Kinetic study on Fermentation of xylose for The Xylitol Production

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Abstract

Xylitol is a natural sugar that has the sweetness level similar to sucrose, but has lower calorie. It is an important sugar alternate for diabetics people. Reduction of xylose is a normally method to produce the xylitol. It conducted through chemical hydrogenation of xylose at high pressures and temperatures by reacting pure xylose with hydrogen gas using a metal catalyst. This process requires pure xylose as the raw material. Alternatively, the reduction process can be carried out through fermentation. This process does not require high purity of xylose as the raw material, and thus the oil palm empty fruit bunch (EFB) hydrolysate, without any prior pretreatment, can be used. In order to scale up the xylitol production by way of fermentation, kinetic study of xylitol fermentation including growth and xylitol formation kinetic using the synthetic xylose as substrate will be required. Data used in the kinetic model development were obtained from series of batch fermentations of Debaryomycess hansenii ITB CCR85 varying the initial xylose and glucose concentrations. Yeast growth could be sufficiently modeled using the Monod kinetics, whereas xylitol production could be reasonably well modelled by Luedeking Piret kinetics.

Keywords: *fermentation, kinetic, model, xylitol, and xylose*

1. INTRODUCTION

Xylitol is a natural five-carbon sugar that is widely consumed by industries, including the food, chemical and pharmaceutical. Xylitol has the sweetness level equal to sucrose, but has low calorie value. Beside it is also an important sugar substitute for diabetics patients. It has a considerably negative heat of solution and low viscosity in Musatto *et.al* [1]. Xylitol is currently produced by the chemical reduction of xylose for example by contacting liquid xylose with pure hydrogen gas in Oh *et. al* [2]. This process is commonly carried out at non-atmospheric conditions in a batch reactor by using pure xylose and noble metal-based catalysts, such as nickel, palladium, ruthenium, and platinum in Parajo *et. al* [3], for example conducted this process at hydrogen partial pressure of 20 bar and temperature of 80-120^{o}C.

Alternatively, xylitol can be produced undergo biotechnologically processed by yeast fermentation [3,9]. Various of microorganisms were investigated for converting xylose into xylitol. Among these, the best xylitol producers are yeasts, mainly belong to the species *Debaryomyces hansenii* in Converti *et.al*, Dominguez *et.al* and Mardawati *et.al* [4, 5, 6]. *D. hansenii* is of particular interest because of the negligible formation of ethanol under semiaerobic conditions, which provide high xylitol yields in Converti *et.al* and Sampaio *et.al* [4,7]. *D. hansenii* metabolize xylose through a two-step oxido-reductive route. First, The enzyme of xylose reductase (XR), in the attendance of NADH and/or NADPH as cofactor, reduces D-xylose to xylitol. In the subsequent reaction, this xylitol is

oxidized to D-xylulose by either NAD+-linked or NADP+-linked xylitol dehydrogenase (XDH) in Parajo *et.al* and Converti *et.al* [3, 4].

Xylitol production by yeasts is influenced by several factors including initial xylose concentration, the strain, presence of other sugars and its ratio, as well as fermentation conditions such as pH, temperature, oxygen supply, and the presence of inhibitor compounds in Parajo *et. al* and in Silva *et. al* [8,9]. The other critical factor in the fermentation of xylitol by yeast is medium. The high concentration of glucose in the medium may repress the activity of the key enzyme in convert the xylose into xylitol. Overall low yield of product is obtained in Silva *et. al* [9]. Oh *et. al*., 1998 reported an optimum xylose to glucose concentration ratio in the substrate to give the maximum xylitol production in Oh *et. al* [2]. Beside that, Oxygen is important for the xylose metabolism by yeasts, it is connected to ATP production, coenzyme regeneration, and sugar transport, throughtout the oxidative phosphorylation. Some yeasts need oxygen for an optimum xylose fermentation in Silva *et. al* [9]. *D. hansenii* is ensures producing high xylitol yields under microaerobic conditions in Converti *et.al* [4]. For that reason, it is a fundamental importance to understand the influence of substrate and cosubstrate factor effect on xylose to xylitol bioconversion for the improvement of an efficient technology for large-scale xylitol production by biotechnological process.

Many researchers reported aspects influencing the xylitol fermentation, but few researches reported the related kinetics aspect. Eventually, to scale-up the xylitol production to pilot scale and finally industrial scale, information on fermentation kinetics, such as specific rate of key reactions, which can only be obtained through kinetics modeling, is required. In this research the kinetics of xylitol fermentation was studied using black box approach. In particular the growth of *D. hansenii* ITB CCR85 on xylose or glucose. Further, xylitol formation kinetics was studied.

