Pak. J. Sci. Ind. Res. 2003 ***46****(6) 465 - 470*

MICROBIAL PRODUCTION OF XYLITOL FROM ACID TREATED CORN COBS

R F Allam

Chemistry of Natural and Microbial Products Department, National Research Centre, Dokki, Cairo, Egypt

(Received December 12, 2001; accepted September 23, 2003)

The fermentation of xylan hydrolyzate corn cobs by different yeast species revealed the formation of different polyalcohol sugars. Both *Schizosaccharomyces japonicus* and *Kluyveromyces bulgaricus* form xylitol as sole product. Relatively high xylitol pro-duction by *S. japonicus* and *K. bulgaricus* was achieved under static fermentation, among other parameters, initial pH 9 and 8, carbon concentrations 88 - 110 g / l, ammonium chloride 1.0, 2.0 g / l and 10, 15 g / l yeast extract for *S. janponicus* and *K. bulgricus*, respectively were obtained. However, maximal xylitol yields were recorded after 4 days of incubation for *S. japonicus* (18 g / l) and *K. bulgaricus* (16 g / l), respectively.

Key words: Corn cobs, Xylitol, Yeast.

Introduction

Hemicellulose (as xylan) comprises up to 20% of the dry biomass of some lignocellulosic materials, such as corn cobs, with xylose as the major (about 94%) constituent sugar (Jeffries 1983; Welther *et al* 2000; Leathers 2003). The extraction and hydrolysis of xylan component, for example with diluted mineral acid, can be achieved more easily than cellulose hydrolysis and can be regarded as a pretreatment step to enhance subsequent cellulose saccharification (Watson *et al* 1984). The fermentation of D-xylose and other pentose sugars will facilitate the exploitation of plant biomass for the production of xylitol and ethanol (Du Preez *et al* 1986).

Xylitol, a five carbon sugar alcohol, is used as a sweetener in foods and may apply to medical purpose as sugar substitute for the treatment of diabetes (Kitpreechavanich *et al* 1984).

Many yeasts possess xylose reductase which catalyzes the reduction of D-xylose to xylitol as first step in xylose metabolism (Bruinenberg 1986; Kim *et al* 2002). This paper deals with production of xylitol from a cheap carbon source (corn cobs), rich in xylose and outlines some factors affecting its production.

Materials and Methods

Yeast strains. The following yeast strains were examined: *Candida albicans, C. utilis, C. lipolytica* CAIM, *C. lipolytica, C. tropicalis, C. kefyr, Cryptococcus laurentii* Y-2536, *Debaryomyces hansenii, Hansenula polymorpha, Kluyveromyces bulgaricus, Lipomyces lipoferus, Metschnikowia pulcherrima, Nadosenia fluvsence, Pachysolen tannophilus* Y-2460, *Rhodotorula rubra, Saccharomyces cerevisiae, S. cerevisiae* var *eulisaudans, S. diastaticus, S. lipolytica* CAIM 26, *S. lipolytica, S. rouxii* CAIM 21, *S. uvarum, Schizosaccharomyces japonicus, Schizosaccharomyces pombe, Trichosporon cutaneum.*

E-mail: allam01@yahoo.com

Xylan corn cobs hydrolyzate: this was prepared according to Whislher (1963).

Medium and fermentation conditions. The organism was routinely maintained on yeast malt agar medium. A loopful of cells taken from mother slant was transferred to a 250-ml Erlenmeyer flask containing 25 ml of inoculum medium of the following composition (g / l): yeast extract, 5; malt extract, 5; NaCl, 1; xylose, 10; pH 5.5 and shaking at 150 rpm for 24 h at 30°C.

Two ml of freshly cultured yeast suspension was inoculated into test tube (20 x 3 cm), each containing 20 ml of a sterilized medium having the following composition (g / l): NH_4Cl, 1; yeast extract, 5; NaCl, 3; $MgSO_4.7H_2O$, 1; K_2HPO_4, 3 and hydrolyzate xylan corn cobs (containing 10 g / l xylose) at pH 5.5 - 6. The tubes were incubated in inclubator at 30°C for 4 days.

