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Fermentation processes by Meyerozyma guilliermondii and Saccharomyces cerevisiae for co-production of xylitol and bioethanol co-Production

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ABSTRACT

The utilization of lignocellulosic biomass in the development of bioproducts has increased significantly in recent years. Biorefinery technologies have focused on process integration for the production of different valuable coproducts to reduce the overall processing cost. In this study, agricultural wastes from rice straw were used for the co-production of bioethanol and xylitol. Bioethanol is produced from the cellulosic fraction and xylitol from the hemicellulose fraction after elimination of lignin using chemical pre-treatments. The chemical treatment was carried out with diluted Sulfuric acid 2.5% at 100 °C for 30 minutes, and then exposed the cellulosic fraction of the solid phase resulting from the chemical process to the enzymatic action of the fungus Trichoderma harzianum for release sugars and fermented at a later stage using *Saccharomyces cerevisiae* for bioethanol production in a simultaneous saccharification and fermentation process. The liquid phase hemicellulose fraction was exposed to the action of *Meyerozyma guilliermondii* strain F22 (*Pichia guilliermondii*) for xylitol production. Resulting was accomplished maximum concentrations and yield was 32.6 g/L 0.39g/g and 20.1 g/L, 0.44g/g for bioethanol and xylitol respectively of the total glucose and xylose presented in rice straw, the co-production of xylitol with ethanol in an integrated biorefinery would create economic benefits making the overall lignocellulose-based process more cost-effective.

1. Introduction

A rapid depletion of conventional energy sources worldwide represents a dire situation that demanding a potential replacement to surmount the current energy crisis. Lignocellulose which represents a logical candidate for biofuel production as its materials being supplied from a variety of resources at a low price. Moreover, it can be used as raw material for chemicals and bio-products [13]. The xylose from bioethanol process by-products might be recovered and used for produce xylitol [45]. Xylitol is a high-value polyalcohol that is produced by reducing (from hemicellulose fraction of lignocellulose) which is among the top 12 value-added chemicals as a building block for organic

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synthesis considered by the Department of Energy/USA and has a high market value in food and pharmaceutical industries [37].

Many yeasts can convert xylose into xylitol. Xylose reductase (XR) catalyzes the initial step of a fungal pathway that permits certain organisms, such as Candida boidinii [6] Candida guilliermondii [39], Candida tropicalis [18] and Candida parapsilosis [22] to metabolize xylose. Pichia stipitis often produces a very low yield of xylitol, but it can be increased after some genetic modifications [40] M. guilliermondii displays potential for being used for xylitol production in biorefineries [10,4 and 58].

Xylitol, one of the polyol sugars, has a wide range of applications, including sugar replacement in food and pharmaceuticals [41]. Xylitol did not require insulin to regulate metabolism in a human's body. Thus, people with diabetes could consume this sugar. Moreover, this sugar also was able to prevent dental caries, so considerably applied in toothpaste [41and 38]. The xylitol production has increased more than forty times in four decades. The manufacturing of xylitol was reported jointly in 1978, with a global total of 6 000 tons. In 2016, the estimated global market for xylitol was 190.9 thousand metric tons, valued at US$725.9 million. The prediction for 2022 is 266.5 thousand metric tons, valued more than US$1 Billion [38]. Xylitol exists in fruits and vegetables, although in quantities of less than 1%, so its direct extraction is economically unfeasible [3]. Xylitol can be produced chemically, and biotechnologically. Chemical processes require catalysts such as ruthenium, palladium, and nickel at high temperatures (80-140 °C) and high pressures (±50 atm). Chemical processes are usually expensive and require high energy and costs. As an alternative, biotechnological replacing traditional chemical processes. Alternatively, lignocellulosic hydrolysate xylose can be fermented to produce xylitol [51]. The xylose in the bioethanol process's by-products might be recovered and used to produce xylitol. Because xylitol has a higher market value than ethanol, the manufacture of xylitol from xylose in hemicellulose should be a better option to fermenting xylose to bioethanol [55 and 11].

Three essential aspects must be considered for economically feasible bioethanol production: feedstock pre-treatment technique, enzymatic hydrolysis, and fermentation [15], the fermentation processes of lignocellulosic hydrolysate such as simultaneous saccharification and fermentation (SSF), separate hydrolysis and fermentation (SHF), consolidated bioprocessing (CBP), and simultaneous saccharification and co-fermentation (SSCF) have to be selected carefully for valuable solutions to the increasing liquid fuel demand. These techniques are expected to play a significant role in replacement of oil-based refineries with lignocellulosic biomass-based biorefineries in the future [46,20 and 54]. Rice straw is one of the most abundant lignocellulosic wastes on the world, with an annual production of 731 million tons. Rice straw, unlike other straws, is not commonly used as animal feed due to its low digestibility, and it appears to have a low social value, thus it is burned openly on the field, polluting the air [56 and43]. The use of cellulose-derived hexosans for ethanol production has been accomplished, although the pentose fraction has remained industrially unviable to date [25]. This work aimed to evaluate the simultaneous co-production of xylitol and bioethanol from rice straw by some fungi.

2. Material and method

2.1 Study area

The study was carried out at Industrial Fermentation unit -Applied Industrial Microbiology Dept. Agriculture Research Directorate / Ministry of Science and Technology, Iraq from January, 2018 to December, 2019).

