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A model of xylitol production by the yeast *Candida mogii*

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Abstract Production of xylitol from xylose in batch fermentations of *Candida mogii* ATCC 18364 is discussed in the presence of glucose as the cosubstrate. Various initial ratios of glucose and xylose concentrations are assessed for their impact on yield and rate of production of xylitol. Supplementation with glucose at the beginning of the fermentation increased the specific growth rate, biomass yield and volumetric productivity of xylitol compared with fermentation that used xylose as the sole carbon source. A mathematical model is developed for eventual use in predicting the product formation rate and yield. The model parameters were estimated from experimental observations, using a genetic algorithm. Batch fermentations, which were carried out with xylose alone and a mixture of xylose and glucose, were used to validate the model. The model fitted well with the experimental data of cell growth, substrate consumption and xylitol production.

Keywords Xylitol · Xylose · Mathematical model · Two-substrate fermentation · *Candida mogii*

Nomenclature

a_{cell}	Specific surface area of the cell (m^2 g DCW^{-1})
C_{glc}	Glucose concentration (g glucose l^{-1})
C_x	Biomass concentration (g DCW l^{-1})
C_{xit}^{ex}	Extracellular concentration of xylitol (g xylitol l^{-1})
C_{xit}^{in}	Intracellular concentration of xylitol (g xylitol l cell volume^{-1})
C_{xyl}	Xylose concentration (g xylose l^{-1})
d_{mem}	Thickness of the cell membrane (m)
D_{mem}	Diffusion coefficient of xylitol in the lipid bilayer (m^2 s^{-1})
HMP	Hexose monophosphate
$K_{i,\ glc}$	Inhibition constant by glucose (g glucose l^{-1})
$K_{i,\ xyl}$	Inhibition constant by xylose (g xylose l^{-1})
$K_{s,\ glc}$	Saturation constant based on glucose (g glucose l^{-1})
$K_{s,\ xit}$	Saturation constant based on xylitol (g xylitol l^{-1})
$K_{s,\ xyl}$	Saturation constant based on xylose (g xylose l^{-1})
K_{par}	Partition coefficient (-)
K_r	Repression constant by glucose (g glucose l^{-1})
min J	Minimum error of the objective function (-)
M	Number of experimentally observable state variables (-)
MW_{xit}	Molecular weight of xylitol (g xylitol mole^{-1})
MW_{xyl}	Molecular weight of xylose (g xylose mole^{-1})
N	Number of sampling points of experimental data (-)
P	Permeability coefficient (m s^{-1})
P_{xit}	Permeability coefficient for xylitol (m s^{-1})
q_{glc}	Specific uptake rate of glucose (g glucose g DCW^{-1} h^{-1})
q_{glc}^{max}	Maximum specific uptake rate of glucose (g glucose g DCW^{-1} h^{-1})
q_{xit}	Specific production rate of xylitol (g xylitol g DCW^{-1} h^{-1})
q_{xyl}	Specific uptake rate of xylose (g xylose g DCW^{-1} h^{-1})
q_{xyl}^{max}	Maximum specific uptake rate of xylose (g xylose g DCW^{-1} h^{-1})
Q_{xit}	Volumetric production rate of xylitol (g xylitol l^{-1} h^{-1})

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Q_{xyl} Volumetric uptake rate of xylose (g xylose l^{-1} h^{-1})
$r_{f,xit}$ Specific rate of formation of xylitol (g xylitol g DCW^{-1} h^{-1})
$r_{u,xit}$ Consumption rate of intracellular xylitol (g xylitol g DCW^{-1} h^{-1})
$r_{t,xit}$ Mass flux of xylitol based on dry cell weight (g xylitol g DCW^{-1} h^{-1})
t Cultivation time (h)
W_i Weighting coefficient (-)
y_{ij} Experimental value of variable (-)
$\hat{y}_{ij}$ Model predicted value of variable (-)
$Y_{x/xit}$ Biomass yield on xylitol (g DCW g xylitol^{-1})
$Y_{x/(xyl+glc)}$ Biomass yield on xylose and glucose (g DCW g sugar^{-1})
$Y_{xit/xyl}$ Xylitol yield on xylose (g xylitol g xylose^{-1})
μ Specific growth rate (h^{-1})
μ_{glc}^{max} Maximum specific growth rate on glucose (h^{-1})
μ_{xit}^{max} Maximum specific growth rate on xylitol (h^{-1})
ρ_x Mass density of cells (g DCW l cell^{-1} volume)
η Energy yield coefficient for biomass production (-)
ξ_p Energy yield coefficient for product formation (-)
σ_{cell} Weight fraction of carbon in biomass (g atom-C g DCW^{-1})
σ_{xit} Weight fraction of carbon in xylitol (g atom-C g xylitol^{-1})
γ_{cell} Reductance degree of biomass (-)
γ_{xit} Reductance degree of xylitol (-)

Introduction

Xylitol is a five-carbon sugar alcohol that is found in small quantities in many fruits and vegetables. Xylitol occurs in the human body as a normal intermediate of carbohydrate metabolism. Sweetening power of xylitol is similar to that of sucrose. Unlike sucrose, xylitol is not metabolized by Streptococci in the mouth and, therefore, xylitol-sweetened foods barely cause any tooth decay in the absence of other carbohydrates that may be susceptible to bacterial action. Furthermore, xylitol is a fully metabolizable sugar substitute for diabetics. Increasing amounts of xylitol are being used as sweetener in the food industry. Xylitol can be produced from *D*-xylose by fermentation with yeasts [1]. Here we discuss the production of xylitol from xylose using the yeast *Candida mogii* ATCC 18364. A mathematical model is developed and experimentally validated to describe xylitol production.