Analytical methods. After removal of the yeast cells by centrifugation, the cell free fermentation broth was analyzed according to Somogyi's method (1952) for reducing sugar and the method of Neish (1952) for polyalcohol contents, respectively.

Isolation and identification of xylitol. After cultivation for 4 days, the culture medium was centrifuged at 500 rpm. The culture filtrate was deproteinized by the addition of 25% zinc sulfate, neutralized to pH 7.5 with 5N NaOH, and then centrifuged. The supernatant was concentrated in vacuum to dryness. The residue was extracted with boiling absolute ethanol and the extract was filtered. The paper chromatography was done to detect the presence of reducing sugars (Moore

Table 1
Screening of some yeasts for xylitol production from hydrolyzed corn cobs

Tested yeasts	Total polyalcohol content (g / l)	Types of detected sugar alcohols			
		Hexitol	Xylitol	Arbitol	Others
Candida albicans	0.20	+	+	+	+
C. utilis	0.27	-	+	-	++
C. lipolytica CAIM	0.26	-	++	+	-
C. lipolytica	0.13	+	++	-	-
C. tropicalis	0.12	-	+	+	+
C. kefyr	0.18	+	+	+	-
Cryptococcus laurentii Y-2536	0.32	+	+	+	+
Debaryomyces hansenii	0.26	+	++	+	-
Hansenula polymorpha	0.20	-	++	+++	+
Kluyveromyces bulgaricus	0.54	-	+++	-	-
Lipomyces lipoferus	0.15	++	-	-	++
Metschnikowia pulcherrima	0.08	+	+	+	-
Nadosenia fluvsence	0.21	-	-	++	+
Pachysolen tannophilus Y-2460	0.30	-	++	+	+
Rodotorula rubra	0.27	+	+	+	-
Saccharomyces cerevisiae	0.23	++	+	-	-
S. cerevisiae var. *eulisaudans*	0.23	-	-	+	+
S. diastaticus	0.24	++	+	+	++
S. lipolytica CAIM 26	0.23	+	-	+ +	+
S. lipolytica	0.24	+	+	+	+
S. rouxii CAIM 21	0.23	+	++	-	+
S. uvarum	0.26	+	++	+	+
Schizosaccharomyces japonicus	0.70	+	+++	-	-
S. pombe	0.25	-	++	+	+
Trichosporon cutaneum	0.23	+	+	+	-

-, negative; +, small amount; + +, medium amount; + + +, large amount.

et al 1960), while xylose and xylitol were analyzed by Shimadzu HPLC (C10) with refractive index detector. The sample (20µl) was injected in shim-pack CLC-NH_2 (6.0 mm i.d.x15 cm) column, the mobile phase acetonitrile / water (7/3) in flow rate: 1.0 ml / min, at 40°C for 30 min.

Results and Discussion

Screening of yeasts for xylitol formation. The tested strains were found to be able to hydrolyze corn cobs as carbon source and produce xylitol and other sugar alcohols, as shown in Table 1. Among them, *Candida utilis, C. lipolytica* CAIM, *Cryptococcus laurentii* Y-2536, *Debaryomyces hansenii, Hansenula polymorpha, Kluyveromyces bulgaricus, Pachysolen tannophilus* Y-2460, *Rhodotorula rubra, Saccharomyces cerevisiae, S. diastaticus, S. uvarum* and *Schizosaccharomyces japonicus* produced total polyalcohols in a relatively good amount. However, *Kluyveromyces bulgaricus* and *Schizosaccharomyces japonicus* were recorded as the best xylitol producers. These two promising yeasts were therefore, selected for further experimentation.

Table 2
Effect of aeration condition on xylitol production

Yeast	Residual sugar (g / l)		Consumed sugar (%)		Xylitol (g / l)	
	Static	Shaked	Static	Shaked	Static	Shaked
Schizosaccharomyces japonicus	16.40	1.50	51.00	95.59	16.30	4.34
Kluyveromyces bulgaricus	10.70	2.60	68.00	92.00	11.90	1.78

- Initial xylose concentration 33.5 g / l.