2.2 Microorganism

The yeast Saccharomyces cerevisiae, Meyerozyma guilliermondii strain F22, and Trichoderma harzianum were used in this study. S. cerevisiae was obtained from the local market, Meyerozyma guilliermondii strain F22 (Pichia guilliermondii) GenBank: MH429782.1 from protoplast fusion between S. cerevisiae and P. stipitis ATCC 58785, which the molecular study using DNA content, RAPD, DNA sequences, and protein profile were applied for improvement of biofuel production from biomass [44 and 14]. T. harzianum were obtained from the biological control Dept. Agriculture Research Directorate / Ministry of Science and Technology, Iraq. S cerevisiae were grown on YPG agar (10 g/L yeast extract, 20 g/L peptones, 20 g/L glucose, 25 g/L agar). Seed cultures were prepared in YPG media at 30 °C, 200 rpm for incubated overnight before use. Meyerozyma guilliermondii strain F22 was grown on a malt agar medium and stored at 4°C. Cells were cultivated on fresh malt agar plates to be used within 24 h of incubation at 30°C. Cells were grown of YPX (1% yeast extract, 2 % peptone, and 3%(w/v) xylose) in an orbital shaker (200 rpm) at 30°Cfor 24 h. T. harzianum was grown on slants of Vogel minimum salts medium [32 and7], xylose (1%), and agar at 4ºC. Vogel's Medium [28] for 1 liter 50× salts: water 755 mL, Na3 citrate.2H2O 125 g, KH2 PO4 250 g, NH4NO3 100 g, MgSO4.7 H2O 10 g, CaCl2. 2H2O 5 g, trace element solution 5 ml, and biotin stock solution 2.5 ml. Enzyme production was carried out under solid-state culture. The microorganism was grown in test tubes containing Vogel minimum salts medium and xylose (1% w/v) as the carbon source, during 7 days, to get spores. These spores were aseptically scraped from the surface of agar plates and re-suspended in sterile Tween 80 solution (0.1% v/v) in physiological saline, and this solution was used as inoculum.

2.3 Pre-treatment of rice straw biomass

Rice straw was obtained from local farmers. The biomass was dried for 24 h at room temperature, cut into particles of ~ 2 cm, and ground. Particle size between 100 and 500 μm was selected by sieve, afterwards, it was dried in an oven at 55 °C until the moisture content was less than 5% (w/w). The temperature of treatment (55 °C for 24 h) was selected due to its high drying efficiency and to prevent microbial growth after drying. It was then stored for further use. Ground rice straw100g as (10% w/v) was pre-treatment with 2.5 % (v/v) dilute sulphuric acid at 121 °C for 30 min for efficient separation cellulose and hydrolyze hemicellulosic sugars [54]. Biomass slurry after pre-treatment was washed and separated by filtration (Whatman No 1 filter paper) into solid cellulose phase and liquid hemicellulosic hydrolysate phase. Solid-phase was washed with distilled water until the washings were of neutral pH and then used for bioethanol fermentation. whereas the liquid hemicellulosic phase was used for the estimation of reducing sugars and used for xylitol fermentation.

2.4 Composition of rice straw.

The cellulose, hemicellulose, and lignin composition of the rice straw were estimated according to the procedure of the National Renewable Energy Laboratory [49 and 36] and the formulas required for calculating cellulose and hemicellulose were according to [35].

2.5 Experiments on liquid phase (hemicelluloses hydrolysate) for xylitol production

During the pre-treatment process of lignocellulose biomass, the production of the inhibitors HMF (from glucose) and furfural (from xylose) [53 and 31] these inhibitor compounds can inhibit the growth of several microorganisms. To detoxify the hemicellulose hydrolysate, the pH of the concentrated hydrolysate was increased to 7 using CaO followed by filtration to remove solid maters, and the pH of the solution was then re-adjusted to 5.5 using sulfuric acid. The obtained solution was centrifuged to remove precipitates. Subsequently, the concentrated hydrolysate was incubated with 3

% activated carbon for 60 min in a 1000-mL container (30 °C and 100 rpm). Then, the liquid the detoxified hydrolysate was separated using filtration. The hemicellulose hydrolysate media was containing ~45g/L xylose was re-filtrated through a 0.22-µm pore filter membrane system. hydrolysates were supplemented with 5 g/l yeast extract supplemented with 5 g/l urea or 5 g/l (NH4)2O4 as a nitrogen source [8]. The inoculum culture was 5 % (v/v) 24 h old inoculum culture of Meyerozyma guilliermondii strain F22 was used to include the detoxified hemicellulose hydrolysate. The fermentation of 250 ml of detoxified hydrolysate was conducted in a 1L fermenter (Lambda minifor-bench-top-laboratory-fermenter) and pH adjustment (pH 5.0) at aerobic conditions at 30 ºC for 60 hr. The rate of agitation was set to 150 rpm and that of aeration to 0.5 vvm. 50% (v/v) silicon antifoam agent was used to check the formation of foam during the process. pH and dO2 (dissolved oxygen) were also checked but not regulated during fermentation.

2.6 Experiments on solid phase (cellulosic hydrolysate) for bioethanol production

Enzymatic cellulose hydrolysate by Trichoderma harzianum.

The production of xylanase was carried out using SSF [16]. In Ten ml of diluents (Vogel's media) was transferred into the 250 ml Erlenmeyer flask containing 10 g of rice straw and mixed well. The flasks were cotton plugged and sterilized in an autoclave at 121 C for 15 min. After cooling the flasks at room temperature, inoculated them with 1.0 ml of fungal conidial suspension under sterile condition and incubated at 30 ºC for seven days. After seven days of fermentation, 50 ml of extractants (distilled water, 0.1% glycerol, 0.1% NaCl, 0.1% tween-80, and citrate buffer pH 5) was added into the fermented mash flask and rotated on a rotary shaker at 150 rpm for 2 h at 30 º C for maximum extraction of enzyme. Then filtered slurry through muslin cloth before being centrifuged at 8000 rpm at 4 C for 10 min to separate fungal spores and tiny particles. The clear supernatant was used as a source of crude xylanase.

Simultaneous Saccharification fermentation (SSF)

The solid-phase cellulosic hydrolysate was hydrolysed by 10 mL crude xylanase with its activity of 750 U/mL and measured with the method of [2]. Enzymatic hydrolysis was performed using a rotary shaker incubator at 60 °C with the agitation of 150 rpm with percentages of solids of 5-10% [5]. The value of pH was controlled with acetic buffer (pH 5.0). Hydrolysis was stopped after 72 h and hydrolysate was collected for monomeric sugar analysis. Around 5 g/L (NH4)2SO4, 1.7 g/L yeast nitrogen base, [54] was added, and 10 % inoculum of S. cerevisiae was inoculated at 30 °C and 150 rpm 1L fermenter (Lambda minifor-bench-top-laboratory-fermenter) for 60 h. Fig. 1 diagram describing rice straw processing steps for co-production of bioethanol and xylitol.

