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Biotechnological production of xylitol by mutant *Candida tropicalis* OMV5: Process optimization using statistical approach

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An orthogonal experimental design L_{16} (2^5) was used to investigate effects of key media components namely xylose, yeast extract, peptone, urea and inoculum size on the production of xylitol by a mutant strain of *Candida tropicalis* (CT-OMV5). Software for automatic design and analysis of the experiments, based on Taguchi approach was used. Optimal levels of key media components were also determined. Among the tested parameters, xylose and urea contributed higher influence and yeast extract concentration also played an important role in the conversion of xylose to xylitol. Application of Taguchi method helped in easy process optimization and higher xylitol yield. The increased yield was possible at lower levels of yeast extract and peptone. The yield of xylitol under these optimal conditions was 0.89g/g of xylose.

Keywords: *Candida tropicalis* OMV5, mutant, orthogonal array, statistical optimization, Taguchi methodology, xylose, xylitol

Introduction

There is an established commercial demand for bulk sugar substitutes that are suitable for diabetics and are non-cariogenic. In this field, one of the most promising sweetener is xylitol. A number of studies have shown the beneficial effects of xylitol as a sweetener when used alone or formulated in combination with other sugars[1-7].

Control of blood glucose, lipids and weight are the three major goals of diabetes management today. Xylitol is slowly absorbed, therefore, when xylitol is used the rise in blood glucose and insulin response associated with the ingestion of glucose is significantly reduced. A further useful property is that it does not need insulin to regulate its metabolism and, therefore, can be used as a sucrose substitute in diabetic foods. The reduced caloric value (2.4 calories/g versus 4.0 for sugar) of xylitol is consistent with the objective of weight[1].

The most notable property of the xylitol is that it is completely safe for the teeth. This has been demonstrated in the repeated clinical and field studies. The usage of xylitol has been associated with a significant reduction of dental caries and plaque formation. All clinical trials carried out on the cariologic effects of xylitol have provided essentially similar findings[8-9]. The results have been similar to the way the caries activity has been expressed, the method of xylitol administration, and the prevalence of the disease. In other words, xylitol has been effective against coronal caries and root-surface caries. The presence of xylitol in foods, chewing gums, soluble dragees or related products as well as in toothpaste has been almost equally effective. Similar results have been obtained while studying relatively healthy populations enjoying systematic prophylactic care and more seriously diseased populations with limited access to preventive and restorative care. The overall effect of the xylitol has been of the same approximate order of magnitude when caries rates or caries indexes have been measured. Several of these observations have been associated with simultaneous reduction in the counts of *Streptococcus mutans* and lactobacilli present in saliva and/or dental plaque and with reduced growth of dental plaque. Xylitol seems to weaken the cariogenicity of dental plaque by diminishing it's adhesivity and acidogenic potential[10-12.]

These ideal characters of this chemical have been recognized and approved as alternative sugar substitute in a varied sector of applications by Food and Drug Administration (FDA), European Union, and Japanese Ministry of Health and Welfare (JMHW) and World Health Organization (WHO)[13-14]. In fact, the National Institutes of Health (NIH), USA, issued a consensus statement as "Non-cariogenic sweeteners have been delivered to teeth as constituents of chewing gum, hard candy, and

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dentifrices. The evidence for sorbitol and xylitol are positive, although the evidence for xylitol is stronger" emerged from a Consensus development conference entitled "Diagnosis and management of dental caries throughout life" (26-28 March 2001 at Bethesda, MD,USA). Xylitol is currently approved for the usage in foods, pharmaceuticals and oral health products in more than 35 countries.

Xylitol is currently produced chemically on a large scale. The chemical method of xylitol production is based on the catalytic hydrogenation of D-xylose or xylose-rich hemicellulose hydrolysate[15]. This chemical synthesis requires extensive purification of substrate, high temperature and pressure[16]. Despite a wide range of applications, the use of xylitol as a sweetener is limited. Comparatively, a high production cost (7$ per kg) seems responsible for its limited market share as a sweetener. This has encouraged the development of improved technologies that are able to reduce the production costs. The biotechnological process may provide an interesting alternative to the chemical process. The existing drawbacks of conventional xylitol production methods motivated researchers to seek alternative ways for its production. Biotechnological production is lately becoming more attractive since the downstream processing is expected to be cheaper[17-18]. Considerable efforts have been focused on the microbial production of xylitol from D-xylose. Special attention is paid to the fundamentals of xylose metabolism by yeast since it is a key factor affecting the feasibility of the most promising biotechnological method for xylitol production.

