

Fermentative Performance of *Candida tropicalis* Kuen 1022 Yeast For D-Xylose and Sunflower Seed Hull Hydrolysate in Xylitol Production

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Abstract

The fermentative ability of *Candida tropicalis* Kuen 1022 yeast to produce xylitol from D-xylose and sunflower seed hull hydrolyste was examined under aerobic conditions. In batch cultures having a synthetic xylose concentrations ranging from 50 gl^{-1} to 100 gl^{-1} , the maximum xylitol yield achieved was 0.26 g/g xylose consumed with a volumetric rate of xylitol production of 0.196 $\text{gl}^{-1}\text{h}^{-1}$ when the initial synthetic sugar concentration was 50 gl^{-1} . The pretreated sunflower seed hull hydrolysate was cultivated with the previously hydrolysate medium adapted yeast cells. The maximum xylitol concentration was 4.72 gl^{-1} , the fermentation time was 96 h, xylitol yield was 0.113 g/g xylose consumed and volumetric productivity was 0.219 $\text{gl}^{-1}\text{h}^{-1}$ for hydrolysate having an initial composition of xylose 66 gl^{-1} and glucose 0.8 gl^{-1} .

Key Words: Xylitol, D-xylose, Sunflower seed hull, *Candida tropicalis*

Candida tropicalis Kuen 1022 Mayasının, Ksilitol Üretimindeki D-Ksiloz ve Ayçiçeği Tohum Kabuğu Hidrolizatını Fermente Etme Performansı

Özet

Bu çalışmada, *Candida tropicalis* Kuen 1022 mayasının aerobik koşullarda D-ksiloz ve ayçiçeği tohum kabuğu hidrolizatından ksilitol üretmedeki fermentasyon yeteneği incelenmiştir. 50 gl^{-1} den 100 gl^{-1} ye değişen bir aralıkta sentetik ksiloz içeren kesikli kültürlerde, erişilen en yüksek ksilitol verimi 0.26 g/g harcanan ksiloz olup, 50 gl^{-1} başlangıç sentetik ksiloz konsantrasyonu ile elde edilmiştir. Bu esnadaki volümetrik ksilitol üretim hızı 0.196 gl^{-1} saat⁻¹ dir. Ön işleme tabii tutulmuş ayçiçeği tohum kabuğu hidrolizatı, önceden hidrolizat ortamına alıştıırılan hücreler ile ekilmiştir. Başlangıçta 66 gl^{-1} ksiloz ve 0.8 gl^{-1} glikoz bileşimine sahip hidrolizattan 96 saat fermentasyon süresinde elde edilen maksimum ksilitol konsantrasyonu 4.72 gl^{-1} , bu sırada, ksilitol verimi 0.113 g/g harcanan ksiloz ve volümetrik üretim hızı ise 0.219 $\text{gl}^{-1}\text{saat}^{-1}$ dir.

Anahtar Sözcükler: Ksilitol, Ayçiçeği tohum kabuğu, D-Ksiloz, *Candida tropicalis*

Introduction

Xylitol, a five carbon sugar alcohol is used as a sweetener in foods and may be used for medical

purposes as a sugar substitute for diabetics (Emodi, 1978). It has received much attention due to its sweetening and anti-cariouss properties. Xylitol is currently manufactured by catalytic chemical reduction of the xylose fraction of hemicellulosic hydrolysates (Hyvönen et al., 1982). Since the hemicellulosic fraction of the raw materials contains the polymers of the other sugars, the process includes extensive purification and separation steps to remove these by-products from xylose and xylitol (Maleja and Hämäläinen, 1977). Recently the biotechnical process for xylitol production was studied with bacteria (Izumori and Tuzaki, 1988), yeasts (Chen and Gong, 1985; Barbosa et al., 1988; Furlan et al., 1991; Meyrial et al., 1991; Roberto et al., 1991; Da Silva and Afschar, 1994; Roca et al., 1996; Felipe et al., 1996). Hemicellulose is one the major constituents of plant cell material comprising up to 40 % of agricultural residues and hardwood (Cowling and Kirk, 1976). Upon hydrolysis with dilute acids, hemicellulose yields a mixture of carbohydrates which are potential substrates for xylitol production. On the other hand, hemicellulosic hydrolysate also contains furfural, acetic acid and phenolic compounds derived from lignin and extractives. All of these have inhibitory effects on microorganisms Sunflower seeds are one of the most abundant renewable materials in Turkey and 1.000.000 ton are produced on average. Sunflower seed hulls are currently used as animal food. Taking into account that the starting