Effect of cultivation technique. Both static and submerged cultivation techniques were examined for xylitol production by the promising yeasts. The data given in Table 2 revealed the superiority of the static technique. Under this condition, relatively more amounts of xylitol were formed, in spite of the

Table 3
Effect of pH regulation on xylitol production by *S. japonicus* and *K. bulgaricus*

Initial pH	Final pH	*S. japonicus*		*K. bulgaricus*	
		Consumed Sugar (%)	Xylitol (g / l)	Consumed sugar (%)	Xylitol (g / l)
3.00	4.37	48	15.00	43	13.70
4.00	4.57	51	17.60	46	14.80
5.00	5.36	54	19.10	50	15.90
6.00	5.91	57	18.70	54	17.70
7.00	5.84	56	20.30	57	18.70
8.00	6.40	62	20.80	60	19.70
9.00	7.00	61	22.50	58	18.80
10.00	7.71	55	19.80	55	16.50

- Initial reducing sugar 44 g / l.

Table 4
Effect of different nitrogen sources and concentrations on xylitol production by the tested yeasts

Nitrogen source	Nitrogen conc. (g / l)	Consumed sugar %		Xylitol (g / l)	
		S. japonicus	*K. bulgaricus*	*S. japonicus*	*K. bulgaricus*
NH_4Cl	0.50	57	73	10.51	5.50
	1.00	43	66	14.00	5.30
	2.00	44	70	10.70	9.00
Urea	0.50	67	74	7.50	5.40
	1.00	54	70	7.40	5.00
	2.00	66	73	6.50	4.80
$(NH_4)_2SO_4$	0.50	47	45	12.80	10.00
	1.00	66	50	9.40	8.00
	2.00	70	60	8.60	5.80
NH_4NO_3	0.50	70	76	4.30	0.40
	1.00	73	73	4.20	1.40
	2.00	70	69	4.30	1.70
$NH_4H_2PO_4$	0.50	45	55	11.00	8.20
	1.00	35	60	12.30	6.70
	2.00	40	70	10.00	5.70

- NH_4Cl, NH_4NO_3, $NH_4H_2PO_4$, $(NH_4)_2SO_4$ and urea were used at 88 g / l xylose.

assimilation of relatively low xylose levels. As the yeast assimilates xylitol after accumulation as a carbon source in submerged culture, therefore, it is safe to conclude that under the static culture condition, xylose was favourably metabolized to xylitol. This may be because the yeast shows little ability to consume xylitol under limited aeration (Jeffries 1985; Ahmed 1991; Sanchez *et al* 1997; Zagustina *et al* 2001; Walthers *et al* 2001). Our results are also in agreement with the results of Ligthelm *et al* (1988) who reported that xylitol, ribitol and glycerol were formed in high yields under oxygen limitation conditions by *Pachysolen tannophilus*,*C. shehatae* and *Pichia stipitis.* Faria *et al* (2002) reported that *C. guilliermondii* gave 0.71 g of xylitol/g xylose consumed at limited aeration. Also Walthers *et al* (2001) stated that high xylitol was produced (0.62 g/g) by *Candida tropicalis* ATCC 96745 under semi aerobic conditions.

pH regulation. The optimum initial pH values for xylitol production by *S. japonicus* and *K. bulgaricus* were found to be 9 and 8, respectively (Table 3). These results are in agreement with Kitpreechavanich *et al* (1984) who found that the

