Journal

J. Biol. Chem. Environ. Sci., 2008, Vol. 3(4):403-420
www.acepsag.org

ETHANOL PRODUCTION FROM SOME LIGNOCELLULOSIC SUBSTRATES USING *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Ethanol production from lignocellulosic substrates, such as sugar cane bagasse and corncobs was evaluated using *Saccharomyces cerevisiae* in this study. The hydrolysates were prepared with 0.5% H_2SO_4 and solid / liquid ratio of 3: 10 (w / v) at 121 °C for 2h. Hydrolysates were supplemented with ammonium sulfate, dipotassium hydrogen phosphate; magnesium sulfate and yeast extract to prepare the growth medium. Fermentation experiments were carried out at 30 °C. The influence of different aeration rates, pH and initial sugar concentration on the growth kinetics and ethanol production were investigated. The highest biomass growth and ethanol production rates were obtained at pH 4.5 and aeration rate of one vv^{-1} m^{-1}. The results obtained with initial sugar concentration were approximately similar with those of 6.9 or 7.5% for sugar cane bagasse and corn cobs hydrolysates, respectively. The highest overall yield coefficient of ethanol on sugar consumed (Yp/s) or on biomass, formed (Yp/x) were 0.46 and 2.88gg^{-1} for yeast strain grown on sugar cane bagasse hydolysate medium.

Key words: Ethanol production, lignocellulosic substrates, sugar cane bagasse, corncobs and *Saccharomyces cerevisiae.*

INTRODUCTION

Worldwide attention has recently turned to bioethanol production as a strategy to combat global warming and to improve global energy security (Lin & Tanaka, 2006 and Vertès *et al* 2006). However, feedstock of current bioethanol production methods are currently derived from edible parts of food crops such as sugar cane and corn. This leads to an undesirable direct competition between

bioethanol production and the food supply (Gray *et al* 2006). A switch to a more abundant lignocellulosic biomass, some of which may be obtained from inedible parts of food crops, should help to reduce pressure on the food crops and possibly generate increased demand for bioethanol (Gray et al 2006 and Lin & Tanaka, 2006).

The utilization of renewable lignocellulosic agro-industrial residues has been attracted interest due to increasing environmental and political pressure (Davis *et al* 2005). When hydrolyzed, these lignocellulosic materials release sugars and several compounds derived from sugar and lignin degradation, such as furfural and 5-hydroxymethyl furfural (5-HMF) (Klinke *et al* 2002). The lignocellulosic hydrolysates can be used as fermentation media to obtain ethanol and other useful products (Mussato, 2003). Bioconversion of lignocellulosic materials to ethanol requires initial dilute acid hydrolysis of cellulose and hemicellulose to sugars followed by fermentation by microorganisms. The ability of Saccharomyces *cerevisiae* yeast to ferment sugars efficiently to ethanol has led to many investigations that use lignocellulosic hydrolysates as fermentation substrate. However, rapid and efficient fermentation of hydrolysates is limited because a range of toxic compounds in addition to monomeric sugars is generated during the hydrolysis of lignocellulosic materials (Palmqvist and Hahn-Hägerdal, 2000). In order to avoid such inhibition, various treatments for detoxification of fermentation inhibitors have been investigated (Klinke *et al* 2004). Various crop residues rich in lignocelluloses, like wheat straw (Nigam, 2001), rice straw (Roberto *et al* 1999), corn cobs (Saraçoğlu-Eken and Arslan, 2000), bagasse (Watson *et al* 1984), have been exploited for ethanol production. The sugar cane and corn crops are deciduous plant whose yearly production has increased and large amounts of bagasse are produced from sugar factories. The principal factors that must be optimized for *Saccharomyces cerevisiae* are aeration rate, pH, and initial sugar concentration in order to obtain maximum productivity and ethanol yield from hydrolysates. In the present study, the potential use of lignicellulosic hydrolysates derived from sugar cane bagasse and corncobs for ethanol fermentation using *Saccharomyces cerevisiae* was investigated. The choice of appropriate aeration rate, pH and initial sugar concentration for the conversion of sugar cane bagasse and corncobs hydrolysates into ethanol are

considered. The effects of these variables on the kinetics of biomass growth and ethanol production have also been investigated.

MATERIALS AND METHODS

1-Microorganism and growth media

Saccharomyces cerevisiae yeast was isolated from a commercial product Hawamdiya baker's yeast. The yeast was grown at 30°C on agar slants composed of 10g of glucose, 3g of malt extract, 3g of yeast yeast, 5g of peptone and 20g of agar per liter and maintained at 4°C . Inocula were prepared by transferring yeast by loop from one-day slants to 250 ml Erlenmeyer flasks containing 100 ml of the above growth medium lacking agar. The yeast was incubated aerobically on incubator shaker at 150 rpm at 30°C for 24 h prior to use.