Yeasts are widely distributed in nature. Only certain groups of microbial species are known to metabolize the xylose as carbon source (Fig 1). In the last two decades, a lot of effort had been put on investigating the fermentation of xylose by different microorganisms[19-22]. The reason is that xylose is one of the most abundant sugar monomers in lignocellulosic biomass. The possibility of large-scale xylitol production from this sugar is therefore of great economic interest.

Although various media have been used to culture xylitol-producing yeasts, a few generalizations can be made. For some yeasts, yeast extract is an important nutrient for xylitol production. For other yeasts, yeast extract has no significant effect on xylitol formation. These yeasts prefer urea or urea and casamino acids.

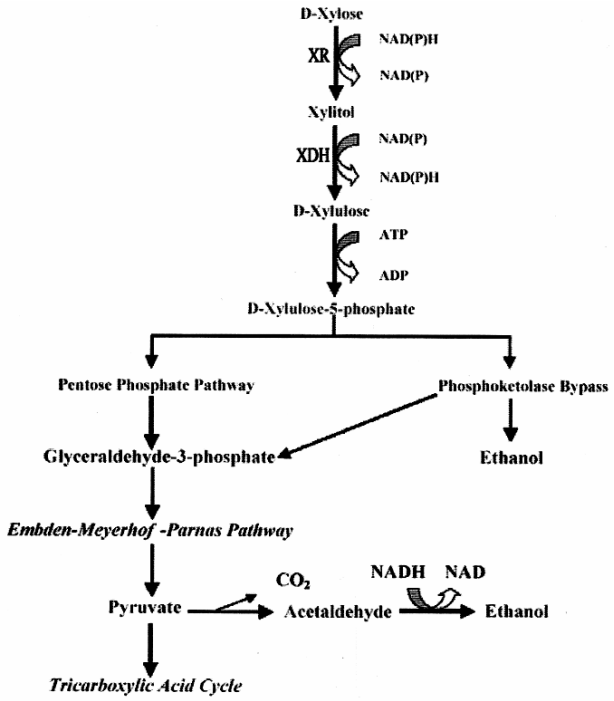

Fig. 1—Simplified scheme of xylose metabolism by yeasts.

Traditional experimental design often uses one factor at a time approach. Only one control factor of a system is allowed to vary, while the other factors are kept fixed during each trial. In this way a lot of experiments are required. Statistical methods have advantages over conventional methodologies in predicting the accurate results basically due to utilization of fundamental principles of statistics, randomization, and replication.

Many Japanese manufacturers have used the Taguchi method and improved product and process qualities with unprecedented success. It created significant changes in several industrial organizations in the USA. The Taguchi method is a statistical method for design of factorial experiments and analysis of the results. It can efficiently reduce the number of experiments[23,38]. In the present investigation, we have optimized xylitol production using a mutant strain of *Candida tropicalis* OMV5 by Taguchi methodology to understand the effects of variables that pose an impact on xylose conversion. The effects of five variables, xylose, urea, yeast extract, peptone and inoculum level were studied on xylitol production using the software for automatic analysis of experimental results. Each variable was tested based on our previous experience over a range and fixed the levels in this design.

Materials and Methods

Microorganism and Culture Conditions

The microorganism used in this study was a mutant *Candida tropicalis* (CT-OMV5). The organism was grown by incubating in an orbital shaker (250 rpm), at 33°C for 48 h and maintained on YM agar slants by subculturing at regular intervals.

All the combination experiments using the assigned parameter values (Table 1) were conducted using YEPX (Yeast extract peptone xylose) media substituted with 10 g/L glucose, 1.0 g/L $(NH_4)_2SO_4$, and 0.5 g/L $MgSO_4.7H_2O$ for inoculum preparation and for fermentation and further incubated in an orbital shaker (250 rpm) at 33°C. The Erlenmeyer flasks of 250 mL capacity, containing 100 mL of fermentation medium (*p*H 5.0) were maintained under agitation of 250 rpm at 33°C for 48 h. After 48 h of fermentation, the culture broth was separated and analyzed for xylitol (Table 1).