Xylose metabolizing yeasts first internalize the extracellular xylose. The internalized xylose is reduced to xylitol with the enzyme xylose reductase in a step that consumes NADPH (Fig. 1). Part of the xylitol produced is excreted and the rest is converted to xylulose through the action of NAD^+-dependent xylitol dehydrogenase. Xylulose is further metabolized to generate cell mass and maintenance energy (Fig. 1). In the presence of an easily metabolized carbon source such as glucose, the cell can

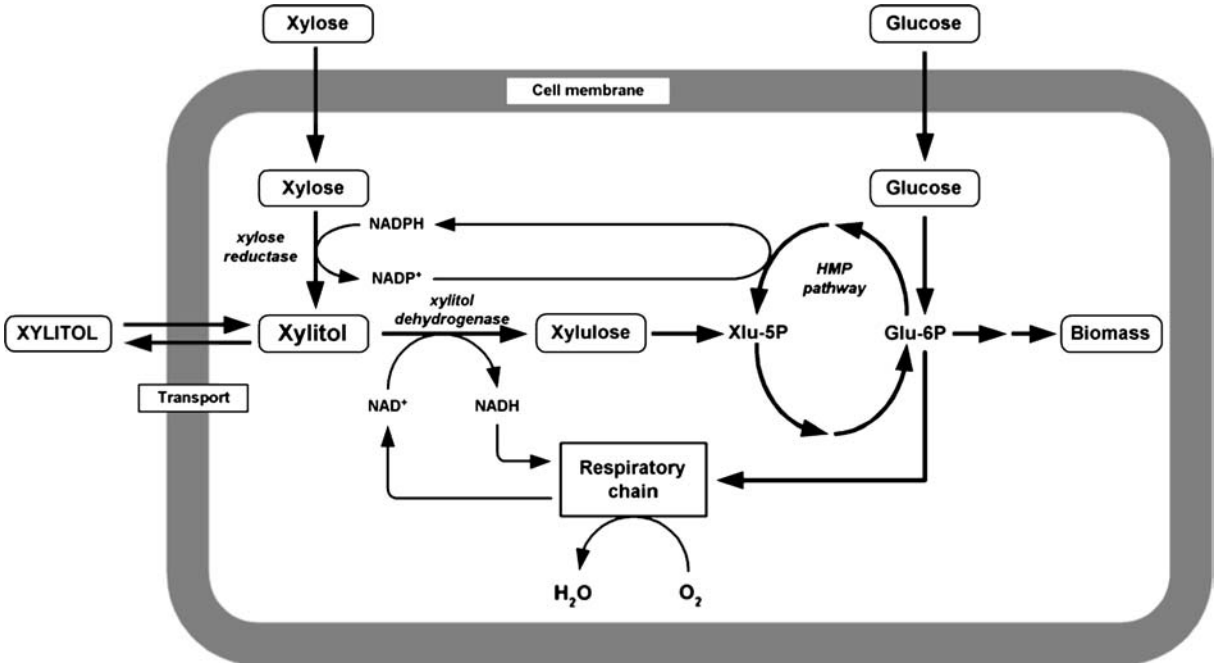

Fig. 1 Simplified representation of xylose and glucose metabolism in *C. mogii*

generate energy and biomass directly from glucose via glucose-6-phosphate (Glu-6P); consequently, hexose monophosphate (HMP) pathway for producing Glu-6P from xylulose via xylulose-5-phosphate (Xlu-5P) slows down. This reduces the consumption of xylitol within the cell and more of it can be excreted.

Ideally, overaccumulation of xylitol inside the cell and its excretion as extracellular product are required to ensure a high yield of xylitol. Total elimination of the flux of xylitol to xylulose is not required, as this would shut down the regeneration of NADPH that is required for converting xylose to xylitol in the first place (Fig. 1). Supplementation with glucose in a xylose fermentation can thus be used to enhance the yield of xylitol. The presence of small amounts of glucose in the medium has indeed improved xylitol yield from xylose in yeast fermentations [2–4]; however, high concentrations of glucose are known to inhibit xylose transport into the cell [5] and repress induction of relevant enzymes by xylose [6, 7].

Modeling of the xylose fermentation in the presence of a cosubstrate such as glucose, can provide answers about how the ratio of glucose-to-xylose influences the yield and rate of production of xylitol. Xylitol production has been mainly studied in the yeasts *Candida tropicalis* [2, 3], *Candida guilliermondii* [6–8], *Candida parapsilosis* [9], and *Debaryomyces hansenii* [4]. Preliminary screening suggests *C. mogii* as a promising microorganism for producing xylitol [10], but little information exists on xylitol production by this yeast [10–12]. No mathematical models are available for fermentation of glucose and xylose mixtures to xylitol by any yeasts.

