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Original Article/Özgün Araştırma

Bioproduction of xylitol by *Candida tropicalis* 13803 from pistachio shell hydrolysate obtained through MW-HPCO$_2$ system

MW-HPCO$_2$ sistemi ile elde edilen antep fıstığı kabuğu hidrolizatından *Candida tropicalis* 13803 kullanılarak ksilitol biyoüretimi

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Abstract

Objective: Biotechnological xylitol production from secondary agricultural residues is a promising approach for a sustainable and environmental purpose. Lignocellulosic biomass is a significant feedstock for biofuel and biochemical production. Its accessibility, cost-effectiveness, renewability, and environmental friendliness make it an attractive alternative to fossil fuels and other conventional sources of energy and chemicals.

Materials and methods: In this study, the conversion of xylan to xylose in a pistachio shell was provided with a novel technology of microwave-assisted high-pressure CO_2/H_2O system. Xylose rich pistachio shell hydrolysate was utilized by *Candida tropicalis* ATCC 13803 for xylitol production. Different concentrations of xylose (50, 100, and 150 g/L) were employed for xylitol production in shake-flask.

Results and conclusion: HMF and furfural were completely removed from xylose-rich hydrolysate by activated charcoal. The improvement in yeast performance was limited with increasing xylose concentration. The highest xylitol produced by *C. tropicalis* from pistachio shell hydrolysate (65.15 g/L) and the maximum yield of xylitol 0.66 g/g with 100 g/L xylose were obtained in shake-flask whereas xylitol produced at 50 g/L and 150 g/L xylose were 0.65 and 0.37 g/g, respectively. Volumetric productivity at 100 g/L of xylose was 1.28 times and 1.84 times higher compared to xylose concentrations of 50 g/L and 150 g/L, respectively. Xylitol production performance (71.73 g/L) of detoxified pistachio shell hydrolysate at 100 g/L of xylose was almost identical to pure xylose. However, the yeast was not able to consume xylose at 150 g/L resulting in no xylitol production.

Keywords: Xylose; microwave-assisted high-pressure CO_2/H_2O hydrolysis; pistachio shell hydrolysate; xylitol; *Candida tropicalis*

Öz

Amaç: İkincil tarımsal kalıntılardan biyoteknolojik yolla ksilitol üretimi, sürdürülebilir ve çevresel bir amaç için umut verici bir yaklaşımdır. Lignoselülozik biyokütle, biyoyakıt ve biyokimyasal üretim için önemli bir hammaddedir. Erişilebilirliği, maliyet etkinliği, yenilenebilirliği ve çevre dostu olması onu fosil yakıtlara ve diğer geleneksel enerji ve kimyasal kaynaklarına karşı cazip bir alternatif haline getirmektedir.

Materyal ve yöntem: Bu çalışmada, Antep fıstığı kabuğundaki ksilanın ksiloza dönüşümü, yeni bir teknoloji olan mikrodalga destekli yüksek basınçlı CO_2/H_2O ile sağlanmıştır. Ksiloz bakımından zengin Antep fıstığı kabuğu hidrolizatı, ksilitol üretimi için *Candida tropicalis* ATCC 13803 tarafından kullanılmıştır. Erlenmayerde ksilitol üretimi için farklı ksiloz konsantrasyonları (50, 100 ve 150 g/L) kullanılmıştır.

Tartışma ve sonuç: HMF ve furfural, aktif kömür ile ksiloz bakımından zengin hidrolizattan tamamen uzaklaştırılmıştır. Maya performansındaki iyileşme, ksiloz konsantrasyonunun artması ile kısıtlanmıştır. Erlenmayerde fıstık kabuğu hidrolizatı kullanılarak *C. tropicalis* tarafından üretilen en yüksek ksilitol (65,15 g/L) ve 0,66 g/g maksimum verim 100 g/L ksiloz konsantrasyonu ile elde edilirken, 50 g/L ve 150 g/L ksiloz ile üretilen ksilitol sırasıyla 0,65 ve 0,37 g/g bulunmuştur. Hacimsel verimlilik 100 g/L ksilozda, 50 g/L ve 150 g/L ksiloz konsantrasyonlarına kıyasla sırasıyla 1,28 kat ve 1,84 kat daha yüksek bulunmuştur. Detoksifiye edilmiş fıstık kabuğu hidrolizatının 100 g/L ksilozdaki ksilitol üretim performansı (71,73 g/L) saf ksiloz ile neredeyse aynı bulunmuştur. Ancak, maya 150 g/L'de ksilozu tüketememiş ve ksilitol üretimi gerçekleşmemiştir.