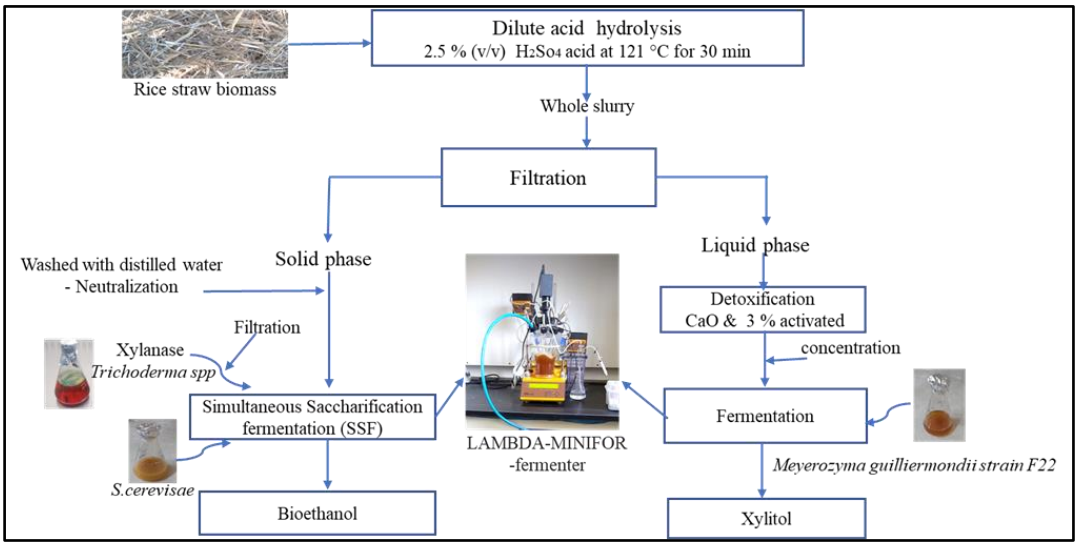

Fig. 1 - Diagram of co-production of bioethanol and xylitol from hydrolase rice straw.

2.7 Extraction products

Bioethanol extraction

To extract the bioethanol, the fermented culture was distilled to collect the ethanol [38]. The ethanol concentration of the fermentation liquid was determined by measuring the specific gravity of the distillate according to [60]. The distillate obtained was used for the analysis of ethanol by Gas Chromatography. The concentrations of the total sugar (glucose, xylose) were determined as glucose equivalent by the Anthrone method as explained by [1].

Xylitol extraction and determination

Xylitol extraction by Liquid-liquid extraction [31]. The filtered fermented broth (50 ml) was extracted with either ethyl acetate, dichloromethane or chloroform (3 · 50 ml). HPLC was used to identify sugars and xylitol in the aqueous fraction with an HPX-87H (300 · 7.8 mm column, at 45 C, using 5 mM sulphuric acid as the eluent at 0.6 ml min)1. The eluate was monitored with an RI detector.

3. Results and discussion

Table 1 listed the carbohydrate and lignin in the rice straw. In untreated rice straw, the glucan content was 34.7%. The content was 20.2 percent of hemicellulose (xylon), + mannan + galactan. The highest amount of the xylose favours the xylitol, their sugars represent only a tiny part of the total biomass sugar. The components, cellulose, hemicellulose, and lignin are relatively different in terms of the time of harvest, cultivation area, and various environmental factors, associated with plant growth [19]. Selective fractionation of hemicellulose and its hydrolysis is possible only when the hydrolysis of biomass is done via dilute acid or hydrothermal treatment. These two popular pre-treatment approaches produce pre-hydrolysate rich in cellulolignin and C5 sugars. Diluted acid reduces the severity of the procedure (to 120 °C) but very high temperatures (To 180 °C) are required in hydrothermal pre-treatments to treat water as an acid [33]. [21] reported that sulfuric acid was chosen from different acids based on the high rice straw saccharification yield. This pre-treatment usually disrupts the glycosidic link between hemicellulose and lignin which results enhances cellulose's enzymatic accessibility to dissolve hemicellulosic sugars.

Table 1. Chemical compositions of untreated and acid-treated rice straw.

Component %	Untreated %	Treated %
Cellulose (glucan)	34.7 ±0.5	36.4 ±03
Hemicellulose (xylan)	20.2±0.8	23.3±0.2
Lignin (AIL+ASL) *	16.8±0.3	2.02 ±0.4
Ash	8.4±0.3	9.4±0.4

Note: * AIL (acid-insoluble lignin); ASL (acid-soluble lignin). The data in the table show the mean value and standard deviation.

3.1 Xylitol production from batch fermentation of hydrolase hemicellulose using Meyerozyma guilliermondii strain F22

The first pre-treatment, which used diluted sulfuric acid to extract the hemicellulose fraction from the biomass, was effective. A high concentration of xylose, was found in the acidic liquid hydrolysate. [19] reported that thermal acidic pre-treatments generate thermally degraded inhibitor products such as furfural (2-furaldehyde), hydroxymethylfurfural (5-hydroxymethyl-2-furaldehyde, 5-HMF), and other aliphatic acids (acetic, formic, and levulinic acid). The inhibitory effects of these compounds were resolved using calcium salt precipitation and a charcoal column in this study, allowing the xylose sugar to be used. Other detoxification techniques include physical methods (evaporation, stream stripping, solvent extraction, phase separation, membrane filtration, and so on), chemical methods (ion-exchange column), and biological methods (enzyme filtration) [29]. [53] expected that detoxification of hydrolysate biomass before fermented Meyerozyma caribbica Y67 to produce xylitol. It can lower inhibitor concentrations, allowing microbial growth will continue unhindered and more products to be made. The liquid hydrolysate used to produce xylitol was rich in xylose, comprising about 83% of total sugars at its concentration of 18 g/L. The hemicellulose fraction is the liquid phase from acid hydrolysis of rice straw composed of sugars mainly xylose and other sugars glucose, mannan, and galactan, with great potential for xylitol production by hydrogenation [12]. The hydrolysate was concentrated to increase the content of xylose before using in fermentation. Table 2 shows that 45.03+0.3 g/ L of xylose was produced. However, some amount of glucose was found in the hemicellulosic hydrolysate

