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Full Length Research Paper

Xylitol production by *Candida tropicalis* under different statistically optimized growth conditions

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Nutritional and environmental conditions of the xylose utilizing yeast *Candida tropicalis* were optimized on a shake-flask scale using a statistical factorial design to maximize the production of xylitol. Effects of the three growth medium components (rice bran, ammonium sulfate and xylose) on the xylitol production were studied, each at three different levels and the highest xylose to xylitol conversion ratio (57.2%) was achieved on using 20 gl^{-1} of xylose, 15 gl^{-1} of rice bran and 1 gl^{-1} of ammonium sulfate. By maintaining the pH of the growth medium at 5.5 using citrate buffer, the xylose to xylitol conversion ratio was greatly enhanced (93.75%) over the use of or unbuffered growth medium and phosphate buffered (88.3 and 83.8%).The least aeration rate was simulated by 40 ml of the growth medium at 250 ml^{-1}. Erlenmeyer flasks result in maximum xylose to xylitol conversion ratio (98%), by using 25 gl^{-1} of xylose, while maximum xylitol production (36.25 gl^{-1}) with a conversion ratio of 72.5% was achieved by using 50 gl^{-1} of xylose.

Key words: xylitol, *Candida tropicalis*, xylose, yeast, pH, aeration.

INTRODUCTION

Xylitol is a naturally occurring sugar alcohol with a similar sweetening power to sucrose (Makinen, 2000). Due to its unique pharmacological properties, world wide de-mand is ever increasing (Emidi, 1978; Makinen, 2000b). Xylitol (C_5 $H_{12}O_5$) stands out from other polyols in preventing dental caries (Biswas and Vashishtha, 1998). Xylitol is also an advantageous sucrose substitute for people suffering from diabetes, obesity and glucose 6-P dehydrogenase deficiency (Grenby and Colley, 1993; Parajo et al., 1998). Xylitol can also be used to prevent middle ear infections in young children (Uhari et al., 1998).

The development of an economic fermentative process for xylitol production involves the selection of yeast strains with high productivity, establishment of conditions that maximize the conversion of xylose into xylitol and optimization of these parameters for scaling up process (Silva et al., 1998). In the conventional production optimization procedures, one parameter is altered at a time, while keeping the other parameters constant; this was to understand the impact of that particular parameter. Factorial designs have been employed for the optimization of the fermentative process because they offer the possibility of studying several variables with a reduced number of experiments (Rodrigues et al., 1998). For this reason, statistical procedures have advantages over conventional methodologies in predicting the accurate results basically due to utilization of fundamental principles of statistics, randomization, replication and duplication (Sreenivas et al., 2004).

For the economical production of xylitol, the initial concentration of xylose should be as high as possible. Aeration also plays an important role in the bioconversion of xylose to xylitol by yeasts (Walther et al., 2001) where a high degree of aeration promotes cell growth, while being detrimental to xylitol accumulation.

Optimum pH for xylitol production by *Candida* sp. was generally ranged from 2.5 to 5.8 (Silva et al., 1998). The pH alteration probably affects the growth by influencing the activity of the permeases present in the cytoplasmic membrane or of the enzymes associated to the cellular

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wall, which tend to coagulate and to precipitate under their isoelectric points (Kampen, 1997). As the gap between the extracellular and the intracellular pH values widens, greater stress is placed on the cells and more energy is expected to maintain the intracellular pH within the range that permits growth and survival of the yeast (Thomas et al., 2002).

Three experiments were designed and carried out to investigate: Firstly, the influence of changing concentrations of the three components of the growth medium (xylose, rice bran and ammonium sulfate); secondly, the influence of maintaining the pH of the growth medium at different values using two buffer systems; thirdly, the influence of aeration rate accompanied by using high xylose concentrations on the conversion of xylose into xylitol by *Candida tropicalis.*

MATERIALS AND METHODS

Microorganism

A xylose utilizing yeast was isolated from an Egyptian cultivated soil (Kaliobia governorate) and identified according to the conventional yeast identification methods based on its morphological and fermentation characteristics as well as its assimilation of different carbon, nitrogen sources (using nitrogen base and carbon base media) and vitamins according to Lodder (1971), Ahearn (1974, 1978) and Barnett et al . (2000).

Preparation of Inoculum

The yeast isolate was grown and sub cultured on a modified Wickerham's agar medium composed of (g l^{-1}): malt extract, 3.0; yeast extract, 3.0; peptone, 5.0; agar, 20 and xylose, 10.0 (pH was adjusted at 5.5 ± 0.1) for 24 h at 30°C.

Inoculum was prepared by transferring a loopful of a four days old yeast culture, which was grown on a modified Wickerham's agar slant into 25 ml of preculture medium with the following composition (g l^{-1}): D- xylose, 25; $(NH_4)_2 SO_4$, 2.0; $CaCl_2.2H_2O$, 0.1 and rice bran, 15 (pH was adjusted at 6.0 ± 0.2) in a 250 ml Erlenmeyer flask plugged with cotton-cloth sandwich and incubated in an orbital shaking incubator at 100 rpm for 24 h at 30°C. The resultant growth was centrifuged, washed with sterile saline, recentrifuged and then, resuspended in a sterile growth medium to get optical density value of 0.3 at 660 nm. 10% was taken as inoculum volume for the fermentation medium, which has the same composition of the preculture medium.

