1997). Acetic acid - friend or foe in anaerobic batch conversion of glucose to ethanol by *Saccharomyces cerevisiae* ?. Chem. Eng. Sci., 52(15): 2653-2659

Trinder, P. (1969). Colorimetric determination of glucose by enzymes. Ann. Clin. Biochem. 6: 24.

Walch E.; Zemann A.; Schinner F.; Bonn G. and Bobleter O. (1992): Enzymatic saccharification of hemicellulose obtained from hydrothermally pretreated sugar cane bagasse and beech bark. Bioresource Technol., 39: 173-177

Walker ,G.M.(1998).Yeast Physiology and Biotechnology. Chapter, 6, pp. 301-320.Chichester New York-Weinham –Brisbane – Singapore-Toronto.

White, J. (1954). Yeast Technology. Chapman and Hall, London.

Wiemken, A.; Matilles, P. and Moor, H. (1969). Vacuolar dynamics in synchronously budding yeast. Arch. Microbiol., 70:89-97.

Wright, J. D. (1988). Ethanol from biomass by enzymatic hydrolysis. Chem. Eng. Prog., 84: 62-74

Yuan, J.Q.; Bellgardet, K.H.; Jiang, W. S. and Deckwer, W. D. (1993). Modification and verification of the dynamic cell cycling model for baker's yeast. Bioprocess Enquiring, 9:173-182.

Zaaky, Hala (2002).Cellulose Production From Fome Fgricultural Fastes. Ph.D. Thesis, Fac. Of Agric., Cairo Univ.

Zahra, M.K.; Veselov, I. Ya.; Gadjieva, L.A and Uvarov, I. P. (1974). Synthesis and use of acetaldehyde upon aerobic cultivation of bakers` yeast. Prikl. Biokhim. Mikrobiol. 1: 24.

التحويل الحيوى لقش الشعير إلى خميرة خباز وكحول

الفت سيد بركات*، سمير عبد الواحد الجيزاوى* ،الباشير محمد أحمـــد عرعـــود و معوض كامل زهرة***

* قسم الميكروبيولوجيا الزراعية، كلية الزراعة ، جامعة القاهرة

** قسم علوم الاغذية ، كلية العلوم التقنية و الهندسية ،جامعة سبها ، ليبيا.**

فى هذه الدراسة تم اختيار قش الشعير كمادة خام لإنتاج خميرة الخباز أو الكحول . لذلك أجريت بعض المعاملات الكيماوية لتحويل الألياف السيليلوزية إلي سكريات بسيطة (الجلوكوز كمكون أساسي) حتى يمكن تنمية الخميرة على ناتج هذا التحلل. وقد تم تقدير التركيب الكيميائي لقش الشعير حيث وجد أن نسبة السيليلوز و الهيميسيليلوز فيه تصل إلى ٤٣ و ٢٣,٩٩ % على التوالي كوزن جاف. وقد تم أجراء التحليل الكيماوي للقش على مرحلتين : معاملة أولية للتخلص من اللجنين والهيميسيليلوز وتسهيل تحليل السيليلوز في المرحلة التالية. وتمت هذه العملية باستخدام تركيزات مختلفة من محلول حامض الكبريتيك أو محلول الصودا الكاوية على درجة حرارة ١٢١°م لمدة ساعة مع تركيزات مختلفة من القش. ولقد وجد إن أفضل معاملة أعطت أعلى كمية من السكريات الكلية (١,٦٦ %) هي باستخدام حامض كبريتيك٤% مع ٦ جرام من القش. ولتحليل السيليلوز المتبقي بعد المعاملة الأولية إلي سكريات بسيطة تم استخدام حامض الكبريتيك أو الصودا الكاوية بتركيزات مختلفة على درجات حرارة مختلفة ولفترات زمنية مختلفة أيضا. وقد وجد أن أفضل نسبة سكريات مختزلة تم الحصول عليها من المعاملات الكيميائية هي ٢١,٣ جرام / لتر باستخدام ٢ % حامض كبريتيك علي حرارة ١٣٦ °م لمدة ساعة.

المرحلة الثانية وتم أستخدام المحلول السكرى الناتج من التحليل الكيماوي للقش في إنتاج خميرة الخباز (*Sacch. cerevisiae 2034*) بطريقة المزرعة علي دفعات لمدة ٩ ساعات. كما تم استخدام ثلاث بيئات مختلفة هي بيئة معدنية تحتوي علي الجلوكوز كمصدر للكربون وبيئة المولاس وبيئة ناتج تحلل السيليلوز من قش الشعير (ناتج التحلل باستخدام الحامض أو الراشح الناتج بعد المعاملة الأولية). وكانت أعلى كفاءة للإنتاج تم الحصول عليها في بيئة الجلوكوز هي ٢٠٩,٢ %. بينما عند استخدام المولاس فكانت ١٠٥,١ % . وعند استخدام بيئة السكريات المحتوية على ناتج تحلل القش بالحامض فكانت كفاءة الإنتاج في نهاية عملية التخمر ٨٠ % . وعند استخدام الراشح الناتج من المعاملة الأولية بالحامض كانت كفاءة الإنتاج منخفضة جدا (٣٢,٦ %) ولذلك تم توجيهه لإنتاج الكحول باستخدام خميرة الخباز من السلالة *Sacch. cerevisiae* O-14. وكانت أعلى نسبة كحول تم الحصول عليها ١٨ جرام/لتر بعد ٢٤ ساعة من التحضين باستخدام ١٠ % لقاح. وكان من الضروري قبل استخدام الراشح الناتج من المعاملة الأولية بالحامض أو ناتج التحلل باستخدام الحامض فى تنمية الخميرة من أجراء عملية لنزع السمية (Detoxification) ولقد استخدم لهذا الغرض أربعة أنواع من المركبات القلوية ووجد أن أفضلها هو هيدروكسيد الكالسيوم .