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PRODUCTION OF ETHANOL FROM AGRO-INDUSTRIAL WASTES:
II - OPTIMIZING VARIABLES FOR ETHANOL PRODUCTION FROM RICE STRAW AND BEET PULP HYDROLYZATES.

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ABSTRACT

Due to increasing commercial significance of ethanol production from lignocellulosic biomass, this study was conducted in an attempt to evaluate the process for ethanol production from the rice straw (RS) and sugar beet pulp (SBP) wastes as a cheap substitute of molasses using *Saccharomyces cerevisiae* thereby, reduce the cost of ethanol production, at the same time eliminates environmental impact of waste via open burning. This investigation has been carried out to study the influences of substrate concentration, pH, nitrogen source and incubation period on ethanol fermentation from (RS) and (SBP) hydrolzates. The results revealed that, the optimum conditions for ethanol production were attained at cultivation conditions being: 100g// (w/v) sugar concentration; ammonium sulfate as nitrogen source, 5.0 pH and 48 hr incubation period at 30 ˚C. At previous fermentation conditions, ethanol yield was 44.42 g// and 42.72 g// with 88.24 and 88.09 **(%)** percentages of the theoretical yield for (RS-H) and (BP-H), respectively. And In this work, a good yield of ethanol was observed after 48 hr of fermentation using rice straw hydrolyzate medium, which shows a satisfactory performance and a potential for lowering ethanol production costs.

Keywords: Ethanol; rice straw; beet pulp, fermentation.

INTRODUCTION

Ethanol is viewed as a potential fuel that is available from biomass and hence new methods to generate ethanol from hitherto inaccessible sources are gaining importance. Currently, ethanol is now produced in Egypt by fermentation of cane and beet molasses. The rapid development in sugar industry production caused a decrease in the molasses amount derived from the process, and because of an increase in demand for ethanol as a biofuel, there was an urgent need for using agro-industrial wastes as an alternative substrate for ethanol production. It achieves many targets such as: elimination the environmental pollution and hazards; securing an economic, cheap source of raw materials due to business development and economic growth; strategic provides an alternative way to replace the refined and costly raw materials (i.e. molasses). Field burning is the major practice for removing agricultural residues, but it increases the air pollution and consequently affects the public health, (Toğrul and Arslan, 2003).

Biotechnology for efficient utilization of lignocelluloses wastes as fuels relies on the utilization of both the cellulosic as well as hemicellulosic portions of the biomass. A low conversion of the fermentable sugars obtained from the lignocellulosic biomass is the critical stage to develop an economically feasible process of ethanol production, since it is well-known that attaining high ethanol concentrations are needed in order to make the

distillation step feasible from an economic point of view (Slverstein *et al*., 2007). Then, further studies are needed to enhance the production of ethanol, for instance, the study of enzymatic hydrolysis of the pretreated solid in order to break the cellulose polymer into more glucose molecules. It is important to take into account the energy consumption associated with the fermentation conditions and the quantity of ethanol that can be obtained from fermentation. Therefore, to fully utilize agricultural wastes such as (rice straw and beet pulp) as a feedstock for ethanol production (Georgieva and Ahring, 2007 and Diep *et al.,* 2012).

Pretreatment, as the first step towards conversion of lignocellulose to ethanol, makes up one-third of the total production costs and remains one of the main barriers preventing commercial success (Shia *et al*. 2009 and Vu˘curovi´c and Razmovski*,* 2012). Bioethanol is regarded as one of the most promising biofuels from renewable sources. Production of bioethanol is increasing every year because of its use as a biofuel and in medicine, cosmetics, and industrial materials. With an increasing oil prices and global environmental concerns, bioethanol production has become a focus of great attention (Mussatto *et al*., 2010 and Lin *et al.* 2012). Taking into account overall economics and energy consumption sugar crops are advantageous raw material for ethanol fermentation in comparison to lignocelluloses and starch crops, because they generally have a high content of readily fermentable sugars. Sugar beet pulp (SBP) is the fibrous by-product left after the extraction of free sugar from commercially grown sugar beets. On a dry weight basis, SBP contains 75–80% polysaccharides, consisting of 22–30% cellulose microfibrils, which, have strong potential for a number of applications (Vu˘curovi´c and Razmovski, 2012). Rice straw is considered to account for the largest portion of available biomass feedstock in the world (e.g., 7.31×10^{14} of dry rice straw per year) (Ko *et al.,* 2009). Therefore, rice straw and sugar beet pulp show promise for use as a feedstock for the production of fermentable sugars. The most commonly used yeast in bioethanol production, *Saccharomyces cerevisiae*, which can ferment glucose to ethanol rapidly and efficiently but it is not natively capable of utilizing xylose which is second most abundant sugar in the lignocellulosic biomass (Alkasrawi *et al,.* 2013 and Karagöz and Özkan, 2014).