Experimental design

A complete factorial design was chosen to do this study and the statistical analysis was carried out using statistical analysis system, SAS user`s guide (2006). Duncan's multiple range tests were applied to classify the mean values as groups.

In the first experiment, effects of changing concentrations of the three components of the fermentation medium (xylose, rice bran and ammonium sulfate) on the xylitol production were examined after 72 h, each at three concentration levels (Table 2). In the second experiment, the effects of maintaining pH at different values (5.0, 5.5 and 6.0) using two different buffer systems (Phosphate and Citrate) on the xylitol production was studied by using a growth medium having the following composition; (gl^{-1}), D-xylose, 20; $(NH_4)_2 SO_4$, 1.0; $CaCl_2.2H_2O$, 0.1 and rice bran, 15.

In the third experiment, the effects of different aeration rates (simulated by different volumes of the growth medium 20, 30 and 40 ml, each in 250 ml Erlenmeyer flasks) in combination with the increment of the xylose concentrations from 25 up to 200 gl^{-1} was studied (Table 8), while the pH of the growth media was adjusted at 5.5. Cultures were grown aerobically under submerged conditions in 250 ml Erlenmeyer flasks containing the experimental designed media. All experiments were carried out on an orbital shaking incubator at 30°C and agitated at 200 rpm.

Analytical methods

Xylose and xylitol concentrations were measured by the methods of Trinder (1975) and Sanchez (1998), respectively. Cell concentration was determined by measuring the optical density at 660 nm.

RESULTS AND DISCUSSION

Yeast identification

The yeast isolate was identified as *C. tropicalis* according to the standard methods for complete yeast identification. Morphologically, white to creamy colonies were raised and reproduce vegetatively by budding elaborating well developed pseudohyphae with blastoconidia in dalmau plate cultures on corn meal agar with no sexual reproduction (Table 1 and Figure 1).

Xylitol production

Effect of alteration in the growth medium components concentrations

The growth medium experiment is a 3^3 incomplete factorial design with three replicates. Xylitol, the main product of the xylose bioconversion by *C. tropicalis* was produced under the prescribed conditions with varying concentrations between 10.42 and 11.93 gl^{-1} (Table 2).

On considering the average effects and the analysis of variances (ANOVA) of the independent variables (xylose and rice bran concentrations), each of them showed a "P" value less than 0.0001 indicating a highly significant response towards the xylitol production (Table 3). For the ammonium sulfate, a significant response was obtained with a "P" value of 0.0045.

On considering the average effects of the factors interactions and the analysis of variances (ANOVA) of the "rice bran x ammonium sulfate" and "xylose x rice bran" interactions, both of them showed a "P" value less than 0.0001, indicating a highly significant responses. For the "xylose x ammonium", sulfate interaction, a non significant response was obtained with a "P" value of 0.1084.

Generally, by increasing the initial xylose concentration, the mean value of the xylitol content was increased significantly, while the xylose to xylitol bioconversion ratios were greatly decreased (Table 4). For the ammonium sulfate (Table 4) a non significant difference

Table 1. Physiological characteristics of the xylose utilizing C. tropicalis isolate.

Carbon assimilation		Carbon fermentation		Vitamin	
D- glucose	+	D- glucose	+	w/o vitamin	+
D- galactose	+	D- galactose	+	w/o pantothenate	+
L- Sorbose	+	Maltose	+	w/o Biotine	+
D- ribose	-	Sucrose	+	w/o Thiamine	+
D-Glucosamine	+	Lactose	-	w/o pyridoxine	+
D- xylose	+	Melibiose	-	w/o Niacin	+
L- Arabinose	+	Cellobiose	-		
D- Arabinose	-	Raffinose	-	**Urea hydrolysis**	-
L- Rhamnose	-	Inuline	-	**Starch formation**	-
Sucrose	+	D- xylose	-		
Maltose	+	Starch	-		
Lactose	-	**Nitrogen assimilation**			
Melibiose	-	Nitrate	-		
Raffinose	-	Nitrite	-		
Inuline	-	Ethyl amine	+		
Starch	+	Creatine	-		
Methanol	-	Creatinine	-		
Glycerol	+	Cadaverine	-		
Ribitol	+	**Cycloheximide sensitivity**			
Xylitol	+	0.01 cycloheximide	+		
D- mannitol	+	0.1 cycloheximide	+		
2keto-D-gluconate	+				
Succinate	+	Citrate	+		