Anahtar kelimeler: Ksiloz; mikrodalga destekli yüksek basınçlı CO_2/H_2O hidrolizi; fıstık kabuğu hidrolizatı; ksilitol; *Candida tropicalis*

1. Introduction

Lignocellulosic biomass, non-edible plant residues generated from forests and energy crops mainly composed of hemicellulose (20-40%), cellulose (35-55%), and lignin (10-25%) together with low quantities of acetyl groups, minerals and phenolic substituents (Okolie et al., 2021). Lignocellulosic biomass is cheap, renewable, and bounteously accessible in nature (Chen et al., 2021). Fermentable sugars (C-5 and C-6) such as glucose and xylose released after deconstruction of the lignocellulosic biomass are valuable feedstocks for biofuels and various biochemicals production through microbial fermentation (Suhartini et al., 2022). The utilization of agricultural residues for industrial purposes is therefore gaining attention as alternative feedstocks replacing petroleum-based resources (Mujtaba et al., 2023). Pistachio shell is a valuable lignocellulosic biomass with its 46.13, 24.50, and 24.83 % of hemicellulose, cellulose, and lignin content, respectively (Hazal et al., 2024). Pistachio shell is potentially one of the most promising feedstocks for microbial xylitol production because of its rich xylan content in hemicellulose fraction (80-90%) (Özbek et al., 2020). Xylan is the primary component of hemicellulose, and it can be depolymerized into xylose, a key precursor for xylitol production (Ergün et al., 2022).

Xylitol is known as a sugar alcohol that offers the same profile of sweetness as sucrose but with approximately 40% fewer calories (Baptista et al., 2020). It is also a favorable natural sweetener with its antimicrobial and anticarcinogenic properties employed for diabetics in the pharmaceutical industry while it is utilized as a direct substitute for sugar in a wide range of food products, including sugar-free or reduced-sugar versions of candies, chewing gum, baked goods, desserts, and beverage (Xu et al., 2019; Selvasekaran and Chidambaram, 2021). Today, xylitol is produced by xylose hydrogenation chemically obtained from various sources with Ni/Al_2O_3 as a catalyst under elevated temperature and pressure conditions (Du et al., 2021). The cost of the chemical process can be relatively high, mostly because of the difficulties related to the purification and separation of xylitol (Xu et al., 2019). An alternative way to chemical processes, there are high-efficiency biotechnological processes in which xylose is reduced to xylitol using yeasts which are environmentally friendly and can utilize lignocellulosic residues as carbon source (Narisetty et al., 2021; Queiroz et al., 2022). Xylitol production via microbial route depends on xylose reductase reducing xylose to xylitol using NADPH as a coenzyme. Afterward, xylitol oxidation to xylulose is catalyzed by an enzyme known as xylitol dehydrogenase (XDH), which depends on the cofactor NAD+ (nicotinamide adenine dinucleotide) (Felipe Hernández-Pérez et al., 2019). Conversion of D-xylose to xylitol can be provided by several yeast species through their metabolic pathways. Some of these yeasts have been studied and used in biotechnology and industrial applications for xylitol production such as *Debaryomyces hansenii*, *Scheffersomyces stipitis (Pichia stipitis), Kluyveromyces marxianus* and recombinant *Saccharomyces cerevisiae* (De Albuquerque et al., 2014; Zhang et al., 2014). Further, certain bacteria, such as *Escherichia coli* (Jin et al., 2018), and various filamentous fungi, like *Aspergillus oryzae* and *Trichoderma reesei* can synthesize xylitol, however, the efficiency of xylitol production was very low. Among these microorganisms, *Candida tropicalis* stands out owing to its natural xylose conversion ability to xylitol with high yield and its strong resistance to inhibitive compounds (furfural, acetate, and phenolic compounds) that are usually released in hemicellulose hydrolysate after pretreatment (Xu et al., 2019). Conventional pretreatment methods are insufficient to promote the sustainable production of bioenergy and bioproducts and may have limitations such as high costs, longer processing times, and potential environmental impacts compared to advanced pretreatment methods such as microwave-assisted pretreatment and high-pressure CO_2 pretreatment (Sidana and Yadav, 2022). Microwave energy provides less reaction time, efficient and quick heating, and higher yield, and it is cost-efficient, environmentally friendly, and energy saving (Mankar et al., 2021). The shorter processing time of microwave-based heating can significantly reduce energy consumption compared to traditional heating methods (Hoang et al., 2021). Under pressure, carbon dioxide molecules can penetrate through the small pores in lignocellulose (Sasaki and Ohsawa, 2021). As pressure increases within high-pressure systems, the solubility of CO_2 in water rises to an optimal level, leading to a decrease in pH and facilitating hydrolysis (Kumar et al., 2021). Still, the presence of inhibitory products in hemicellulosic hydrolysate derived from lignocellulosic biomass after pretreatment can be a significant challenge for biotechnological xylitol production. These inhibitory compounds, often byproducts of the biomass pretreatment and hydrolysis process, can hinder microbial fermentation and lead to incomplete or prolonged