In batch fermentation kinetics of Meyerozyma guilliermondii strain F22 of hemicellulosic hydrolysate rice straw, Glucose was initially used for cell growth and was quickly consumed within 12 h. The presence of glucose in the hydrolysate at low concentration is helpful because this easily metabolized carbon source can be utilised by the yeast cell for growth, increasing the xylose available for conversion to xylitol after glucose depletion, xylose utilization in bacteria, fungi and yeast faces significant obstacles due to the preference of microbes for glucose as a carbon substrate over xylose, commonly known as carbon catabolite repression which causes largely ignored biochemical route for the xylitol production [47]. According to [57], concentrations of monomeric sugars in the hydrolysed liquor, used as a fermentation medium, may cause osmotic stress. This can either inhibit the generation of the enzyme xylose reductase, or cause bioethanol to be produced. Glucose [9] can significantly affect the xylitol production by M. guilliermondii. Xylose reductase and xylitol dehydrogenase are both inhibited in the presence of hexoses. When using hemicellulosic hydrolysate as substrates, glucose catabolite repression will be a major factor prevention xylose from being converted to xylitol due to the presence of glucose. Oxygen is a critical component for xylose uptake since the shift to anaerobic conditions stopped both xylose utilisation and metabolic activity. In a related study, the maximum xylitol yield for Candida tropicalis under semi-aerobic conditions, was 0.62 g/g xylose,

while under microaerobic conditions, the maximum yield was 0.36 g/g [57]. Our previous study in protoplast fusion between S. cerevisiae and P. stipitis obtained fusant, fusant which appear decrease in bioethanol production compare with a parent were selected for current study on xylitol production. [4] reported that Meyerozyma guilliermondii, a non-conventional yeast, is being considered as a biotechnological candidate for xylitol processing. This is in agreement with the aforementioned [33] about the improvement of xylitol production from sugarcane bagasse by using phylogenetically identified as Pichia fermentans isolated from a chemical mutagenesis using ethyl methanesulfonate (EMS). This mutant produced maximum xylitol titers and yields of 34.0 g/L and 0.68 g/g, respectively compared with the control wild type strain 27.0 g / L xylitol with a conversion yield of 0.45 g / g under the same production conditions. Pichia stipitis FPL-YS30, a xyl3-Δ1 fusant that metabolizes xylose under aerobic and oxygen-limited culture conditions produced a negligible amount of bioethanol and converted xylose mainly into xylitol with comparable yields 0.30 and 0.27 g /g respectively [40].

xylitol concentration 20.1 g/L in this study was comparable with the reported value of 25.1 g/L by co-culture of S. cerevisiae and C. tropicalis in [59] with lower productivity. In this study was attained 0.44g/g compare with 0.58 g/g which could be a result of different composition of hydrolysate rice straw and detoxification /concentration steps implemented led to maximum sugar yield 81 % conversion and continuous co-production process used in a membrane bioreactor. The maximum xylitol production by Meyerozyma caribbica Y67 on sugarcane hydrolysates at 24 hr showed 3.2 g/L, whereas the xylitol production on corncobs showed 3.4 g/L [53]. M. guilliermondii shows the ability for xylitol production in biorefineries [10,4 and 58]). Candida guilliermondii, the anamorph stage of Pichia guilliermondii, is one of several Candida species capable of producing xylitol, and since certain Candida species are pathogenic, M. guilliermondii (Pichia guilliermondii) has been marketed as the favored yeast species for use in the food and health industries [42]. The maximum production of xylitol obtained in this study was higher than those obtained using C. tropicalis in corncob hydrolysate (17.1 g/L) and wheat straw hydrolysate (15.8 g/L) reported previously in [42 and 8], respectively. the conversion of xylose to bioethanol can be stimulated to the levels of other sugars present.

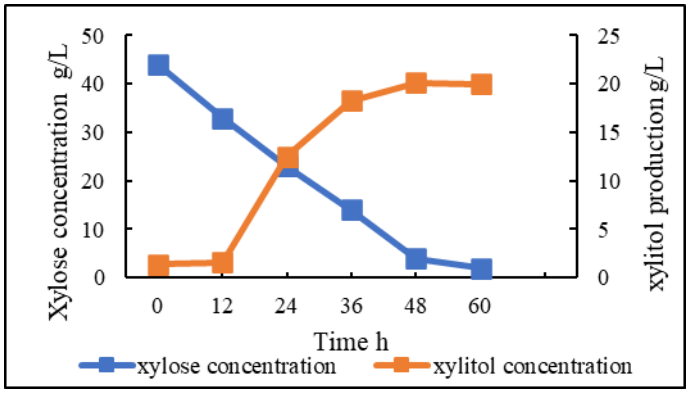

Figure 2: Batch fermentation of the xylose extract by the Meyerozyma guilliermondii strain F22 using hemicellulosic hydrolysate rice straw at 30 °C and 150 rpm for 60 h.

Yeast cell growth was slow until 12 h, according to a lag phase, and then increased exponentially before entering a stationary phase between 36 and 50 h (Figure 2). The concentration of xylose decreased gradually due to cell growth. All of the xylose in the culture broth was depleted at 60 h. The highest xylitol concentration was 20.2 ± 0.8 g/L at 60 h, giving a xylitol yield of 0.44 g/g. In the current study, yeast strains Meyerozyma guilliermondii strain F22 (Pichia guilliermondii) resulting

from the genetic modification of the protoplast were used in the study, as many studies indicated that yeasts that consume xylose to produce ethanol when exposed to the genetic modification inhibit this pathway and produce xylitol.

Many yeasts convert xylose to xylitol, but it increases following some genetic modifications [40] using D-xylulokinase mutant of Pichia stipitis converted the stover hydrolysate into xylitol with the highest xylitol yield (0.61 g/g and volumetric productivity (0.18 g/Lh). Also, many recent studies have employed genetic modification with reliable engineering to improve xylitol productivity [8,5,12,40 and 17]. [19] was obtained 35.2± 0.8 g/L xylitol concentration within 61 h for a yield of 0.44 g/g during batch fermentation empty palm fruit bunch fibre using the adapted Candida tropicalis and the post-pre-treated xylose solution,

liquid¬_liquid extraction was used to recover xylitol from a fermented medium. The unwanted impurities were extracted from the broth using ethyl acetate according to recommendations [30] for preference in the extraction process over chloroform or dichloromethane. Because of the low product concentration and complex composition of the fermented broth, xylitol recovering and purification is the most difficult step in this process. However, no method for efficiently purifying and recovering xylitol, which is required for the production of xylitol to become economically competitive. Liquid–liquid extraction is used to purify solutions in a variety of industrial applications in recovering dissolved chemicals or remove unwanted impurities. It is simple, clean, fast, and easy owing to its low boiling points [30].