cost of these residues is very low, using such xylose rich material in the production of xylitol might be an alternative way to utilize these residues. The objective of this study was to investigate the production of xylitol from D-xylose and sunflower seed hull derived hydrolysate using *Candida tropicalis* Kuen 1022 yeast under aerobic conditions.

Materials and Methods

Substrates

D-xylose was purchased from the Merck Chemical Company. Hemicellulosic hydrolysate was prepared as follows: 10-20 mesh sunflower seed hulls were hydrolyzed with 0.5 M H_2SO_4 in a glass batch reactor at 98°C. The reaction time was 3 hours and a solid/liquid ratio of 1/3 was used. These reaction conditions were selected to maintain a furfural concentration below 1 g l^{-1} in hydrolysate preliminary kinetic studies (Çavuşoğlu, 1996). Following filtration the pH of the hemicellulosic hydrolysate was first adjusted to 10 by adding $Ca(OH)_2$ and then HCl was added to bring the pH to 6. This overtitration method was advised for the removal of toxic phenolic compounds present in hydrolysate (Roberto et al., 1991). Filtration was done after each pH adjustment to remove precipitates. The hemicellulosic hydrolysate had a xylose concentration of 66 g l^{-1} and a glucose concentration of 0.8 g l^{-1}

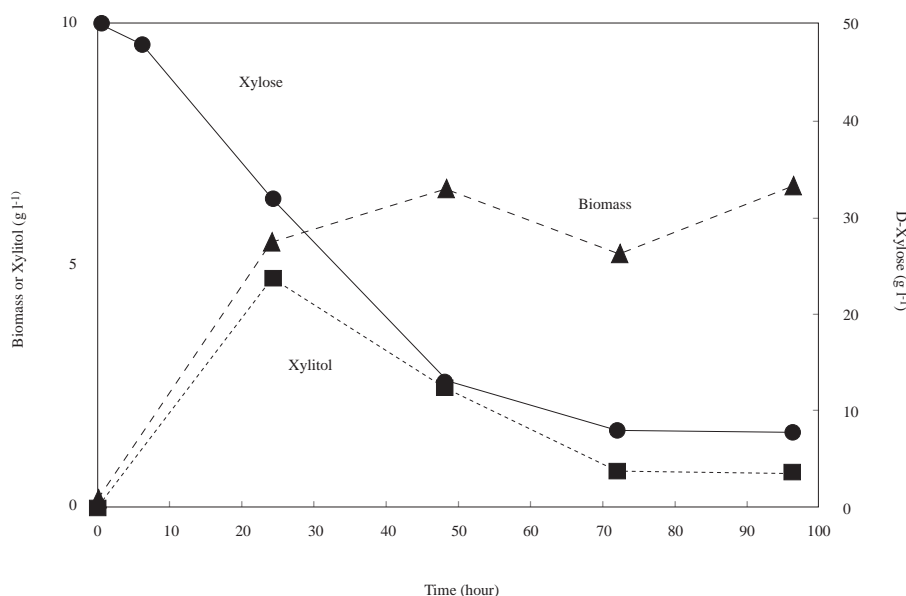


Figure 1. Cell Growth and Xylitol Production by *Candida tropicalis* when initial D-xylose concentration was 50 g l^{-1}

Microorganism

Candida tropicalis Kuen 1022 species was kindly supplied by the Microbiology Department of İstanbul University in a lyophilized form. *Candida tropicalis* was transferred to YP-xylose agar slants containing 2% D-xylose, 0.5% polypeptone, and 0.5% yeast extract (w/v) and incubated at 29°C for 25 hours. To improve the hydrolysate tolerance, acclimatization of yeasts to hemicellulose hydrolysate was carried out. The YP-agar plates prepared with the neutralized and diluted hydrolysate in place of xylose were used for hydrolysate fermentation. The incubation time was 48 hours.

