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XYLITOL BIOPRODUCTION FROM TOBACCO STALK

(TÜTÜN SAPINDAN KSİLİTOLÜN BİYOÜRETİMİ)

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ABSTRACT

The aim of this study was to produce xylose from tobacco stalk and conversion of xylose to xylitol production by Candida tropicalis. Xylitol is a five-carbon sugar alcohol. Tobacco stalk was hydrolyzed with dilute sulphuric acid to extract xylose. Fermentation ofhydrolysate was performed by Candida tropicalis. The hydrolysate, oxygen and inoculum concentration were optimized for the production of xylitol with high yield. Under the optimum conditions, the hydrolysate was fermented to xylitol with 59% yield and 0.18 g/l-hr volumetric production rate.

Keywords: Xylose, Xylitol, Tobacco stalk, Optimization

ÖZ

Bu çalışmanın amacı tütün sapından ksiloz eldesi ve ksilozun Candida tropicalis ile ksilitole dönüşümüdür. Ksilitol, beş karbonlu şeker alkolüdür. Ksilozu ekstrakte etmek için tütün sapı seyreltik sülfirik asit ile hidroliz edilmiştir. Hidrolizat Candida tropicalis ile fermente edilmiştir. Yüksek verimlilikte ksilitol üretimi için hidrolizat, oksijen ve inokülüm konsantrasyonu optimize edilmiştir. Optimum koşullar altındahidrolizat %59 verimlilikte ve 0.18 g/l-sa volümetrik üretim hızında ksilitole fermente olmuştur.

Anahtar Kelimeler: *Ksiloz, Ksilitol, Tütün sapı, Optimizasyon*

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1. INTRODUCTION

Xylitol is a five-carbon sugar alcohol, equivalent to sucrose in sweetness and occurs widely in nature. It is anticariogenic and natural sweetener. It gives a pleasant cool and fresh sensation due to its high negative heat of solution. It has been quite popular sweetener due to its positive effects on human health. It is absorbed more slowly than other sugars by body and does not change of blood sugar quickly because its metabolism is independent from insulin pathway. Therefore, it is recommended for diabetic patients [1, 2]. Xylitol is used in various food products such as bakery products, spices and relishes, jams, jellies, marmalades and desserts [1]. It does not react in Maillard reaction so it is useful for using as additive [2].

Despite many advantages of xylitol, the use of xylitol as sweetener is limited because of its comparatively high production cost. Although it is occured in many fruits and vegetables, it is not economical to extract of xylitol from these sources because of high cost and low yield of the process [1, 2]. Therefore, the studies have focused on finding different sources for the production of xylitol. In recent years, lignocellulosic materials such as corn cobs, barley bran, wheat and rice straws have been investigating for xylitol production [3-7]. Agricultural wastes, which are rich in lignocellulosic materials and widely available in Turkey, can serve as an ideal source for the production of xylitol. These wastes are usually left to rot or burned in the field after harvesting [8]. Nowadays, utilization of them for industrial purposes receive enormous attention due to their huge amount of carbohydrates (cellulose and hemicellulose) contents, low cost, wide availability and reduction of environmental pollution. One of these agricultural wastes is tobacco stalk which is widely available in Turkey.

Xylitol can be produced by a chemical process based on the catalytic hydrogenation of xylose, which is a high-cost method. It requires extensive xylose purification steps and results in a relatively expensive final product. Alternatively, it can be produced by biotechnological methods which are more simple, specific, and more economic. Biotechnological method is performed by microorganisms. Although some kind of bacteria, yeast and mold convert xylose to xylitol [2], generally, yeasts such as *Candida* species known for the best producer of xylitol among [1].

The aim of this study was to produce xylose from tobacco stalk and to convert xylose to xylitol by *Candida tropicalis*. The present study determined the effect of aeration, inoculum and xylose concentration on the yield of xylitol from tobacco stalk. Response surface methodology was used as a statistical design to optimize the formation of xylitol in the hydrolysate.

2. METHOD

2.1. Xylose Production

Hydrolysis of tobacco stalk was performed in a 1 l stainless-steel pressure batch reactor, at 140°C for 15 min reaction time with sulfuric acid concentration of 6%. After the reaction was completed, the hydrolysate was filtrated, neutralized with $CaCO_3$ and concentrated by vacuum evaporation below 50°C to increase the initial xylose content.

