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Fermentative Production of Xylitol: A First Trial on Xylose Bifurcation

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Abstract

Background/Objectives: Xylitol production through chemical processes pathway involves high energy usage and production cost. Alternative method via microbial biotransformation and biocatalyst offer more sustainable and environmental friendly feedstock to be used for xylitol production. **Methods:** Production of xylitol by Aspergillus niger PY11 using different conditions on 2 carbon source, glucose and xylose, were done for the development of this research. Batch fermentation of A. niger PY11 was conducted for 4 days or 96 hours in temperature set at 30ºC and agitation speed of 200 rpm. Samples were taken at 12 hours interval, filtered and analyzed for cell biomass, remaining sugar and D-xylitol concentration. The production of biomass and xylitol was monitored through dry-mass weight of mycelium and by HPLC, respectively. **Findings:** From the results of the utilization of single carbon source, fermentation of D-xylose produced the highest xylitol yield, which was 0.101 g xylitol/g D-xylose consumed, with the xylitol titre of 1.139 g/l was obtained (equivalent to 0.482 g xylitol/ g biomass). However, the highest cell growth was observed when fermentation were conducted using a mixture of D-xylose and D-glucose at the ratio of 3:1, which resulted the biomass yield of 0.239 g biomass/g D-xylose (equivalent to 0.211 g xylitol/g biomass). Total amount of 44.94% of added D-xylose was consumed during the fermentation. **Applications/Improvements:** This paper shown that the addition of glucose had resulted higher biomass growth of A.nigerPY11, thus subsequently increased the bioconversion of xylose to xylitol.

Keywords: Aspergillus niger, Co-Substrate, Fermentation, Xylitol

1. Introduction

Xylitol was reported in 1891 when it was first produced from birch trees by catalytic hydrogenation of xylose. The consumption of xylitol had increased through its usage as sweetener for diabetic patients in Europe. Xylitol is a five-carbon polyalcohol or sugar alcohol (alditol) that has the same degree of sweetness as of sucrose[1]. Owing to have lower caloric value of 2.4 cal/g as it is compared to sucrose that has 4 cal/g[2], xylitol is considered to be as a safe sweetener by the U.S. FDA. Xylitol has good water solubility and is endothermic character, causing soothing effect in oral care products[3]. Naturally, xylitol can be found in fruits and vegetables at low quantity, thus the extraction is not economically feasible[4]. Nowadays, xylitol is widely used in confectionery and chewing gum industry due to its non-cariogenic property[1]. Common application such as non-sugar chocolate and fondant are added together with xylitol, and often used with other sweetener to achieve the required sweetness[5]. In addition to that, xylitol also plays a vital role in oral care, especially in prevention of dental plaque. Daily intake of xylitol at 7 – 10 g reduces dental plaque at 50%[6]. Furthermore, xylitol can be used as carbon source in parenteral nutrition since glucose is not suitable

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for the diabetic patient and patients suffered from severe degree of burn and shock[7]. Not to mention too that xylitol can prevent acute otitis media (AOM) in children[8].

In conventional approach, xylitol are produced through hydrogenation of xylose, which converts the sugar (an aldehyde) into a primary alcohol, from hemicellulose hydrolysate[9]. Acid hydrolysis and purification process of the hydrolysate could generate 80-85% of the xylose sugar. Hydrogenation is then carried out in a reactor at the temperature of 80-150°C and pressure up to 50 atmosphere with the presence of Raney nickel catalyst[4]. Nonetheless, the conversion of xylose to xylitol by chemical synthesis could reach is only 50-60% of xylan, that contributed to the high cost of purification[10]. Despite latter research on ruthedium catalyst on NiO supported TiO_2 showed higher conversion, the high operating cost causing biosynthesis of xylitol more preferable[11].

Xylitol production by fermentation and bioprocess has the advantages as the operations involve mild temperature and pressure during operations, low energy consumption, high yield, lower cost in separation process and cleaner production[12]. There are several on-going researches on the xylitol production focusing on utilizing bacteria, yeast and fungi as the producer. Among many others, Candida sp. has the highest yield of producing xylitol from xylose[13]. In spite of using yeast, the interest of focusing on the fungi, precisely Aspergillus sp. is due to that they are good in hydrolyzing lignocellulosic biomass, which is lacking in yeast enzyme system[14]. Biomass hydrolysate from woody materials can be used as a carbon source feedstock in fungi fermentation. Recently, water hyacinth biomass from acid hydrolysis can be used as the xylose source[15].