fermentation, making the process inefficient. To overcome this, detoxification of hemicellulosic hydrolysate is needed to remove toxic substances for biotechnological xylitol production. There is a variety of detoxification studies in the literature such as pH adjustment (Sjulander and Kikas, 2020), ion-exchange resins (Vardhan et al., 2024), and activated charcoal (Ahuja et al., 2022). The utilization of activated charcoal in the detoxification process is vast due to its simple application and is economically feasible to minimize the amounts of phenolic compounds, acetic acid, aromatic compounds, furfural and hydroxymethylfurfural (HMF) generally present in hemicellulosic hydrolysate (Arminda et al., 2021). One of the factors affecting the product yield in microbial xylitol production is the selection of microorganisms as well as the determination of appropriate fermentation conditions. The ambient temperature, dissolved oxygen concentration, pH, initial substrate concentration, the amounts of nitrogenous substances in the medium, and agitation speed are the parameters to be taken into account in microbial fermentation. As a result of proper control of these factors, high efficiency of the target product can be achieved (Manaf et al., 2023). The initial concentration of xylose in the medium is the key factor affecting xylitol yield produced by yeast. However, at high xylose concentrations, microorganism growth and product formation are inhibited due to osmotic stress (Hoppert et al., 2022).

Conventional methods, primarily employing acid hydrolysis, were used to obtain pistachio shell hydrolysate for xylitol production (Sasaki et al., 2011; Domínguez et al., 2012). This is the first research to hydrolyze pistachio shells before fermentation using an eco-friendly technique. Therefore, the aim of this work was to develop a biotechnological xylitol production from pistachio shell hydrolysate obtained with a sustainable strategy. In this context, the pistachio shell was depolymerized into C-5 sugars with microwave-assisted high-pressure CO_2/H_2O hydrolysis (MW-HPCO_2) application in the first stage. Xylose-enriched hydrolysate was detoxified using activated charcoal followed by the concentration of the hydrolysate to attain desired concentrations. Subsequently, the effect of the initial xylose concentrations on the yield of xylitol by *Candida tropicalis* was studied in flasks. Further, non-detoxified pistachio shell hydrolysate and pure xylose were compared to detoxified hydrolysate from pistachio shell for culture medium at optimum xylose concentration to investigate xylitol production.

2. Materials and methods

2.1. Materials

Pistachio shells were supplied from Gaziantep, Turkey. The shells were milled to 1-2 mm size by Brook Crompton Series 2000 (Huddersfield, UK). All chemicals and standards were supplied from Sigma Aldrich (St. Louis, MO, USA).

2.2. Preparation of pistachio shell hydrolysate by microwave-assisted high-pressure CO_2/H_2O hydrolysis

MW-HPCO_2 system was conducted by using a microwave (Milestone SynthWave, Italy) equipped with a chiller and CO_2-supplying tube. Pistachio shell and distilled water (5°C) were added into a 1 liter polytetrafluoroethylene (PTFE) vessel. The system was pressurized by CO_2 to 55 bars. Microwave power was set to 1500 W for each run. Preliminary runs were carried out by Hazal et al. (2023). The experiments were performed under optimum conditions at 190°C for 30 min previously reported by Hazal et al. (2024). Liquid (hemicellulose-enriched sugars) and solid fractions were separated by vacuum filtration. The liquid part was kept at −20°C for further use. Concentrations of fermentable sugars (xylose, arabinose, and glucose), acetyl groups (acetic acid and formic acid), HMF, and furfural in the hydrolysate were identified by HPLC.