Growth Medium

The composition of the synthetic growth medium was 3 g l⁻¹ Bacto-yeast extract, 3 g l⁻¹ Bacto-peptone and 10 g l⁻¹ D-xylose. The pH was adjusted to 5 with 0.5 M HCl. The yeast was grown in a 250 ml flask containing 100 ml of growth medium at 30°C on a magnetic stirrer for 10 h. Growth medium for hemicellulosic hydrolysate was prepared with 1/3 strength diluted hydrolysate growth medium. Cells which grew in YP-hydrolysate agar were transferred into hydrolysate growth medium. The remaining components were added to yield the same compo-

sition given for synthetic medium. Inoculum for hydrolysate fermentation was incubated for 24 hours.

Fermentation

Shake flask experiments were conducted in 250 ml Erlenmeyer flasks each containing 100 ml fermentation medium with 20 g l⁻¹ yeast extract and a D-xylose concentration of 50-100 g l⁻¹ for synthetic medium, 66 g l⁻¹ for hydrolysate. In each case, the pH was 6. A part of the corresponding growth medium was inoculated into fermentation medium to achieve an initial dry cell amount of ~ 0.1 g l⁻¹ for synthetic medium and ~ 1 g l⁻¹ for hydrolysate. All flasks were placed in a shaking water bath (140 rpm) at 30°C for about 96 hours under aerobic conditions. Samples were collected after the 6th, 24th, 48th, 96th hours for the measurements of cell, xylose and xylitol concentrations.

Analytical methods

Xylose was determined using reducing dinitrosalicylic acid (Miller, 1959). Xylitol was measured by the method of Bassler (Bassler, 1974). Dry cell amount was estimated with a calibration curve made from the relationship between the absorbance at 620 nm and dry cell weight.

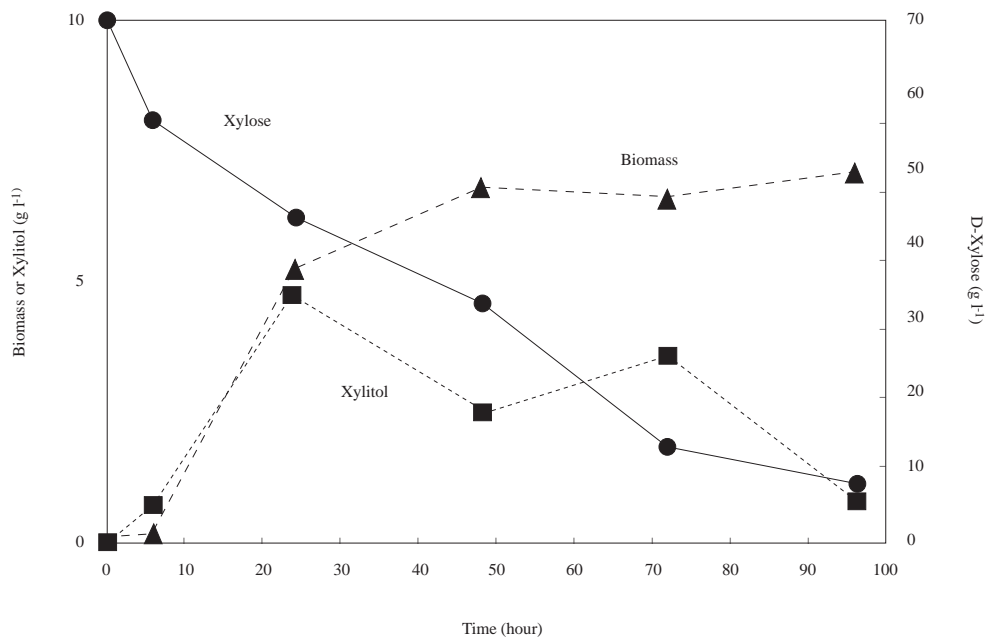


Figure 2. Cell Growth and Xylitol Production by *Candida tropicalis* when initial D-xylose concentration was 70 g l⁻¹