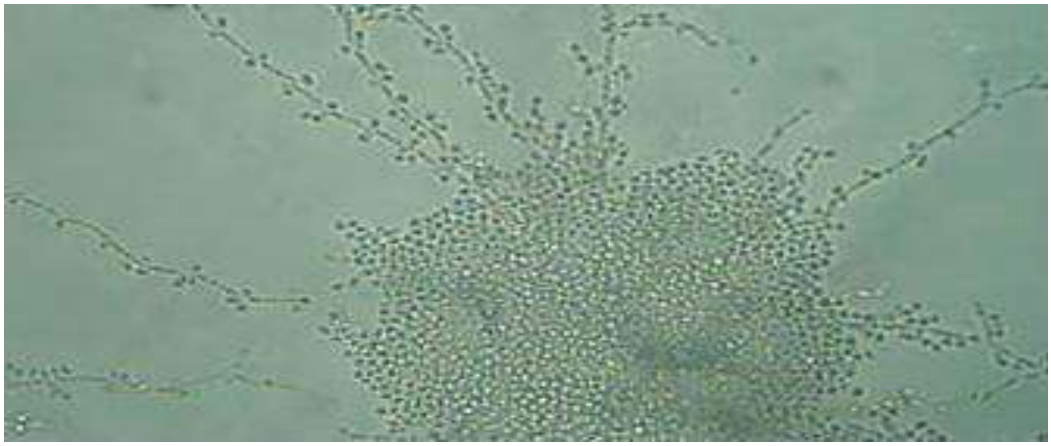

Figure 1. Scanned electron micrograph showing a branched pseudomycelium and blastospores of *C.tropicalis*.

was found by using the three chosen concentrations (1, 2 and 3 gl^{-1}). A non significant difference of the xylitol mean value was also found on using 10 and 15 gl^{-1} of rice bran, while 5 g/l gave a lower mean value with a significant difference (Table 4).

On studying the "rice bran x ammonium sulfate" interactions (Table 4), it was found that at rice bran concentration of 10 and 15 gl^{-1} and by increasing the ammonium sulfate concentration, the xylitol mean value was decreased gradually. On using the rice bran at concentration of 5 gl^{-1} and by increasing the ammonium sulfate concentration, an increase in the xylitol contents

Table 2. Experimental design of the growth medium composition with alteration in the xylose, rice bran and ammonium sulfate concentrations and their effects on xylitol production.

Xylose (g^{-1})	Ammonium sulfate (g^{-1})	Rice bran (gl^{-1})	Initial pH	Final pH	Optical density (OD)	Xylitol (gl^{-1})
20	1	5	6.12	4.11	0.059	10.42
20	2	5	5.96	4.08	0.078	10.79
20	3	5	6.08	3.88	0.082	11.10
20	1	10	5.79	2.6	0.087	11.07
20	2	10	6.0	4.2	0.090	10.87
20	3	10	5.91	2.76	0.111	10.79
20	1	15	5.88	4.55	0.119	11.44
20	2	15	6.22	4.10	0.074	10.90
20	3	15	6.05	4.01	0.078	10.92
25	1	5	5.9	4.3	0.990	10.89
25	2	5	6.02	3.92	0.083	10.72
25	3	5	5.82	3.80	0.095	11,11
25	2	10	5.89	4.22	0.110	11.51
25	3	10	6.10	3.84	0.110	11.28
25	1	15	5.99	2.25	0.110	11.13
25	2	15	5.98	4.02	0.103	11.00
25	3	15	6.10	3.93	0.099	11.41
30	1	5	6.10	3.97	0.099	10.63
30	1	10	6.2	4.37	0.100	11.93
30	2	10	6.38	4.1	0.103	11.66
30	3	10	5.95	3.82	0.103	11.74
30	1	15	6.05	4.25	0.123	11.25
30	2	15	6.03	3.96	0.123	11.29
30	3	15	6.17	3.81	0.099	11.58

pH initial was adjusted at 6.0 ± 0.2, xylitol concentration was estimated after 72 h of incubation.

Table 3. ANOVA for xylitol production as a function of alteration in the xylose, rice bran and ammonium sulfate concentrations.

Source	DF	Mean square	P > F
Xylose	2	1.26926068	<.0001
Ammonium sulfate	2	0.13056698	0.0045
Rice bran	2	1.20228660	<.0001
Xylose and ammonium sulfate	4	0.04340681	0.1084
Xylose and rice bran	4	0.26864824	<.0001
Ammmonium sulfate and rice bran	4	0.36279847	<.0001
Error	53	0.02174217	
Correct total	71	11.69008750	

DF, Degree of freedom

was observed. In conclusion, the effect of the ammonium sulfate on the xylitol production was greatly affected by its combination with the rice bran, whereas, the effect of the rice bran on the xylitol production was not influenced by its combination with the effect of the ammonium sulfate.

On studying the effect of “xylose x rice bran“ interactions (Table 4), it was found that the general effect of the rice bran was not influenced by changing the xylose concentrations, whereas, the general effect of the xylose was greatly reduced on using 5 gl^{-1} of rice bran with 30 gl^{-1} of xylose.

So, it can be concluded that changing of the growth medium composition led to an increment of the xylitol production and the optimum composition could be

Table 4. Mean values of xylitol production as a function of xylose, rice bran, and Ammonium sulfate concentrations and their interactions.