The purpose of this research was to obtain high ethanol production with high productivity by using rice straw (RS-H) and sugar beet pulp (SBP-H) hydrolyzates. The effect of initial sugar concentration, pH value, nitrogen source and fermentation period on the production of ethanol by *Saccharomyces cerevisiae* was optimized to improve the ethanol fermentation performance, reduce the cost of ethanol production instead of molasses as a conventional carbon source and decrease the environmental hazards.

MATERIALS AND METHODS

Materials

Rice straw and beet pulp:

Rice straw was obtained from Sakha Research Station, Agriculture Research Center, Sakha, Kafr El-Sheikh, Egypt, during the summer season

of 2012. While sugar beet pulp was obtained from Delta Sugar Company located in Elhamol city Kafr El-Sheikh governort, Egypt.

Organism:

Saccharomyces cerevisiae was isolated from commercial baker's yeast, which is produced by Starch and Yeast Company, Alexandria.

All the required analysis were performed in the Food Technology Department, Faculty of Agriculture, Kafrelshiekh University.

Methods:

Dilute acid pretreatment of rice straw and sugar beet pulp

The whole waste was dried at 80˚C to a constant weight, then milled in kitchen blender to give powder (80 mesh), which was used for further investigation. Acid pretreatment was carried out according to Ammar and Elsanat, (2014) as follow: The milled rice straw and beet pulp were soaked in 4% (w/v) H_2SO_4 (Solid: liquid ratio 1:20 w/v) for 36 h, then autoclaved at 121°C for 90 min. to form a slurry. This slurry was homogenized for 5 min to ensure that the solids were uniformly dispersed. The filtered liquid hydrolyzate fraction was neutralized with $CaCO_3$ to reduce the acetic acid concentration, then vacuum evaporation to increase the sugar concentration and decreased of furfural concentration. Finally, adsorption of the hydrolyzates on activated charcoal to reduce the inhibitors concentration and remove of lignin. The clear liquid fraction of the rice straw (RS-H) and sugar beet pulp (SBP-H) hydrolyzates was used for ethanol production.

Inoculum preparation:

Saccharomyces cerevisiae isolate was grown in 250 ml Erlenmeyer flasks containing 100 ml sterilized medium (glucose 20 g/*l*, peptone 10 g/l, yeast extract 10 g/l; pH 4.5). It was cultured at 30 °C for 24 h. in a rotary incubator (120 rpm). The content was transferred into 2 L Erlenmeyer flask, containing 1 L of the same medium and cultured for another 24 h at 30 °C. Yeast cells were separated by centrifugation at 3000 rpm for 10 min and suspended in sterilized 0.9% NaCl. From this suspension, an adequate volume was taken to attain the desired inoculum final concentration in experimental media.

Fermentation media and conditions:

Rice straw (RS) and sugar beet pulp (SBP) were used as fermentation medium. To study the effect of sugar concentration the hydrolyzates were used after the dilution with distilled water to give a total sugar concentration of 100 and 120 g/*l*, respectively and pH was adjusted to 5.0 pH by addition of 10% H_2SO_4 (v/v). The optimum pH for the fermentation was adjusted to different pH values (5.0, 5.5 and 6.0). The media pH was adjusted to 5.0, when the effect of nitrogen source (0.15% ammonium sulfate, 0.05% urea and 0.1% rice bran) (urea solution was sterilized by filtration) was subjected to study, and urea was replaced with various of nitrogen sources. The media were sterilized by autoclaving at 121˚C for 20 min. All experiments were carried out in 125 ml Erlenmeyer flasks containing 50 ml of the fermentation medium. The flasks were inoculated with *S. cerevisiae* to a cell concentration of 10 g/*l* at 30˚C with shaking (100 rpm) for ethanol production. Samples were removed at 24, 48 and 72 h intervals and analyzed for ethanol, residual sugar, cell biomass concentrations and pH as were monitored with respect to

time. All the experiments were conducted in triplicate and the data reported are the average of three replications.