Ethanol production by *Pichia stipitis* on mixtures of glucose and xylose has previously been modeled using modified Monod kinetics [13, 14]. The diauxic conversion of glucose/xylose mixture was described by competitive inhibition of the uptake of one sugar by the other substrate. Other work that has modeled xylitol production by yeasts has focused only on single-sugar fermentations [8, 9]. Previous work involving cosubstrates [2–4] has neither modeled the fermentation process nor focused on the yeast that is of interest in the present work.

Fermentation model

The biomass growth rate was modeled using the well-known autocatalytic growth equation, as follows:

$$\frac{\mathrm{d}C_x}{\mathrm{d}t} = \mu \cdot C_x, \tag{1}$$

where C_x is the biomass concentration at time t and μ is the specific growth rate. Equation 1 disregards cell death because, under optimal growth conditions, the specific growth rate far exceeds the specific rate of cell death.

The yeast does not use xylose directly. Xylose is first converted to xylitol inside the cell before it can be used for metabolic purposes. The observed specific growth rate μ in the presence of the two substrates, was modeled as consisting of contributions of glucose in the medium and xylitol inside the cell, as follows:

$$\mu = \mu_{\mathrm{glc}}^{\max}\frac{C_{\mathrm{glc}}}{K_{\mathrm{s,glc}} + C_{\mathrm{glc}}} + \mu_{\mathrm{xit}}^{\max}\frac{C_{\mathrm{xit}}^{\mathrm{in}}}{K_{\mathrm{s,xit}} + C_{\mathrm{xit}}^{\mathrm{in}}} \cdot \frac{K_{\mathrm{r}}}{K_{\mathrm{r}} + C_{\mathrm{glc}}}. \tag{2}$$

Here $\mu_{\mathrm{glc}}^{\max}$ is the maximum specific growth rate on glucose alone, $\mu_{\mathrm{xit}}^{\max}$ is the maximum specific growth rate on xylitol alone, C_{glc} is the glucose concentration, $C_{\mathrm{xit}}^{\mathrm{in}}$ is the concentration of xylitol within the cell, $K_{\mathrm{s,glc}}$ is Monod saturation constant for glucose, $K_{\mathrm{s,xit}}$ is the Monod saturation constant for xylitol, and K_{r} is the repression constant by glucose. The amount of xylitol that goes into generating cell mass and energy depends on the activity of xylitol dehydrogenase. This enzyme is repressed by glucose [6] and K_{r} accounts for this repression. Repression of xylitol dehydrogenase by glucose results in yeast utilizing glucose and intracellular xylitol in a sequential manner.

The first step of xylose fermentation is the uptake of sugar through the yeast cell membrane. Xylose uptake by *C. mogii* follows Michaelis-Menten kinetics and this is indicative of a carrier-mediated facilitated diffusion through the cell membrane [10]. In mixed-substrate fermentation, the presence of glucose affects the uptake of xylose and vice versa because transport of both sugars uses the same facilitated diffusion system [5]. Considering this, the specific uptake rates of xylose and glucose were modeled as if the carrier enzyme was competitively inhibited [15] by the two substrates; thus, the specific uptake rates of glucose (q_{glc}) and xylose (q_{xyl}) were expressed as follows:

$$q_{\mathrm{glc}} = q_{\mathrm{glc}}^{\max}\frac{C_{\mathrm{glc}}}{C_{\mathrm{glc}} + K_{\mathrm{s,glc}}\left(1 + \frac{C_{\mathrm{xyl}}}{K_{\mathrm{i,xyl}}}\right)}, \tag{3}$$

$$q_{\mathrm{xyl}} = q_{\mathrm{xyl}}^{\max}\frac{C_{\mathrm{xyl}}}{C_{\mathrm{xyl}} + K_{\mathrm{s,xyl}}\left(1 + \frac{C_{\mathrm{glc}}}{K_{\mathrm{i,glc}}}\right)}, \tag{4}$$

where $q_{\mathrm{glc}}^{\max}$ and $q_{\mathrm{xyl}}^{\max}$ are the maximum specific uptake rates of glucose and xylose, respectively; $K_{\mathrm{i,xyl}}$ is a constant relating to inhibition of glucose uptake by xylose; $K_{\mathrm{i,glc}}$ is a constant for inhibition of xylose uptake by glucose; C_{xyl} and C_{glc} are concentrations of xylose and glucose, respectively, in the medium. The saturation constant $K_{\mathrm{s,glc}}$ in Eq. 3 was assumed to be identical to that in Eq. 2 because glucose uptake is the rate-limiting step in its metabolism.

The rates of glucose and xylose uptake are related to the biomass concentration (C_x) as follows:

$$\frac{\mathrm{d}C_{\mathrm{glc}}}{\mathrm{d}t} = -q_{\mathrm{glc}} \cdot C_x \tag{5}$$

$$\frac{dC_{xyl}}{dt} = -q_{xyl} \cdot C_x. \quad (6)$$

Equations 5 and 6 result from a simple mass balance and are well known.