Analytical Methods

Sugar and sugar alcohols in the culture broth were measured by HPLC using an ion moderated partition chromatography sugar column SHODEX SC 1011 (8 mm X 300 mm). The samples were eluted with HPLC water at a flow rate of 0.5 mL/min at 80°C and detected with a differential refractometer (WATERS 410).

Experimental Design with Taguchi Method

Some of the operating or physical parameters have been previously verified for their values in enhancing the output of xylitol production i.e. temperature, *p*H, agitation, concentration of nutrients. These have been fixed as constant for experimentation purpose. The following nutrients determined the cost effectiveness of the production of xylitol. Five variables like xylose, urea, yeast extract, peptone, and inoculum with two levels have been considered for experimentation to find out their effect on the xylitol yield and maximize the out put (Table 2).

Five variables with factorial design of experiments need 32 experiments. On the contrary, Taguchi methods suggest the use of L_8 or L_{16} for variables with two levels each, where we need to conduct initially only 8 or 16 experiments, respectively. Though L_8 offers only 8 experiments, but the second order interactions will be overlapped by another second order interaction and also the main variables will be confounded by the second order interactions which are not desirable and the accuracy will be suffered.

Since the second order interactions are common among this type of processes, it is required to know the effect of all second order interactions. Hence L_{16} has been used which is resolution V, where all second order interactions can be analyzed clearly and the main effect of variables is not confounded by the second order interaction. Further, four replicates also planned at mid points to find out the significance of interactions and variables.

The experimental data collected with the above design was fitted to the following equation to create response surface:

$$Y = \beta_0 + \sum_{i=1}^{5} \beta_i x_i + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \beta_{ij} x_i x_j \quad \ldots (1)$$

Where Y is the response (yield of xylitol), β_0 is the constant, β_i is the linear term coefficient, β_{ij} is the coefficient of the interaction and X represents the input variables. The experimental data collected is given in Table 1 and used to evaluate the model coefficients using SIGMA TECH, a software

Table 1—Taguchi method L_{16} for planning experiments for data collection

Experiment No.	1	2	3	4	5	Xylitol yield (g/g of xylose)
1	1	1	1	1	2	0.83
2	1	1	1	2	1	0.87
3	1	1	2	1	1	0.83
4	1	1	2	2	2	0.77
5	1	2	1	1	1	0.87
6	1	2	1	2	2	0.87
7	1	2	2	1	2	0.86
8	1	2	2	2	1	0.87
9	2	1	1	1	1	0.71
10	2	1	1	2	2	0.84
11	2	1	2	1	2	0.74
12	2	1	2	2	1	0.73
13	2	2	1	1	2	0.84
14	2	2	1	2	1	0.81
15	2	2	2	1	1	0.77
16	2	2	2	2	2	0.79

Table 2—Factors and their levels for experimentation

No.	Factors	Lower level 1	Higher level 1	Midpoint 0
1	Xylose (% w/v)	3.0	5.0	4.0
2	Urea (% w/v)	0.5	1.0	0.75
3	Yeast extract (%w/v)	0.5	1.0	0.75
4	Peptone (%w/v)	1.0	1.5	1.25
5	Inoculum (% v/v)	5.0	7.0	6.0

package. The F test was also done to find out the significance of the interactions and variables on the process, while fitting the model.

Results and Discussion

Analysis of Experimental Data

The analysis of experimental data indicated that xylose, urea, yeast extract are very prominent in affecting the yield, because xylose, urea and yeast extract individually gave maximum sum of squares (SS%) more than other factors and their interactions (Table 3). Though the inoculum effect was not substantial, but its interaction with xylose was prominent and contributed to 13% sum of the squares (SS%) and all other interactions were very small compared to the coefficients of main variables, hence the effect of main variables was predominant[24]. To be more precise, the F test was also conducted and the results are given in Table 4. Except xylose × urea, xylose × yeast extract and urea × inoculum interactions all other interactions had some impact on the yield.