2. MATERIAL AND METHODS

2.1 Microorganism

The yeast strain of *D. hansenii* ITB CCR85 was used in this study and obtained from Microbiology and Bioprocess Technology Laboratory of Chemical Engineering ITB. The yeast was grown in glucose yeast extract agar (GYE) for 3 days at 30^{o}C before further used.

2.2 Fermentation

Fermentation process was carried out in a bioreactor with the volume of 1.3 L (New Brunswick BF115). The bioreactor is equipped with pH, temperature, level, dissolved oxygen (DO), and foam control system. Batch fermentation was executed in 700 mL working volume and 450 rpm mixing speed at pH 5 and temperature 30^{o}C. Inoculum used was 10% of working volume. The fermentation was sampled over time during the fermentation time. Fermentation is terminated when the culture reached the stationary phase. To study the effect of substrate type variation, aeration level was maintained at 0.5 vvm during the fermentation process. The system was aerated in 0.5 vvm aeration rate to simulate semi-aerobic environment.

2.3 Analysis

Sample was analysed for biomass as cell concentration by the turbidimetric-gravimetric method using UV-Vis spectrophotometer. Xylose, glucose, and xylitol concentrations were determined using high performance liquid chromatography (HPLC) type BioRad Aminex HPX-87H column and 5 mM H_2SO_4 as eluent as was described in Mardawati [6].

2.4 Experimental Design

To acquire sufficient data for growth kinetics model development, 8 runs were executed using either glucose or xylose as substrate at various concentration, between 7.5 and 20 g/L (Table 1). Meanwhile, to estimate product formation kinetics only xylose was used as substrate. Further, the effect of xylose concentrations on xylitol yield were also studied.

Table 1. Summary of runs conducted in this research.

Run Number	Glucose Concentration (g/L)	Xylose Concentration (g/L)
1	7.5	0
2	10	0
3	15	0
4	20	0
5	0	7.5
6	0	10
7	0	15
8	0	20

2.5 Data Interpretation

2.5.1 Estimation of Growth Kinetics Parameters

Growth of microorganisms were parameterised as the specific growth rate that was calculated from biomass concentration data during the logarithmic phase, following equation 1.

$$\frac{dX}{dt} = \mu X \quad (1)$$

μ is referred as specific growth rate (1/hours), the μ value depends on substrate concentration. Many models are used to describe the relationship between μ and substrate concentration (denoted as S); however, the simplest model is formulated by Monod, as expressed in Equation 2 and The kinetics parameters were estimated using the Lineweaver-Burk plot in Shuler and Kargi [10].

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S} \quad (2)$$

where:
X = cell concentration (g/L)
t = fermentation time (h)
μ = specific growth rate (h^{-1})
S = substrate concentration (g/L)
μ_{max} = maximum specific growth rate (h^{-1})
K_s = Monod constant (g/L)

2.5.2 Xylitol Production for *D. hansenii* ITBCCR85 Growth on xylose substrate

In order to measure the performance of a fermentation system, the yield values are used. The general statement of yield can be described as ratio of formed product (product or cell) to used substrate, as stated on Equation 3 - 5.

$$Y_{P/S} = \frac{(P - P_0)}{-(S - S_0)} \quad (3)$$

$$Y_{P/X} = \frac{(P - P_0)}{(X - X_0)} \quad (4)$$

$$Q_P = \frac{(P - P_0)}{t} \quad (5)$$

where:
P = xylitol product concentration (g/L)
T = Total fermentation time (hours)
$Y_{P/S}$ = Product-from-substrate yield (g xylitol/g xylose)
$Y_{P/X}$ = product-from-cell yield (g xylitol/g cell)
Q_P = The volumetric productivity (g/L/h)

2.5.3 Estimation of Product Formation Kinetic Parameter

Product formation is commonly stated in its specific rate form. Definition for specific product formation rate (q_P) is expressed in Equation 6.