Evaluation of fermentation parameters

Sugar utilization (SU%) was calculated as the ratio of utilized sugar to the initial and multiplying by 100. The ethanol yield (Yp/s, g/g) was calculated as grams of ethanol produced per gram of utilized sugar. Also a percentage of the maximal theoretical ethanol yield (Ep/s, %) was calculated based on 51% of the total fermentable carbohydrates. The volumetric ethanol productivity (Qp, g/l h) were calculated as grams of ethanol produced per liter per hour (Vu˘curovi´c and Razmovski, 2012).

Analytical methods:

Samples of fermented liquids were analyzed for ethanol, sugar and yeast biomass. The samples were centrifuged at 3000 rpm for 15 min. The sample of supernatant was hydrolyzed in 33% HCl at 100 °C for 10 min and neutralized with NaOH solution and sugars were than determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Ethanol was determined by using the spectrophotometric dichromate reaction method of Caputi *et al.* (1968). Cell dry weight was determined according to the method of Rocha and Olsson, (2003). The free cell concentration was determined by optical density (OD) measurement of the samples using Spectrophotometer (Jenway 6100) at 620 nm, using the standard calibration curve of OD versus dry cell weight.

Statistical analysis:

Data were analyzed according to Steel and Torrie, (1980). A one way analysis of variance for main (ANOVA) using the general linear models (GLM) procedure was used to test for main effects where more than two variables being compared. Differences with P values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The pretreatment of lignocellulosic material results in the release of sugars from the hydrolysis and dissolution of cellulose and hemicellulose. Therefore, the fermentation of the hydrolyzate obtained from acid hydrolysis was conducted to evaluate the ferment ability of cellulose-derived sugars aiming the production of ethanol. The rice straw (RS-H) and beet pulp (BP-H) hydrolyzates obtained under the optimal pretreatment conditions of (soaked at 4% (w/v) H_2SO_4 at Solid: liquid ratio 1:20 (W/V) and then autoclaved for 90 min at 121°C) were fermented by *S. cerevisiae* for the production of ethanol.

Influence of sugar concentrations on bio-ethanol production

Improved ethanol fermentation activity can be achieved by controlling various parameters. The batch experiment was performed with two initial sugar concentrations (100 and 120 g*/l*) tested at 30°C to develop ethanol production. The experimental conditions and the results summarized in Table (1) show the changes in ethanol concentrations, the specific ethanol production rate and ethanol conversion rate at different initial sugar concentrations after 24 hour's incubation. The data presented in Table (1)

show that the production of ethanol was affected by the substrate concentration where higher substrate concentrations may achieve higher ethanol production at a temperature of 30°C. Ethanol concentrations obtained were 27.05, 31.66g// and 26.23, 30.60 g// for rice straw and beet pulp hydrolysats for initial sugar concentration of 100 and 120 g//, respectively.

Results show that a higher substrate concentration may prevent the ethanol fermentation process occurring. One of the reasons may be the accumulation of high concentrations of ethanol and by-products which make the pH change. Moreover, higher initial sugar concentration may have actually decreased the ethanol conversion efficiency, since the higher substrate and production concentrations may have inhibited the process of ethanol fermentation. The data of the same table illustrates that higher initial sugar concentration may decrease the ethanol conversion efficiency. The maximum sugar conversion after 24 hour's incubation was observed at 79.55, 79.95%, and 79.49, 79.71% for 100 and 120 g// of initial sugar for (RS-H) and (SBP-H), respectively. More substrate did not improve the specific ethanol production rate. The growth of yeast was not increased at a significant rate with the increase of sugar concentrations. The decline of biomass production at higher sugar concentration is due to that *S. cerevisiae* is crabtree positive yeast, meaning that fermentative growth can happen even at aerobic conditions. This phenomenon gives a lowered biomass yield as well as an increase the ethanol production (Fiechter and Seghezzi, 1992 and Lin *et al.* 2012).

Table (1): Effect of sugar concentration on the fermentation of ethanol using rice straw (RS) and beet pulp (BP) hydrolyzates.