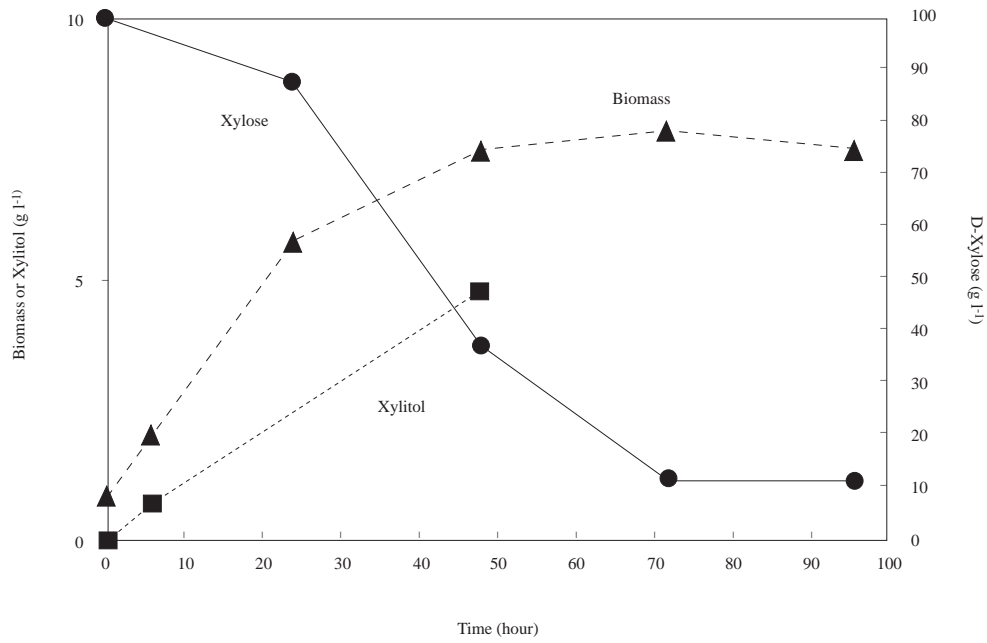


Figure 3. Cell Growth and Xylitol Production by *Candida tropicalis* when initial D-xylose concentration was 100 g l^{-1}

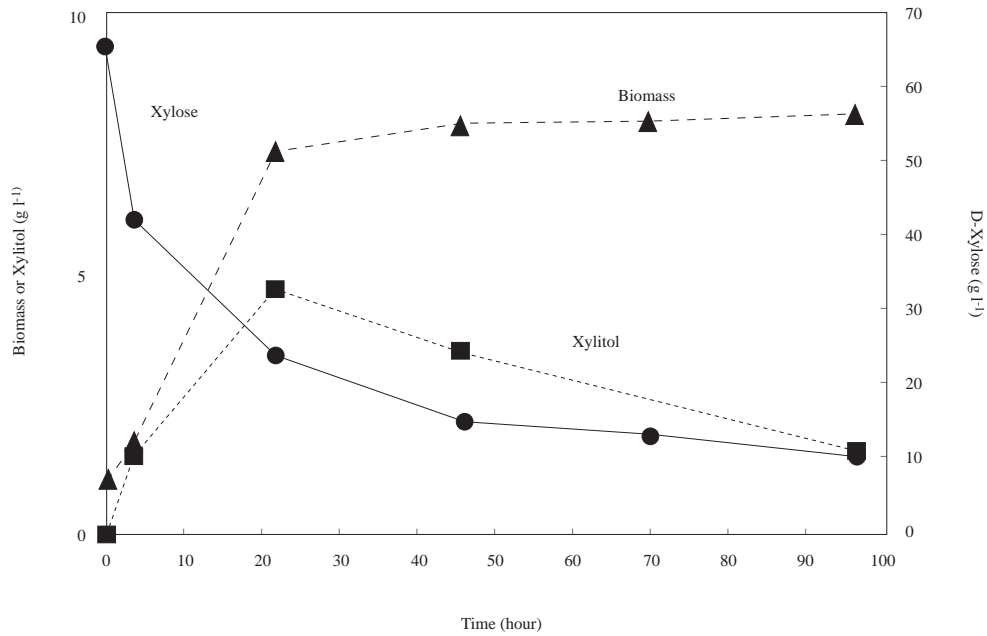


Figure 4. Cell Growth and Xylitol Production by *Candida tropicalis* when initial D-xylose concentration was 66 g l^{-1} in sunflower seed hull hydrolysate