2.2. Xylitol Fermentation

Candida tropicalis was inoculated into a culture medium containing 30 g/l of xylose and the following nutrients (g/l): 10 g/l yeast extract, 20 g/l peptone, 0.5 g/l K_2HPO_4, 0.5 g/l

KH_2PO_4, 0.5 g/l $MgSO_47H_2O$, 2 g/l $(NH4)_2SO_4$, and grown on a rotary shaker (180 rpm) at 30°C for 48 hr. The cells were then collected by centrifugation (2000xg) for 10 min, resuspended in sterile distilled water [9]. An adequate volume was taken from this suspension to attain an inoculum concentration of 0.5-1.5 g/l (dry weight) in the fermentation medium. Fermentation media was prepared from the concentrated hydrolysates, treated with activated charcoal, sterilized by filtration, and supplemented with nutrients above except xylose.The fermentations were performed in a 1.5-l fermentor with agitation, aeration, temperature, pH, and dissolved oxygen control. Experiments were carried out at 30°C with 0.5 l of fermentation medium and 300 rpm.

For optimization study, the effects of air (volumetric flow rate), substrate (xylose concentration) and cell concentration on the production of xylitol yield and volumetric xylitol production rate were investigated. Response surface methodology (RSM) was used for the optimization of xylitol production conditions. In Table 1, concentrations of independent variables used in experiments are shown.

Table 1. Values of independent variables used in experiments.

Independent variables	**Symbol**	**-α**	**-1**	**0**	**+1**	**+α**
Xylose concentration (g/l)	X_1	5.73	18	36	54	66.27
Cell concentration (g/l)	X_2	0.16	0.5	1	1.5	1.84
Air concentration (vvm)	X_3	0.16	0.5	1	1.5	1.84

2.3. Experimental design and optimization study

A 2^3 rotatable central composite design (CCD) was used to fit a second order model, and the design consisted of 20 sets of experiments. Xylose concentration (5.73-66.27 g/l), inoculum level (0.16-1.84 g/l) and volumetric flowrate (0.16-1.84 g/l) were investigated as experimental factors (Table 1). Xylose yield and volumetric xylitol production rate were taken as the dependent variables. The quadratic model was selected for predicting the optimal point and is expressed as

$$Y= b_0+ b_1X_1+ b_2X_2+ b_3X_3+ b_{11}X_1^2+ b_{22}X_2^2+ b_{33}X_3^2+ b_{12}X_1X_2+ b_{13}X_1X_3+ b_{23}X_2X_3$$

where Y represents response variables (xylitol yield and volumetric production rate); b_0 is the interception coefficient; b_1, b_2 and b_3 are the linear terms; b_{11}, b_{22} and b_{33} are the quadratic terms; and X_1 (xylose concentration), X_2 (volumetric air flow rate) and X_3 (inoculums concentration) represent the variables studied.

The Design Expert v. 7 (Stat-Ease Inc., Minneapolis) was used for regression and graphical analyses of the data obtained. The type of model equation was determined by Fischer's test, whereas the statistical significance of regression coefficients was determine by the Student's t-test. The optimum concentrations of the variables were obtained by the numerical analysis using the Design-Expert program.

2.4. Analytic Methods

The concentration of xylose, glucose, arabinose, acetic acid, ethanol and xylitol in the hydrolysate and during fermentation were analysed using HPLC on Aminex HPX 87H (300 × 7.8 mm). They were eluted with 5 mmol/l H_2SO_4 from the column at 45°C and a flow rate of 0.5 ml/min. Specific xylose consumption was defined as the differences in xylose concentration divided by the initial dry cell weight. Xylitol yield and volumetric xylitol production rate were determined as the function of xylose consumption and the function of time, respectively.

3. RESULTS

3.1. Hydrolyzation of Tobacco Stalk

Acid hydrolysis of tobacco stalk was done according the method provided by Akpinar et al [10]. Since hydrolysis of the lignocellulosic material produces a hydrolysate containing different sugars besides xylose and several toxic compounds such as acetic acid, furfural, phenolic that act as inhibitors of microbial metabolism and reduce cell growth and product yield [7], the hydrolysate can not be used directly for the fermentation. To prepare and improve of the bioconversion of the hydrolysate to xylitol, it was concentrated and treated by activated charcoal to reduce its toxicity. After the detoxification process, the tobacco stalk hydrolysate was composed of xylose (90 g/l), glucose (16 g/l), arabinose (2 g/l) and acetic acid (9 g/l).