$$q_P = \frac{1}{X}\frac{dP}{dt} \quad (6)$$

Generally, microbial products are classified into three categories (Shuler & Kargi, 2002), which are:

a. Growth-associated product
For a growth-associated product, product formation rate is proportional to growth rate.
$$q_p = \alpha\mu \quad (7)$$
b. Non-growth-associated product
For a non-growth-associated product, product formation rate is unaffected by growth rate.
$$q_p = \beta \quad (8)$$
Non-growth-associated products include secondary metabolites.
c. Mixed-growth-associated product
This product formation model is a combination of growth-associated product (Equation 7) and non-growth-

associated product (Equation 8), which is:

$$q_p = \alpha\mu + \beta \quad (9)$$

3. Result and Discussions

3.1. Estimation of Monod Kinetics Parameters for *D. hansenii* Growth

Xylitol is produced from xylose substrate by yeast, but yeast needs the glucose as carbon source for their growth so that in fermentation media may also contain co-substrate. Xylose-to-xylitol fermentation mechanisms involve glucose as co-substrate. The effect of co-substrate existence is noteworthy, especially if fermentation is to be executed by using lignocellulosic hydrolysate, which consists of primarily xylose and glucose. In order to study the influence of each substrates on the cell growth, fermentation were conducted using either glucose or xylose as substrate. Specific growth rates (μ) of each runs were determined using Eq.2. Followingly, the calculated specific growth rates for glucose and xylose as substrate are presented in Fig.1.

Fig.1 shows that the high concentrations of either glucose or xylose in the medium increase their consumption by the yeasts and consequently, enhances the μ value. According to data from initial glucose concentrations of 7.5 g/L, 10 g/L, 15 g/L, and 20 g/L in Fig.1 a trend of μ increase is observed in response to initial glucose concentration. The trend is the same with initial xylose concentrations effect on specific growth rate in Figure 1. In general Figure 1 shows that the specific growth rate of *D. hansenii* in xylose is lower than in glucose. This shows that *D. hansenii* ITBCCR85 preferred glucose over xylose as the substrate for cells growth. Glucose is easier to consume than xylose. Tochampa *et.al* in [11] explained that cells can produce energy and biomass directly utilize glucose through glucose 6 phospaphate (Glu-6P), and consequently the HMP pathway slow down. In contrast, xylose metabolism by yeasts first supplementary xylose excreted into D-xylulose through an oxidoreductive route consisting of two chronological reactions in Tochampa *et.al* [11]. The route of xylose is longer than glucose. Followingly, the Monod kinetic parameters were presented in Table 2.

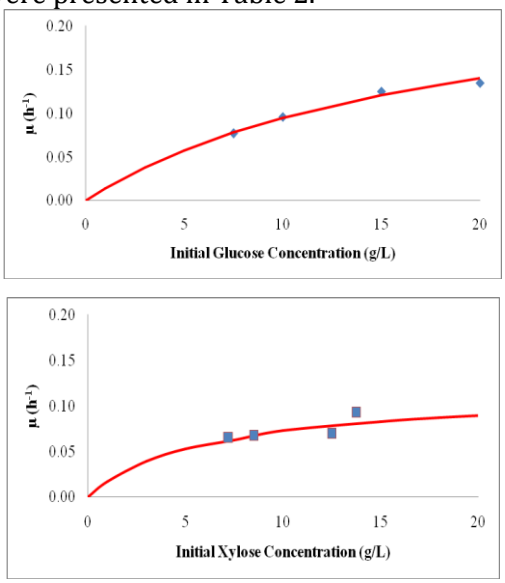


Figure 1. The calculated specific growth rate values from *D. Hansenii* using xylose and glucose as substrate (Experimental data and model)

Table 2. The Monod kinetics parameter of *D. hansenii* growth.

	Glucose	Xylose
μ max(h^{-1})	0.27	0.11
K_s (g/L)	18.55	5.89

The values of K_S and μ_{max} were found to be 18.55 g/L and 0.270 h^{-1} for glucose, 5.89 g/L and 0.11 h^{-1} for xylose. Data showed that K_S value of *D. hansenii growth on* glucose as substrate is higher than on xylose. It explains that *D. hansenii* ITBCCR85 has low affinity to glucose, while its maximum growth rate is even higher than xylose.

Tochampa *et.al* in [11] studied *Candida mogii* growth in the aerobic condition on glucose and xylose, and found that K_S= 9.998 g/L and μ_{max} = 0.66 h^{-1} for glucose, and K_S= 11.761 g/L and μ_{max} = 0.19 h^{-1} for xylose [11] . Both the μ_{max} and K_S value is substantially different; it finely explains

that *D. hansenii* ITBCCR85 has low affinity to glucose, while its maximum growth rate is even lower than *C. mogii*.