Xylose (gl^{-1})	Mean value of xylitol content	Ammonium Sulfate (gl^{-1})	Mean value of xylitol content	Rice bran (gl^{-1})	Mean value of xylitol content	Ammonium sulfate + Rice bran (gl^{-1})	Mean value of xylitol content	Xylose + Ammonium sulfate ($gl^{-1)}$	Mean value of xylitol content	Xylose and Rice bran (gl^{-1})	Mean value of Xylitol content
30	11.50381 ^{A}	3	11.26583 ^{A}	10	11.42292 ^{A}	1 x 10	11.56667 ^{A}	30 x 3	11.66167 ^{A}	30 x10	11.8200 ^{A}
25	11.22500 ^{B}	1	11.16542 ^{A}	15	11.28593 ^{A}	2 x 10	11.44889 ^{B}	30 x 2	11.55833 ^{A}	25 x10	11.5566 ^{B}
20	10.93148 ^{C}	2	11.15750 ^{A}	5	10.82190 ^{B}	1 x 15	11.40556 ^{B}	30 x 1	11.36222 ^{B}	30 x15	11.4577 ^{B}
						3 x 10	11.30111 ^{B}	25x 3	11.31667 ^{B}	25 x15	11.2988 ^{C}
						2 x 15	11.12889 ^{C}	25 x 2	11.22000 ^{BC}	20x15	11.1011 ^{D}
						3 x 5	11.12667 ^{C}	25 x 1	11.09500 ^{CD}	20 x10	10.9366 ^{E}
						2 x 5	10.76333 ^{D}	20 x 1	11.01556 ^{D}	25 x 5	10.9300 ^{E}
						1 x 5	10.65778 ^{D}	20 x 3	10.95111 ^{ED}	20 x 5	10.7566 ^{F}
								20 x 2	10.82778 ^{E}	30 x 5	10.6933 ^{F}

Means with the same letters are not significantly different.

obtained by decreasing the ammonium sulfate concen-tration from 2.0 to 1.0 gl^{-1} and rice bran from 15.0 to 10.0 gl^{-1}.

Effects of pH maintaining at different values by using different buffer systems

Two buffer systems were used to maintain the pH of the growth medium at three different values (5.0, 5.5 and 6.0) in comparison with the unbuffered medium. Maximum xylitol production (18.75 gl^{-1}) was achieved after 72 h using a growth medium buffered by citrate at pH 5.5 (Table 5). Rodrigues et al. (2003) studied the effect of different pH values (3.5, 5.5 and 7.5) on *Candida guilliermendii* FTI 20037 and found that the maximum values of xylitol volumetric productivity and xylose volumetric consumption were attained at pH 5.5. Abdel-Aziz et al., 2005) showed that, the optimum value of the initial pH depends on the employed type of yeast, where xylitol production was enhanced at pH 5.5 for *C. tropicalis*, Debaromyces *hansenii* and *C. guilliermendii*, while the production enhanced at pH 4.5 for *Candida shehatae.* These results are in accordance with that obtained from this work, where the pH value of 5.5 was the optimum for xylitol production.

The final pH was decreased by a mean value (ΔpH) of 2.813 for the unbuffered medium, by 0.716 for the phosphate buffered medium and finally, by 0.412 for the citrate buffered medium (Table 5). A direct correlation was found between the xylitol production and the decrease in pH values where it was increased by decreasing ΔpH values. This decrease in pH values was also reported by Silva et al. (1998) in fermentation medium without pH control and by Abdel-Aziz et al. (2005), where they found a decrease in the pH values by about 2 to 4° in the case of *C. shehatae* under unbuffered growth conditions. Sanchez et al. (1997) reported that, the pH of the fermentation medium determines the metabolic activity of the cell, thus, a modification in the pH might cause some micronutrients to precipitate and so become unavailable for assimilation. Raising the external pH closer to the intracellular pH places less stress on cells and less energy is wasted in maintaining the internal pH within a range optimal for growth (Thomas et al., 2002)

Average effects and the analysis of variances (ANOVA) of using different buffer systems, pH values, incubation times and their interactions, all showed a “P” value of less than 0.0001, indicating a highly significant response (Table 6). The highest xylitol content was obtained on using citrate buffer, followed by phosphate buffered and finally, by unbuffered growth media with a significant difference (Table 7). Effect of the incubation periods was also studied and the highest xylitol content was obtained after 72 h followed by 96 h and finally after 48 h with a

Table 5. Experimental design of the initial pH of the growth medium, the buffer system and the incubation time and their effects on xylitol production.