Table 3—Analysis of the experimental data

No.	Designated	Coefficient	SS%
1	Constant	0.8125	
2	Peptone	0.0063	1.0
3	Yeast extract	-0.0175	11.0
4	Peptone x yeast extract	-0.0113	5.0
5	Urea	0.0225	18.0
6	Urea x peptone	-0.0062	1.0
7	Urea x yeast extract	0.0050	1.0
8	Xylose x inoculum	0.0187	13.0
9	Xylose	-0.0338	41.2
10	Xylose x peptone	0.0075	2.0
11	Xylose x yeast extract	-0.0038	0.6
12	Urea x inoculum	0.0000	0.0
13	Xylose x urea	0.0013	0.2
14	Yeast extract x inoculum	-0.0100	4.0
15	Peptone x inoculum	0.0063	1.0
16	Inoculum	0.0050	1.0

Model Fitting and Optimization Condition

The coefficients of the model given by equation – (1) were determined by fitting the experimental data. The statistical analysis showed that the coefficient of determination (R^2) of the model was 0.99, which indicated an adequate precision that the model could be used to predict the output. The best fitting model for the yield of xylitol is,

Table 4—F test and interactions

No	Factors	Coefficients	SS=MS	F value	F std 0.05	F value/F std	SS%
1	Constant	0.8125					
2	Xylose	-0.03375	0.018225	546.75	10.1	54.13366	41.13995
3	Urea	0.0225	0.0081	243	10.1	24.05941	18.28442
4	Yeast extract	-0.0175	0.0049	147	10.1	14.55446	11.06095
5	Peptone	0.00625	0.000625	18.75	10.1	1.856436	1.410835
6	Yeast extract x peptone	-0.01125	0.002025	60.75	10.1	6.014851	4.571106
7	Urea x peptone	-0.00625	0.000625	18.75	10.1	1.856436	1.410835
8	Urea x yeast extract	0.005	0.0004	12	10.1	1.188119	0.902935
9	Xylose x inoculum	0.01875	0.005625	168.75	10.1	16.70792	12.69752
10	Xylose x peptone	0.0075	0.0009	27	10.1	2.673267	2.031603
11	Xylose x yeast extract	-0.00375	0.000225	6.75	10.1	0.668317	0.507901
12	Urea x inoculum	0	0	0	10.1	0	0
13	Xylose x urea	0.00125	0.000025	0.75	10.1	0.074257	0.056433
14	Yeast extract x Inoculum	-0.01	0.0016	48	10.1	4.752475	3.611738
15	peptone x inoculum	-0.00625	0.000625	18.75	10.1	1.856436	1.410835
16	inoculum	0.005	0.0004	12	10.1	1.188119	0.902935
			0.0443				
						Insignificant	100

Table 5—Simulation by steepest ascent method

No.	X1 Xylose	X2 Urea	X3 Yeast extract	X4 Peptone	X5 Inoculum	Xylitol yield (g/g of xylose)
1	3.5	0.84	0.68	1.27	6.09	0.8412
2	3.0	0.92	0.61	1.28	6.18	0.8650
3	2.5	1.00	0.54	1.30	6.28	0.8850
4	2.0	1.09	0.48	1.32	6.36	0.9007
Actual	2.0	1.09	0.48	1.32	6.36	0.8900

$$Y = 08125 - 0.0337\,X1 + 0.0225\,X2 - 0.0175\,X3 + 0.0063\,X4 + 0.0050\,X5 + 0.0075\,X1X4 + 0.0185\,X1X5 + 0.0050\,X2X3 - 0.0063\,X2X4 - 0.0113\,X3X4 - 0.01\,X3X5 - 0.0063\,X4X5. \quad \ldots (2)$$

Where X1, X2, X3, X4 & X5 represent xylose, urea, yeast extract, peptone and inoculum, respectively.

Model Simulation and Confirmation

The model (2) was used to simulate through the steepest ascent method and the predicted yields along with the parameters are given in Table 5. A maximum xylitol yield 0.9007 (g/g of xylose) was predicted with the parameters at xylose 2%, urea 1.09%, yeast extract 0.48% and peptone 1.32% and inoculum 6.36%. On verification by conducting experiments with the same parameters the yield of xylitol obtained was 0.89 g/g of xylose. Xylose to xylitol conversion started after 10 h of inoculation and maximum of yield reached by 48 h. Most of the experiments showed a similar pattern (Fig. 2).