Fermentation parameter	Sugar concentration (g/L)			
	Rice straw hydrolyzate[A]		Beet pulp hudrolysate[B]	
Initial reducing sugar concentration (S_0) (g//)	100	120	100	120
Initial cell concentration (X) (g//)	10.00			
Final sugar (g//)	20.45[b]	24.24[a]	20.49[b]	24.35[a]
Sugar utilization, Su (%)	79.55[b]	79.95[a]	79.49[b]	79.71[ab]
Ethanol produced (g//)	27.05[b]	31.66[a]	26.23[c]	30.60[a]
Ethanol productivity, Qp (g/l h)	1.12[c]	1.32[a]	1.09[c]	1.27[b]
Ethanol yield coefficient, Yp/s (g/g)	0.34[a]	0.33[b]	0.33[b]	0.32[c]
Percentage of the theoretical yield, Ep/s (%)	66.66[a]	64.70[b]	64.70[b]	62.74[c]
Cell biomass (g//)	12.75[c]	13.10[a]	12.62[d]	13.04[b]
pH value	4.95[a]	4.90[b]	4.94[a]	4.96[a]

Fermentation conditions: Volume medium/volume system ratio (v/v) =50/125, shaker speed 100 rpm, nitrogen source: 0.15% urea (w/v), 30˚C, pH_O = 5.0, fermentation time = 24h.

A and B: comparison of means of ethanol by waste type.

a and b: comparison of means of ethanol by sugar concentration.

Means within a column not sharing superscript are significantly different (P <0.01, Tukey test) (N=3).

Theoretical value is considered as 0.51g ethanol/g sugar consumed (efficiency, %) as described by Kim and Lee (2005).

Influence of pH on bio-ethanol production

In addition to temperature and substrate concentration, pH is also a key factor that affects ethanol fermentation Kasemets and Nisamedtinov (2007). pH plays a vital role in the activities of various chemical and biological reactions. As almost all enzymes show a maximum activity with a specific pH ranges, in this study changes in ethanol were investigated to estimate the activity of the ethanol production ability with changes in pH. The system was evaluated for bio-ethanol production at different pH values (5.0 – 6.0). Table (2) shows the results of the batch fermentation used to investigate the effect of pH on ethanol production. When the pH value was 5.0, the quantity of ethanol produced substantially decreased. Therefore a pH 5.0 may be regarded as the operational limit for the anaerobic ethanol production process. Although there was some ethanol produced, the ethanol fermentation yield was still reduced by the acetic acid production. The highest ethanol yield coefficient was 0.36 g/g, with an ethanol conversion efficiency of 70.59%. In addition, the ethanol concentration did not decrease after the nutrient was consumed. This may indicate that the ethanol could not be utilized as the carbon source under anaerobic condition.

Table (2): Effect of pH value on the production of ethanol using rice straw (RS) and beet pulp (BP) hydrolyzates.

Fermentation parameter	pH value					
	Rice straw hydrolyzate[A]			Beet pulp hudrolysate[B]		
	5.0	5.5	6.0	5.0	5.5	6.0
Initial sugar concentration (S_0) (g/*l*)	100					
Initial cell concentration (X) (g/*l*)	10					
Final sugar (g/*l*)	20.45[a]	18.05[d]	18.45[b]	20.49[a]	18.17[c]	18.25[b]
Sugar utilization, Su (%)	79.55[c]	81.95[a]	81.55[b]	79.49[c]	81.83[a]	81.75[a]
Ethanol produced (g/*l*)	27.05[c]	29.50[a]	28.54[b]	26.23[d]	28.64[b]	27.95[c]
Ethanol productivity, Qp (g/l h)	1.12[d]	1.23[a]	1.19[b]	1.09[e]	1.19[b]	1.16[c]
Ethanol yield coefficient, Yp/s (g/g)	0.34[c]	0.36[a]	0.35[b]	0.33[d]	0.35[b]	0.34[c]
Percentage of the theoretical yield, Ep/s (%)	66.66[c]	70.59[a]	68.63[b]	64.70[d]	68.62[b]	66.66[c]
Cell biomass (g/*l*)	12.75[d]	13.12[a]	13.05[b]	12.62[e]	13.01[b]	12.95[c]
pH value	4.45[d]	4.95[b]	5.25[a]	4.44[d]	4.83[c]	5.28[a]

Fermentation conditions: Sugar conc. 100 g/*l*, volume medium/volume system ratio(v/v) =50/125, shaker speed 100 rpm, nitrogen source: 0.15% urea w/v, 30˚C, fermentation time = 24h.