Buffer system	pH initial	Incubation time(h)	pH final	ΔpH	Mean value of ΔpH	Optical density (OD)	Xylitol (g/l)
		48	4.34	0.66		0.125	10.65
	5.0	72	4.4	0.60		0.152	15.35
		96	4.8	0.20		0.127	15.07
		48	4.5	1.00		0.152	12.43
Phosphate	5.5	72	4.56	0.94		0.147	16.09
		96	5.08	0.42		0.156	15.27
		48	5.04	0.96		0.241	14.28
	6.0	72	5.08	0.92		0.253	16.77
		96	5.4	0.60	0.716	0.260	15.74
		48	4.2	0.80		0.136	15.9
	5.0	72	4.52	0.48		0.131	16.2
		96	4.81	0.19		0.140	15.58
		48	4.93	0.57		0.202	15.51
Citrate	5.5	72	5.0	0.50		0.209	18.75
		96	5.24	0.26		0.199	16.31
		48	5.42	0.58		0.290	16.98
	6.0	72	5.3	0.70		0.314	17.26
		96	5.65	0.35	0.412	0.305	16.74
		48	2.49	2.50		0.324	9.79
	5.0	72	2.56	2.44		0.436	11.99
		96	3.27	1.73		0.180	10.05
		48	2.24	3.36		2.107	11.99
Unbuffered	5.5	72	2.02	3.46		2.474	17.67
		96	2.36	3.14		2.525	14.47
		48	3.0	3.00		0.221	9.34
	6.0	72	3.1	2.90		0.246	10.31
		96	3.19	2.99	2.813	0.562	9.07

Initial xylose concentration=20 g/l.

significant difference. On studying the combined effects of using “different buffer systems × different pH values“ (Table 7), it was found that the citrate buffer at pH value of 6.0 gave the highest xylitol content followed by that obtained at pH 5.5 with no significant difference.

The xylitol contents of the medium buffered at pH 6.0 were decreased by extending the incubation time for 96 h (Table 7). This could be attributed to the conversion of xylitol into xylulose by xylitol dehydrogenase (Ikeuchi et al., 1999). Incubation period of 72 h, favored the pH 5.5 over pH 6.0 with no significant difference.

On studying the combined effects of the “buffer systems × incubation periods” (Table 7), citrate buffer gave the highest xylitol contents after 72 h followed by 96 and 48 h and the same effects were also noticed on using phosphate buffered and unbuffered media. These results indicated the favored effect of the buffer types over the incubation time.

Effects of using high xylose concentrations and different volumes of the growth medium

Since xylitol formation in yeasts is most sensitive to substrate concentration and aeration rate, the effect of the initial xylose concentration was further investigated by testing concentrations higher than 30 to 200 gl^{-1} in combination with the effect of using different aeration rates.

Maximum xylitol production (36.25 g/l) was achieved after 72 h by using 50 gl^{-1} of xylose and 40 ml of growth medium, while maximum xylose to xylitol conversion ratio

Table 6. ANOVA for the xylitol production as a function of alteration in the buffer system, pH initial and incubation time and their interactions.

Source	DF	Mean square	P > F
Buffer	2	173.2203420	<.0001
pH	2	26.5399494	<.0001
Incubation time	2	42.6240198	<.0001
Buffer and pH	4	22.1227531	<.0001
Buffer and incubation time	4	5.3555123	<.0001
pH and incubation time	4	4.3396475	<.0001
Error	62	0.2255209	
Correct total	80		

Table 7. Mean values of xylitol production as a function of the buffer types, pH initial and incubation time and their interactions.

Buffer type	Xylitol content	pH initial	Xylitol content	Incubation time (h)	Xylitol content	Buffer type and pH initial	Xylitol content	Buffer type and incubation time(h)	Xylitol content	pH and incubation time(h)	Xylitol content
Citrate	16.577 ^{A}	pH 5.5	15.3422 ^{A}	72	15.5319 ^{A}	Citrate x pH 6.0	16.981 ^{A}	Citrate x 72	17.406 ^{A}	pH 5.5 x 72	17.295 ^{A}
Phosphate	14.658 ^{B}	pH 6.0	14.0589 ^{B}	96	14.2415 ^{B}	Citrate x pH5.5	16.857 ^{A}	Citrate x96	16.225 ^{B}	pH 5.5 x 96	15.316 ^{B}
Unbuffered	11.557 ^{C}	pH 5.0	13.3915 ^{C}	48	13.0193 ^{C}	Citrate x pH 5.0	15.892 ^{B}	Citrate x48	16.098 ^{B}	pH 6.0 x 72	14.748 ^{C}
						Phosphate x pH 6.0	15.587 ^{B}	Phosphate x 72	16.047 ^{B}	pH5.0 x 72	14.551 ^{C}
						Phosphate x pH 5.5	14.698 ^{C}	Phosphate x 96	15.353 ^{C}	pH 6.0 x 96	13.855 ^{D}
						Unbuffered x pH 5.5	14.470 ^{C}	Unbuffered x72	13.141 ^{D}	pH 6.0 x 48	13.572 ^{D}
						Phosphate x pH5.0	13.687 ^{D}	Phosphate x48	12.573 ^{E}	pH 5.0 x 96	13.5522^{D}
						Unbuffered x pH 5.0	10.594 ^{E}	Unbuffered x 96	11.145 ^{F}	pH 5.5 x 48	13.414 ^{D}
						Unbuffered x pH 6.0	9.607 ^{F}	Unbuffered x 48	10.385 ^{G}	pH5.0 x 48	12.071 ^{E}

Means with the same letters are not significantly different.