Some of the xylitol formed in the cell is further metabolized for growth; therefore, intracellular xylitol is assumed to be the growth-limiting substrate. In yeasts such as *P. stipitis*, uptake of xylose has been shown to be the rate-limiting step in aerobic metabolism [16]. Assuming a similar mechanism for *C. mogii*, intracellular conversion of xylose to xylitol would be limited by the uptake rate of xylose. Intracellular consumption of xylitol for maintenance energy and coenzyme regeneration was assumed to be negligible in comparison with xylitol's consumption for biomass growth. All reactions in the HMP pathway were lumped together and assumed to be reflected in the cell growth term.

Each mole of xylose taken up by the cell was assumed to be quantitatively converted to xylitol. Therefore, the specific rate of formation of intracellular xylitol ($r_{f,xit}$) and the specific rate of uptake of xylose (q_{xyl}) were related as follows:

$$r_{f,xit} = \frac{MW_{xit}}{MW_{xyl}} \cdot q_{xyl}. \quad (7)$$

Here MW_{xit} and MW_{xyl} are the molar masses of xylitol and xylose, respectively.

As mentioned earlier, any xylitol consumed within the cell was assumed to generate cell mass (i.e. maintenance term was negligible compared to growth). Therefore, the rate of consumption of intracellular xylitol for growth ($r_{u,xit}$) was related to the specific growth rate on xylitol μ_{xit} as follows:

$$r_{u,xit} = \frac{\mu_{xit}}{Y_{x/xit}}, \quad (8)$$

where $Y_{x/xit}$ is the biomass yield on xylitol.

Transport of acrylic polyols such as xylitol from outside to inside the cell, is known to occur by passive diffusion in bakers' yeast *Saccharomyces cerevisiae* [17]. Assuming an equivalent mechanism for transmembrane xylitol transport in *C. mogii*, the rate of molecular diffusion can be estimated from a modification of Fick's law [18], such that the mass flux of xylitol ($r_{t,xit}$) across the membrane is directly proportional to the permeability coefficient of the membrane (P_{xit}), the difference between intracellular and extracellular concentrations of xylitol (i.e. $C^{in}_{xit} - C^{ex}_{xit}$) and the specific surface area of the cell (a_{cell}), as follows:

$$r_{t,xit} = 3.6 \times 10^6 \frac{D_{mem} K_{par}}{d_{mem}} a_{cell} \left(C^{in}_{xit} - C^{ex}_{xit}\right) = 3.6 \times 10^6 P_{xit} a_{cell} \left(C^{in}_{xit} - C^{ex}_{xit}\right). \quad (9)$$

Here d_{mem} is the thickness of the cell membrane, D_{mem} is the diffusion coefficient of xylitol in the lipid bilayer membrane, and K_{par} is the partition coefficient. The factor of 3.6×10^6 accounts for conversion of units.

By mass balance, the rate of change of concentration of intracellular xylitol C^{in}_{xit} is given by the following equation:

$$\frac{dC^{in}_{xit}}{dt} = \rho_x \left(r_{f,xit} - r_{u,xit} - r_{t,xit}\right) - \mu \cdot C^{in}_{xit}, \quad (10)$$

where ρ_x is the density of the yeast cell, $r_{f,xit}$ is the specific rate of formation of xylitol, $r_{u,xit}$ is the consumption rate of intracellular xylitol, and $r_{t,xit}$ is the mass flux of xylitol based on dry cell weight. Similarly, the change in concentration of the extracellular xylitol is given as follows:

$$\frac{dC^{ex}_{xit}}{dt} = r_{t,xit} \cdot C_x, \quad (11)$$

where C_x is the biomass concentration.

Equations 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 comprised the fermentation model for *C. mogii*.

Materials and methods

Microorganism, media and inoculum

The yeast *Candida mogii* ATCC 18364 was the microorganism used. It was maintained on potato dextrose agar (PDA) at 4 °C. The minimal medium used for growth contained (per liter of solution): 18.75 g KH_2PO_4, 6 g $(NH_4)_2HPO_4$, 1.13 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$, 36.5 mg myo-inositol, 18.2 mg calcium pantothenate, 3.66 mg thiamine-HCl, 0.9 mg pyridoxal-HCl, 0.018 mg biotin, 9.1 mg $FeCl_3$, 6.4 mg $MnSO_4 \cdot H_2O$, 5.46 mg $ZnSO_4 \cdot 7H_2O$, 1.46 mg $CuSO_4 \cdot 5H_2O$ and specified carbon sources.

Yeast cells were suspended in the medium to obtain an optical density of 0.8 at 620 nm. This suspension (1 ml per flask) was used to inoculate two 250-ml Erlenmeyer flasks. Each new flask contained 20 ml of minimal medium that had been supplemented with 20 g l^{-1} of glucose. Flasks were incubated aerobically at 30 °C for 24 h on a rotary shaker (250 rpm). These flasks were then used to inoculate two 500-ml Erlenmeyer flasks. Each of the latter contained 180 ml of minimal medium that had been supplemented with 20 g l^{-1} of glucose. The flasks were incubated for 24 h at the conditions specified above.