3.2. Fermentation of Xylose to Xylitol

Xylitol production was performed by xylose reductase (XR) and xylitol dehydrogenase (XDH) enzymes. XR reduced xylose to xylitol and XDH oxidized xylitol to xylulose. Ratio of XR/XDH of yeasts must be more to produce xylitol efficiently [11]. To keep this ratio high, 20 different experiments were performed. The results of xylitol yield, specific xylose consumption rate, increase of cell weight, volumetric xylitol production rate, ethanol and acetic acid concentration obtained from these experiments are shown in Figure 1. It was found that xylose concentration, air flow rate and yeast cell concentration were important parameters affecting xylitol yield and volumetric xylitol production rate. Xylitol yield and volumetric xylitol production rate changed according to selected conditions. Maximum xylitol yield and volumetric xylitol production rate were obtained at 36 g/l xylose concentration, 1.84 vvm and 1 g/l yeast concentration. Maximum cell weight increase was occured at 18 g/l xylose concentration, 1.5 vvm and 0.5 g/l yeast concentration. Maximum xylose consumption was obtained at 36 g/l xylose concentration, 1 vvm ve 0.16 g/l yeast concentration. Due to the fermentation of glucose, the maximum ethanol accumulation was observed at 54 g/l xylose concentration, 1.5 vvm and 0.5 g/l yeast concentration. The experiment that had lower xylose concentration, xylitol yield and xylitol production rate was higher due to the low concentration of the inhibitors in hydrolysate.

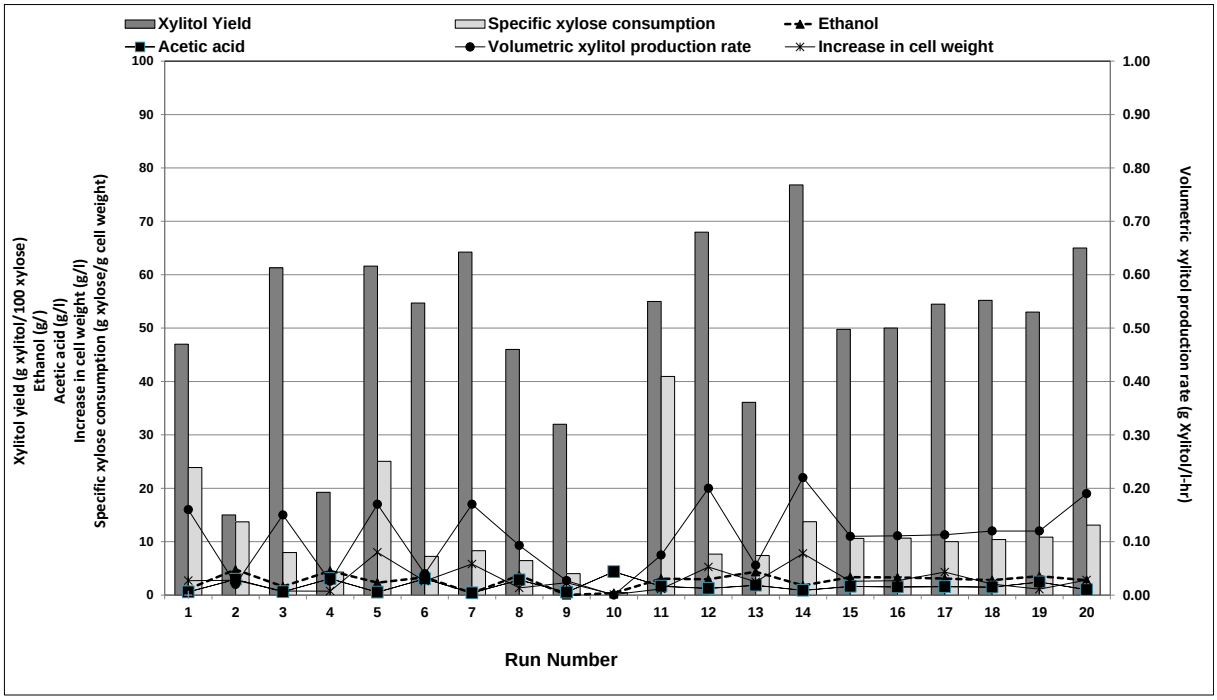

Figure 1. Xylitol yield, volumetric xylitol production rate, specific consumption of xylose, amount of acetic acid, increase in cell weight, ethanol fermentation of tobacco stalk hydrolysate to xylitol