2-Preparation of acid hydolysates

The acidic hydrolysis of sugar cane bagasse and corncobs was carried out by the following the procedure: five liter Erlenmeyer flasks containing substrate and 0.5% sulfuric acid at solid/ liquid ratio of 3:10 (w/v) was used. The flasks were autoclaved at 121°C for 2h. The hydrolysates were filtered through Watman filter paper No. 1 to remove the suspended particles. The filtered hydrolysates were neutralized using $CaCO_3$,then precipitate of calcium sulfate was removed. The hydrolysates were again filtered through active carbon to remove coloring compounds present in the hydrolysates. The sugar content was standardized after completion of hydrolysis by concentrating the solution through evaporation process at 100°C for one hour. The hydolysates of sugar cane bagasse and corncobs had 69 and 75g total sugar per liter.

3-Fermentation conditions

Sugar cane bagasse and corncobs were hydrolyzed with sulfuric acid and the resulting hydrolysates were used for ethanol production with the yeast *Sacch. cerevisiae.* All experiments were performed as a batch culture using 3L capacity fermentor (Cole- Parmer E-29200-10) with 0.7 L working volume. During the experiments, temperature and agitation rate were controlled at 30°C and 450-rpm min^{-1}.and airflow rate of 1 vvm was used. Fermentation medium contained 10g of $(NH_4)_2SO_4$, 1.5g of K2HPO4, 0.5 of $MgSO_4$, 3g of yeast extract and one liter of hydrolysate. This medium was inoculated with 0.8g of yeast cells. The pH was controlled by addition of 2M NaOH

throughout the experiments. To study the effect of pH, aeration rate and sugar concentration, experiments were conducted with two values of pH (4.5 and 5.5), aeration rates of 0.5 and 1.5 v $v^{-1}m^{-1}$ and 5% total sugar). Samples were taken periodically during fermentation time for analysis of biomass weight, sugar and ethanol concentration.

4-Analytical methods

Samples of 10 ml were aseptically removed at 6-h intervals and analyzed for cell dry weight, total sugar (as glucose) and ethanol concentration. Cell dry weight was detemined, samples were taken from the growth medium, centrifuged, washed with distilled water and dried at 90°C for 24 h. The filtrates were used to determine total sugar (as glucose) by somogyi-semi micro method (1945) and ethanol concentration bydichromateoxidationmethod (AOAC, 1990).

The relationship between yeast biomass weight, sugar consumption and ethanol concentration were calculated throughout the entire and the end of fermentation time.

Nomenclature

X	biomass concentration, gl^{-1}
Xt	biomass concentration at time t, gl^{-1}
T	time, h.
P	ethanol concentration, gl^{-1}
Pt	produced ethanol concentration at time t, gl^{-1}
Pp	ethanol productivity at time t, $gl^{-1}h^{-1}$
Px	biomass productivity at time t, $gl^{-1}h^{-1}$
P_{formed}	produced ethanol concentration at time t, gl^{-1}
S	sugar concentration, gl^{-1}
St	sugar concentration at time t, gl^{-1}
$S_{consumed}$	consumed sugar concentration at time t, gl^{-1}
μ	specific growth rate, h^{-1}
Rx	cell growth rate, g l^{-1} h^{-1}
Rs	sugar consumption rate at time t, g $l^{-1}h^{-1}$
Rp	ethanol formation rate at time t, g $l^{-1}h^{-1}$
$Y_{x/s}$	yield coefficient of biomass on sugar consumed (g biomass/ g sugar consumed)
$Y_{p/s}$	yield coefficient of ethanol on sugar consumed (g ethanol/ sugar consumed)
$Y_{p/x}$	yield coefficient of ethanol on produced biomass (g ethanol/ g biomass)

RESULTS AND DISCUSSION

Dilute-acid sugar cane bagasse or corncobs hydrolysates were used as carbon and energy sources for cultivation of *Sacch. cerevisiae* to produce ethanol. All experiments started with the seeding of 0.8 gl^{-1} in 0.7 L of hydrolysates media containing of 69 and 75 gl^{-1} of total sugar for sugar cane bagasse and corncobs hydrolysates, respectively. The cultures were sparged with airflow rate of 1.0 $vv^{-1}m^{-1}$. Fermentation was carried out at 30°C.

1-Effect of initial pH on alcohol fermentation

The effect of pH has a significant influence on yeast growth and alcohol production, as well as its effect on fermentation inhibitors such as furfural and HMF. Therefore, experiments were carried out with sugar cane bagasse (SBH) and

corncobs (CCH) hydrolysates containing 69 and 75 g l^{-1} total sugar with aeration rate of 1.0 $vv^{-1}m^{-1}$ and two pH values of 4.5 and 5.5 in order to evaluate the efficiency of *Sacch. cerevisiae* towards ethanol production. The experimental data for the variation of pH on biomass growth, sugar consumption and ethanol production as a function of time are shown in Tables (1 to 4). It is interesting to note that biomass growth, sugar consumption and ethanol production varied considerably with changes in pH, whereas the rate of cells formation increase with decrease in pH from 5.5 to 4.5. The maximum of biomass concentration(X) and Productivity (Px) were 12.1 g l^{-1} and 0.45 g l^{-1} h^{-1} at pH 4.5 on SBH medium during the fermentation time.

Moreover, accelerated sugar utilization rate during the entire fermentation was obtained at pH 4.5 whereas the highest figures of sugar consumed of 10.5 and 9.6 g l^{-1} were recorded at pH 4.5 on CCH and SBH media, respectively.