Bioreactor cultures

Aerobic batch fermentations were carried out in a 5-l stirred-tank fermenter (Biostat B, B. Braun Biotech International, Germany) that contained 3.7 l of minimal medium supplemented with glucose (10 g l^{-1}) and xylose (5 g l^{-1}). The culture temperature was 30 °C. The pH was automatically controlled at 4.5 by adding 6 M NaOH as needed. The aeration rate was kept constant at 1 vvm. The agitation speed was adjusted in the range of 600–800 rpm to maintain a dissolved oxygen concentration of above 75% of air saturation. After 24 h, the

fermentation was switched to xylitol production phase, with the agitation speed and aeration rate adjusted to maintain a dissolved oxygen concentration of 20% of air saturation. The pH in this phase was controlled at 6.0. At the beginning of the xylitol production phase, 300 ml of an aqueous solution containing 30 g l^{-1} of xylose and 0, 3 or 4.2 g l^{-1} of glucose, was added to the fermenter to achieve initial molar ratios of glucose-to-xylose of 0, 10 or 14% in separate experiments.

Analytical methods

The broth was sampled periodically. Cells were recovered by centrifugation, washed twice in distilled water and dried to constant weight at 105°C to obtain dry cell weight. The supernatant of the broth samples was used to measure xylose, xylitol and glucose. Xylose and xylitol were determined by the methods of Deschatelets and Yu [19] and Adler and Gustafsson [20], respectively. Glucose concentration was determined with an enzymatic test kit (catalog number 510; Sigma, St. Louis, MO, USA).

Estimation of model parameters

The kinetic parameters (i.e. μ_{glc}^{max}, μ_{xit}^{max}, q_{xyl}^{max}, q_{glc}^{max}, $K_{s,xyl}$, $K_{s,glc}$, $K_{s,xit}$, $K_{i,xyl}$, $K_{i,glc}$, K_r, P_{xit}) of the model were estimated from the experimental data. The estimation procedure varied the values of the parameters to minimize the difference between the model-predicted fermentation profiles and the experimentally observed profiles. The parameter values that best reproduced the measured data were the optimal values. The mean of weighted square error (*min J*) of the objective function used for data fitting was estimated as follows:

$$min\ J = \frac{1}{N}\sum_{i=1}^{M} W_i \sum_{j=1}^{N} \left(\hat{y}_{ij} - y_{ij}\right)^2, \qquad (12)$$

where M is the number of experimentally observable state variables, N is the number of sampling points of experimental data, W_i is the weighting coefficient (i.e. square of the reciprocal maximum value of a dependent variable), y_{ij} is the experimental value and $\hat{y}_{ij}$ is the model-predicted value. The genetic algorithm optimization toolbox (GAOT), implemented in MATLAB® (The MathWorks, Inc., Natick, MA, USA) was used as a search algorithm to estimate the model parameters [21]. The model parameters were the variables to be estimated by the genetic algorithm and became the elements of the algorithm chromosome.

Results and discussion

Effects of glucose on xylitol production

The concentration profiles of biomass, xylose, glucose and xylitol for batch fermentations carried out with three different initial glucose/xylose ratios, are shown in Fig. 2. In the absence of glucose (Fig. 2a), *C. mogii* consumed xylose to produce biomass and xylitol. Xylitol production commenced early in the fermentation compared to when glucose was present (Fig. 2b, c). When both xylose and glucose were used as substrates, glucose was consumed much more rapidly than xylose (Fig. 2b, c). Increasing concentrations of initial glucose, delayed accumulation of extracellular xylitol but higher concentrations of xylitol were eventually achieved (Fig. 2b, c) compared to when xylose was the sole carbon source (Fig. 2a). Clearly, a glucose/xylose ratio of 10%