(98%) was achieved after 96 h by using 25 gl^{-1} (Table 8).

On considering the average effects and the analysis of variances (ANOVA) of the independent variables (initial volume, xylose concentration and incubation time), each of them showed a “P” value of less than 0.0004 indicating a highly significant response towards the xylitol produc-tion. Also, the average effects of the different factors inte-ractions, all have a “P” values less than 0.0001 indicating a highly significant responses towards the xylitol production (Table 9).

Small change in oxygen availability induce changes in yeast metabolism and consequently in xylitol excretion (Martinez et al., 2000), this influence is likely to be related to the generation of co-factors essential for the activity of xylose reductase and xylitol dehydrogenase as well as for ATP production during oxidative phosphor-ylation. Generally, by increasing the volume of the growth medium, the xylitol content was increased(Table 10), with a significant difference on using 40 ml against 30 ml, while a non significant difference was found by using 30 ml

Table 8. Experimental design of the growth medium volume, xylose concentration and incubation time and their effects on xylitol production.

Volume of growth medium (ml)	Initial xylose concentration (gl^{-1})	Incubation time(h)	pH final	Optical density (OD)	Xylitol concentration (gl^{-1})
20	25	72	4.65	2.451	10.05
		96	4.84	2.433	19.75
	50	72	4.67	2.013	27.85
		96	4.91	2.408	30.17
	75	72	4.8	1.641	21.24
		96	4.6	2.397	22.15
	100	72	4.82	1.721	11.36
		96	4.55	2.324	17.64
	125	72	4.75	1.453	16.54
		96	4.47	2.310	16.54
	150	72	4.55	1.609	15.53
		96	4.22	2.181	12.83
	175	72	4.42	1.723	11.54
		96	4.76	2.12	13.79
	200	72	4.56	1.51	8.77
		96	4.6	2.00	8.54
30	25	72	4.87	2.165	20.23
		96	4.74	2.88	16.15
	50	72	4.68	2.268	20.14
		96	4.89	2.453	25.91
	75	72	4.66	1.776	19.2
		96	4.68	2.367	28.45
	100	72	4.96	1.735	14.55
		96	4.55	2.198	17.69
	125	72	4.65	1.759	14.11
		96	4.82	2.177	14.5
	150	72	4.77	1.574	13.63
		96	4.26	2.258	16.17
	175	72	4.55	1.862	10.93
		96	4.58	2.05	11.84
	200	72	5.54	1.661	10.86
		96	4.62	2.017	11.68

Table 8. Contd.

40	25	72	4.82	2.155	17.04
		96	4.82	2.325	24.51
	50	72	4.68	2.218	24.42
		96	4.7	2.407	36.25
	75	72	4.7	2.064	21.24
		96	4.64	2.436	30.31
	100	72	4.72	1.858	15.19
		96	4.86	2.151	19.59
	125	72	4.62	1.565	9.09
		96	4.63	2.199	16.04
	150	72	4.69	1.686	9.92
		96	4.73	2.09	15.12
	175	72	4.65	1.839	11.93
		96	4.65	2.022	12.44
	200	72	4.53	1.825	11.61
		96	4.51	1.981	10.12

Table 9. ANOVA for xylitol production as a function of alteration in the volume of growth medium xylose concentration and incubation time.

Source	DF	Mean square	P > F
Volume	2	26.696527	0.0004
Xylose	7	630.744890	<.0001
Incubation time	1	411.68	<.0001
Volume and xylose	14	29.807146	<.0001
Volume and incubation time	2	41.409394	<.0001
Xylose and incubation time	7	29.293110	<.0001
Error	110	3.206521	
Corrected total	143	5938.179300	

against 20 ml. In related studies, Nolleau et al. (1993) and Winkelhausen et al. (1996) reported that reduced aeration levels favored xylitol over ethanol production by *C. boidinii, C. guilliermondii* and C. *parapsilosis.*

The maximum xylitol production was obtained by using 50 gl^{-1} followed by 75 and 25 gl^{-1}, with a significant difference between the obtained results (Table 10). Rosa et al. (1998) investigated the effect of initial xylose concentration on *C. guilliermondii* FTI20037 and found that, xylose concentration of 15 to 60 gl^{-1} was the best for the production of xylitol. The correlation between xylitol accumulation and xylose concentrations may be a consequence of an oxygen reduction, resulting from high cell densities of highly concentrated substrates (Silva and Afschar, 1994).

By studying the effect of increasing xylose concentrations more than 100 g/l (up to 200 g^{-1}), the xylitol content values were decreased with no significant difference (Table 10). Extremely high initial xylose concentrations would be detrimental to the xylitol yield and this could be attributed to the generated osmotic stress, which could be induced in the microorganisms by the excess amount of sugar in the medium (Walther et al., 2001). Vongsuvanlert and Tani (1989) observed that, in cultures of *C. boidinii* there was a significant reduction in the xylitol concentration (from 36 to 18 gl^{-1}) when the xylose concentration was increased from 100 to 150 g^{-1}, a fact probably caused by osmophilic effects or substrate

Table 10. Mean values of the xylitol contents as a function of volume of growth medium, xylose concentration and incubation time and their interactions.