Suryadi *et al*[25] tested four yeasts for xylitol production from D-xylose. *Hansenula polymorpha* was found to be the better strain, out of 4 strains tested, which produced 43.2 g/L xylitol from 100g/L D-xylose in 4 d of cultivation with 1% (v/v) methanol supplementation. Urea, $(NH_4)_2SO_4$ and NH_4NO_3 gave the highest yields of xylitol with this yeast.

The effects of different nitrogen sources on xylitol production from D-xylose by *Candida* sp. L-102 had been studied[26]. Maximum xylitol production was obtained with urea as the nitrogen source and a yield of 87% was reported.

The culture media for *Candida parapsilosis* ATCC 28474[27], *C. boidinii* no. 2201[28], *C. guilliermondii* NRC 5578[29] and *C. tropicalis* IFO 0618[30] contained yeast extract in concentrations ranging from 10 to 20 g L^{-1}. Yeast extract at a maximum concentration of 10 g L^{-1} was sufficient for *C. tropicalis* DSM 7524. Concentrations higher than 15 g L^{-1} blocked the conversion of D-xylose to xylitol[31]. Increased concentrations of yeast extract of 5 and 10 g L^{-1} increased the biomass production of *C. guilliermondii* FTI 20037, but sharply decreased its xylitol productivity[32]. Similarly the addition of yeast extract and peptone to the defined medium for *C. mogii* ATCC 18364 enhanced cell growth but had no significant effect on the yield and specific productivity of xylitol[33].

These studies indicate that, understanding the basic needs or optimization of parameters is an important factor in achieving maximum output. The dependence of economic yield and productivity is based on the medium composition especially xylose, yeast extract and peptone. For calculation purposes, the cost of each nutrient and the selling price of xylitol depend on these variables[39]. These variables are important for optimization of xylitol production.

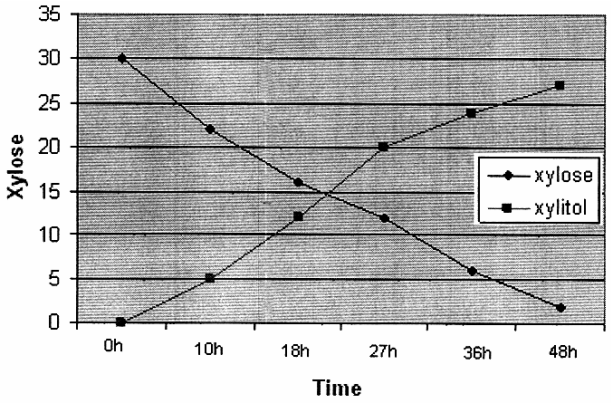

Fig. 2—D-xylose (g/L) utilization and xylitol production (g/L) using YEPX media and incubated in an orbital shaker (250 rpm) at 33°C.

Depending on the microbial system used, xylitol yield ranges from 0.62 to 0.87 g/g of xylose. Sirisansaneeyakul *et al*[33] working with *C. mogii* reported a xylitol yield of 0.62 g/g of xylose. In another study, the xylitol yield was 0.80 g/g of xylose with *C. guilliermondii*[34]. Lu *et al* reported a xylitol yield of 0.87 g/g of xylose with *Candida* sp. L-102 strain. Working with *C. tropicalis*, Kim *et al*[35] reported only 0.75 g/g of xylose. It was observed in the present investigation that after optimization, xylitol yield was found to improve from 0.77 g/g with wild *C. tropicalis*[36-37], to 0.89g/g with mutant CT-OMV5.