A and B: comparison of means of ethanol by waste type.

a, b, c, d and e: comparison of means of ethanol by pH value.

Means within a column not sharing superscript are significantly different (P <0.01, Tukey test) (N=3).

Theoretical value is considered as 0.51g ethanol/g sugar consumed (efficiency, %) as described by Kim and Lee (2005).

Also, Table (2) shows competition for the substrate, glucose, by the microorganisms, and may suggest a change in the main fermentation pathway at various pH ranges. The results show that at 30 °C with the initial sugar concentration of 100g/*l*, with the pH value higher than 5.0, much sugar was consumed and converted to ethanol, so conversion efficiency was greatly increased. So if the pH was set at a suitable value, the efficiency

might be somewhat increased. The maximum specific ethanol production rates were observed at pH 5.5, and the ethanol fermentation yields were 70.59% and 68.62% of the maximum theoretical value for (RS-H) and (SBP-H), respectively. The changes in the operational pH in the ethanol production process may have induced a change in the main fermentation pathway. Beyond this value, the formation of by-products, such as acetic acid and butyric acid may have consumed some of the substrate and reduced the efficiency of ethanol fermentation.

The activities of the yeast glycolytic and fermentation enzymes were highly specific for a defined pH range, and their activities were inhibited at both acidic and basic pH. Indeed, significant quantities of bio-ethanol were only produced at pH 5.5 (29.50 g/*l*) and 6.0 (28.54 g/*l*), findings that are supported by the results of Khattak *et al.*, (2014). Ethanol production recorded the highest value at pH 5.5 compared with the lowest value pH 5.0 of (RS-H) and (SBP-H). This behavior can be related to consumption of acetic acid, present in the hydrolyzate, by the yeast which may also have contributed to raise the final pH value in the medium. These results are in accordance with those reported by Asli, (2010) and Lin *et al.,* (2012).

Influence of nitrogen source on bio-ethanol production

Nitrogen source is considered as the second contributor of the medium which affects the yeast growth, thereby investigation of cheap renewable nitrogenous materials like food-processing by-products was considered (Torija *et al*., 2003) Various organic and inorganic nitrogen sources were investigated for their stimulating yeast growth. The results in Table (3) reveal that the yeast could utilize all tested nitrogen sources with variable favouration.

Table (3): Effect of nitrogen source on the production of ethanol using rice straw (RS) and beet pulp (BP) hydrolyzates.

Fermentation parameter	Nitrogen source					
	Rice straw hydrolyzate[A]			Beet pulp hudrolysate[B]		
	AS[a]	UR[c]	RB[b]	AS[a]	UR[c]	RB[b]
Initial sugar concentration (S_0) (g/*l*)	100.00					
Initial cell concentration (X) (g/*l*)	10.00					
Final sugar (g/*l*)	12.50[e]	18.05[b]	15.42[c]	13.06[d]	18.17[a]	16.03[b]
Sugar utilization, Su (%)	87.50[a]	81.95[d]	84.05[c]	86.94[b]	81.83[d]	83.97[c]
Ethanol produced (g/*l*)	33.25[a]	29.50[e]	31.10[c]	32.17[b]	28.64[e]	30.22[d]
Ethanol productivity, Qp (g/l h)	1.39[a]	1.23[d]	1.30[c]	1.34[b]	1.19[e]	1.25[d]
Ethanol yield coefficient, Yp/s (g/g)	0.38[a]	0.36[c]	0.37[b]	0.37[b]	0.35[d]	0.36[c]
Percentage of the theoretical yield, Ep/s (%)	74.50[a]	70.59[c]	72.54[b]	72.54[b]	68.62[d]	70.59[c]
Cell biomass (g/*l*)	15.43[a]	13.12[c]	14.28[b]	15.34[a]	13.01[c]	14.21[b]
pH value	5.05[c]	5.12[a]	5.08[b]	5.07[b]	5.13[a]	5.09[b]

AS: Ammonium sulfate, UR: Urea and RB: Rice bran.

Fermentation conditions: Sugar conc. 100 g/*l*, volume medium/volume system ratio (v/v) =50/125, shaker speed 100 rpm, 30˚C, pH_o = 5.0, fermentation time = 24h.

A and B: comparison of means of ethanol by waste type.

a, b, c, d and e: comparison of means of ethanol by nitrogen source.