2.3. Detoxification of pistachio shell hydrolysate by activated charcoal

Activated charcoal at different concentrations (1, 2, and 3% w/v) and temperatures (25-45°C) was mixed with the hydrolysate of the pistachio shell to remove inhibitive products (acetic acid, furfural, and HMF) for the bioconversion process. The suspension of charcoal was stirred at 200 rpm for 60 min using an orbital shaker incubator. The removal of the charcoal from the liquid part was provided through vacuum filtration. The further concentration of the detoxified hydrolysate using a rotary vacuum evaporator to obtain specific concentrations of xylose (50, 100, and 150 g/L) was applied at 70°C. The xylose, acetic acid, HMF, and furfural composition in the hydrolysate after detoxification and their concentrations were analyzed by HPLC.

2.4. Microorganism and inoculum preparation

Candida tropicalis 13803 was supplied from ATCC. Lyophilized cells were grown on Yeast Malt (YM) agar slants at 4°C. Yeast cells were cultivated in the medium containing 50 g/L xylose, 5 g/L yeast extract, 5 g/L $(NH_4)_2SO_4$, 2 g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$, and 1 g/L peptone

to prepare inoculum for xylitol production in 250 mL Erlenmeyer flasks containing 50 mL of medium (Cheng et al., 2009). A loopful of *C. tropicalis* grown on YM agar was passed to the medium. The cells were harvested at 200 rpm and 30°C for 48 h using an orbital shaker incubator (New Brunswick Innova 40R Incubator Shaker, USA). After completion of the inoculum culture, the medium was centrifuged at 4°C, 4000 × g for 10 min, and yeast cells were washed twice with sterilized water. The cells were suspended in 5 mL of the fresh medium containing 5 g/L yeast extract, 5 g/L $(NH_4)_2SO_4$, 2 g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$ and 1 g/L peptone (without xylose). The cell density of the suspension was measured by counting under the microscope using a hemocytometer to attain $1x10^7$ cells/mL.

2.5. Xylitol production in shake-flask

All shake flask experiments were carried out in 50 mL of the same medium as used in the inoculum culture: 5 g/L yeast extract, 5 g/L $(NH_4)_2SO_4$, 2 g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$ and 1 g/L peptone containing pistachio shell hydrolysate at 50, 100 and 150 g/L in 250 mL flasks at 30°C and 200 rpm using an orbital shaker incubator (Morais Junior et al., 2019; Zhang et al., 2019). The inoculum was added to the medium at $1x10^7$ cells/mL. The pH of the medium was modified by 1 N KOH to 6 using and not controlled throughout the bioconversion process. Sugar was sterilized with 0.22 µm sterile filter whereas other nutrients were autoclaved at 121°C for 15 min. Samples were withdrawn periodically at 12 h intervals until all xylose was consumed.

2.6. Determination of fermentation parameters

QP (gL^{-1} h^{-1}) volumetric xylitol yield is the ratio of xylitol produced to fermentation time, $Y_{P/S}$ (g/g) maximum xylitol yield is the ratio of xylitol produced (g/L) to xylose consumed (g/L) and q_P (g xylitol g $cell^{-1}$ h^{-1}) specific productivity is the ratio of xylitol concentration produced to cell mass during fermentation time.

2.7. Analytical methods

All samples were withdrawn regularly at 12 h intervals to analyze the microbial growth, dry cell weight (DCW), xylose consumption, and xylitol yield. For DCW samples were withdrawn at 12 h intervals and then were centrifuged at 4°C, 4000 × g for 10 min. The samples were washed twice to remove the residues of the medium. The yeast cells were then dried in the oven at 105°C. The microbial cells were counted using a hemocytometer for the microbial growth.

Xylose, acetyl groups (acetic acid), furfural, HMF, and xylitol concentrations (g/L) were determined through HPLC using an Aminex HPX-87H column (Biorad, USA) described by the method previously by Hazal et al (2023).

3. Results and discussion

3.1. Microwave-assisted CO_2/H_2O hydrolysis and detoxification of the pistachio hydrolysate using activated charcoal

As CO_2 dissolves in water, carbonic acid is formed that primarily supports the dissolution of the biomass because of decreasing in the pH linked to an increase in hydronium ion, which also contributes to the decomposition of hemicellulose into monomeric sugars, just like in acid hydrolysis (Hunt et al., 2010; Morais et al., 2015). The pH of a mixture of water (H_2O) and carbon dioxide (CO_2) has decreased to approximately 2.9. A pH of 2.9 is considered strongly acidic. The presence of such acidity in the environment is mentioned to facilitate biomass hydrolysis (Sasaki and Ohsawa, 2021). In addition, the acidity of the hydrolysis medium does not pose an environmental problem since it is easily eliminated by releasing the pressure in the process medium. The carbon dioxide will leave the reaction medium as a gas and so the medium will be free from the carbonic acid (Morais et al., 2015). In this study, the pH of the medium decreased minimum level of 2.9 through high-pressure CO_2 and the carbonic acid formation provided by dissolution of CO_2 in water. With decreasing pH and temperature supplied by microwave, xylan in the hemicellulose fraction of the pistachio shell was degraded to monomer sugars such as xylose and glucose.