In general, the increase in xylose concentration in the substrate resulted in an increase in the produced xylitol concentration and xylitol yield ($Y_{P/S}$), except for the run at initial xylose concentration of 15 g/L. In particular, the maximum product yield on substrate achieved is 0.310 g/g, which corresponds to xylitol volumetric productivity of 0.022 g/L/h. This value could still be improved because the xylose utilization is still low (max = 58%).

The productivity volumetric,Q_P, productivities as well as the product yield on starting substrate, $Y_{P/S}$, were very low under conditions of substrate of 7.5 g/L, therefore the yeast could have not taken enough energy to grow and preferred to consume the carbon source for respiration and ATP production in Converti *et.al* [4]. The calculation of xylitol yield base on xylitol concentration per cell biomass ($Y_{P/X}$) has the same phenomena, that the increase in xylose concentration resulted in the increase xylitol yield ($Y_{P/X}$), which is the maximum ($Y_{P/X}$) of 0.860 g xylitol/g cell.

3.2. Estimation of Xylitol Formation Kinetics Parameter

Xylitol production was observed from fermentation of *D. Hansenii* on xylose, because glucose can not be converted to xylitol. In Table 3, the yield of xylitol from each experiments are presented.

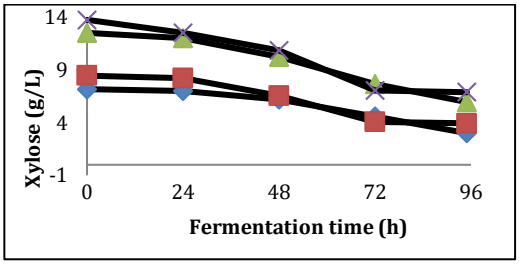
Similar phenomena was observed in literaters. Microbial production of xylitol by *D. Hansenii* NRRL Y-7426 in Converti *et.al* [4], the result showed that at relatively low starting xylose substrate concentration (S_0 = 50 g/L), xylitol formation was very slow (Y p/s = 0.24 g/g, QP = 0.058 g/L/h; On the other hand, another initial xylose concentration range (90–125 g/L), $Y_{P/S}$ was rather high (0.82–0.83 g/g). This phenomena in which constitutes a very interesting result for a potential industrial development of this fermentation process. It also stated that the rate of xylitol production increased similar with the increasing initial xylose in Dominguez *et.al* [5]. Xylitol fermentation for the same strain resulted yield of 0.79 g/g at initial xylose concentration 279 g/L. A high concentration of xylose (279 g/L) was converted rapidly and efficiently to produce xylitol with Q_p of 4.6 g/L/h.

Table 3. The Effect of Xylose on Xylitol Yield.

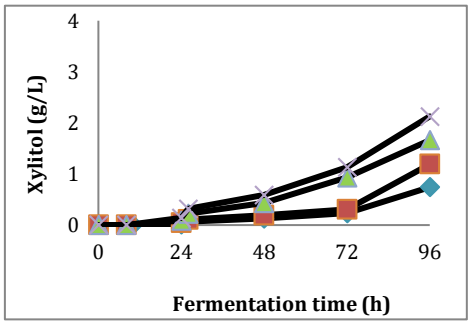
Xylose (design) (g/L)	Actual Xylose (g/L)		Xylose Utilization (%)	Biomassa (g/L)		Yx/s (g/g)	Xylitol (g/L)	Yp/s (g/g)	Yp/x (g/g)
	t_{0h}	t_{96h}		t_{0h}	t_{96h}				
7.5	7.167	2.998	58	0.440	2.255	0.435	0.742	0.178	0.409
10	8.443	3.933	53	0.347	2.482	0.473	1.190	0.264	0.557
15	12.500	5.940	52	0.227	2.671	0.373	1.662	0.253	0.680
20	13.736	6.873	50	0.229	2.926	0.393	2.127	0.310	0.789

Note : Initial xylitol concentration = 0 g/L

The profile of xylitol, biomass production and xylose utilization as long as fermentation time are presented in Fig.2. These figures show that xylitol production



(a)



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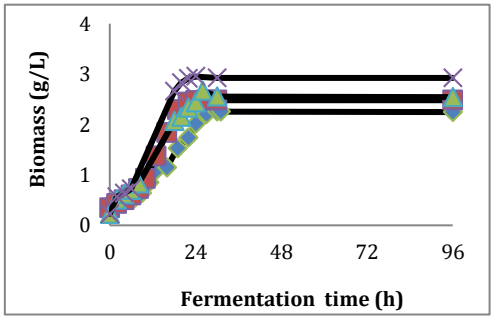

(c)

Figure 2. The Profile of final substrate (a), xylitol product (b) and biomass (c) were produced by *D. hansenii* for each substrate concentration. (◆: 7.5 g/L; ■: 10 g/L; ▲: 15 g/L; X : 20 g/L)

was started during the growth phase and the concentration reaches its maximum in the stationary phase. The calculation of specific product formation rate (q_P) at each specific growth phase is presented in Table 4.