There were very few studies conducted on xylitol production by fungi, started by the work carried out in year 1960 on Penicillium chrysogenum metabolism[16,17] able to obtain 39.8 g/L xylitol when Petromyces albertensis were grown on a medium consisting 100 g/L xylose for 10 days. They successfully obtained the highest yield from 11.50 g/L of xylose by Penicillium crustosum, with xylitol titre of 0.52g/L. The consumption of xylose was 76%[12]. Another study done by different research groups using Aspergillus niger had found xylitol yield was relatively low. Microbial screening works conducted by Mudaliyar and co-worker[18] on agricultural waste found A. niger NCIM 1015 able to give the highest yield of 0.75 g/g. Summary of other xylitol producers are listed in Table 1.

Consumption on xylitol keep on increasing in recent years, causing the demand of xylitol exceeds 125,000 tonnes annually. The current price of xylitol stands ranging from RM14.30 to RM17.50/kg in the commodity-trading price, while in retail sale it was around RM63.60/kg[19].

In this study, experimental trials were carried out on production of xylitol via fermentation by fungi Aspergilus niger PY11. The combination of D-xylose and D-glucose were used in the medium to investigate the effects of different ratio of 2-carbon source fermentability by A. niger PY11 on xylitol yield. The ratio of the two carbon source reflected the value of major composition of sugar monomers in ligno-cellulosic biomass.

Table 1. Xylitol producers with different fermentative conditions

Producer	Carbon source	Fermentation condition	Yield	Reference
Corynebacteriumsp.	D-xylose	–	0.69 g/g	13
Enterobacterliquefaciens	D-xylose	–	0.33 g/g	13
Mycobacteriumsmegmatis	D-xylulose, D-mannitol	Anaerobiccondition	0.7 g/g	13
Petromycesalbertensis	D-xyloseandmethanol	InitialpHat 7.0	0.39 g/g	13
Candidatropicalis HXP2	D-xylose	Aerobiccondition, 30°C	0.96 g/g	13
Candida guilliermondii FTI-20037	D-xylose	Aerobiccondition, 30-35°C	0.77 g/g	13
Hansenulapolymorpha	D-xyloseandglycerol	pH 8	0.52 g/g	13
Debaryomyceshansenii UFV-170	D-xylose	Micro-aerobiccondition	0.54 g/g	12
Aspergillusoryzae P5	D-xylose	Aerobiccondition, 30°C	0.43 g/g	14
Aspergillusniger DMB2	D-xylose	Aerobiccondition, 30°C	0.04 g/g	12
Aspergillusniger NCIM 1015	Agriculturalwaste	as nitrogen source, initial pH at 5.0	0.06-0.75 g/g	18

2. Materials and Methods

2.1 Source of Microbes

The microorganism, Aspergillus niger PY11, was obtained from Microbiology Laboratory of Faculty Science and Technology, UniversitiKebangsaan Malaysia. The strain has been modified by adding vector ANIpCBH1 that consisted of glucoamylase promoter (GlaP) and gene pyrG as auxotroph indication. This strain has the ability to release large amount of protein into the culture medium and hence had been used as the host for cellulose enzyme production.

2.2 Culture and Fermentation Medium

The media composition used to revive A. niger from stock culture was as follow (in the unit per litre): salt solution; 50mL, $MgSO_4 \cdot 7H_2O$ (2.25M); 5mL, dextrose; 10g, trace elements solution; 1mL, peptone; 2g, yeast extract; 1g, casamino acid; 1g, and a vitamin solution; 1mL respectively. Once the media in the container were cooled prior to autoclaving, the vitamins were added.

The fermentation process was carried out in a series 250mL Erlenmeyer flask with medium working volume of 100mL. The fermentation medium comprised (g/L) KH_2PO_4; 0.5, K_2HPO_4; 0.5, $MgSO_4.7H_2O$; 0.05, $(NH_4)_2SO_4$; 2, yeast extract; 10, and peptone; 20. D-glucose and D-xylose representing main carbon source with total concentration of 30g/L were added separately after they were autoclaved at 121°C. The experiments were repeated for different ratio of xylose to glucose as follow: 1:0, 10:1 and 3:1. After 10% v/v of inoculum was mixed to the medium, the culture was incubated at 30°C and agitation at 200 rpm rotation speed. The pH of the medium was maintained at pH 5.0 with 1M HCl. A series of Erlenmeyer flask fermentations representing batch cultivation of A. niger were performed for 4 consecutive days and samples were taken every 12 hours interval of fermentation for analysis.