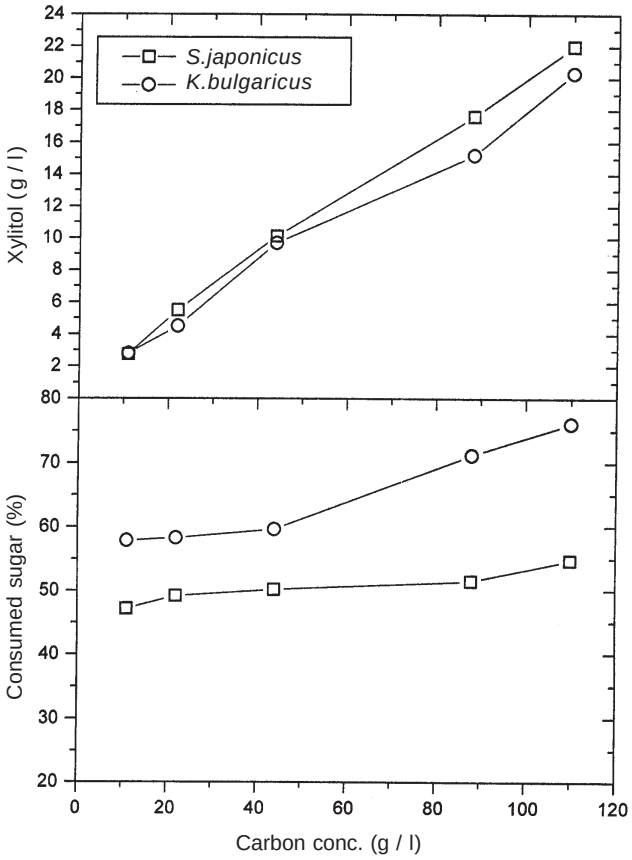

Fig. 1 Effect of hydrolyzed xylan corn cobs concentration on xylitol production.

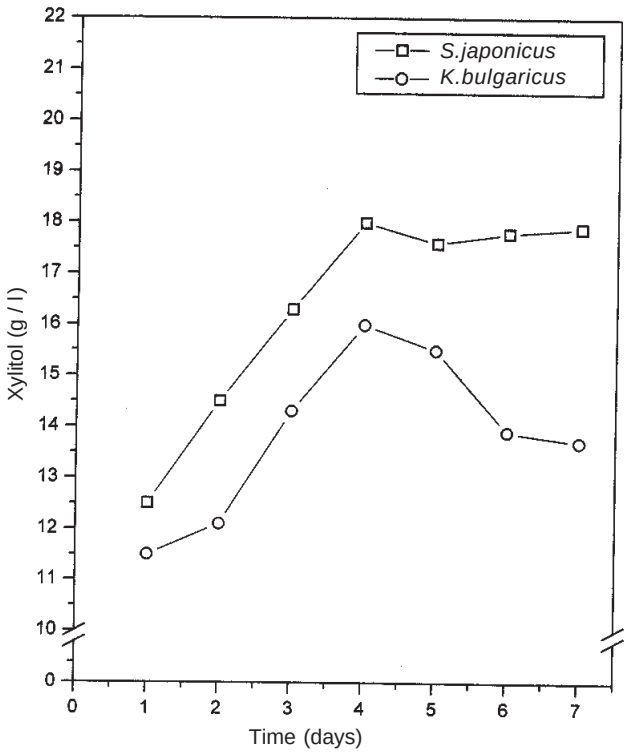

Fig. 2 Time course of xylitol production.

conversion of xylose to xylitol is about 90% at pH 7.5 by *C. pelliculosa.* On the other hand, Du Preez *et al* (1986) reported that *C. shehatae* produced considerable amounts of xylitol at pH 3.5 - 4.5.

Carbon source. The data illustrated in Fig 1 clearly indicates that the formation of xylitol was steadily increased with the increase of the corn cobs. Maximum xylitol outputs were recorded with *S. japonicus* and *K. bulgaricus* at the highest hydrolyzate level namely 110 g / l. Our results are in agreement with the results of Yoshitake *et al* (1973) who reported that the concentration of xylitol increased with the increase of xylose up to 10% in culture medium of *Enterobacter.* Du Preez *et al* (1986) reported that at concentration of 100 g/l they obtained 31.9 g / l xylitol and Zagustina *et al* (2002) reported tht 150 g /l concentration and limited aeration favours the reduction of xylose.