Incubation time (h)	Xylitol content	Xylose Concentration	Xylitol content	Initial volume	Xylitol content	Initial volume (ml) and xylose + concentration (gl-1)	Xylitol content	Xylose concentration (gl^{-1}) + incubation time (h)	Xylitol content	Initial volume + incubation time	Xylitol content
9	18.9150 A	50	27.6817 A	40	18.0817 A	40 x 50	30.678 A	50 x 96	31.025 A	40 x 96	20.845 A
72	15.5333 B	75	24.0883 B	30	16.8640 B	20 x 50	29.170 A	75 x 96	27.256 B	30 x 96	18.020 B
		25	18.2267 C	20	16.7269 B	40 x 75	26.142 B	50 x 72	24.337 C	20 x 96	17.879 B
		100	16.2928 D			30 x 75	24.175 BC	75 x 72	20.920 D	30 x 72	15.707 C
		125	14.7511 E			30 x 50	23.197 DC	25 x 96	20.501 D	20 x 72	15.574 C
		150	14.0833 E			20x 75	21.948 DE	100 x 96	18.668 E	40 x 72	15.318 C
		175	12.2261 F			40x 25	21.008 E	25 x 72	15.952 F		
		200	10.4433 G			30 x 25	8.497 F1	125 x 96	15.82 F		
						40 x 100	17.747 F	150x 96	14.957 GF		
						20 x 125	16.83 FG	100 x 72	13.916 GH		
						30 x 100	6.507 GFH	125 x 72	13.682 GH		
						20 x 25	15.175 GIH	150 x 72	13.208 GHI		
						30 x 150	15.150 GIH	175 x 96	12.774 HI		
						20 x 100	625 GJIH	175 x 72	11.677 JI		
						30 x 125	14.502 JIH	50 x 96	10.571 J		
						20 x 150	14.360 JIH	75 x 96	10.315 J		
						20 x 175	12.955 KJI				
						40 x 125	12.922 KJI				
						40 x 150	12.740 KJ				
						40 x 175	12.295 KJ				
						30 x 200	11.457 K				
						30 x 175	11.428 K				
						40 x 200	11.122 K				
						20 x 200	8.752 L				

Means with the same letters are not significantly different.

repression of xylose-metabolizing enzymes. In contrast to our results, Gong et al. (1981) noticed that *C. tropicalis* HXP2 produced more xylitol when the xylose concentration was increased from 5 to 20%.

The mean value of the xylitol production was increased significantly by increasing the incubation time from 72 to 96 h (Table 10). Generally, by increasing the growth medium volume within a xylose concentration ranged from 25 to 100 g^{-1}, an increase of the xylitol production was recognized (Table 10). By using xylose concentrations of 50 and 75 g^{-1} within a growth medium volume ranged from 20 to 40 ml, maximal xylitol production values were achieved.

Mean value of the xylitol production was increased significantly by increasing the incubation time from 72 to 96 h and the volume of growth medium from 20 to 40 ml (Table 10). There is no significant difference in the mean values of xylitol production on using different volumes of growth

medium at incubation time of 72 h, while after 96 h incubation period, there is a significant difference on using 40 ml growth medium in comparison with 30 and 20 ml (Table 10).

After incubation times of 72 and 96 h, increasing the xylose concentrations led to a decrease in the xylitol production, with the exception of 25 gl^{-1} that gives lower xylitol content than that of 50 and 75 gl^{-1} (Table 10).

Conclusions

Applying of the statistical factorial design experiments leads to improving the tolerance of the *C. tropicalis* yeast to the high xylose concentrations with enhanced xylose to xylitol conversion ratios. Maintaining the pH value at 5.5 by using citrate buffer leads to a raise in the xylose to xylitol conversion ratio from 57.2 to 93.75% by using 50 gl^{-1} of xylose. Further enhancement (98%) could be achieved by decreasing the aeration rate by using 40 ml as a volume of medium and decreasing the xylose concentration to 25 gl^{-1}.

REFERENCES

Abdel-Aziz ABE, Elzawahry YA, El-Fouly MEZ, El-Mongy TM, Mehany A (2005). Effect of certain environmental factors and radiation on xylitol production from rice straw by yeasts. PhD thesis. Microbiology dept., National Center For Radiation Research and Technology (NCRRT).

Ahearn DG (1974). Identification and Ecology of yeasts of medical importance. In Prier JE and Fried man (eds), opportunistic pathogens. Baltimore, University, Park presses, pp. 129-146.

Ahearn DG (1978). Medically important yeasts. Ann. Rev. Microbiol. 32: 59-68.

Barnett JA, Payne RW, Yarrow D (2000). Yeasts: characteristics and identification, 2nd Ed. Cambridge Univ. Press Cambridge.

