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Dynamic mathematical modelling of reaction kinetics for xylitol fermentation using *Candida tropicalis*

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ABSTRACT

Xylitol is an extracellular sugar alcohol produced by the biological conversion of xylose through the fermentation process. The present study investigates the reaction kinetics of xylitol fermentation by considering the effect of substrate and product concentration on the microbial growth rate. A 3.5-L batch fermentation produced xylitol at different xylose concentrations and agitation speeds. The experimental data showed that the xylose concentration limit was less than 100 g/L and that increasing the xylose concentration reduced the xylitol and cell yields. The optimum agitation speed was 400 rpm with a k_La value of 32.6 h^{-1}. The experimental data were used to estimate the unknown parameters with gPROMS software. The Monod model was modified to include the inhibitory effect of the substrate and the limitation effect of dissolved oxygen on cell growth.

1. Introduction

Xylitol is a five-carbon sugar alcohol usually used as a sugar substitute in food preparation and pharmaceutical products. Xylitol is often consumed by diabetic patients and those with a glucose-6-phosphate dehydrogenase deficiency because it does not require insulin for its digestion. Because of its anticariogenic properties, xylitol is an excellent substance for reducing plaque and cavity formation. It is not fermented by *Streptococci* in the mouth, the bacteria that cause tooth decay. Xylitol appears to inhibit the attachment of pneumococci and prevent the acute otitis media [1]. The major application of xylitol is in oral hygiene products such as toothpaste, mouthwash and sugar-free chewing gums.

Research on xylitol production using biological methods has been extensively studied to replace the expensive chemical methods requiring more energy and hence imposing higher processing costs [2]. The biological method has good prospects as a process because it uses microbials as a catalyst, operates at low pressure and temperature, and requires only one purification step, instead of the two required in the chemical process [3]. The development of this economical biological process using xylose generated from lignocelluloses has generated great interest globally.

Some limitations can be found when dealing with a biological process, such as low productivity and yield. However, it is possible to improve the production of xylitol by understanding the variables that affect the metabolism of xylose-consuming yeasts, such as *Candida* sp. Extracellular xylitol accumulation is regulated by the activity of two enzymes in the yeast, xylose reductase (XR) and xylitol dehydrogenase (XDH). Conversion by these enzymes requires two reaction steps. First, xylose is reduced to xylitol by the presence of the cofactor nicotinamide adenine dinucleotide phosphate (NADP) or nicotinamide adenine dinucleotide (NAD^+)-dependent XR, then the second step is the oxidation of xylitol to xylulose in the presence of NAD^+ dependent XDH [4]. Xylulose is phosphorylated and incorporated into the pentose phosphate pathway in the metabolic fluxes of yeast.

Various kinetics parameters for xylitol production have been published previously. The effects of process parameters on the fermentation system for xylitol production have been reviewed by some researchers [5,6]. Most of the effects of the process parameters, such as pH, temperature, inoculum level and medium composition on the kinetics parameters were investigated in the conical flasks [7–10]. Few fermentation studies in the bioreactor on reaction kinetics were previously reported [11,12] but the kinetic modelling of the respective systems was not described. Kinetic modelling is important for providing an understanding of the reaction mechanism to improve xylitol production as well as its process design. Information on the kinetic modelling of xylitol