There are many factors including oxygen content, pH, cell weight in the fermentation media that affect the adaptation and the development of the yeast and the production of xylitol. To monitor the change of these factors during the fermentation of tobacco stalk hydrolysate, the experiment which had 54 g/l xylose, 0.5 g/l yeast, 1.5 vvm air concentration was choosen (Figure 2). As seen from the figure, dissolved oxygen (DO) is one of the important parameters affecting the production of xylitol. It was seen that decreasing of DO incerased xylitol production and production rate. Xylitol fermentation was began when the DO decreased from 73% to 0.5%, its production was carried out at DO level of 0.1%-0.4%. The xylitol production is performed best under limited oxygen conditions, because under this condition, XR enzyme activity linked NAD(P)H is high and the yeast can transform xylose to xylitol efficiently [2]. As seen in the Figure 2, xylitol production was low while DO was decreasing from high concentration to oxygen-limited environment, because during this part of the fermentation, xylose was used for the cell growth.

The amount and composition of substrate are also important for yeast adaptation and decreasing DO. If there is low inhibitors in the medium, yeasts would grow faster and decrease DO. As can be seen in the figure, during the first part of fermentation (aerobic condition), called lag or adaptation period, yeast only used xylose to ensure its own development and xylitol production did not take place. The microorganism, used in this study at the selected condition, showed 24 hr of delay; after this stage, the yeast adapted to the medium and started the transformation of xylose to xylitol. The result showed that xylitol production and acetic acid consumption occurred together, acetic acid concentration decreased to 1.7 g/l while pH increased to 5.4.