Xylose (14.05±0.26 g/L) was clearly the major component in the hydrolysate. Furfural and acetic acid were 1.57±0.06 and 2.96±0.05 g/L respectively, whereas HMF (0.05±0.00 g/L) was only found in very low concentrations. Özbek et al. (2021) investigated how microwave-assisted alkali pre-treatment influences the deconstruction of pistachio shells. They found that under optimal conditions determined by response surface methodology (RSM), consisting of a microwave power of 224 W, NaOH concentration of 1.96 N, and pre-treatment time of 2.63 min, the process yielded 2.62 g/L xylose, 3.47 g/L acetic acid, and 0.30 g/L furfural. In comparison to our study, the temperature (142°C) generated by the microwave system in their study did not sufficiently break down hemicellulose into xylose. With the use of high-pressure CO_2/H_2O pretreatment for pistachio shell, xylose was obtained as 1.7 g/L at conditions of 182°C, 0 min and liquid to solid ratio of 6.16 (Özbek et al., 2018). The results indicated that the

combined use of microwave and high-pressure CO_2 resulted in a significant increase in xylose yield. The use of hemicellulosic hydrolysates in bioconversion processes can result in the formation of some inhibitive substances such as furfural, HMF, and acetic acid because of harsh process conditions. Among these substances, acetic acid has showed a strong inhibitive effect on xylose metabolism by cells (Felipe et al., 1995). The fermentation of the hemicellulosic hydrolysate can be improved by the removal or minimizing of these inhibitive compounds (Lee et al., 2011). The results of the detoxification process applied by activated charcoal are summarized in Table 1. Less than 3% of activated charcoal was not very effective in removing inhibitive compounds.

Table 1. Concentrations of xylose, acetic acid, HMF, and furfural after hydrolysis by MW-HPCO_2 and detoxification

	Temperature (°C)	Activated charcoal	Concentration (g/L)			
			Xylose	Acetic acid	HMF	Furfural
Raw hydrolysate			14.05±0.26	2.96±0.05	0.05±0.00	1.57±0.06
Detoxified hydrolysate	25	1%	12.27±0.16	2.74±0.05	-	0.21±0.01
		2%	12.24±0.11	2.60±0.04	-	0.07±0.00
		3%	12.12±0.14	2.55±0.05	-	-
	30	1%	12.23±0.14	2.85±0.04	-	0.23±0.01
		2%	12.10±0.09	2.67±0.04	-	0.07±0.01
		3%	11.55±0.12	2.62±0.05	-	0.02±0.00
	45	1%	12.25±0.21	3.06±0.06	-	0.25±0.01
		2%	12.11±0.12	2.77±0.06	-	0.08±0.00
		3%	12.03±0.11	2.67±0.04	-	0.04±0.00

Higher activated charcoal content (>3%) used in the pretreated hydrolysate decreased xylose concentration which necessity is essential for xylitol bioconversion, and the change in acetic acid was not considerable. The effect of the detoxification method changes in terms of the type of hemicellulosic hydrolysate (raw material composition and pretreatment conditions) due to differences in their toxicity (Carvalho et al., 2006). Although higher temperatures of 30 and 45°C were studied rather than 25°C, a decrease was observed in the xylose concentration. Interestingly acetic acid was more absorbed at 3% activated charcoal at 25°C. Therefore, in the detoxification process, 3% of activated charcoal was utilized in the hydrolysate at 25°C with a rotary incubator for 60 min. The xylose concentration was found as 12.12 g/L whereas HMF and furfural were completely removed from the hydrolysate, however, activated charcoal was not strongly effective in the removal of acetic acid.