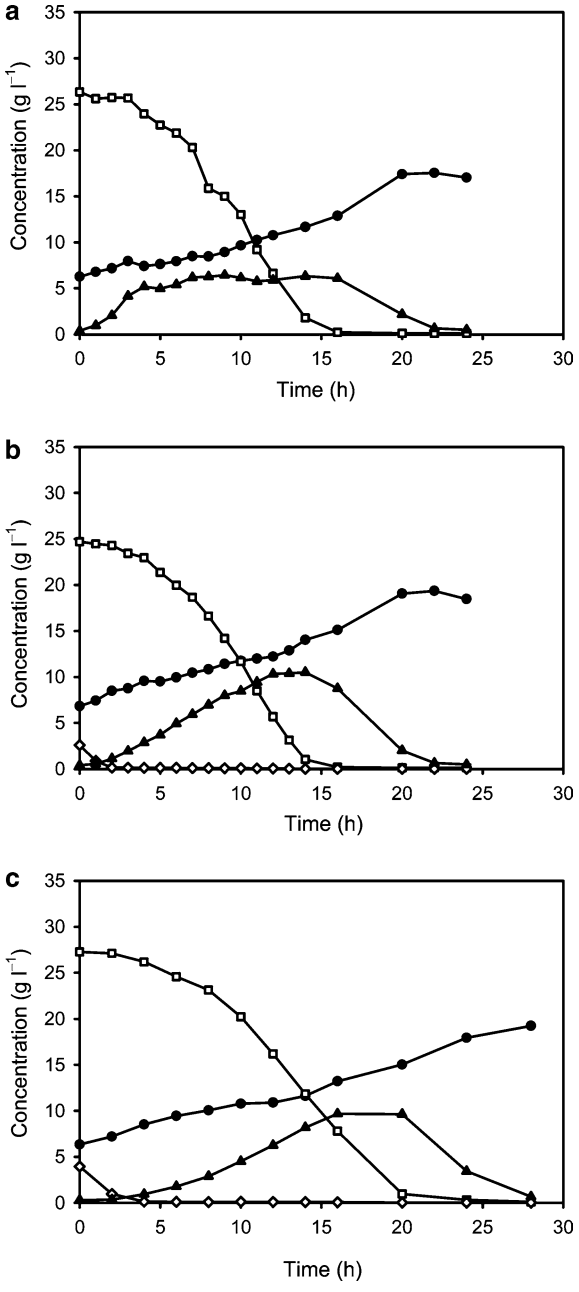

Fig. 2 Batch fermentation profiles for initial glucose/xylose concentration ratios of **a** 0%, **b** 10% and **c** 14%. Biomass (*filled circle*), xylose (*open square*), glucose (*open diamond*), xylitol (*filled triangle*)

(Fig. 2b) was better for maximizing xylitol productivity when compared with the use of higher level of glucose (Fig. 2c) and no glucose (Fig. 2a).

The increasing lag in initial accumulation of xylitol with increasing initial concentration of glucose can be explained as follows: the yeast can take up xylose in the presence of glucose, but this xylose accumulates in the cell because glucose is a known inhibitor of the intracellular enzyme responsible for catabolism of xylose to xylitol [22]. Once glucose has been consumed, the enzyme for converting xylose to xylitol is activated and xylitol production accelerates.

Xylitol concentration peaks just before xylose is nearly all consumed (Fig. 2a–c). If the fermentation is allowed to progress further, the cells use increasing amounts of xylitol for generating biomass and sustenance; therefore, the concentration of extracellular xylitol declines until all of it is consumed by the cells. Because xylitol is the desired product, fermentation should be interrupted just before xylose is exhausted. A proper prediction of the optimal harvest time is essential for conducting this fermentation.

Ability of *C. mogii* to take up and use xylitol as carbon source for cell growth, is similar to that of *C. guilliermondii* [8]. Utilization of xylitol as a carbon source is also found in *Pichia sipitis*, where xylitol utilization is strictly respiratory and biomass and carbon dioxide are the only products formed [23]. In contrast, the metabolism of xylitol by *Pichia guilliermondii* (*C. guilliermondii*, asexual state) is related to production of riboflavin by the cells without new biomass being produced [24]. In addition to biomass, other unmeasured metabolic products such as glycerol or acetate may be produced from xylitol to lower its yield.

When xylitol concentration reached the maximum, the specific rates and yields were evaluated as shown in Table 1. The specific growth rate in minimal medium containing only xylose as a carbon source, under the controlled dissolved oxygen concentration of 20% of air saturation, was similar to that reported for highly aerobic conditions [10]. This suggests that a dissolved oxygen concentration corresponding to 20% of air saturation is not an oxygen-limited condition for this yeast. Yeasts generally grow well in glucose-containing media; therefore, increased specific growth rate in the presence of glucose (Table 1) is not surprising.

When xylose was the sole carbon source, the xylitol yield obtained was 0.534 g g^{-1}, or 59% of theoretical yield [25]. Xylitol yield is known to depend on the initial xylose concentration and dissolved oxygen level [10]. Under oxygen-limited conditions in optimized complex media supplemented with high initial xylose concentrations (e.g. 50–60 g l^{-1}), xylitol yields have approached 0.7 g g^{-1} in cultures of *C. mogii* [10–12]. For the purpose of evaluating the xylitol production model, this work used a synthetic medium, relatively low concentrations of xylose and the culture conditions used were generally aerobic.

Adding glucose increased the biomass yield compared with the use of xylose as the sole carbon source, but the xylitol yield from xylose decreased. This suggests that in the presence of glucose, an increased quantity of xylitol is diverted into producing biomass. In *P. stipitis*, in contrast, adding glucose to the xylose medium leads to respiration to produce carbon dioxide rather than biomass [23].

Compared with the use of xylose alone, adding a small amount of glucose, i.e. initial glucose/xylose concentration ratio of 10%, did not affect the specific uptake rate of xylose. Nevertheless, the specific production rate of extracellular xylitol decreased noticeably. Because the specific rate of xylitol production is directly correlated with the uptake rate of xylose, a decrease in xylitol production rate suggests that more of the intracellular xylitol was being diverted to synthesis of biomass. The specific uptake rate of xylose was inhibited by glucose only when the initial glucose/xylose ratio exceeded 10%. Glucose inhibition of xylose uptake led to a significant decrease in the specific rate of production xylitol.

Adding a small amount of glucose improved the volumetric productivity of xylitol. This was because more total biomass was produced, but the negative effect of glucose had not come into play at low levels of glucose. Higher concentration of glucose increased the attainable biomass concentration but glucose inhibition of xylose uptake greatly reduced xylitol productivity. The maximum xylitol productivity obtained under aerobic conditions using initial glucose/xylose concentration ratio of 10% was 0.827 g l^{-1} h^{-1}. This productivity was higher than previously attained with *C. mogii* in ideal oxygen-limited conditions using xylose as the sole carbon source [12] or using concentrated rice straw hydrolysate as a substrate [11].