Nitrogen nutrition. Different sources and concentrations of nitrogen were tested in relation to their effect on the production of xylitol from hydrolyzate xylan corn cobs by the experimental organism. As shown in Table 4, the tested ammonium salts and urea except ammonium nitrate are more suitable for xylitol production. The optimum concentration of NH_4Cl seems to be 1.0, 2.0 g / l, wherein relatively high xylitol yields were maintained at this N_2 level in case of *S. japonicus*

Table 5
Effect of yeast extract concentration on xylitol production

Yeast extract conc. (g / l)	Consumed sugar %		Xylitol g/l	
	S. japonicus	*K. bulgaricus*	*S. japonicus*	*K. bulgaricus*
2.50	47.16	63.52	5.86	4.30
5.00	64.00	66.59	8.63	5.70
10.00	67.85	67.61	12.40	5.90
15.00	74.43	66.59	11.60	9.21

- Yeast extract ranged from 2.5 - 15 g / l; - corn cobs hydrolyzate 88 g / l; - pH 9 and 8 for *S. japonicus* and *K. bulgaricus*, respectively.; - 30˚C for 4 days.

and *K. bulgaricus* wherein $(NH_4)_2SO_4$ the concentration of 0.5 g / l favours xylitol production in both *S. japonicus* and *K. bulgaricus.* Holzer and Witt (1960) reported that ammonium salts seem to stimulate the oxidative pentose-phosphate pathway in *S. cerevisiae.*

Effect of organic nitrogen sources on xylitol production. The results in Table 5 reported the superiority of the medium, corn cobs hydrolyzate and containing of different concentrations of yeast extract for xylitol production. Where, relatively higher xylitol yields were obtained both in case of *S. japonicus* and *K. bulgaricus.* However, maximum yields were obtained by *S. japonicus* at 10 g / l yeast extract and that of 15 g / l for *K. bulgaricus.* In agreement with our results, Ahmed (1991) and Hottori and Suzuki (1974) described that 10 g/l gave the optimal concentration, of mannitol and erythritol. Contrary to these results, Hajany (1964) found that at highest concentration, a very poor arbitol production was obtained.

Time course of xylitol production. The time course of xylitol production by the tested yeasts using the most favourable medium composed of (g / l): NH_4Cl, 1; yeast extract, 5; NaCl, 3; $MgSO_4.7H_2O$, 1; KH_2PO_4, 3 and hydrolyzate xylan corn cobs (unpublished data) was studied (Fig 2). The xylitol production reached maximum after 4 days for both *S. japonicus* (18 g / l) and *K. bulgaricus* (16 g / l) at carbon source xylose (88 g / l) from hydrolyzate corn cobs. In agreement with his results Cao *et al* (1994) found that maximum xylitol was obtained after 96 h from 260 g / l xylose.

Assessment of the presence of xylose and xylitol. Both paper chromatography and HPLC were used to analyze the product present in the alcoholic extract.

The spots were visualized on paper chromatogram by spraying with KIO - benzidine reagent. The spots were closely agreed with authentic xylitol for both strains. While, xylose was detected by aniline hydrogen phthalate reagent. The HPLC analysis assessed the presence of xylose and xylitol which were separated at different time intervals of 7 and 13 min, respectively, identical to the authentic samples.

Conclusion

Schizosaccharomyces japonicus and *Kluyveromyces bulgaricus* were the most potent microorganisms to produce xylitol from hydrolyzate xylan corn cobs using static technique at pH 9, 8 for *S. japonicus* and *K. bulgaricus,* respectively. The maximum productivity was reached on using 110 g / l carbon source and ammonium chloride after four days fermentation. Xylose and xylitol were identical with authentic samples when analyzed using HPLC.

References

Ahmed Y M 1991 Biochemical studies on the use of farm by-products for the production of ketosugars and sugar alcohols by microorganisms. Ph.D. Thesis, Faculty of Agric., Cairo University, Egypt.

Bruinenberg P 1986 The NADP(H) redox couple in yeast metabolism. *Anto. Van Leeuwenhoek* **52** 411 - 429.