3.2. The influence of initial xylose concentration on xylitol production

Initial xylose concentration in the fermentation process plays a crucial and game-changing role in xylitol production. Xylose is particularly used for cell development at low initial concentrations. Increased concentration in xylose is necessary for both microbial development and xylitol production (Winkelhausen and Kuzmanova, 1998). Nevertheless, too high xylose concentrations can cause substrate inhibition and negatively affect xylitol production (Xu et al., 2019). In this context, different xylose concentrations were used in the ranges of 50, 100, and 150 g/L for the production of xylitol by *C. tropicalis*.

The change of DCW concentration in detoxified hydrolysate with concentrations of 50, 100, and 150 g/L of xylose depending on the cultivation time is presented in Figure 1-3. The lag phase was observed at 12 h in the culture medium with 50 g/L hydrolysate whereas this phase lasted 12 h and 24 h for 100 and 150 g/L, respectively. The highest dry cell concentration for 50 g/L was 11.70±0.12 g/L, however, this value started to decline after 60 h. The cell density showed a continuous increase during the cultivation period of 168 h at 150 g/L. Despite the depletion of xylose in the culture medium, yeast growth continued. When xylose in the medium was exhausted after 168 h, xylitol was therefore utilized for cell growth (Buhner and Agblevor, 2004). When looking at the culture medium at 100 g/L, a decrease was observed after 158 h. The highest concentration of dry cells reached 31.70±0.23 g/L at 100 g/L. Similar results have been obtained in the literature (Buhner and Agblevor, 2004; Ko et al., 2006) when compared with studies on the production of xylitol with *C. tropicalis.*

Higher xylose concentrations (100-150 g/L) enhanced yeast growth, however, *C.tropicalis* could not metabolise sole carbon source xylose until 12 and 24 h for 100 and 150 g/L respectively. At 50 g/L, xylose was consumed rapidly within 60 h while yeast growth was highest at 96 h for 100 g/L and 120 h for 150 g/L then decreased dramatically. The increasing xylose concentration resulted in the depletion of xylose consumption by *C. tropicalis*. Higher substrate concentration in a hydrolysate medium decreased xylitol production by *C. tropicalis* due to an unfavourable effect on yeast growth (Li et al., 2012).

A high initial xylose concentration, especially when using a small amount of xylose for cell growth, can be advantageous for xylitol production. This means that a larger portion of the xylose is available for conversion into xylitol, potentially leading to higher xylitol yields. However, there is a limit to how much xylose concentration can be increased. If the xylose concentration becomes too high, it can lead to substrate inhibition. Substrate inhibition occurs when the high concentration of the substrate (xylose, in this case) starts to negatively affect the metabolic activity of the microorganisms, leading to reduced xylitol yield (Mussatto and Roberto, 2008).

The consumption of xylose and xylitol concentration in the detoxified pistachio shell hydrolysate of 50 g/L by *Candida tropicalis* were demonstrated in Figure. 1. Whilst xylose was consumed rapidly by the yeast between 12 and 24 h, 20.18±1.76 g/L of xylitol was produced. This increase remained up to 60 h and the xylitol concentration reached to 31.96±1.50 g/L. After this, xylose was almost completely depleted, and xylitol yield also decreased. In the literature, the fermentation carried out using pure xylose (50 g/L) with *C. tropicalis* 20336, 33.6 g/L xylitol was obtained (Ko et al., 2006). Compared to the current study, almost the same amount of xylitol production was obtained. This, in turn, has proved that pistachio shell hydrolysate is promising for the production of xylitol.

The highest xylitol production was obtained at 65.15±1.5 g/L for 100 g/L of concentrated xylose of the pistachio shell hydrolysate after 96 h with xylose consumption of 98.00±0.91 g/L shown in Figure 2. Results were similar to the report by Xu et al. (2019) who investigated xylitol fermentation by *Candida tropicalis* from sugarcane bagasse hydrolysate and reached the highest xylitol concentration of 62.98 g/L after 54 h.

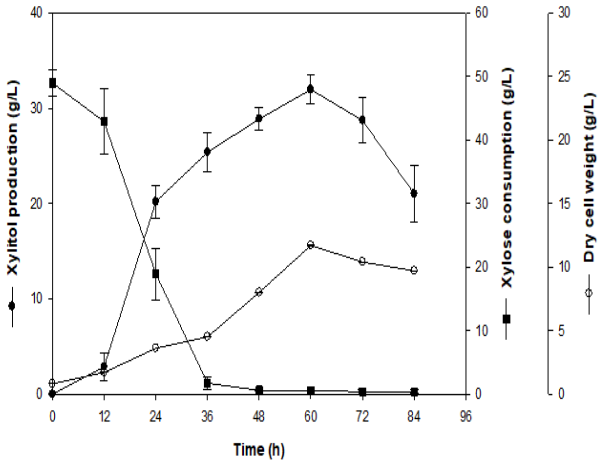

Figure 1. Shake flask by *C. tropicalis* for xylitol production (g/L), xylose consumption (g/L), and DCW (g/L) using concentrated initial xylose concentrations of 50 g/L