Kinetic parameters of xylitol production

The model required values of the cell density and specific surface area of the yeast. Cell density of *C. mogii* ATCC

Table 1 Observed fermentation parameters of xylitol production by *C. mogii*

Initial [Glc]/[Xyl](%)	Yields		Specific rates			Volumetric rates	
	$Y_{x/(xyl+glc)}$ (g g^{-1})	$Y_{xit/xyl}$ (g g^{-1})	μ (h^{-1})	q_{xyl} (g g^{-1} h^{-1})	q_{xit} (g g^{-1} h^{-1})	Q_{xyl} (g l^{-1} h^{-1})	Q_{xit} (g l^{-1} h^{-1})
0	0.235	0.534	0.040	0.166	0.089	1.260	0.673
10	0.249	0.522	0.046	0.166	0.087	1.585	0.827
14	0.294	0.481	0.042	0.124	0.060	1.216	0.586

18364 was obtained from the literature, as 120 g DCW l^{-1} cell volume [26]. Dimensions of the cell were determined by direct measurements of cell length and width using an eyepiece micrometer under a microscope. The specific surface area of the cell was calculated to be 7.6 m^2 g DCW^{-1}. This was for cells grown in the minimal medium with xylose as the sole carbon source. The calculated surface area was similar to values of 7.73–7.86 m^2 g DCW^{-1} reported for the yeast *Candida albicans* at various phases of growth and in different nutrient media [27].

The biomass yield on xylitol ($Y_{x/xit}$) was estimated using the following equation [28, 29]:

$$Y_{x/xit} = \eta \frac{\sigma_{xit}\gamma_{xit}}{\sigma_{cell}\gamma_{cell}}, \quad (13)$$

where η is the energy yield coefficient for biomass formation; γ_{cell} and γ_{xit} are the reductance degrees of biomass and xylitol, respectively; and σ_{cell} and σ_{xit} are the weight fractions of carbon in biomass and xylitol. Values of γ_{cell}, γ_{xit}, σ_{cell}, and σ_{xit} are 4.19, 4.40, 0.49 and 0.39, respectively. For this calculation, growth was assumed to occur exclusively on xylitol as the carbon source and ammonia as the nitrogen source. The biomass was assumed to have the molecular formula of $CH_{1.79}O_{0.5}N_{0.2}$ [29].

For aerobic processes, the thermodynamic efficiency is the sum of η and the energy yield coefficient for product formation (ξ_p). Thermodynamic efficiency ranges between 0.55 and 0.6 [28]. Because xylitol use is strictly by respiration, biomass and carbon dioxide were assumed to be the only metabolic products [23]; hence, ξ_p was nil. Roels [29] has suggested that η is 0.58 for organic substrates with reductance degree ≤ 4.67. Therefore, the estimated value of $Y_{x/xit}$ was 0.48.

The other model parameters in Eqs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 were estimated from experimental data obtained during xylitol production phase of batch fermentations. The real-code genetic algorithm with population size of 80 and 1000 generations was used for the estimation. The algorithm chromosome contained 11 elements that represented the 11 model parameters as described above. The estimated values of parameters were obtained by minimizing the mean weighted square error between the experimental data and model-simulated values. The estimated model parameter values are given in Table 2.

The estimated value of the maximum specific growth rate on glucose (μ_{glc}^{max}) is greater than that on xylitol (μ_{xit}^{max}) (Table 2). This is consistent with a lower value of the saturation constant for glucose ($K_{s,glc}$) compared to the saturation constant for xylitol ($K_{s,xit}$) .(A high saturation constant for a substrate indicates a low affinity for the substrate by the converting enzyme.) Glucose is generally known to promote more rapid growth of yeast than does xylitol.

The saturation constant for xylose ($K_{s,xyl}$) is bigger than the saturation constant for glucose (Table 2), confirming a higher affinity for glucose uptake than for xylose uptake by the cell. Consistent with this, the maximum specific uptake rate of glucose (q_{glc}^{max}) is nearly tenfold higher than that of xylose (q_{xyl}^{max}) (Table 2). The competitive inhibition of glucose and xylose uptake rates depends on the value of the corresponding inhibition constants (i.e.$K_{i,glc}$ and $K_{i,xyl}$). The values of inhibition constants in Table 2 imply that the uptake rate of xylose by *C. mogii* is highly influenced by glucose, but xylose has a relatively minor effect on glucose uptake rate. The mutual inhibitory effect of glucose and xylose on their uptake appears to depend on the type of yeast. For example, for *Candida shehatae*, the values of $K_{i,glc}$ and $K_{i,xyl}$ are similar at around 3.5 g sugar l^{-1} [30]. In comparison with this, the $K_{i,xyl}$ value for *C. mogii* is much higher (Table 2) but even higher values have been observed in other yeasts. For example, the $K_{i,xyl}$ of *D. hansenii* has been reported as 26.25 g xylose l^{-1} [31].