With regard to ethanol production, pH had remarkable effect on ethanol production where there was a considerable variation in ethanol yield. The maximum ethanol concentration was obtained at pH 4.5 on CCH medium followed SBH medium being 27.5 and 26.5 g l^{-1}, respectively. The same trend was observed with productivity (Pp) throughout the fermentation time, the corresponding values were 0.78and 0.70 g l^{-1} at pH 4.5 on SBH and CCH media, respectively. However, these values measured experimentally fell below those values estimated theoretically, whereas these values represented 80 and 83.3% of ethanol concentration estimated theoretically.

Table (1) daynamic growth of *Saccharomyces cerevisiae* grown on sugarcane bagasse hydrolysate medium and ethanol production at pH 5.5

Time(h)	Biomass concentration, $X(gh^{-1})$		Substrate concentration $S(gl^{-1})$		Ethanol Concentration,$P(gh^{-1})$		Ethanol Concentration theoretically $P(gh^{-1})$	Biomass productivity $Px(gl^{-1}h^{-1})$	Ethanol productivity $Pp(gl^{-1}h^{-1})$	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	69.0	-	-	-	-	-	-	-	-	-
6	1.2	0.4	64.0	5.0	1.5	1.5	2.56	0.07	0.25	0.08	0.30	3.75
12	2.1	0.9	59.0	5.0	3.7	2.2	2.56	0.15	0.37	0.18	0.44	2.44
18	2.6	0.5	54.0	5.0	6.1	2.4	2.56	0.08	0.40	0.10	0.48	4.80
24	4.2	1.6	48.3	5.7	8.9	2.8	2.91	0.27	0.47	0.28	0.49	1.75
30	5.4	0.8	43.7	4.6	11.1	2.2	2.35	0.13	0.37	0.17	0.48	2.82
36	5.9	0.5	39.1	4.6	13.0	1.9	2.35	0.08	0.32	0.11	0.41	3.73
42	6.3	0.4	34.5	4.6	15.1	2.1	2.35	0.07	0.35	0.09	0.46	5.11
48	7.2	0.9	29.0	5.5	17.2	2.1	2.81	0.15	0.35	0.16	0.38	2.38
54	8.3	1.1	22.1	6.9	19.0	1.8	3.52	0.18	0.30	0.16	0.26	1.63
60	8.1	-0.2	15.4	6.7	20.1	1.1	3.42	-	0.18	-	0.16	-
66	7.5	-0.6	14.2	1.2	20.5	0.4	0.61	-	0.07	-	0.33	-

Table (2) Daynamic growth of *Saccharomyces cerevisiae* grown on sugarcane bagasse hydrolysate medium and ethanol production at pH 4.5

Time(h)	Biomass concentration,$X(gh^{-1})$		Substrate concentration $S(gl^{-1})$		Ethanol Concentration,$P(gh^{-1})$		Ethanol Concentration theoretically $P(gh^{-1})$	Biomass productivity $Px(gl^{-1}h^{-1})$	Ethanol productivity $Pp(gl^{-1}h^{-1})$	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	69.0	-	-	-	-	-	-	-	-	-
6	1.4	0.6	65.6	3.4	1.4	1.4	1.74	0.10	0.23	0.18	0.41	2.28
12	2.3	0.9	60.4	5.2	3.9	2.5	2.66	0.15	0.42	0.17	0.48	2.82
18	2.9	0.6	53.8	6.6	7.0	3.1	3.73	0.10	0.52	0.09	0.47	5.20
24	3.6	0.7	48.1	5.7	9.8	2.8	2.91	0.12	0.47	0.12	0.49	4.08
30	4.5	0.9	43.2	4.9	12.4	2.6	2.50	0.13	0.43	0.18	0.53	2.94
36	6.4	1.9	35.2	8.0	16.4	4.0	4.09	0.32	0.67	0.24	0.50	2.08
42	7.8	1.4	30.4	4.8	18.6	2.2	2.45	0.23	0.37	0.29	0.46	1.59
48	8.6	0.8	22.0	8.4	20.1	1.5	4.29	0.13	0.25	0.10	0.18	1.80
54	11.3	2.7	12.4	9.6	24.8	4.7	4.90	0.45	0.78	0.28	0.49	1.75
60	12.1	0.8	8.20	4.2	26.5	1.7	2.15	0.13	0.28	0.19	0.40	2.11
66	12.0	-0.1	8.00	-	25.0	-	-	-	-	-	-	-

Table (3) Daynamic growth of *Saccharomyces cerevisiae* grown on corncobs hydrolysate medium and ethanol production at pH 5.5