The phenomenon involved in this process can be acetic acid concentration in the fermentation broth because the amount of acetic acid in the fermentation medium did not affect much xylose consumption when it was around 2 g/L, but when this value reached 4 g/L, the concentration of xylitol in the medium dramatically dropped by 72% (Cheng et al., 2009).

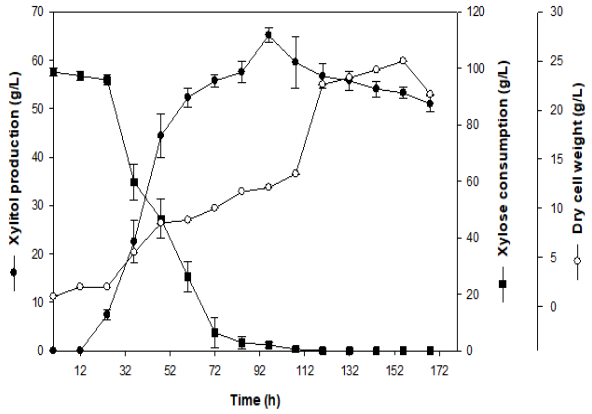

Figure 2. Shake flask by C. tropicalis for xylitol production (g/L), xylose consumption (g/L), and DCW (g/L) using concentrated initial xylose concentrations of 100 g/L

In this study, even though the detoxification process was performed for 150 g/L of concentrated pistachio shell hydrolysate, the amount of acetic acid was maintained at a very high concentration (9.56±2.62 g/L), and acetic acid was not well absorbed by activated charcoal. This could be one of the reasons why the concentration of xylitol remained low in bioconversion trials where the initial concentration was 150 g/L (Figure 3). Zhang

et al. (2012) investigated the effects of initial xylose concentration on xylitol production from 100 to 300 g/L using *Candida athensensis* SB18 in flasks. They reported that 69.02±0.84 g/L xylitol was produced by 100 g/L initial xylose concentration after 96 h. Similarly, maximum xylitol concentration was obtained at 96 h in this study. However, different bioconversion capacities of yeasts and the type of the hydrolysate can impact the xylitol production. The differences in behavior observed among different yeasts when metabolizing xylose can indeed be attributed to variations in the initial stage of xylose metabolism. The enzyme responsible for this stage is xylose reductase. The activity of xylose reductase is known to depend on the initial concentration of xylose (Mussatto and Roberto, 2008). When the concentration of xylose is high, the enzyme tends to exhibit higher activity, resulting in efficient conversion of xylose to xylitol. On the other hand, at low concentrations of xylose, the activity of xylose reductase may be limited, leading to reduced conversion of xylose and, consequently, lower xylitol accumulation (Gurpilhares et al., 2009).

The effect of the initial xylose concentrations on the xylitol yield represented in Table 2 was subjected to $Y_{P/S}$ (g xylitol/g xylose) maximum xylitol yield, q_P (g xylitol g cell^{-1} h^{-1}) specific xylitol production rate and Q_p (gL^{-1} h^{-1}) volumetric xylitol yield.

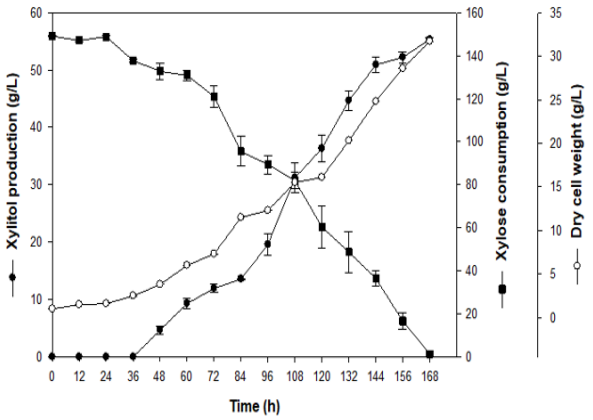

Figure 3. Shake flask by *C. tropicalis* for xylitol production (g/L), xylose consumption (g/L), and DCW (g/L) using concentrated initial xylose concentrations of 150 g/L**.**

Xylose was almost completely consumed in all three concentrations. Although the xylitol yield for 50 and 100 g/L of xylose concentrations was constant after completion of the bioprocess, the highest volumetric xylitol yield and specific xylitol production rate were 0.68±0.02 gL^{-1}h^{-1} and 0.06±0.00 for 100 g/L, respectively. Volumetric productivity was higher as compared to the results reported by Tamburini et al. (2015) who studied xylitol production using pure xylose in the synthetic medium.