2.3 Analytical Method

The quantity of glucose, xylose and xylitol in the medium were analyzed using high performance liquid chromatography (HPLC), Agilent 1200 Series HPLC System (Agilent Technologies, USA). Rezex RPM column (Phenomenex, USA) was used as stationary phase in column temperature set at 60°C. Deionized water used as the mobile phase and was set at flow rate of 0.6 mL/min. The samples were detected using refractive index (RI) detector. Method of dried-weight mass basis was used to calculate biomass of the fungi mycelium.

3. Results and Discussion

As mentioned previously, each Erlenmeyer flask was taken for analysis at every 12 hours. Dry mycelium mass analysis were determined by dry weight cell and calculated for biomass yield while the supernatant of the spin liquid samples were tested for xylitol, D-glucose and D-xylose by using HPLC. The retention times for the targeted reducing sugars were as follow in unit of minute: glucose; 15.62, xylose; 16.84, and xylitol; 43.19. The mycelium growth started to increase during the fermentation in initial lag phase of 48 hours. However, acceleration phase can be seen after 48 hours fermentation when a plateau was observed to reach after 72 hours.

As shown in Figure 1, 2 and 3, it was found that xylitol was produced after 48 hours fermentation and xylose consumed was correlated with the growth of fungi, which attributed to low xylitol at initial stage. The presence of glucose also influenced the xylitol production rate where it can be seen that glucose was consumed prior to xylose. Highest biomass yield can be found when co-fermentation of glucose and xylose was done at the ratio of 3:1 that resulted in biomass yield of 0.239g/g xylose. The highest xylitol yield 0.101g xylitol/g xylose in the fermentation was found when only xylose was added. The concentration of xylitol was found in titre of 1.139 g/L. The yield obtained were comparable values with[12,18] works as indicated in Table 1. Albeit with highest yield,

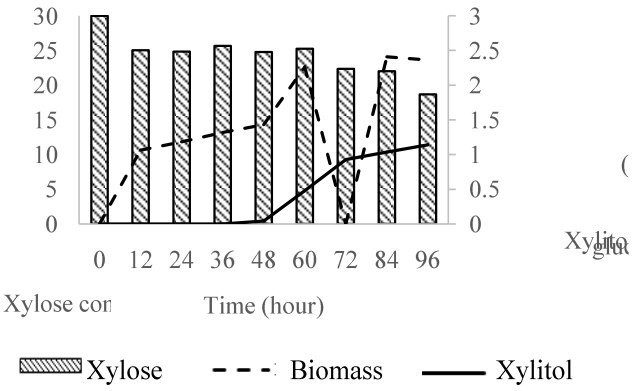

Figure 1. Fermentation profile for production of xylitol by Aspergillus niger PY11 on single carbon source, xylose.

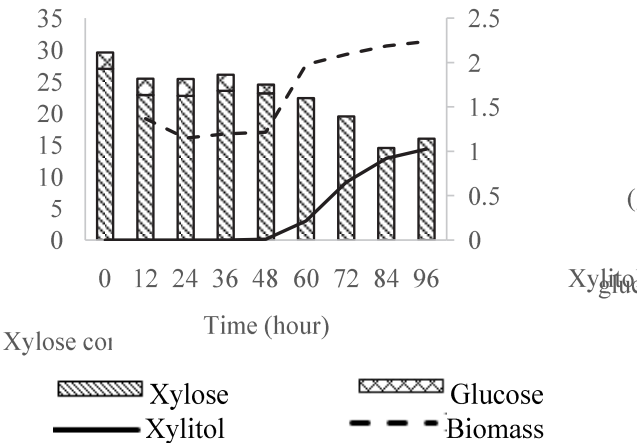

Figure 2. Fermentation profile for Aspergillus niger PY11 in the production of xylitol at the ratio of xylose to glucose at 10:1.