Time(h)	Biomass concentration,X(gh^{-1})		Substrate concentration S(gl^{-1})		Ethanol Concentration,P(gh^{-1})		Ethanol Concentration theoretically P(gh^{-1})	Biomass productivity Px($gl^{-1}h^{-1}$)	Ethanol productivity Pp($gl^{-1}h^{-1}$)	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	75	-	-	-	-	-	-	-	-	-
6	0.9	0.1	72.5	2.5	0.5	0.5	1.28	0.017	0.08	0.04	0.20	5.00
12	1.0	0.1	70	2.5	1.2	0.7	1.28	0.017	0.12	0.04	0.28	7.00
18	2.2	1.2	67.5	2.5	2.4	1.2	1.28	0.200	0.20	0.48	0.40	0.83
24	3.1	1.9	61.9	5.6	4.4	2.0	2.86	0.150	0.33	0.16	0.36	2.25
30	3.9	0.8	54.8	7.1	7.2	2.8	3.63	0.130	0.47	0.11	0.39	3.55
36	5.9	2.0	48.2	6.6	10.2	3.0	3.37	0.330	0.50	0.30	0.45	1.50
42	6.2	1.6	42.8	5.4	13.8	3.6	2.76	0.270	0.60	0.30	0.67	2.23
48	7.2	1.0	36.4	6.2	16.3	2.5	3.17	0.170	0.42	0.16	0.40	2.5
54	5.4	-2.1	35.1	1.3	16.6	0.3	0.66	-	0.05	-	0.23	-
60	5.5	-0.9	35.0	0.1	15.6	-	0.05	-	-	-	-	-
66	5.1	-0.4	34.0	1.0	14.1	-	0.51	-	-	-	-	-

Table (4) Daynamic growth of *Saccharomyces cerevisiae* grown on corncobs hydrolysate medium and ethanol production at pH 4.5

Time(h)	Biomass concentration,X(gh^{-1})		Substrate concentration S(gl^{-1})		Ethanol Concentration,P(gh^{-1})		Ethanol Concentration theoretically P(gh^{-1})	Biomass productivity Px($gl^{-1}h^{-1}$)	Ethanol productivity Pp($gl^{-1}h^{-1}$)	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	75.0	-	-	-	-	-	-	-	-	-
6	1.0	0.2	71.3	3.7	1.5	1.5	1.89	0.03	0.25	0.05	0.41	8.2
12	1.6	0.6	65.0	6.3	4.7	3.2	3.22	0.10	53	0.10	0.51	5.1
18	2.1	0.5	58.7	6.3	7.3	2.6	3.22	0.08	0.43	0.08	0.41	5.13
24	3.4	1.3	52.5	6.2	10.3	3.0	3.17	0.22	0.5	0.21	0.48	2.29
30	4.8	1.4	45.0	7.5	14.0	3.7	3.83	0.23	0.62	0.19	0.49	2.18
36	6.1	1.3	37.5	7.5	16.8	2.8	3.83	0.22	0.47	0.17	0.37	1.85
42	6.4	0.3	31.5	6.0	19.0	2.2	3.07	0.05	0.37	0.05	0.37	7.4
48	7.2	0.8	25.5	6.0	21.8	2.8	3.07	0.13	0.47	0.13	0.47	3.62
54	7.0	-0.2	15.0	10.5	26.0	4.2	5.37	-	0.70	-	0.40	-
60	6.2	-0.8	11.3	3.70	27.0	1.0	1.89	-	0.17	-	0.27	-
66	6.0	-0.2	7.50	3.80	27.5	0.5	1.94	-	0.08	-	0.13	-

There are three important parameters used to evaluate the efficiency of alcohol production by yeast strain throughout the fermentation time: yield coefficient of biomass (Yx/s), yield coefficient of ethanol on sugar consume (Yp/s) or biomass formed (Yp/x). The first parameter was lower than the estimated value (0.567 g cells dry wt /g sugar consumed). Moreover, it was also observed that Yp/s was closed to the estimated figure theoretically (0.511 g ethanol /g sugar consumed unlike Yp/x was far from the theoretical figure (17.03g ethanol /g biomass formed).

Data of specific growth rates, ethanol production rates, percentage of sugar consumed and overall yields at the end of fermentation presented in Table (11), revealed that the highest values of specific growth rate (μ) and cell growth rate (Rx) were obtained at pH 4.5 on SBH medium compared with those at pH 5.5, being 0.049 h^{-1} and 0.19 g l^{-1} h^{-1}, respectively. Similar trend was observed with percentage of sugar consumed, overall Yx/s, overall Yp/s and overall Yp/x.

From the aforementioned data, it could be noticed that the lower ethanol yield and sugar conversion obtained with higher pH value was possibly due to the formation of undesired productes like glycerol, ogranic acids at the expense of ethanol (Pramanik 2003). On the other hand, the higher ethanol yield may be attributed to that cell growth and ethanol production in lignocellulosic hydrolysates dependent on pH due to the degree of toxicity whereas at low pH the available forms of furfural and HMF minimize (Palmqvist and Hahn-Hägerda, 2000). These results in this study are in agreement with the study conducted by Parmanik (2003 and 2005) who studied the effect of pH on fermentation kinetics of grape waste by *Saccharomyces cerevisiae* in batch culture. Therefore, from the pH study, pH 4.5 was formed to be the optimum pH value for ethanol fermentation using *Saccharomyces cerevisiae* grown on SBH and CCH media.

2-Effect of aeration rate on alcohol fermentation

The potentiality of *Sacch. cerevisiae* to ferment sugar cane bagasse and corn cobs acid hydolysates to ethanol was evaluated under different levels of aeration,i.e., 0.5 and 1.5 $vv^{-1}m^{-1}$. Assays in 0.7 L of concentrated hydrolysates containing 69 and 75 g l^{-1} total sugar for SBH and CCH media, respectively.