Conclusion

A combination of factors and their levels involved in the production of xylitol by *C.tropicalis* OMV5 were identified for maximum yield as indicated in Table 2 (Experment nos 2, 5, 6 and 8). The DOE using the Taguchi approach has proved to be economical in optimization of xylitol production. From the analysis, it is evident that the xylose, urea, and yeast extract contributions were found to be significant. It can be seen from Table 2 that four observations (2, 5, 6 and 8) show same yield (0.87%). That means there is more than one feasible solution. This could be obtained because of the planned experimentation. Out of four observations, experiment

no.5 was more beneficial, in view of low concentrations of nutrients used. Further, steepest ascent path has shown (Table 5) that even at 2% of xylose and 1.32% peptone the yield was higher by about 3%. This could not have been possible to achieve in the conventional method. Mutant strain of *C. tropicalis* OMV5 used in this investigation with Taguchi approach of optimization gave more xylitol (0.89 g/g of xylose) than the parent strain (0.77 g/g of xylose).

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References

1 Fran Gare, The sweet miracle of xylitol, *ISBN* (2003) 1-59120-038-5.

2 Pepper T & Olinger P M, Xylitol in sugar-free confections, *Food Technol*, 10 (1988) 98-106.

3 Makinen K K, Can the pentitol-hexitol theory explain the clinical observations made with xylitol?, *Medical Hypothesis*, 54 (2000a) 603-613.

4 Makinen K K, The rocky road of xylitol to its clinical application, *J Dent Res*, 79 (2000b) 1352-1355.

5 Hayes C, The effect of non-cariogenic sweeteners on the prevention of dental caries: Review of the evidence, *J Dent Educ*, 65 (2001) 1106-1109.

6 Jannesson L, Renvert S, Kjellsdotter P, Gaffar A, Nabi N & Birkhed D, Effect of a triclosan-containing toothpaste supplemented with 10% xylitol on *mutans streptococci* in saliva and dental plaque. A 6-month clinical study, *Caries Res*, 36 (2002) 36-39.

7 Lynch H & Milgrom P, Xylitol and dental caries: An overview for clinicians, *J Calif Dent Assoc*, 31 (2003) 205-209.

8 Maguire A & Rugg-Gunn A J, Xylitol and caries prevention-is it a magic bullet? *Br Dent J*, 194 (2003) 429-436..

9 Richter P & Chaffin J, Army's "look for xylitol first" program, *Dent Assist*, 73 (2004) 38-40.

10 Makinen K K, Makinen P L, Pape H R, Peldyak J, Hujoel P *et al*, Conclusion and review of the Michigan xylitol programme (1986-1995) for the prevention of dental caries, *Int Dent J*, 46 *(*1996) 22-34.

11 Peldyak J & Makinen K K, Xylitol for caries prevention, *J Dent Hyg*, 76 (2002) 276-285.

12 Hildebrandt G & Sparks B S, Maintaining *mutans streptococci* suppression with xylitol chewing gum, *JADA*, 131 (2000) 909-916.

13 Scheinin A, Banoczy J, Szoke J, Esztari I, Pienihakinen K *et al*, Collaborative WHO xylitol field studies in Hungary. I. Three-year caries activity in institutionalized children, *Acta Odontol Scand*, 43 (1985) 327-347.

14 Szoke J, Pienihakkinen K, Esztari I, Banoczy J & Schenin A, Collaborative WHO xylitol field studies in Hangary. V. Three-year development of oral hygiene, *Acta Odontol Scand*, 43 (1985) 371-376.

15 Jyri-Pekka M, Tapio S & Rainer S, Effect of solvent polarity on the hydrogenation of xylose, *J Cheml Tecnhol Biotechnol*, 76 (2001) 90-100.

16 Hyoenen, Koivistoninen L & Voirol H, Food technological evaluation of xylitol, *Food Res*, 28 (1993) 373-403.

17 Whistler R L & Bemiller R D, *Hemicellulose*, in *Industrial gums, polysacchanides and their derivatives* (Academic Press, San Diego) 1993, 295-308.

18 Kim J H, Ryu V W & Seo J H, Analysis and optimization of a two-substrate fermentation for xylitol production using *Candida tropicalis*, *J Ind Microbiol Biotechnol*, 22 (1999) 181-186.

19 Jeffries T W, Utilization of xylose by bacteria, yeasts and fungi, *Adv Biochem Bioeng Biotechnol*, 27 (1983) 1-32.

20 Skoog K & Han-Hagerdal B, Xylose fermentation, *Enzyme Microb Technol*, 10 (1988) 66-80.

21 Bernard A P, Stephanus G K & James C P, Fermentation of D-xylose by the yeasts *Candida shehatae* and *Pichia stipitis*, *Process Biochem*, 24 (1989) 21-31.