Means within a column not sharing superscript are significantly different (P <0.01, Tukey test) (N=3).

Theoretical value is considered as 0.51g ethanol/g sugar consumed (efficiency, %) as described by Kim and Lee (2005).

In the medium containing $(NH_4)_2SO_4$ as a nitrogen source, the ethanol concentration was 33.25 g/*l*, with an ethanol yield of 0.38 g ethanol/g sugar and a productivity of 1.39 g/*l*.h. It can be observed from these results that the supplementation of ammonium sulfate was found to be the best nitrogen source in the production of ethanol. It is usually the case that the addition of medium components to the hydrolyzate can influence the process kinetics and the process economics, but in this study, the nutrients present in the hydrolyzates were sufficient to allow for cell growth and ethanol production**.** On the other hand rice bran extract was favorite organic nitrogen source. This could be attributed to its content of other nutrients such as minerals, vitamins, amino acids etc. In general, the organic nitrogen source (rice bran extract) induced higher ethanol production as compared with urea. Ammonium sulfate gave most the highest significantly parameters such as ethanol and dry cell biomass (Yu and Zhang, 2003)

Influence of incubation period on the yield of biomass production:

To verify the cultivation period at which the maximum ethanol was produced, the yeast was cultivated under the estimated optimal conditions (sugar conc. 100g/*l*, ammonium sulfate 0.15% and inoculum size 10 g/*l* w/v). Table (4) show the effect of incubation period on ethanol production. Results showed that reducing sugars consumption, ethanol production was positively influenced and increased as a function of time. More than 90% of reducing sugars in medium was consumed after 24 h of fermentation. Ethanol concentration increased accordingly and reached 44.42 and 42.72 g/*l* bio-ethanol at the end of the 48 hour fermentation process using 100 g/*l* sugar in rice straw and beet pulp hydrolzates, respectively. The fermentation process was performed continuously for 72 h; there was a significant increase in bio-ethanol production in the initial 24 h, followed by a slow increase up to 48 h and then a negligible increase to the end of the experiment. The decrease in growth after 48 hr could be attributed to exhaustion of the nutrients and oxygen in the cultivation media and accumulation of metabolism by-products due to cell autolysis **(**Kays and Vanderzant, 1980 and Nancib *el al.,* 1997).

As expected the concentration of sugar decreased during the fermentation, coinciding with an increase in produced ethanol and CO_2. The cells almost completely utilized the present sugar (100 g/*l*) after fermentation in the case of both media (Su over 97% in average) indicating that cells retained a very high metabolic activity. Very high efficiency of *S. cerevisiae* was reported previously (Plessas *et al*., 2007). The values of Su in rice straw and beet pulp hydrolyzates were 97.85 and 97.49% for rice straw and beet pulp hydrolyzates, respectively. The Qp, Yp/s and Ep/s was in accordance with the sugar utilization. The Ep/s maintained almost constant for two hydrolyzats fermentation and ranged from 88.24% and 88.09% for rice straw and beet pulp hydrolyzates, respectively. In the fermentation of rice straw hydrolyzate maximum ethanol concentration of 44.42 g/l, ethanol yield of 0.44 g/g (equal to 88.24% of its theoretical value) was achieved, with almost complete utilization of sugar (97.85%).

Table (4): Effect of incubation period on the production of ethanol using rice straw (RS) and beet pulp (BP) hydrolyzates.

Fermentation parameter	Incubation period (h)					
	Rice straw hydrolyzate[A]			Beet pulp hudrolysate[B]		
	24	47	720	24	48	72
Initial sugar concentration (S_0) (g/*l*)	100					
Initial cell concentration (X) (g/*l*)	10					
Final sugar (g/*l*)	12.50[a]	2.15[c]	0.0[b]	13.06[a]	2.51[b]	0.0[c]
Sugar utilization, Su (%)	87.50[c]	97.85[b]	100[a]	86.94[c]	97.49[b]	100[a]
Ethanol produced (g/*l*)	33.25[d]	44.42[b]	49.80[a]	32.17[e]	42.72[c]	49.75[a]
Ethanol productivity, Qp (g/l h)	1.39[a]	0.93[b]	0.70[c]	1.34[a]	0.89[b]	0.69[c]
Ethanol yield coefficient, Yp/s (g/g)	0.38[c]	0.45[b]	0.46[a]	0.37[c]	0.44[b]	0.45[b]
Percentage of the theoretical yield, Ep/s (%)	74.50[c]	88.24[b]	90.19[a]	72.54[c]	88.09[b]	88.24[b]
Cell biomass (g/*l*)	15.43[b]	19.70[a]	19.65[a]	15.34[b]	19.62[a]	19.24[a]
pH value	5.05[b]	5.12[a]	4.97[c]	5.07[b]	5.15[a]	4.95[c]