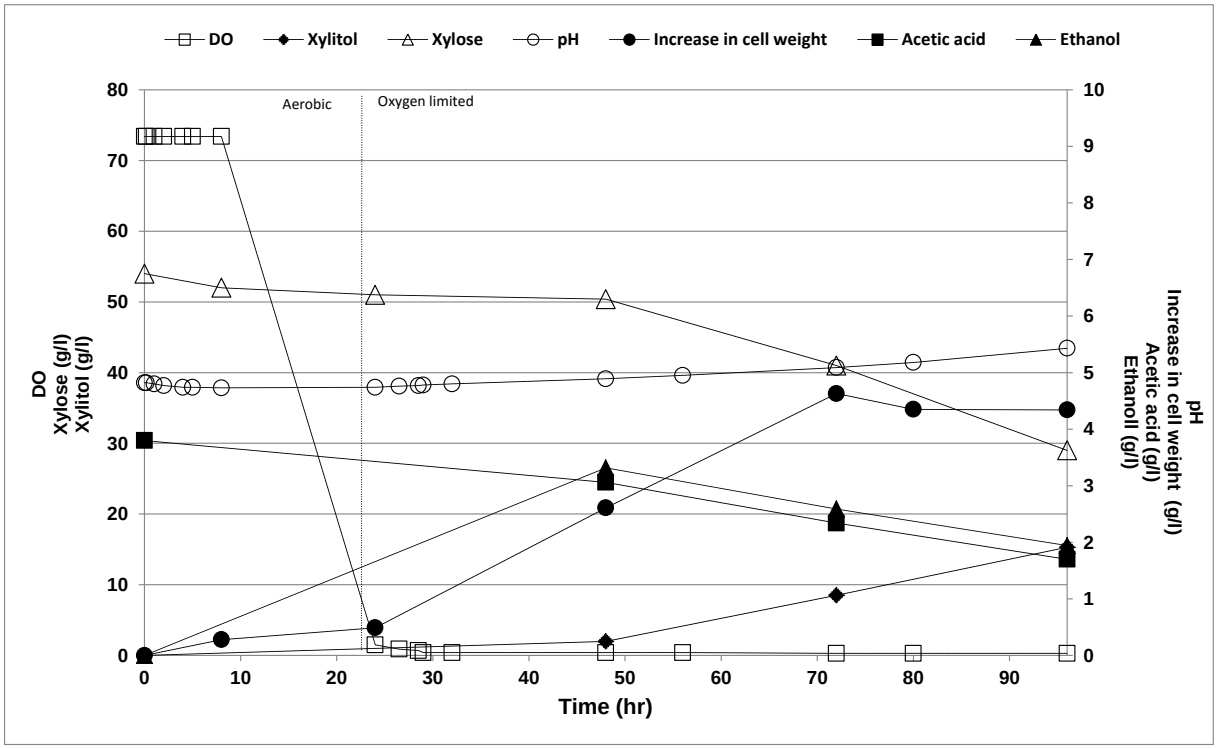

Figure 2. The changes in the fermentation medium when 54 g/l of xylose concentration, 1.5 vvm of air flow rate and 0.5 g/l of inoculum level were used

3.3. Statistical Modeling

Results of xylitol yield (Y_1) and volumetric xylitol production rate (Y_2) of dependent variables are presented in Table 2. The quadratic models with coded variables are shown in Eqs. (1) and (2), which represent the xylitol yield (Y_1) and volumetric xylitol production rate (Y_2) as function of xylose concentration (X_1), volumetric air flow rate (X_2) and inoculum concentration (X_3).

$$Y_1=54.5-11.3X_1+2.5X_2+11.2X_3-13.3X_1^2+2.9X_2^2-1.1X_3^2-2.7X_1X_2+6.1X_1X_3-3.1X_2X_3 \quad \text{Eqs.(1)}$$

$$Y_2=0.1-0.004X_1+0.02X_2+0.03X_3-0.04X_1^2+0.006X_2^2+0.006X_3^2+0.009X_1X_2+0.007X_1X_3+0.007X_2X_3 \quad \text{Eqs.(2)}$$

Figures 3 and 4 show the response surfaces to estimate the xylitol yield and volumetric xylitol production rate over the independent variables of xylose concentration (X_1), air flow rate (X_2) and inoculum level (X_3). When the air flow rate was set at 1 vvm as the center point (Figure 3A), the maximum xylitol yield (64%) was obtained, working with 27 g/l of xylose concentration and 1g/l of inoculum level. When xylose concentration was set at 36 g/l as the center point (Figure 3B), the maximum xylitol yield (70%) was obtained with 1.5 vvm and 0.5 g/l inoculum level. The maximum xylitol yield (67%) was found at 33 g/l of xylose concentration and 1.5 vvm when inoculum level was 1g/l (Figure 3C).

Table 2. Experimental design and results for independent variables obtained by fermentation of tobacco stalk hydrolysate to xylitol

	Variables			**Results**	
	X_1	**X_2**	**X_3**	**Y_1**(100x xylitol/ xylose consumption)	**Y_2**(xylitol/ time)
1	-1	-1	-1	47.00	0.16
2	1	-1	-1	15.00	0.02
3	-1	1	-1	61.30	0.15
4	1	1	-1	19.26	0.03
5	-1	-1	1	61.61	0.17
6	1	-1	1	54.70	0.04
7	-1	1	1	64.24	0.17
8	1	1	1	46.00	0.09
9	-α	0	0	32.00	0.03
10	A	0	0	0.00	0.00
11	0	-α	0	55.00	0.08
12	0	α	0	67.98	0.20
13	0	0	-α	36.11	0.06
14	0	0	α	76.82	0.22
15	0	0	0	49.77	0.11
16	0	0	0	50.00	0.11
17	0	0	0	54.50	0.11
18	0	0	0	55.20	0.12
19	0	0	0	53.00	0.12
20	0	0	0	65.00	0.19

Y1 (xylitol yield)= 100 x (Xylitol/Xylose consumption); Y2 (volumetric xylitol production g/l-h) = Xylitol/Time

The results of ANOVA are shown in Table 3. Regression coefficient for xylitol yield and volumetric xylitol prduction rate was found 0.97 and 0.75 and regression found statistically significant between 95% confidence interval.