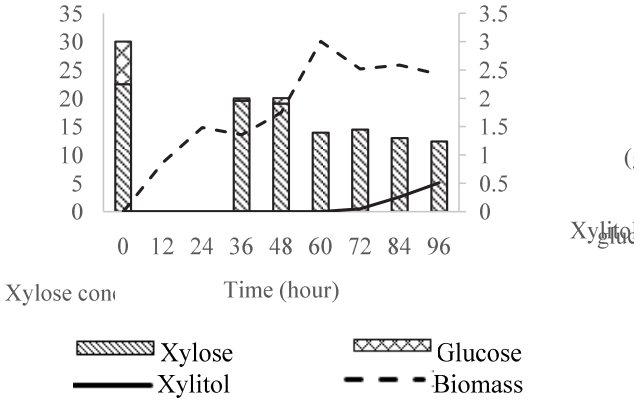

Figure 3. Fermentation profile for Aspergillus niger PY11 in the production of xylitol at the ratio of xylose to glucose at 3:1.

the consumption of xylose was the lowest where in that case only 37.74% of xylose was consumed. The same trend of xylose consumption can be observed for the biomass growth in pure xylose fermentation.

As far as xylose metabolism is concerned, the utilization of xylose is mainly for the growth during the fermentation. Xylitol production in fungi are relatively low compared to the production by xylitol producing yeast is due to the aerobic condition favours respiration than fermentation. Xylose that acts as a sole carbon source will be utilized as energy source for growth, thus once xylitol is being produced, it is more likely to be converted into xylulose hereafter by Pentose Phosphate Pathway (PPP) as shown in Figure 4.

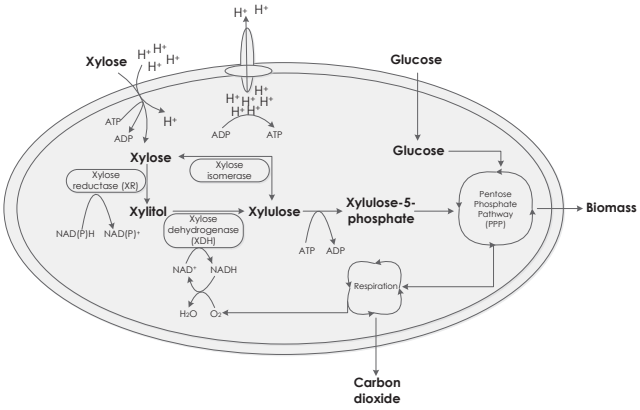

Figure 4. Schematic diagram for the metabolism of Aspergillus niger PY11[3,5,9,20-21].

The reduction of xylose to xylitol is coupled with the oxidation of NAD(P)H to NAD(P)$^+$ by xylose reductase (XR). Xylitol is then oxidized to xylulose by xylitol dehydrogenase, along with the electron acceptor of NAD$^+$ to form NADH. Regeneration of NAD$^+$ and NADPH is done through transhydrogenase enzyme for electron balance, where NAD(P)$^+$ + NADH → NAD(P)H + NAD$^+$ in the cell[22]. Another explanation is that the regeneration of cofactor can be found in aerobic conditions, where oxygen is used to oxidize NADH. In the case of xylose fermentation, bifurcation of xylose occurred when part of the xylose is converted into xylulose by oxido-reductase and subsequently to xylulose-5-phosphate (X5P) by xylulokinase. In addition to that, in Aspergillus sp., xylose can be directly converted through xylose isomerase into xylulose[23]. X5P is further used for biomass production in PPP before entering back into Embden–Meyerhof Pathway (EMP)[9]. Co-fermentation of xylose with glucose enhanced the biomass yield due to the selectivity of glucose as carbon source for fungal growth. Glucose that present in the fermentation medium was mainly consumed in the EMP, therefore resulting highest biomass growth shown in Figure 3.

4. Conclusion

Xylitol is a five-carbon polyalcohol that has the same sweetness as sucrose. Fermentation was performed by Aspergillus niger PY11 to produce xylitol from xylose by manipulating different ratio of 2-carbon source, D-glucose and D-xylose, against xylitol yield. The highest yield of xylitol was achieved at 0.101 g/g when only xylose

was used as carbon source. However, the yield on 2 carbons source were much higher as xylose bifurcation into biomass can be proven from the growth medium at initial stage of fermentation, and part of it were further reduced into xylitol at the latter stage of fermentation. In conclusion, trials showed the addition of glucose could mitigate the bifurcation of xylose, allowing better conversion of useful product such as xylitol.

5. Acknowledgement

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