3.2 Bioethanol production from Simultaneous Saccharification fermentation (SSF)of cellulosic hydrolysed

Biological conversion of carbohydrates in lignocelluloses to ethanol can be realized by SSF of the pre-treated raw material. Lignocellulolytic enzyme complexes play a crucial role in the hydrolysis of lignocellulosic biomass [28]. Reduced number of process reactors is one of the features of SSF, which integrates enzymatic hydrolysis and fermentation in one reactor. In SSF, the released sugars from enzymatic hydrolysis are simultaneously utilised by the fermenting microorganism as S. cerevisiae during fermentation avoiding effect inhibition of enzymes and also lowering the risk of contamination [20 and 54]. Table 2 shows that 82.02+0.3 g/ L of glucose was produced from cellulosic hydrolysate phase rice straw after concentration. SSF kinetics is shown in Fig. 3 S. cerevisiae completely utilized glucose within 48 h and produced the maximum bioethanol production of 33.4 ± 0.5 g/L with the yield and production values of 0.40 ± 0.01 g/g and 1.34 ± 0.01 g/L-h, respectively. The removal of hemicellulose in the first pre-treatment stage which used diluted sulfuric acid increased the biomass's pore sizes. As a result, it improved cellulase accessibility and digestibility [19]. After rice straw was pretreated with acid, the solid cellulose phase has been saccharified to produce sugar reduction using the crude enzyme secreted by the Trichoderma harzianum to produce reducing sugars. Pre-treated solid with glucose composition was utilized as substrate for ethanol production in (SSF) using S. cerevisiae and enzyme cellulase from Trichoderma as found superior as compared to the other fungal strains [34]. The microbial cellulase system is responsible for the generation of glucose from cellulose. Endoglucanases, exoglucanases, and β-glucosidases are the three main groups of cellulases [27 and 50]. Recently commercial research has numerously expanded towards low-cost, easily available, and relevant feedstock for large-scale industrial of biofuels [21].

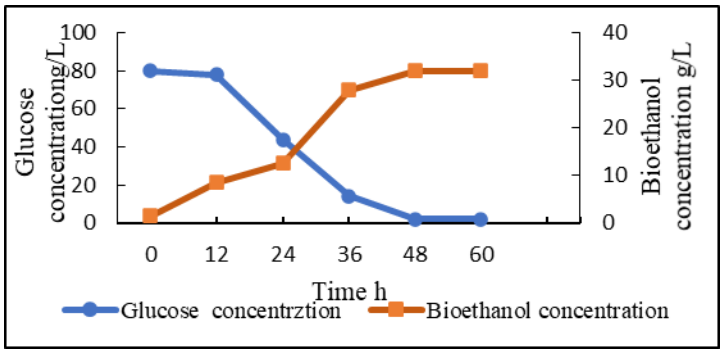

Figure 3: profile of glucose utilization for bioethanol production in simultaneous saccharification and fermentation (SSF) using S. cerevisiae and enzyme cellulase from Trichoderma harzianum using cellulosic hydrolysed rice straw at 30 °C and 150 rpm for 60 h.

A low level of glucose accumulation indicated a proper balance between enzyme and yeast cell performances during SSF. Yeast cell growth was observed in the first 24 h. The pre-treated solid with a glucose composition of 82.02+0.3g/L was utilized as substrate for bioethanol production in (SSF) using S. cerevisiae and enzyme cellulase. [48] reported that the SSF and rice straw released a high amount of reducing sugars (728.45 mg/g) at 60°C and pH 5.0 after 48 h using cellulolytic enzymes of Myceliophthora thermophila at resulted in 18.07 g/L ethanol after 72 h fermentation by S. cerevisiae.

Table 2: Kinetic parameters for production of bioethanol and xylitol from rice straw. maximum production and production for ethanol and xylitol.

	Reducing sugars g/L		Fermentation kinetics					
	Glucose	Xylose	Bioethanol			Xylitol		
			g/L	Yield g/g	QP g/Lh	Concentration g/L	Yield g/g	QP g/Lh
Solid-phase	82.02+0.3		32.6	0.39	1.34			
Liquid-phase	3.12+0.12	45.03+0.3				20.1	0.44	0.42

Solid-phase was cellulosic hydrolysate
Liquid-phase was hemicelluloses hydrolysate
Bioethanol production by S. cerevisiae by fermenting the enzymatic hydrolysate of cellulosic fraction
Xylitol production by Meyerozyma guilliermondii strain F22 by fermenting the hemicellulosic hydrolysate obtained by the acid hydrolysis

Table (2) showed the maximum concentrations of bioethanol and xylitol in batch cultures and SSF were recorded at 32.6 g/L (0.39 g/g yield) and 20.1 g/L (0.44 g/g yield), respectively, comparable to the result of [49]as the ethanol and xylitol productions were recorded at 31.5 g/L (0.42 g/g yield) and 26.5 g/L (0.58 g/g yield), in the single cultures co-production of ethanol and xylitol from rice straw hydrolysate. Author studies in co-production process ethanol and xylitol as 7.3 g/L (0.28 g/g yield) and 15.8 g/L (0.33 g/g yield) from wheat straw hydrolysate using C. tropicalis and 33.4 g/L (0.44 g/g yield) [26] and 25.1 g/L (0.55 g/g yield) from rice straw using S. cerevisiae, C. tropicalis [8]. Simultaneous xylitol and bioethanol production was achieved using rapeseed straw hydrolysate, comparable to this work, with 0.42 g/g and 0.12 g/g, respectively [23]. Coproduction of bioethanol and other value-added

by-products from waste biomass is a cost-effective technique for enhancing the cost competitiveness of bioethanol production. One of the most researched products has been the production of xylitol by biotechnological application. The microbial pathway to xylitol processing is less energy-intensive and environmentally friendly.