Table 3. ANOVA table

Source	**Sum of squares**		**Degrees of freedom**		**Mean square**		***F*-value**		***P*-value**	
	Y_1	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**
Model	6764.67	0.061	9	9	751.63	0.0067	34.10	3.28	<0.0001	0.0391
Residual	220.45	0.021	10	10	22.04	0.0021				
Lack of fit	64.87	0.016	5	5	12.97	0.0032	0.42	3.29	0.8205	0.1084
Pure error	155.58	0.0048	5	5	31.12	0.00096				
Total	6985.12	0.082	19	19						
R^2	0.97	0.75								

When the air flow rate was set at 1 vvm as the center point (Figure 4A), volumetric xylitol production rate (0.16 g/l-hr) was obtained, working with 30 g/l of xylose concentration and 1.5 g/l of inoculum level. When xylose concentration was set at 36 g/l as the center point (Figure 4B), volumetric xylitol production rate (0.19 g/l-hr) was obtained with 1.5 vvm and 1.5 g/l inoculum level. Maximum volumetric xylitol production rate (0.17 g/l-hr) was found at 30 g/l of xylose concentration and 1.5 vvm when inoculum level was 1 g/l (Figure 4C).

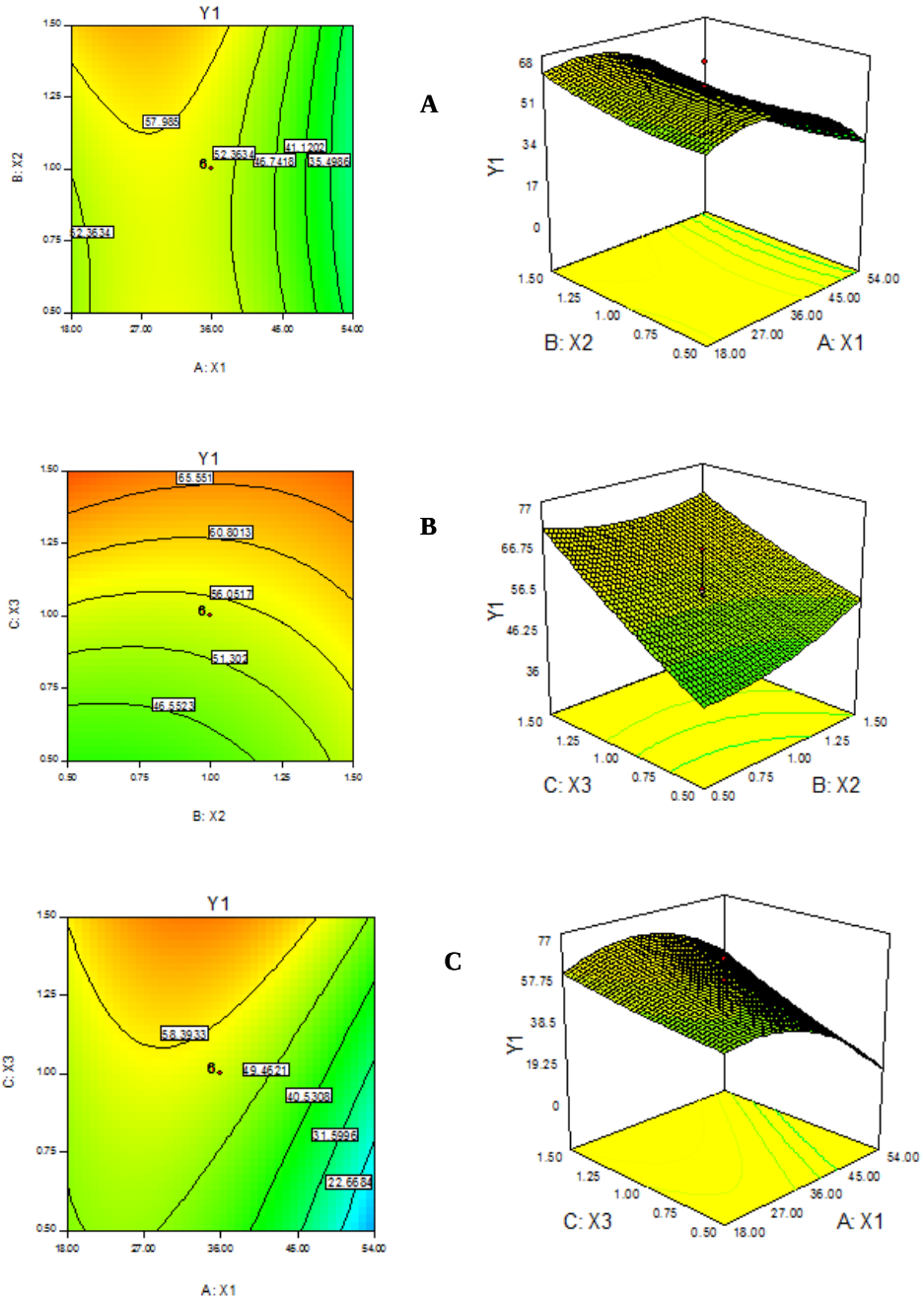