Table 2. The effect of the initial xylose concentration on xylitol yield by *Candida tropicalis*

Initial xylose concentration (g/L)	Incubation time (h)	Xylose consumption (g/L)	Xylitol concentration (g/L)	Xylitol productivity (gL^{-1} h^{-1})	Xylitol yield (%)	Specific productivity (g xylitol g cell^{-1} h^{-1})
50	60	49.67±0.29	31.96±1.50	0.53±0.03	64.34±2.64	0.05±0.01
100	96	98.00±0.91	65.15±1.49	0.68±0.02	67.00±0.90	0.06±0.00
150	168	148.34±0.30	55.15±0.52	0.33±0.00	37.17±0.27	0.01±0.00

3.3. Comparison of xylitol production from hydrolysate, detoxified hydrolysate, and pure commercial xylose

On the contrary, pure xylose and undetoxified pistachio shell hydrolysate were used at an initial concentration of 100 g/L xylose (optimum xylose concentration found in detoxified pistachio shell hydrolysate) in the culture medium to evaluate the xylitol production by *C. tropicalis*. The use of undetoxified hydrolysate did not allow the yeast to consume xylose because of the inhibitive compounds (acetic acid, HMF, and furfural) formation after pretreatment. The results of xylitol production, xylose consumption, and DCW at 100 g/L using pure xylose are shown in Figure 4.

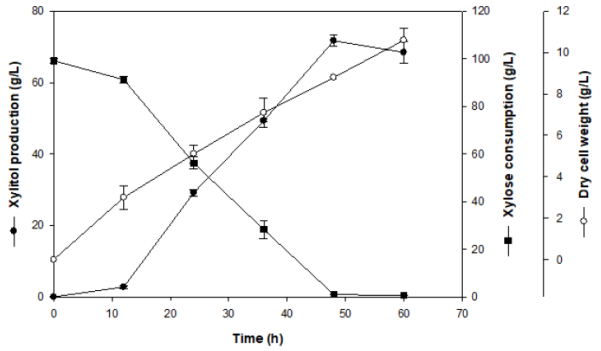

Figure 4. Xylose consumption, xylitol production DCW (g/L) at 100 g/L of pure xylose

Xylose was consumed by *C. tropicalis* until 48 h and 71.73±1.61 g/L xylitol was produced. After that, a depletion occurred in the xylitol production. Xylose consumption was highly effective on yeast growth leading to 10.6±0.57 g/L of DCW. Prakash et al. (2011) studied *Debaryomyces hansenii* to produce xylitol using D-xylose and sugarcane bagasse hydrolysate. They reported that maximum xylitol yield and volumetric productivity were 0.76 g/g and 0.44 g/L/h by D-xylose while using sugarcane bagasse hydrolysate the yield of xylitol and volumetric productivity were 0.69 g/g and 0.28 g/L/h.

4. Conclusion

In summary, a green method, microwave integrated with CO_2 system, was applied for depolymerization of lignocellulosic biomass into reducing sugars. The removal of inhibitive compounds through detoxification facilitated the bioconversion to produce xylitol. Highest xylitol yield of 67% was achieved after the bioconversion of the pistachio shell hydrolysate at 100 g/L of xylose by *C. tropicalis* ATCC 13803. *C. tropicalis* had the capacity to ferment xylose for xylitol production. However, the conversion of xylose into xylitol was inhibited by the existence of acetic acid, HMF and furfural in the undetoxified pistachio shell hydrolysate after pretreatment. When the concentration of xylose in the culture medium increased, the xylose consumption was strongly affected by the yeast, resulting in decrease in the xylitol production. Therefore, initial xylose concentration should be optimized for the efficiency of the xylitol production. With this work, a green and sustainable system revealed that pistachio shell hydrolysate is a promising carbon source for xylose assimilating yeast to produce xylitol. This study can pave the way for new approaches in terms of xylitol production and valorisation of value-added products from lignocellulosic wastes.

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6. Kaynaklar

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