Cao N J, Tang R, Gong C S, Chem L F 1994 The effect of cell density on the production of xylitol from D-xylose by-yeast. *Appl Biochem Biotechnol* **45 - 46** 515 - 519.

Du Preez J C, Bosch M, Prior B A 1986 Xylose fermentation by *Candida shehatae* and *Pichia stipitis:* Effect of pH, temperature and substrate concentration. *Enzyme Microb Technol* **8** 360 - 364.

Faria L F, Gimenes M A, Nobrega R, Pereira N Jr 2002 Influence of oxygen availability on cell growth and xylitol production by *Candida guilliermondii*. *Appl. Biochem Biotechnol* **98 - 100** 449 - 458.

Hajany G J 1964 D-Arabitol production by *Endomyces chodati. Appl Microbiol* **12** 87 - 92.

Hattori K, Suzuki T 1974 Production of erythritol by n-alkalne grown yeasts. *Agric Biol Chem* **38** 581 - 586.

Holzer H, Witt I 1960 Bschleunigung des oxidativen pentosephosphatcyclus in Hefezellen durch moniumsalze. *Biochim Biophys Acta* **38** 163 - 164.

Jeffries J E 1983 Utilization of xylose by bacteria, yeasts and fungi. *Adv Biochem Engin Biotechnol* **27** 1 - 32.

Jeffries T W 1985 Emerging technology for fermentation D-xylose. *Trends Biotechnol* **3** 208 - 212.

Kim J H, Han K C, Koh Y N, Ryn, Y W, Sea J H 2002 Optimization of fed batch fermentation of xylitol production by *Candida tropicalis. J Ind Microbiol Biotechnol* **29** 16 - 19.

Kitpreechavanich V, Hayashi M, Nishio N, Nagaishi S 1984 Conversion of D-xylose into xylitol by xylose reductase from *C. pelliculose* coupled with the oxidoreductase system of methanagen strain. *Hu Biotechnol Lett* **6** 651 - 656.

Leathers T D 2003 Bioconversion of maiz residues to value added co-products using yeast like fungi. *FEM Yeast Res* **3** 133 - 140.

Ligthelm M E, Prior B A, Du Preez J C, Brandt V 1988 The oxygen requirement of yeasts for the fermentation of D-xylose and D-glucose to ethanol. *Appl Microbiol Bio-technol* **28** 63 - 68.

Moore W E, Effland M J, Johnson D B, Daughorty M N, Schwerdtfeger E J 1960 Chromatographic analyses of sugar alcohols and glycols. *Appl Microbiol* **8** 169 - 173.

Neish A C 1952 *Analytical Methods for Bacterial Fermentation.* 2nd rev. Natl. Research Council Can., Praire Regional Lab., Saskatoon, Saskatchewan N.R.C. Canada.

Ssnchez S, Bravo V, Castro E, Moya A J, Camacho F 1997 Influ-

ence of pH and aeration rate on the fermentation of D-xylose by *Candida shehatae*. *Enzyme Microb Technol* **21**(5) 355 - 360.

Somogyi M 1952 Notes on sugar determination. *J Biol Chem* **195** 19 - 23.

Walthers T, Hensirisak P, Agblervor F A 2001 Model compound studies: Influence of aeration and hemicellulosic sugars on xylitol production by *Candida tropicalis*. *Appl Biochem Biotechnol* **91 - 93** 423 - 435.

Watson N E, Prior B A, Lategan P M 1984 Factors in acid treated bagasse inhibiting ethanol production from D-xylose by *Pachysolen tannophilus*. *Enzyme Microb Technol* **6** 451 - 456.

Whislher R L 1963 *Methods Carbohydrate Chem.*, **1** 88 - 90.

Yoshitake J, Ishizaki H, Shimamura M, Imai T 1973 Xylitol production by *Enterobacter* sp. *Agric Biol Chem* **35** 905 - 911.

Zagustina N A, Rodonova N A, Mestechkina N M, Secherbukhin V D, Bezborodov A M 2001 Fermentation of xylitol in *Candida guilliermondii* 2581 culture. *Prikl Biokim Mikrobiol* **37** 573 - 577.