Figure 3. Response surface and countour plots for xylitol yield during the fermentation of tobacco stalk hydrolysate to xylitol. A: Effect of xylose concentration and incoulum level on xylitol yield when air flow rate was set 1 vvm as the center point, B: Effect of air flow rate and incoulum level on xylitol yield when xylose concentration was set at 36 g/l as the center point, C: Effect of xylose concentration and air flow rate on xylitol yield when inoculums level was set at 1 g/l as the center point

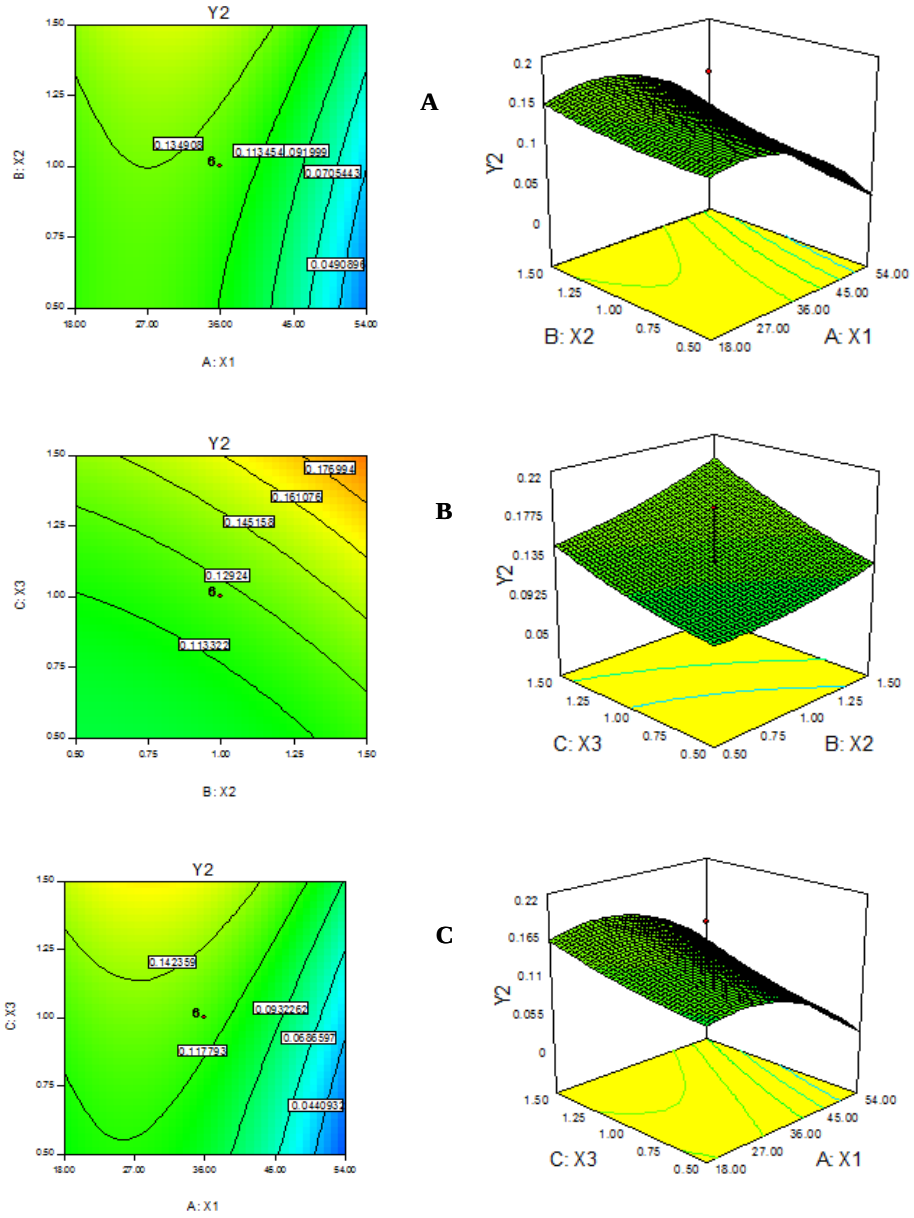

Figure 4. Response surface and countour plots for xylitol yield during the fermentation of tobacco stalk hydrolysate to xylitol. A: Effect of xylose concentration and incoulum level on xylitol yield when air flow rate was set 1 vvm as the center point, B: Effect of air flow rate and incoulum level on xylitol yield when xylose concentration was set at 36 g/l as the center point, C: Effect of xylose concentration and air flow rate on xylitol yield when inoculums level was set at 1 g/l as the center point