The experimental data for aeration rate on the biomass growth, sugar consumption and ethanol production as a function of time are shown in Tables (5to 8). It seems from the results that both biomass growth and ethanol formation are sensitive to the amount of oxygen supplied, whereas air flow rate of 0.5 vv^{-1} m^{-1}, caused low biomass. During the other experimental runs, i.e., 1.5 $vv^{-1}m^{-1}$, an oxygen supply stimulated biomass growth depending on aeration rate.

With regard to sugar consumption throughout the fermentation time, the peak of value was obtained at air flow rate 1.5 vvm, being 12.2 g l^{-1} on CCH medium. In addition to, the same figure was observed at airflow rate of 0.5 vvm on SBH medium.

On the contrary, during the fermentation time, the highest ethanol formation and ethanol productivity (Pp) were 13.7g l^{-1} and 0.65 g l^{-1} h^{-1} at air flow rate 0.5 vvm on SBH medium.

Table (11) summarizes the main estimated results obtained for the effect of aeration rate on alcoholic fermentation. The highest specific growth rate of 0.063 h^{-1} was obtained at air flow rate of 1.5 $vv^{-1}m^{-1}$ in SBH medium. The highest values of sugar consumption rate and the percentage of sugar consumed were 1.01 gl^{-1} h^{-1} and 89% at air flow rate 1.5 vvm on CCh medium. On the contrary, the peak of values for Rp, overall Yp/s and overall Yp/x were 0.33 gl^{-1} h^{-1}, 0.25 gg^{-1} and 1.79 gg^{-1} at airflow rate of 0.5 vvm on SBH medium.

It was cleared from the aforementioned data that further increase in flow rate produced less ethanol. This suggests that the degree of aeration has to be at a certain threshold level before ethanol production is diminished. As seen from the aforementioned results there is a negative correlation between ethanol formation and aeration rate, which indicates that excessive aeration reduces the ethanol yield because of either product oxidation or cell growth.In addition to, increase the rate of aerationleads to an increase in pH (Okur and Saracoğlu, 2006) This observation is in agreement with same previous results obtained by Grootjen *et al* 1990;Varela *et al* 1992 ; Nigam, 2002 and Alfenor *et al* 2004.

Table (5) Effect of aeration rate on ethanol production in sugar cane bagasse hydolyzate medium at 0.5 vvm using Saccharomyces cerevisiae

Time(h)	Biomass concentration,X(gh^{-1})		Substrate concentration S(gl^{-1})		Ethanol Concentration,P(gh^{-1})		Ethanol Concentration theoretically P(gh^{-1})	Biomass productivity Px($gl^{-1}h^{-1}$)	Ethanol productivity Pp($gl^{-1}h^{-1}$)	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	69.0	-	-	-	-	-	-	-	-	-
6	1.4	0.6	63.0	6.0	1.20	1.2	3.07	0.10	0.20	0.10	0.20	2.00
12	2.1	0.7	56.2	6.8	3.10	2.1	3.47	0.12	0.35	0.16	0.31	3.10
18	3.3	1.2	45.6	10.6	5.30	2.6	5.42	0.20	0.43	0.11	0.25	2.27
24	4.2	0.9	37.1	8.50	9.20	3.5	4.34	0.15	0.58	0.11	0.41	3.73
30	5.6	1.4	24.9	12.2	13.1	3.9	6.23	0.23	0.65	0.11	0.32	2.91
36	6.9	1.3	21.3	3.60	13.5	0.4	1.84	0.22	0.07	0.36	0.11	0.31
42	7.4	0.5	19.1	2.20	13.7	0.2	1.12	0.08	0.03	0.23	0.09	0.31
48	7.8	0.4	17.2	1.90	12.5	-1.2	0.97	0.07	-	0.21	-	-
54	8.1	0.3	16.1	1.10	12.1	-0.4	0.56	0.05	-	0.27	-	-
60	8.2	0.1	15.3	0.80	11.2	-0.9	0.41	0.02	-	0.13	-	-
66	6.1	-2.1	14.7	0.60	10.5	-0.7	0.31	-	-	-	-	-

Table (6) Effect of aeration rate on ethanol production in corn cobs hydolyzate medium at 0.5 vvm using Saccharomyces cerevisiae

Time(h)	Biomass concentration,X(gh^{-1})		Substrate concentration S(gl^{-1})		Ethanol Concentration,P(gh^{-1})		Ethanol Concentration theoretically P(gh^{-1})	Biomass productivity Px($gl^{-1}h^{-1}$)	Ethanol productivity Pp($gl^{-1}h^{-1}$)	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	75.0	-	-	-	-	-	-		-	-
6	1.2	0.4	70.0	5.0	0.55	0.55	2.56	0.07	0.09	0.08	0.11	1.38
12	1.8	0.6	63.0	7.0	1.46	0.91	3.58	0.10	0.15	0.09	0.13	1.44
18	3.0	1.2	53.6	9.4	3.06	1.60	4.80	0.20	0.27	0.13	0.17	1.31
24	4.3	1.3	45.3	8.3	5.86	2.80	4.24	0.22	0.47	0.16	0.34	2.13
30	6.1	1.8	35.3	10.0	6.60	0.74	5.11	0.30	0.12	0.18	0.07	0.39
36	7.9	1.8	29.1	6.2	8.30	1.70	3.17	0.30	0.28	0.29	0.27	0.93
42	9.5	1.6	22.0	7.1	10.2	1.90	3.63	0.27	0.32	0.23	0.27	1.17
48	10.1	0.6	13.9	8.1	11.3	1.10	4.14	0.10	0.18	0.07	0.14	2.0
54	10.7	0.6	11.8	2.1	9.20	-2.10	1.07	0.10	-	0.29	-	-
60	8.9	-1.8	10.3	1.5	8.10	-1.10	0.77	-	-	-	-	-
66	8.0	-0.9	9.80	0.5	7.30	-0.80	0.26	-	-	-	-	-