Table 1. Kinetic Parameters of D-Xylose fermentation by *Candida tropicalis* Kuen 1022

Initial Xylose (g l ⁻¹)	μ (h ⁻¹)	Q_p (g l ⁻¹ h ⁻¹)	q_p (g g ⁻¹ h ⁻¹)	$Y_{P/S}$	$Y_{X/S}$
50	0.205	0.196	0.097	0.260	0.301
70	0.185	0.196	0.092	0.180	0.196
100	0.073	0.098	0.024	0.075	0.118
66 (Hydrolysate)	0.225	0.219	0.057	0.113	0.176

Results and Discussion

Data for the utilization of D-xylose for the production of xylitol and the increase in the cell mass of for synthetic medium and hydrolysate are shown in Figures 1-4. The results indicated that D-xylose was not completely consumed by yeast in either case during 96 hours fermentation under aerobic conditions. The overall xylose consumption was 85%-89% for synthetic medium and 84% for hydrolysate. Xylitol production started without a lag period and xylitol production and cell growth occurred simultaneously. That is why the xylose-xylitol conversion by *Candida tropicalis* Kuen 1022 yeast could be considered a growth-associated product formation process. Xylitol concentrations usually decreased after a maximum value. This could be a result of the assimilation of xylitol by yeast after extent of xylose consumption. Various kinetic parameters calculated for the exponential growth are shown in Table 1. Cell and xylitol yields are based on total xylose consumed at maximum xylitol concentrations achieved. The results showed that an increase in the initial synthetic sugar concentration from 50 g l⁻¹ to 100 g l⁻¹ led to a significant decrease in the kinetic parameters and yield values. This indicates that the xylitol production process and growth process were inhibited by an increase in the xylose concentration. The highest fermentative performances of *Candida tropicalis* Kuen 1022 yeast were obtained with the lowest initial xylose concentration (50 g l⁻¹). The highest xylitol yield of 0.260 g g⁻¹ was obtained with an average specific rate of 0.097 g g⁻¹h⁻¹. These values are still lower than some reported values obtained with some other high xylitol producing yeasts (Gong et al., 1981; Chen and Gong, 1985; Meyrial et al., 1991). The maximum xylitol concentration obtained for hydrolysate was 4.72 gl⁻¹. The kinetic values for hydrolysate (initial xylose concentration = 66gl⁻¹) usually fell into the range of parameters obtained by varying synthetic xylose concentrations (50 - 100 gl⁻¹). This is an indication of the positive effect of the pretreatment of hemicellulose hydrolysate

and also the adaptation of the microorganism on hydrolysate medium. During the preliminary runs without pretreatment of hydrolysate and adaptation of the microorganism and with lower medium pH values (4.5), no metabolism of xylose and no xylitol production was observed. These results could be due to the presence of toxic substances in the hydrolysate which might interfere with the sugar catabolism (van Zyl et al., 1988). Acetic acid is one of the important fermentation inhibitors reducing the rate and yield. Its toxic effect is basically due to its undissociated form. It was reported in the literature (Ferrari et al., 1992) that acetic acid inhibition for ethanol production is highest at pH 5. That is why a pH value of 6 was chosen for the fermentation medium. From the data presented in this study, *Candida tropicalis* Kuen 1022 is characterized as having a limited potential for the production of xylitol under aerobic conditions. However, when considering the mild condition of pressure and temperature held during fermentation, efforts should be continued in the utilization of these low cost agricultural residues. In addition, for agricultural countries like Turkey it is quite important from an ecological point of view to find new applications for lignocellulosic residues like sunflower seed hull.

Notation

q_p	average specific xylitol production rate (g gl ⁻¹ h ⁻¹)
Q_p	volumetric xylitol productivity calculated for the maximum xylitol concentration reached (gl ⁻¹ h ⁻¹)
$Y_{P/S}$	xylitol yield calculated when the xylitol concentration was maximal (g xylitol/g xylose consumed)
$Y_{X/S}$	biomass yield calculated when the xylitol concentration was maximal (g biomass/g xylose consumed)
μ	specific growth rate calculated during exponential phase (h ⁻¹)

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