Based on the models, numerical optimization was carried out with Design Expert program, and the optimal working condition based on high xylitol yield and volumetric xylitol production was chosen using the following criteria: xylitol yield>50% and volumetric xylitol production rate >0.15g/l-hr; 33 g/l of xylose concentration, 0.9 g/l of inoculum level and 1.4 vvm were chosen from several optimum working conditions predicted by the program. The fermentation was carried at this optimum condition, and the xylitol yield and xylitol production rate were found to be 59% and 0.18 g/l-hr.

There are several studies in the literature that report the production of xylitol from different agricultural waste. Xylitol yield was found 43-45% for the fermentation of corn fiber and sugar cane by *Candida tropicalis* [9]. Fermentation of sunflower seed husk by *Candida*

tropicalis was resulted in 11.3% xylitol yield [12]. Xylitol yields of these studies were found lower than this study. However, when pure xylose solution (100 g/l, 1 g/l inoculum level, 1 vvm) were used, xylitol yield and volumetric xylitol production rate were obtained 81% and 5.06 g/l-hr [13]. Other studies done with different yeast reported that xylitol yield and volumetric xylitol production rate were 57%, 0.88 g/l-hr for wheat stalk hydrolysate (39.3 g/l xylose, 0.5 g/l inoculum level ve 0.4 vvm) fermentation by *Candida guilliermondii* [14].

4. CONCLUSIONS

In the literature, studies on xylitol production from different agricultural wastes are available, but bioconversion of tobacco stalk to xylitol has not been studied in detail. This study presents the information about fermentation progress of tobacco stalk hyrolysate and optimum production condition. The results show that the tobacco stalk that does not have an important economic value, can be utilized into high value-added products and can serve as a potential renewable source for the production of xylitol.

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UFUK BÖLÜKBAŞI; Prof. Dr. (Prof. Dr)

She got her bachelors' degree in the Chemical Engineering Department at Middle East Technical University, Ankara/Turkey in 1983, her master degree in the Food Engineering Department at Middle East Technical University, USA in 1985, PhD degree in the Chemical Department at Iowa State University, USA in 1993. She is still an academic member of the Chemical Engineering Department at Middle East Technical University.

Lisans derecesini 1983'de Orta Doğu Teknik Üniversitesi Kimya Mühendisliği Bölümü'nden, Yüksek Lisans derecesini 1985'de Orta Doğu Teknik Üniversitesi Gıda Mühendisliği Bölümü'nden, Doktora derecesini 1993 yılında Iowa State Universitesi Kimya Mühendisliği Bölümü'nden aldı. Hala Orta Doğu Teknik Üniversitesi

Kimya Mühendisliği Bölümü'nde öğretim üyesi olarak görev yapmaktadır.

LEVENT YILMAZ; Prof. Dr. (Prof. Dr)
He got his bachelors' degree in the Chemical Engineering Department at Middle East Technical University, Ankara/Turkey in 1980, his master degree in the Chemical Engineering Department at Middle East Technical University, USA in 1982, PhD degree in the Chemical Department at Illinois University, USA in 1987. He is still an academic member of the Chemical Engineering Department at Middle East Technical University.

Lisans derecesini 1980'de Orta Doğu Teknik Üniversitesi Kimya Mühendisliği Bölümü'nden, Yüksek Lisans derecesini 1982'de Orta Doğu Teknik Üniversitesi Kimya Mühendisliği Bölümü'nden, Doktora derecesini 1987 yılında Illinois Universitesi Kimya Mühendisliği Bölümü'nden aldı. Hala Orta Doğu Teknik Üniversitesi Kimya Mühendisliği Bölümü'nde öğretim üyesi olarak görev yapmaktadır.