Biswas S, Vashishtha N (1998). Xylitol and business opportunities. Chem. Eng. World, 33: 103-108.

Emidi A (1978). Xylitol, its properties and food applications. Food Technol. 32: 20-32.

Gong C, Chen LF, Tsao GT (1981). Quantitative production of xylitol from D-xylose by a high-xylitol producing yeast mutant *Candida tropicalis* HXP2. Biotechnol. Lett. 3(3): 125-130.

Grenby TH, Colley J (1993). Dental effects of xylitol compared with other carbohydrates and polyols in the diet of laboratory rats. Arch. Oral Biol. 28(8): 745-758.

Ikeuchi T, Azuma M, Kato JO (1999). Screening of microorganisms for xylitol production and fermentation behavior in high concentrations of xylose. Biomass Bioeng. 16: 333-339.

Kampen WH (1997). Nutritional requirements in fermentation process.In Fermentation and Biochemical Engineering Handbook. Principles, Process Design and Equipment. 2nd edn. William Andrew Publishing/Noye.

Lodder J (1971). The yeast:A taxonomic Study.2nd edn.North Holland Publ. Co. Amesterdam 12. Makinen KK (2000a). Can the pentitol-hexitol theory explain the clinical observations made with xylitol. Med. Hypothesis, 54: 603-613.

Makinen KK (2000b). The rocky road of xylitol to its clinical application. J. Dental Res. 79: 1352-1355.

Martinez EA, Silva SS, Felipe MGA (2000). Effect of the oxygen transfer coefficient on xylitol production from sugarcane bagasshydrolysate by continuous stirred-reactorfermentation. Appl. Biochem. Biotechnol. 84/86: 633-641.

Nolleau V, Preziosi-Belloy l, Delgenes JP, Navarro JM (1993). xylitol production from xylose by two yeast strans:sugar tolerance. Curr. Microbiol. 27: 191-197.

Parajo JC, Dominguez H Domingguez JM (1998). Biotechnological production of xylitol. Part1: Interest of xylitol and fundamentals of its biosynthesis. Bioresour. Technol. 65: 191-201.

Rodrigues DC, Silva SS, Felipe MGA (1998). Using response-surface methodology to evaluate xylitol production by *Candida guilliermondii* by fed-batch process with exponential feeding rate. J. Biotechnol. 62: 73-77.

Rodrigues DC, Silva SS, Ahmeida E, Silva JB, Vitolo M (2003). Xylose reductase activity of *Candida guilliermondii* during xylitol production by Fed-fermentation. Appl. Biochem. Biotechnol. 98-100: 783-785.

Rosa SA, Filipe MGA, Silva SS, Vitolo IC (1998). Xylose reductase production by *Candida guilliermondii*. Appl. Biochem. Biotechnol. 70-72: 127-160.

SAS. Statistical analysis system, SAS user`s guide statistics. SAS Institute 2006. Inc. Editors, Gary, NC.

Sanchez J (1998). Colorimetric assay of alditol in complex biological samples. J. Agric. Food Chem. 46: 157-160.

Sanchez S, Bravo V, Castro E, Moya AJ, Camacho F (1997). The influence of pH and aeration rate on the fermentation of D-xylose by *Candida shehatae*. Enzyme Microbial Technol. 21: 355-360.

Silva SS, Afschar AS (1994). Micorbial production of xylitol from D-xylose using *Candida tropicalis*. Process Biochem. 11: 129-134.

Silva SS, Felipe MGA, Mancilha IM (1998). Factors that affect the biosynthesis of xylitol by xylose-fermenting yeasts. Appl. Biochem. Biotechnol. 70-72: 331-339.

Sreenivas Roa R, Prakasham RS, Krishna Prasad K, Rajesham S, Sarma PN, Venkateswar Rao L (2004). Xylitol production by *Candida* sp: parameter optimization using Taguchi approach. Process Biochem. 39: 951-956.

Thomas KC, Hynes SH, Ingledew WM (2002). Influence of medium buffering capacity on inhibition of *Saccharomyces cerevisiae* growth by acetic and lactic acids. Appl. Environ. Microbiol. 68(4): 1616-1623.

Trinder P (1975). Micro-determination of xylose in plasma. Analyst. 100 (1186): 12-15.

Uhari M, Kontiokari Niemela T, Novel MA (1998). Use of xylitol sugar in preventing acute otitis media. Pediastrics, 102: 879-884.

Vongsuvanlert V, Tani Y (1989). Xylitol production by methanol yeast *Candida boidinii* (klocckera Sp) No. 2201. J. Ferment. Bioeng. 67: 35-39.

Walther T, Hensirisak P, Agblevor FA (2001) . The influence of aeration and hemicellulosic sugars on xylitol production by *Candida tropicalis*.Bioresour. Technol. 76(3): 213-220.

Winkelhausen E, Pittman P, Kuzmanova S, JeffriesTW (1996). Xylitol formation in oxygen limited chemostat culture. Biotechnol. Lett. 18: 753-758.