22 Hahn-Hagerdal B, Jeppsson H, Skoog K & Prior B A, Biochemistry and physiology of xylose fermentation by yeasts, *Enzyme Microb Technol*,16 (1994) 933-943.

23 Sreenivas Rao R, Prakasham R S, Krishna Prasad K, Rajesham S, Sharma P N & Venkateswar Rao L, Xylitol production by *Candida* sp.: Parameter optimization using Taguchi approach, *Process Biochem*, 39 (2004) 951-956.

24 Davies O L, *The design and analysis of industrial experiments* (Oliver and Boyd, London) 1967, 513-514.

25 Suryadi H, Katsuragi T, Yoshida N, Suzuki S & Tani Y, Polyol production by culture of methanol utilizing yeast, *J Biosci Bioeng*, 89 (2000) 236-240.

26 Lu J, Larry B, Gong C S & Tsao G T, Effect of nitrogen sources on xylitol production from D-xylose by *Candida* Sp.L-102, *Biotechol Lett*, 17 (1995) 167-170.

27 Furlan S A & Boutflond de Castro H F, Influence of oxygen on ethanol and xylitol production by xylose fermenting yeasts, *Process Biochem*, 29 (1994) 657-662.

28 Vongsuvanlert V & Tani Y, Xylitol production by a methanol yeast *Candida boidinii (Kloeckera sp.)* no. 2201, *J Ferment Bioeng*, 67 (1989) 35-39.

29 Meyrial V, Delgenes J P, Moletta R & Navarro J M, Xylitol production from D-xylose by *Candida guilliermonddi:* Fermentation behaviour, *Biotechnol Lett*, 11 (1991) 281-286.

30 Horitsu H, Yahashi Y, Takamizawa K, Kawai K, Suzuki T & Watanabe N, Production of xylitol from D-xylose by *Candida tropicalis:* Optimization of production rate, *Biotechnol Bioeng*, 40 (1992) 1085-1090.

31 Silva S S & Afschar A S, Microbial production of xylitol from D-xylose using *Candida tropicalis*, *Bioprocess Eng*, 11 (1994) 129-134.

32 Silva S S, Quesada-Chanto A & Vitolo M, Upstream parameters affecting the cell growth and xylitol production

by *Candida guillienmondii* FTI 20037, *Z Naturforsch*, 52 (1997) 359-363.

33 Sirisansaneeyakul S, Staniszewski M & Rizzi M, Screening of yeasts for production of xylitol from D-xylose, *J Ferment Bioeng*, 6 (1995) 564-570.

34 Preziosi-Belloy L, Nolleau V & Navarro J M, Xylitol production from aspenwood hemicellulose hydrolysate by *Candida guilliermondii*, *Biotechnol Lett*, 22 (2000) 239-243.

35 Kim J H, Han K C, Koh Y H, Ryu V W & Seo J H, Optimization of fed-batch fermentation for xylitol production by *Candida tropicalis*, *J Ind Microb Biotechnol*, 29 (2002) 16-19.

36 Sreenivas Rao R, Pavan Jyothi Ch, Prakasham R S, Sarma P N & Vekateswar Rao L, Xylitol production from corn fiber and sugar cane bagasse hydrolysates by *Candida tropicalis*, *Bioresour Technol*, 97 (2005) 1974-1978.

37 Sreenivas Rao R, Pavan Jyothi Ch, Prakasham R S, Subba Rao Ch, Sarma P N & Vekateswar Rao L, Xylitol strain improvement of *Candida tropicalis* for the production of xylitol: Biochemical and physiological characterization of wild and mutant strain CT-OMV5, *J Microbiol*, 44 (2006) 113-120.

38 Taguchi, G. *System of experimental design: Engineering methods to optimize quality and minimize cost* (UNIPUB/Kraus International: White Plains, NY) 1987.

39 Bustos G, Moldes A B, Alonso J L & Vazquez M, Optimization of D-lactic acid production by *Lactobacillus coryniformis* using responce surface methodology, *Food Microbiol*, 21 (2004) 143-148.