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Nomenclature

$\overline{A}$	Average of observed value
A	Numerical constant
$C_{eq,O2}$	Concentration of oxygen at equilibrium, g/L
$C_{g,O2}$	Concentration of oxygen gas at fermentation medium, g/L
C_{O2}	Concentration of dissolved oxygen in fermentation medium, g/L
C_S	Concentration of xylose in fermentation medium, g/L
C_X	Concentration of cell in fermentation medium, g/L
C_P	Concentration of product in fermentation medium, g/L
D	Impeller diameter, m
K_{eq}	Equilibrium constant, g/L
$K_{in,O2}$	Oxygen inhibition constant, g/L
$K_{in,P}$	Product inhibition constant, g/L
$K_{in,S}$	Substrate inhibition constant, g/L
k_La	Volumetric oxygen transfer coefficient, h^{-1}
$K_{li,O2}$	Dissoved oxygen limitation constant, g/L
$K_{li,P}$	Product limitation constant, g/L
$K_{li,S}$	Substrate limitation constant, g/L
K_δ	Product decay constant, g/L
K_{O2}	Dissolved oxygen constant, g/L
$m_{g,O2}$	Mass of oxygen gas, g
m_{O2}	Mass of dissolved oxygen, g
N	Impeller tip rotational speed, rpm
N_{CD}	Impeller rotational speed at complete gas dispersion, rpm
NE	Number of experiment performed
N_M	Total number of measurements taken during experiments
NM_{ij}	Number of measurements of the jth variables in the ith experiments
N_{O2}	Oxygen transfer rate, g/L h
NV_i	Number of variablesmeasured in ith experiments
n	Number of samples
O_r	Observed value of profile r
P	Xylitol production, g/L
P_r	Predicted value for profile r
r_P	Xylitol production rate, g/L h
r_S	Xylose consumption rate, g/L h
r_X	Cell growth rate, g/L.h
S	Substrate concentration, g/L
T	Vessel inside diameter, m
t	Time, h
U_g	Superficial gas velocity, (m/s)
V	Liquid volume, L
vvm	volume of gas per volume of liquid per minute
X	Cell concentration, g/L
Y_{O2}	Yield of oxygen per cell, g/g
Y_{PS}	Yield of product per unit substrate, g/g
Y_{XS}	Yield of cells per unit substrate, g/g
$\tilde{z}_{ijk}$	Kth measured value of variables j in experiment i
z_{ijk}	kth (model) predicted value of variable j in experiment i

Greek letters

μ	Specific growth rate, h^{-1}
μ_{max}	Maximum specific growth rate, h^{-1}
δ	Product decay rate, g/L h
δ_0	Initial product decay rate, h^{-1}
ε	Ratio of gas volume to liquid volume inside the bioreactor
$\upsilon_{g,}$	Oxygen gas flow rate in the liquid phase, g/L h
σ_{ijk}	Variance of the kth measurements of variable j in experiment i

production is scarce. To date, a stoichiometric model has been developed by analysing the metabolic fluxes and the reaction rate of *Candida parapsilosis* [13] and a model of the same species under oxygen-limited conditions [14]. Another growth model was developed for the presence of a co-substrate such as glucose to facilitate the production of xylitol [15] and a prediction of growth model using non-linear methods [16].

Substrate concentration and oxygen concentration are essential for xylitol fermentation systems, which must be taken into account for describing the cell growth and production process. Literature review reported that high xylose concentration may leads to high xylitol accumulation. The experimental studies on *Candida tropicalis* showed that for higher xylose concentration in optimal aeration rate, a significant of cell growth occurred at the beginning and the xylitol production rate is considerably improved [17]. Therefore, kinetic modelling of substrate and oxygen concentration is important to describe the xylitol production.

The objective of the present study is to develop a reaction kinetic model to represent xylitol production by fermentation using *C. tropicalis*. The kinetic parameters of the models were expressed as a function of the initial xylose concentration and oxygen concentration that related to the agitation speed.

2. Model description

The unstructured dynamic model equations of reaction kinetics for xylitol production was proposed based on the following assumptions; (1) the growth of yeast is represented under substrate limited and controlled by oxygen limiting conditions; (2) the mixing in the reactor are considered to be homogenous; and (3) mass balance within the reactor.

2.1. Growth rate model

In xylitol fermentation process, the cell growth rate (r_X) of *C. tropicalis* can be described as:

$$r_X = \frac{dC_X}{dt} = \mu \times C_X. \quad (1)$$

where μ is the specific growth rate, and c_X represents the concentration of cell in the fermentation broth. The relationship of specific growth rate (μ) to substrate or xylose concentration (C_S) is assumed to form the limiting or saturation kinetics. This kinetics can be represented by the Monod equation:

$$\mu = \mu_{max} \times \frac{C_S}{C_S + K_{li,S}}. \quad (2)$$

where $\mu_{\max}$ is the maximum specific growth rate (h^{-1}) and $K_{li,S}$ is the substrate limiting constant (g/L).

The experimental results showed that the dynamic growth of *C. tropicalis* was limited by oxygen and inhibited by a high xylose concentration. A high xylose concentration inhibits cell growth owing to the osmotic stress established across the cell membrane, thus decreasing the reaction rate. The DO level drops below the critical level for *C. tropicalis* after several hours of fermentation (approximately 6–12 h) and xylose is completely consumed approximately after 50 h of fermentation. This multiple substrate limitations is