Table (7) Effect of aeration rate on ethanol production in sugar cane bagasse hydolyzate medium at 1.5 vvm using Saccharomyces cerevisiae

Time(h)	Biomass concentration,X(gh^{-1})		Substrate concentration S(gl^{-1})		Ethanol Concentration,P(gh^{-1})		Ethanol Concentration theoretically P(gh^{-1})	Biomass productivity Px($gl^{-1}h^{-1}$)	Ethanol productivity Pp($gl^{-1}h^{-1}$)	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	69.0	-	-	-	-	-	-	-	-	-
6	1.9	1.1	60.0	9.0	0.6	0.6	4.60	0.18	0.10	0.12	0.07	0.58
12	3.4	1.5	52.5	7.5	1.1	0.5	3.83	0.25	0.08	0.20	0.07	0.35
18	5.9	2.5	46.3	6.2	2.3	1.2	3.17	0.42	0.20	0.40	0.19	0.48
24	7.9	2.0	38.2	8.1	4.5	2.2	4.14	0.33	0.37	0.25	0.27	1.08
30	9.4	1.5	32.1	6.1	6.4	1.9	3.11	0.25	0.32	0.25	0.31	1.24
36	10.2	0.8	21.2	10.9	8.2	1.8	5.57	0.13	0.30	0.07	0.17	2.43
42	11.1	0.9	16.5	4.7	9.2	1.0	2.40	0.15	0.17	0.19	0.01	0.05
48	9.4	-1.7	12.2	4.3	8.2	-	2.20	-	-	-	0.21	-
54	8.2	-1.2	10.8	1.4	7.4	-	0.72	-	-	-	-	-
60	8.1	-0.1	8.3	2.5	7.2	-	1.28	-	-	-	-	-
66	7.5	-0.6	8.00	0.3	7.1	-	0.15	-	-	-	-	-

Table (8) Effect of aeration rate on ethanol production in corn cobs hydolyzate medium at 1.5 vvm using Saccharomyces cerevisiae

Time(h)	Biomass concentration,X(gh^{-1})		Substrate concentration S(gl^{-1})		Ethanol Concentration,P(gh^{-1})		Ethanol Concentration theoretically P(gh^{-1})	Biomass productivity Px($gl^{-1}h^{-1}$)	Ethanol productivity Pp($gl^{-1}h^{-1}$)	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	75.0	-	-	-	-	-	-	-	-	-
6	1.5	0.7	71.0	4.0	0.3	0.3	2.04	0.12	0.05	0.18	0.08	0.44
12	2.3	0.8	62.4	8.6	1.7	1.4	4.39	0.13	0.23	0.09	0.16	1.78
18	4.2	1.9	50.2	12.2	2.1	0.4	6.23	0.32	0.07	0.16	0.03	0.19
24	6.3	2.1	42.4	7.80	3.8	1.7	3.99	0.35	0.28	0.27	0.22	0.81
30	8.2	1.9	40.5	1.90	4.1	0.3	0.97	0.32	0.05	1.00	0.16	0.16
36	10.1	1.9	32.3	8.20	5.3	1.2	4.19	0.32	0.20	0.23	0.15	0.65
42	14.3	4.2	20.4	11.9	6.2	0.9	6.08	0.70	0.15	0.35	0.08	0.23
48	15.7	1.4	16.2	4.2	7.5	1.3	2.14	0.23	0.22	0.33	0.31	0.94
54	16.3	0.6	14.5	1.7	7.2	0.3	0.87	0.10	-	0.35	-	-
60	14.1	-2.2	10.1	4.4	6.3	0.9	2.25	-	-	-	-	-
66	12.2	-1.9	8.20	1.9	4.1	-2.2	0.97	-	-	-	-	-

3-Effect of initial sugar concentration on alcohol fermentation

An interesting research field in alcoholic fermentation is the study of yeast strains able to utilize sugar solutions more concentrated than those generally fermented in usual practice (Converti et al 1985) and hence it is important to establish the limits of ethanol tolerance of yeast strain (Shiyuan et al 1087). According to the definition of Crabtree effect, in Sacch. *cerevisiae*, high sugar concentration trigger alcohol fermentation, even under fully aerobic conditions (De Deken, 1966;Petrick *et al* 1983 and Watson *et al* 1984) and to reduce the adverse impact of inhibitors in the hydrolysates on biomass growth and therefore, on the production of ethanol. This has been investigated by carrying out fermentation experiments with initial sugar concentration of 5% and airflow rate 1 vvm.