Fermentation conditions: Sugar conc. 100 g/*l*, volume medium/volume system ratio (v/v) =50/125, shaker speed 100 rpm, nitrogen source: 0.15% ammonium sulfate w/v, 30˚C and pH_O= 5.0.

A and B: comparison of means of ethanol by waste type.
a , b, c and d: comparison of means of ethanol by incubation period.
Means within a column not sharing superscript are significantly different (P <0.01, Tukey test) (N=3).
Theoretical value is considered as 0.51g ethanol/g sugar consumed (efficiency, %) as described by (Kim and Lee, 2005).

The decrease of beet pulp hydrolyzate fermentation parameters was due to decrease of sugar utilization ability along with the fermentation capacity of yeast cells. It is important to note that the yeast cells in the beet pulp medium gradually changed the color to dark brown along with the repeated fermentation cycles, while it was not the case for rice straw hydrolyzate. It is well known that the *S. cerevisiae* can be used as biosorbent for different color compounds (Aksu and Donmez, 2003 and Nikoli´c *et al*., 2009). The higher initial color concentration in beet pulp than in rice straw presented above enhances the adsorption process. Hence, the decrease in ethanol productivity for beet pulp hydrolyzate fermentation may be caused by accumulation of nonsucrose and color compounds from beet pulp hydrolyzate by yeast cells. The amounts of these compounds depend on the raw material used Vu˘curovi´c and Razmovski, 2012 and Rocha *et al*., 2014).

CONCLUSION

Owing to the fermentation results, by comparing the obtained process parameters for examined media it can be pointed out that rice straw and beet pulp hydrolyzates are a convenient industrial medium for ethanol production, and can be used with some nutrient supplementation. Statistical optimization of conditions still has to be done, and investigations on scale-up are

necessary to evaluate economic feasibility of the process. The development of a fermentation medium based on industrial substrates is economically desirable. Hence, in the light of the rapidly increasing costs of liquid fuel, the production of ethanol from rice straw and beet pulp hydrolyzates could be an attractive economic possibility and also an alternative to improve the efficiency of sugar–ethanol factory.

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انتاج الايثانول من بعض مخلفات التصنيع الزراعي :
٢- الظروف المثلى لإنتاج الإيثانول من متحللات قش الأرز ولب البنجر

أمين كمال عمار وسمير يوسف السناط

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نظرا لتزايد الأهمية التجارية لإنتاج الإيثانول من المخلفات اللجنوسليلوزية ، فقد أجريت هذه الدراسة في محاولة لتقييم عملية إنتاج الإيثانول من متحللات قش الأرز ولب بنجر السكر كبدائل رخيصة للمولاس الناتج من صناعة السكر باستخدام خميرة الخباز وبالتالي خفض تكلفة إنتاج الإيثانول. وفي نفس الوقت إزالة الأثر البيئي السيئ الناتج من حرق هذه المخلفات في الهواء الطلق. وقد تم دراسة تأثيرات التركيز الإبتدائي للسكر، ودرجة الـpH، ومصدر النيتروجين وفترة الحضانة على إنتاج الايثانول بالتخمر من متحللات قش الأرز ولب بنجر السكر. اظهرت النتائج أن الظروف المثلى التي تحقق أعلى إنتاج للإيثانول تحت ظروف التجربة كانت تركيز سكر ١٠٠ جرام / لتر (وزن/وزن)، باستخدام سلفات الأمونيوم كمصدر للنتروجين، درجة pH ٥.٠ وفترة تحضين ٤٨ ساعة على ٣٠ م°. في ظروف التخمر السابقة، كان عائد الإيثانول ٤٤.٤٢ جرام / لتر و ٤٢.٧٢ جرام / لتر وكفاءة تخمر قدرها ٨٨.٢٤ و ٨٨.٠٩ ٪ لكلا من متحلل قش الأرز ومتحلل لب البنجر على الترتيب.