Conflict of interest:

The authors declare that there is no conflict of interest.

References

[1] Ahsen, M. A., Naqvi, S. A., Jaskani, M. J., Waseem, M., Khan, I. A., Hussnain, K., Mahmood, K., & Khan, M. M. (2019). Evaluation of exotic citrus rootstocks against Fusarium spp. Journal of Global Innovations in Agricultural and Social Sciences, 7(4), 151–156. doi.org/10.22194/JGIASS/7.874.

[2] Anand, G., Leibman-Markus, M., Elkabetz, D., & Bar, M. (2021). Method for the Production and Purification of Plant Immuno-Active Xylanase from Trichoderma. International Journal of Molecular Sciences, 22(8), 4214. doi.org/10.3390/ijms22084214

[3] Arcano YD, Valma ˜ na Garc ˜ ´ıa OD, Mandelli D,Carvalho WA, Magalhaes Pontes LA, Xylitol: A Review on the Progress ˜and Challenges of its Production by Chemical Route, Catalysis Today (2018), doi.org/10.1016/j.cattod.2018.07.060

[4] Atzmüller, D., Ullmann, N., & Zwirzitz, A. (2020). Identification of genes involved in xylose metabolism of Meyerozyma guilliermondii and their genetic engineering for increased xylitol production. AMB Express, 10, 1-11.

[5] Baptista SL, Cunha JT, Romaní A, Domingues L Xylitol production from lignocellulosic whole slurry corn cob by engineered industrial Saccharomyces cerevisiae PE-2.Bioresour Technol. 2018 Nov;267:481-491. doi.org/10.1016/j.biortech.2018.07.068

[6] Bedő, S., Fehér, A., Khunnonkwao, P., Jantama, K., & Fehér, C. (2021). Optimized Bioconversion of xylose derived from pre-treated crop residues into xylitol by using Candida boidinii. Agronomy, 11(1), 79:1-18. doi.org/10.3390/agronomy11010079

[7] Cai, P., Wang, B., Ji, J., Jiang, Y., Wan, L., Tian, C., & Ma, Y. (2015). The putative cellodextrin transporter-like protein CLP1 is involved in cellulase induction in Neurospora crassa. Journal of Biological Chemistry, 290(2), 788-796. doi.org/10.1074/jbc.M114.609875

[8] Chang, Z., Liu, D., Yang, Z., Wu, J., Zhuang, W., Niu, H., & Ying, H. (2018). Efficient xylitol production from cornstalk hydrolysate using engineered Escherichia coli whole cells. Journal of agricultural and food chemistry, 66(50), 13209-13216. doi.org /10.1021 /acs.jafc.8b04666

[9] Clara Vida G. C., et al., 2019. Xylitol Production: Identification and Comparison of New Producing Yeasts. Microorganisms. 7(11), 484.

[10] da Silveira, F. A., Fernandes, T. A. R., Bragança, C. R. S., Balbino, T. R., Diniz, R. H. S., Passos, F. M. L., & da Silveira, W. B. (2019). Isolation of xylose-assimilating yeasts and optimization

of xylitol production by a new Meyerozyma guilliermondii strain. International Microbiology, 1-10. doi: 10.1016/j.jbiosc.2009.07.013

[11] Damião Xavier, F., Santos Bezerra, G., Florentino Melo Santos, S., Sousa Conrado Oliveira, L., Luiz Honorato Silva, F., Joice Oliveira Silva, A., & Maria Conceição, M. (2018). Evaluation of the simultaneous production of xylitol and ethanol from sisal fiber. Biomolecules, 8(1), 2 doi.org/10.3390/biom8010002

[12] Dasgupta, D., Junghare, V., Nautiyal, A. K., Jana, A., Hazra, S., & Ghosh, D. (2019). Xylitol production from lignocellulosic pentosans: a rational strain engineering approach toward a multiproduct biorefinery. Journal of agricultural and food chemistry, 67(4), 1173-1186. https://doi.org/10.1021/acs.jafc.8b05509

[13] Espinoza-Acosta, J. L. (2020). Biotechnological production of xylitol from agricultural waste//Producción biotecnológica de xilitol a partir de residuos agrícolas. Biotecnia, 22(1), 126-134. doi.org/10.18633/biotecnia.v22i1.1160

[14] Fawzia Jassim Shalsh, Noor Azlina Binti Ibrahim, Mohammed Arifullah, &Anis Shobirin Binti Mero Hussin. Evaluation bioethanol production from fusant of S. cerevisiae and P. stipitis. Asia-Pacific power and Energy Engineering conference (APPEEC)-19-21 April-Xiamen –China. In book Emerging Developments in the Power and Energy Industry – Dufo-López, Krzywanski & Singh (eds)© 2019 Taylor & Francis Group, London, ISBN 978-0-367-27169-5.

[15] Hargono, H., Jos, B., Purwanto, P., Sumardiono, S., & Zakaria, M. F. (2021). A Comparison of the Bioethanol Production from Suweg (Amorphophallus campanulatus) through Separate Hydrolysis and Fermentation as well as Simultaneous Saccharification and Fermentation Using Saccharomyces cerevisiae. In IOP Conference Series: Materials Science and Engineering .1053(1) p. 012036. IOP Conference Series: Materials Science and Engineering, Volume 1053, International Conference on Chemical and Material Engineering (ICCME 2020) 6th-7th October 2020, Semarang, Indonesia

[16] Irfan, M., Gulsher, M., Abbas, S., Syed, Q., Nadeem, M., & Baig, S. (2011). Effect of various pretreatment conditions on enzymatic saccharification. Songklanakarin Journal of Science & Technology, 33, 397e404.