Tables 9 and 10 show the effect of initial sugar concentration on the cell growth kinetics, sugar consumption and ethanol formation using *Sacch. cerevisiae* on SBH and CCH media throughout the fermentation time.Cell mass growth and biomass productivity gave the maximum values of 7.43gl^{-1} and 0.23 gl^{-1} h^{-1} on SBH medium, respectively.

The behavior of *Sacch. cerevisiae* to consume sugars at 5% was closed with the higher sugar concentrations,i.e., 69 and 75 g l^{-1} in SBH and CCH media, respectively where the percentage of sugar consumption was 84% on SBH medium(Table 11).

It was observed that ethanol concentration and ethanol productivity during the fermentation time had high values, being 19.2gl^{-1} and 0.52 gg^{-1} on SBH medium

Table (11) show specific growth rate of yeast strain grown on SBH medium had the value of 0.04 h^{-1} and this was closed with those of yeast strain grown on high sugar concentrations (6.9 and 7.5%). On the other hand, Sacch. *cerevisiae* had the lower of μ, 0.34 h^{-1} on CCH medium containing 5% than those of yeast strain obtained with experiments containing 69 and 75 g l^{-1} . The overall yields, Yp/s, Yp/x and Pp were approximately equal to the corresponding values obtainted with yeast strain grown on other sugar concentrations,whereas the figures of Yp/s and Yp/x were 0.46 and 2.88 gg^{-1}, respectively on SBH medium.

Table (9) Effect of initial sugar on ethanol production in sugar cane bagasse hydolyzate medium at 50 gl^{-1} using Saccharomyces cerevisiae

Time(h)	Biomass concentration,X(gh^{-1})		Substrate concentration S(gl^{-1})		Ethanol Concentration,P(gh^{-1})		Ethanol Concentration theoretically P(gh^{-1})	Biomass productivity Px($gl^{-1}h^{-1}$)	Ethanol productivity Pp($gl^{-1}h^{-1}$)	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	50.0	-	-	-	-	-	-	-	-	-
6	1.27	0.47	46.4	3.6	1.9	1.9	1.84	0.08	0.32	0.13	0.53	4.08
12	1.75	0.48	42.5	4.0	3.6	1.7	2.04	0.08	0.28	0.12	0.43	3.58
18	2.27	0.52	38.2	4.3	5.5	1.9	2.20	0.09	0.32	0.12	0.44	3.67
24	3.27	1.00	32.4	5.8	7.6	2.1	2.96	0.17	0.35	0.17	0.36	2.12
30	3.87	0.60	26.3	6.1	10.0	2.4	3.12	0.10	0.40	0.10	0.39	3.9
36	4.57	0.70	20.5	5.8	13.1	3.1	2.96	0.12	0.51	0.12	0.53	4.42
42	5.97	1.40	14.9	5.6	15.5	2.4	2.86	0.23	0.40	0.25	0.43	1.72
48	6.57	0.60	12.8	2.1	17.8	2.3	1.07	0.10	0.38	0.29	1.10	3.79
54	7.03	0.46	10.1	2.7	18.3	0.5	1.38	0.08	0.08	0.17	0.19	1.12
60	7.43	0.40	8.00	2.1	19.2	0.9	1.07	0.07	0.15	0.19	0.43	2.26
66	6.03	-1.40	-	-	18.0	-1.2	-	-		-	-	-

Table (10) Effect of initial sugar concentration on ethanol production in corn cobs hydolyzate medium at 50 gl^{-1} using Saccharomyces cerevisiae

Time(h)	Biomass concentration,X(gh^{-1})		Substrate concentration S(gl^{-1})		Ethanol Concentration,P(gh^{-1})		Ethanol Concentration theoretically P(gh^{-1})	Biomass productivity Px($gl^{-1}h^{-1}$)	Ethanol productivity Pp($gl^{-1}h^{-1}$)	Yx/s gg^{-1}	Yp/s gg^{-1}	Yp/x gg^{-1}
	Xt	Xformed	St	Sconsumed	Pt	Pformed						
0	0.8	-	50.0	-	-	-	-	-	-	-	-	-
6	0.92	0.12	48.2	1.8	0.5	0.5	0.92	0.02	0.08	0.07	0.28	4
12	1.10	0.18	45.0	3.2	1.7	1.2	1.64	0.03	0.2	0.06	0.38	6.33
18	1.40	0.30	43.2	1.8	2.2	0.5	0.92	0.05	0.08	0.17	0.28	1.65
24	1.90	0.50	40.5	2.7	2.6	0.4	1.38	0.08	0.07	0.19	0.15	0.79
30	2.98	1.08	36.0	4.5	5.1	2.5	2.30	0.18	0.42	0.24	0.56	2.33
36	2.88	0.90	31.2	4.8	7.2	2.1	2.45	0.15	0.35	0.19	0.44	2.32
42	3.88	0.90	26.6	4.6	9.2	2.0	2.35	0.15	0.33	0.20	0.43	2.15
48	5.02	0.24	24.0	2.6	9.8	0.6	1.33	0.04	0.10	0.09	0.23	2.56
54	5.54	0.52	20.1	3.9	11.1	1.3	1.99	0.09	0.22	0.13	0.33	2.54
60	6.19	0.65	15.4	4.7	13.1	2.0	2.40	0.11	0.33	0.14	0.43	3.07
66	6.29	0.10	14.2	1.2	13.3	0.2	0.61	0.02	0.03	0.08	0.17	2.13