Table 4. Estimation of product formation kinetics parameters.

Initial substrate concentration (g/L)	**7.5**	**10**	**15**	**20**
r_p growth (g xylitol/L.h)	0.0010	0.0030	0.0050	0.0090
r_p stationer (g xylitol/L.h)	0.0090	0.0140	0.0210	0.0260
q_p growth (g xylitol/g X.h)	0.0001	0.0003	0,0004	0.0009
q_p stationer (g xylitol/g X.h)	0.0041	0.0058	0.0081	0.0087
β average	0.0070			
α average	0.0060			

The calculation showed that both alpha and beta are non zero. This indicates that xylitol is produced both at the growth and stationary phase, which agrees with the observation. Validation of model was done with simulate this parameter to estimate the xylitol production in the fermentations time, further compared with the experimental result (Fig.3).

CONCLUSIONS

The results showed that glucose is significantly easier to consume than xylose during the fermentaion of *D. hansenii* ITBCCR85. The growth was observed to follow Monod kinetics model, with estimated parameters (K_S and μ_{max}) subsequently 18.55 g/L and 0.27 hour^{-1} for glucose, 4.6 gL^{-1} and 0.10 hour^{-1} for xylose. Xylitol formation was categorized as mix growth product, with product formation kinetics estimated to be q_p = 0.006μ + 0.007. The maximum xylitol yield obtained in this work was 0,310 g/g which corresponds to volumetric productivity of xylitol of 0.022 g/L/h. These values were obtained from experiment with initial xylose concentration of 20 g/L.

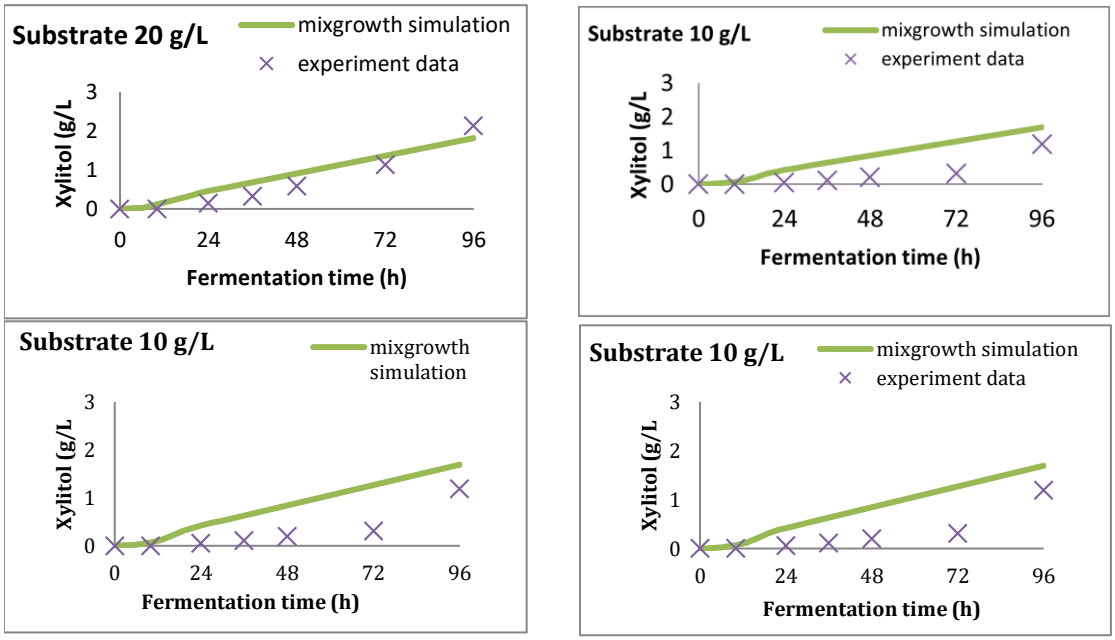

Figure 3. Xylitol products from experiment and model simulation on the fermentations time for each initial xylose concentration

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