[17] Jin, Y. S., Cruz, J., & Jeffries, T. W. (2005). Xylitol production by a Pichia stipitis D-xylulokinase mutant. Applied microbiology and biotechnology, 68(1), 42-45

[18] Kim TB, Oh DK (2003) Xylitol production by Candida tropicalis in a chemically defined medium. Biotechnol Lett 25(24): 2085–2088

[19] Kim, S. (2019). Xylitol production from byproducts generated during sequential acid-/alkali-pretreatment of empty palm fruit bunch fiber by an adapted Candida tropicalis. Frontiers in Energy Research, 7, 72. doi.org/10.3389/fenrg.2019.00072

[20] Koppram, R., & Olsson, L. (2014). Combined substrate, enzyme and yeast feed in simultaneous saccharification and fermentation allow bioethanol production from pretreated spruce biomass at high solids loadings. Biotechnology for biofuels, 7(1), 1-9.

[21] Kumar, A., Jain, K. K., & Singh, B. (2020). Process optimization for chemical pretreatment of rice straw for bioethanol production. Renewable Energy, 156, 1233-1243. doi10.1016/j.renene.2020.04.052

[22] Lee, J. K., Koo, B. S., & Kim, S. Y. (2003). Cloning and characterization of the xyl1 gene, encoding an NADH-preferring xylose reductase from Candida parapsilosis, and its functional expression in Candida tropicalis. Applied and environmental microbiology, 69(10), 6179-6188. doi.org/10.1128/AEM.69.10.6179-6188.2003

[23] Linares, J.C.L.; Romero, I.; Cara, C.; Castroa, E.; Mussatto, E.I. Xylitol production by Debaryomyceshansenii and Candida guilliermondii from rapeseed straw hemicellulosichydrolysate. Bioresour. Technol. 2018, 247, 736–743. doi.org/10.1016/j.biortech.2017.09.139

[24] Mardawati, E., Maharani, N., Wira, D. W., Harahap, B. M., Yuliana, T., & Sukarminah, E. (2020). Xylitol Production from Oil Palm Empty Fruit Bunches (OPEFB) Via Simultaneous Enzymatic Hydrolysis and Fermentation Process. Journal of Industrial and Information Technology in Agriculture, 2(1). doi.org/10.24198/jiita.v2i1.25064

[25] Mariana A.G Tiburcio1and Inês C. Roberto. 2019 Xylitol production from rice Straw hemicellulosic hydrolysate using cells of Candida guilliermondii permeabilized with Triton X-100. CE12. The 12th European Congress of Chemical Enginering Florence 15-19 September 2019

[26] Mattam, A.J., Kuila, A., Suralikerimath, N., Choudary, N., Rao, P.V., Velankar, H.R., 2016. Cellulolytic enzyme expression and simultaneous conversion of lignocellulosic sugars into ethanol and xylitol by a new Candida tropicalis strain. Biotechnol Biofuels. 26 (9), 157.

[27] Meng, Q. S., Zhang, F., Liu, C. G., Bai, F. W., Zhao, X. Q. (2021). Measurement of Cellulase and Xylanase Activities in Trichoderma reesei. In Trichoderma reesei (pp. 135-146). Humana, New York, NY.

[28] Mesa L, González E, Romero I, Ruiz E, Cara C, Castro C (2011) Comparison of process configurations for ethanol production from two-step pretreated sugarcane bagasse. Chem Eng J 175:185–191. doi:10.1016/j.cej.2011.09.092

[29] Mpabanga, T. P., Chandel, A. K., da Silva, S. S., and Singh, O. V. (2012). "Detoxification strategies applied to lignocellulosic hydrolysates for improved xylitol production," in D-xylitol, eds S. S. da Silva and A. K. Chandel (Berlin; Heidelberg: Springer-Verlag), 63–82

[30] Mussatto, S. I., Santos, J. C., Ricardo Filho, W. C., & Silva, S. S. (2005). Purification of xylitol from fermented hemicellulosic hydrolyzate using liquid–liquid extraction and precipitation techniques. Biotechnology letters, 27(15), 1113-1115.

[31] Mussatto, S.I., D. Giuliano and I.C.R. Dragone. 2005. Influence of the toxic compounds present in brewer's spent grain hemicellulosic hydrolysate on xylose-to-xylitol bioconversion by Candida guilliermondii. Process Biochemistry 40: 3801–3806.

[32] Perkins, D. D. How to choose and prepare media Perkins.2006.

[33] Prabhu, A.A., Bosakornranut, E., Amraoui, Y. et al. Enhanced xylitol production using non-detoxified xylose rich pre-hydrolysate from sugarcane bagasse by newly isolated Pichia fermentans. Biotechnol Biofuels 13, 209 (2020). doi.org/10.1186/s13068-020-01845-2

[34] Prasad, S., Kumar, S., Yadav, K. K., Choudhry, J., Kamyab, H., Bach, Q. V., Gupta, N. (2020). Screening and evaluation of cellulytic fungal strains for saccharification and bioethanol production from rice residue. Energy, 190 116422. doi.org/ 10.1016/j. energy.2019.116422

[35] Qiu, Aita, G.M., Walker, M.S., 2012. Effect of ionic liquid pretreatment on the chemical composition, structure and enzymatic hydrolysis of energy cane bagasse. Bioresour. Technol. 117, 251–256. doi.org/10.1016/j.biortech.2012.04.070.

[36] Ramesh, S., Muthuvelayudham, R., Kannan, R. R., & Viruthagiri, T. (2021). Statistical optimization of medium composition and process variables for xylitol production from rice straw hemicellulose hydrolysate by debaryomyces hansenii var hansenii. Journal of Microbiology, Biotechnology and Food Sciences, 2021, 2332-2339.

[37] Ravella, S. R., Gallagher, J., Fish, S., and Prakasham, R. S. (2012). "Overview on commercial production of xylitol, economic analysis and market trends," in D-xylitol, eds S. S. da Silva and A. K. Chandel (Berlin; Heidelberg: Springer-Verlag, 291–306. Google Scholar

[38] Research & Market. Xylitol- a global market overview. The report reviews, analyzes and projects: the global market for Xylitol for the period 2014-2022." www.grandviewresearch.com/industryanalysis/xylitol-market).