Table (11) Summary of the main results obtained for the different experiments

Experiment	Source S	μ (h^{-1})	Rx ($gl^{-1}h^{-1}$)	Rs($gl^{-1}h^{-1}$	Rp($gl^{-1}h^{-1}$)	Sconsumed %	Overall Yx/s	Overall Yp/s	Overall Yp/x
pH 4.5	SBH	0.049	0.19	0.92	0.44	88	0.19	0.43	2.26
	CCH	0.046	0.13	1.02	0.42	90	0.09	0.41	4.56
pH 5.5	SBH	0.043	0.14	0.83	0.32	79	0.14	0.38	2.71
	CCH	0.046	0.13	0.62	0.31	55	0.16	0.40	2.50
airflow rate (0.5vvm)	SBH	0.043	0.12	0.82	0.33	79	0.14	0.25	1.79
	CCH	0.048	0.18	0.99	0.24	87	0.15	0.17	1.13
airflow rate (1.5vvm)	SBH	0.055	0.21	0.92	0.19	88	0.17	0.15	0.88
	CCH	0.056	0.29	1.01	0.16	89	0.23	0.11	0.48
initial sugar concentration 50 gl^{-1}	SBH	0.040	0.11	0.70	0. 32	84	0.16	0.46	2.88
	CCH	0.034	0.08	0.54	0.20	72	0.15	0.37	2.47

SBH: sugar cane bagasse hydrolysate media
CCH: corncobs hydrolysate media

From the aforementioned results, it could be noticed that adjust the minimum sugar concentration in lignocellulosic hydrolysates media was the desirable amount to obtain the maximum biomss growth and ethanol production. This positive data was the result of reducing the harmful effect of the inhibitory substances, such as furfural and HMF (Palmqvist *et al* 1999; Zaldivar *et al* 1999; Zaldivar and Ingram 1999 and Klinke *et al* 2004).

CONCLUSION

Ethanol can be produced easily from lignocellulosic hydrolysates using *Sacch. cerevisiae* and it could be concluded the following:

1-This study shows that Such. cerevisiae assimilate sugar (as glucose) extracted from sugar cane bagasse and corn cobs aerobically and it is able produce ethanol from reducing sugars. However, the aeration rate is an important factor to obtain a reasonable yield of ethanol from hydrolysate sugars.

2-The adjustment of pH at 4.5 and initial sugar concentration of 50 g l^{-1} in hydrolysates media are the best to minimize the harmful effect of the inhibitory substances produced in the hydrolysates, such as furfural anf HMF.

3-In comparison between sugar cane bagasse and corncobs hydrolysates media, the later gave the highest figures of growth kinetics and determined parameters of ethanol production.

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انتاج كحول الايثانول من المواد اللجنوسليلوزية باستخدام خميرة سكارواميسيس سرفيسيا

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استخدمت فى هذه الدراسة مواد لجنوسليلوزية مثل مصاصة قصب السكر وقوالح الذرة لانتاج كحول الايثانول باستخدام خميرة سكارواميسيس سرفيسيا تم تحضير مهضوم المواد اللجنوسليلوزية باضافة ٣٠٠جرام من كل مادة على حدة الى لتر من حامض الكبريتيك تركيز ٥,٥% فى دوارق سعة ٥ لتر والتى وضعت فى الاوتوكلاف على درجة حرارة ١٢١ °م لمدة ساعتان وبعد ترشيح المهضوم من خلال ورق ترشيح وايتمان ومعادلته بمادة كربونات الكالسيوم،استخدم الراشح مع اضافة الاملاح اللازمة لنمو خلايا الخميرة وانتاج كحول الايثانول. اجريت جميع الاختبارات على درجة حرارة 30 درجة منوية.

صممت اختبارات لدراسة تاثير رقم pH و معدلات التهوية وتركيز السكر فى بداية التخمرلقياس وتقدير نمو خلايا الخميرة واستهلاك السكر وانتاج الكحول خلال مدة التخمر بالاضافة الى القياسات فى نهايةفترة التخمر.اوضحت النتائج ان افضل نمو لخلايا الخميرة واستهلاك السكروانتاج كحول الايثانول كان عند درجةpH 4.5ومعدل تهوية بمعدل حجم هواء الى حجم بيئة فى الدقيقة. اوضحت النتائج ايضا ان استخدام تركيزسكر فى بداية التخمر 5% كانت نتائجه متقاربة مع تلك النتائج المتحصل عليها مع تركيزات سكر 6.9 و 7.5 فى بيئات مهضوم مصاصة قصب السكر وقوالح الذرة على التوالى . كما اوضحت النتائج تسجيل اعلى معامل لانتاج كحول الايثانول على اساس السكر الممثل او على اساس الخلايا المتكونة 0.46 جرام ايثانول لكل جرام سكر ممثل و2.88 جرام ايثانول لكل جرام خلايا متكونة على التوالى.