[39] Rodrigues RC, Sene L, Matos GS, Roberto IC, Pessoa A Jr, Felipe MGA (2006) Enhanced xylitol production by precultivation of Candida guilliermondii cells in sugarcane bagasse hemicellulosic hydrolysate. Curr Microbiol 53(1):53–59

[40] Rodrigues, R. C., Kenealy, W. R., & Jeffries, T. W. (2011). Xylitol production from DEO hydrolysate of corn stover by Pichia stipitis YS-30. Journal of industrial microbiology & biotechnology, 38(10), 1649-1655. doi.org/10.1007/s10295-011-0953-4

[41] Salli, K., Lehtinen, M. J., Tiihonen, K., & Ouwehand, A. C. (2019). Xylitol's health benefits beyond dental health: a comprehensive review. Nutrients, 11(8), 1813. doi.org/10.3390/nu11081813

[42] Santos D.T., Sarrouh B.F., Rivaldi J.D., Converti A.,Silva S.S., Use of Sugarcane bagasse as biomaterialfor cell immobilization for xylitol production,J. Food Eng., 86, p. 542 (2008).

[43] Sarkar N, Aikat K. Kinetic study of acid hydrolysis of rice straw. ISRN Biotechnol 2013. doi.org/10.5402/2013/170615

[44] Shalsh, F. J., Ibrahim, N. A., Arifullah, M., & Hussin, A. S. B. M. (2018). Molecular Genetic Characterization of Fusants from Protoplast Fusion of S. cerevisiae and P. Stipitis ATCC 58785. Biomedical Journal of Scientific & Technical Research, 12(3), 9335-9342. doi: 10.26717/BJSTR.2019.12.002272

[45] Shankar, K., Kulkarni, N. S., Sajjanshetty, R., Jayalakshmi, S. K., & Sreeramulu, K. (2020). Co-production of xylitol and ethanol by the fermentation of the lignocellulosic hydrolysates of banana

and water hyacinth leaves by individual yeast strains. Industrial Crops and Products, 155, 112809. doi.org/10.1016/j.indcrop.2020.112809

[46] Shawky, B. T., Talaat, N. B., & Mohapatra, S. (2021). Enzymes in Fuel Biotechnology. In Bioprospecting of Enzymes in Industry, Healthcare and Sustainable Environment (pp. 201-217). Springer, Singapore. Bioprospecting of Enzymes in Industry, Healthcare and Sustainable Environment pp 201-217| doi.org/10.1007/978-981-33-4195-1_10

[47] Sievert C, Nieves LM, Panyon LA, Loefer T, Morris C, Cartwright RA, et al. Experimental evolution reveals an efective avenue to release catabolite repression via mutations in XylR. Proc Natl Acad Sci USA. 2017; 114:7349–54. doi.org/10.1073/pnas.1700345114

[48] Singh, B., & Kumar, A. (2020). Process development for sodium carbonate pretreatment and enzymatic saccharification of rice straw for bioethanol production. Biomass and Bioenergy, 138, 105574. doi.org/10.1016/j.biombioe.2020.105574

[49] Sluiter, Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Nrel, D.C., 2012. Determination of Structural Carbohydrates and Lignin in Biomass Determination of Structural Carbohydrates and Lignin in Biomass. Laboratoty Analytical Procedures (LAP). National Renewable Energy Laboratory (NREL) 2012; Golden, CO Revised version Jul.

[50] Soni, M., Mathur, C., Soni, A., Solanki, M. K., Kashyap, B. K., & Kamboj, D. V. (2021). Xylanase in Waste Management and Its Industrial Applications. Waste to Energy: Prospects and Applications, 393.

[51] Subroto, E., & Hayati, F. (2020). Chemical and biotechnological methods for the production of xylitol: a review. Int J, 8(6). Available Online at http://www.warse.org/IJETER/static/pdf/file/ijeter49862020.pdf

[52] Swain, M. R., & Krishnan, C. (2015). Improved conversion of rice straw to ethanol and xylitol by combination of moderate temperature ammonia pretreatment and sequential fermentation using Candida tropicalis. Industrial Crops and Products, 77, 1039-1046

[53] Thontowi, A., Mayangsari, W., Kholida, L. N., Kanti, A., Wardani, A. K., & Hermiati, E. (2020, February). Evaluation of Addition The Activated Charcoals and pH Adjustment in The Treatment of Lignocellulosic Hydrolisates for Xylitol Production. In IOP Conference Series: Earth and Environmental Science (Vol. 439, No. 1, p. 012023). IOP Publishing. doi:10.1088/1755-1315/439/1/012023

[54] Unrean, P., & Ketsub, N. (2018). Integrated lignocellulosic bioprocess for co-production of ethanol and xylitol from sugarcane bagasse. Industrial crops and products, 123, 238-246. doi.org/10.1016/j.indcrop.2018.06.071

[55] Vallejos, M.E.; Chade, M.; Mereles, E.B.; Bengoechea, D.I.; Brizuela, J.G.; Felissia, F.E.; Area, M.C. (2016). Strategies of detoxification and fermentation for biotechnological production of xylitol from sugarcane bagasse. Ind. Crop. Prod., 91, 161–169. [CrossRef]

[56] Valles, A., Álvarez-Hornos, F. J., Martínez-Soria, V., Marzal, P., & Gabaldón, C. (2020). Comparison of simultaneous saccharification and fermentation and separate hydrolysis and fermentation processes for butanol production from rice straw. Fuel, 282, 118831.

[57] Walther. T., P. Hensirisak and F.A. Agblevor. 2001. The influence of aeration and hemicellulosic sugars on xylitol production by Candida tropicalis. Bioresource Technology 76: 213-220.

[58] Yan, W., Gao, H., Qian, X., Jiang, Y., Zhou, J., Dong, W., & Jiang, M. (2020). Biotechnological applications of the non-conventional yeast Meyerozyma guilliermondii. Biotechnology Advances, 107674.doi.org/10.1002/bbb.2196

[59] Zahed, O., Jouzani, G.S., Abbasalizadeh, S., Khodaiyan, F., Tabatabaei, M., 2016. Continuous co-production of ethanol and xylitol from rice straw hydrolysate in a membrane bioreactor. Folia Microbiol. (Praha) 61 (3), 179–189. P. Unrean, N. Ketsub Industrial Crops & Products 123 (2018) 238–246.

[60] Zoecklein, B., Fugelsang, K. C., Gump, B. H., & Nury, F. S. (2013). Wine analysis and production. Springer Science & Business Media.