The diffusion of polyols such as xylitol across the cell membrane depends on their molecular size. The estimated value of the permeability coefficient for xylitol (P_{xit}) in Table 2 is lower than that for smaller polyols such as methanol ($P = 2.80\times10^{-4}$ m s^{-1}), glycerol ($P = 2.10\times10^{-7}$ m s^{-1}) and erythretol ($P = 1.40\times10^{-8}$ m s^{-1}) [32]; however, it is higher than the *P*-value for larger polyols such as sorbitol ($P = 1.70\times10^{-9}$ m s^{-1}) and mannitol ($P = 3.30\times10^{-10}$ m s^{-1}) [33]. No directly measured data exist on P_{xit} in *C. mogii*, but the evidence suggests that the model estimated value is within the expected bounds.

Table 2 The estimated values of the model parameters

Parameters	Values	Units
μ_{glc}^{max}	0.662	h^{-1}
μ_{xit}^{max}	0.189	h^{-1}
q_{xyl}^{max}	0.342	g xylose·g DCW^{-1} h^{-1}
q_{glc}^{max}	3.276	g glucose g DCW^{-1} h^{-1}
$K_{s,xyl}$	11.761	g xylose l^{-1}
$K_{s,glc}$	9.998	g glucose l^{-1}
$K_{s,xit}$	16.068	g xylitol l^{-1}
$K_{i,xyl}$	14.780	g xylose l^{-1}
$K_{i,glc}$	0.100	g glucose l^{-1}
K_r	0.100	g glucose l^{-1}
P_{xit}	7.591×10^{-9}	m s^{-1}

Model validation

The model was validated by application to fresh batch fermentations that had not been used for estimating the model parameters. The kinetic model represented by Eqs. 1, 2, 3, 4, 5, 6, 7, 8, 9,10 and 11 with the parameter values listed in Table 2, was used to predict the state variables (i.e. concentrations of biomass, glucose, xylose and xylitol) during cultivation. A comparison between the measured data and the model predictions is shown in Fig. 3 for two fermentations.

Figure 3a compares the experimental data and model predictions in a batch cultivation without glucose.

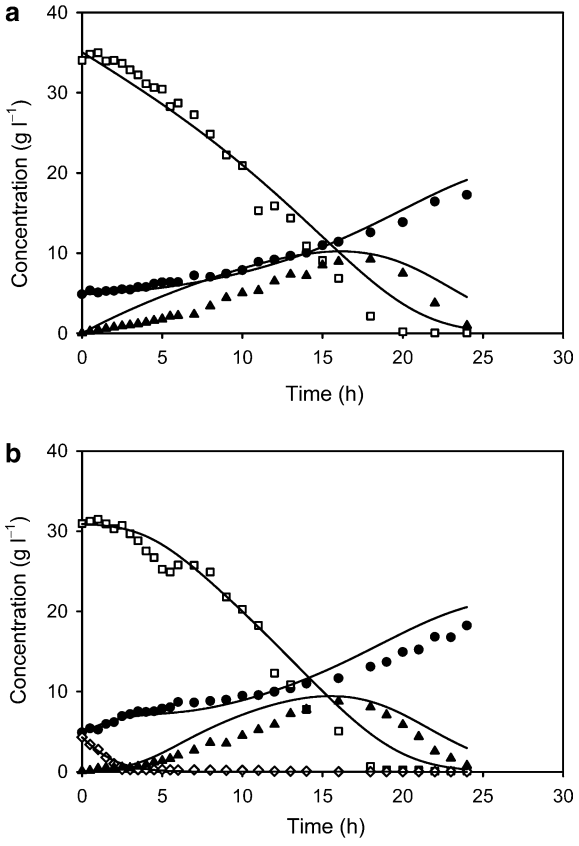

Fig. 3 Comparison between experimental observations and model predictions for batch cultures: **a** without glucose and **b** with added glucose. Biomass (*filled circle*), xylose (*open square*), glucose (*open diamond*), xylitol (*filled triangle*), model prediction (solid lines)

The model accurately predicts the biomass concentration profile. The xylose concentration is predicted reasonably well, except in the last 10 h of culture when the predicted values are slightly higher than the measured data. The predicted xylitol concentrations are always a little higher than the measured values; nevertheless, the model does predict well the trend of xylitol concentration profile. The maximum value of the xylitol concentration is predicted well. In the presence of glucose as cosubstrate such that the initial ratio of glucose-to-xylose was 14%, the model again reproduced the observed behavior of the fermentation quite closely, as shown in Fig. 3b.

The model assumed that xylose was consumed exclusively for generation of biomass. This explains the slightly high predicted values of xylose concentration in comparison with the measurements near the end of the fermentation. Clearly, a small fraction of xylose is being consumed for cell maintenance and this is not reflected in the model.

Conclusions

In xylose fermentations by the yeast *C. mogii* ATCC 18364, biomass growth rate and yield were enhanced by adding glucose as a cosubstrate. Initial glucose/xylose ratios of 10% enhanced volumetric productivity of xylitol compared to when xylose was the sole carbon source; however, higher glucose levels suppressed production of xylitol. A mathematical model developed to predict the state variables of the fermentation, was confirmed to be effective for fermentations with and without the cosubstrate. Xylose uptake by *C. mogii* was shown to be highly influenced by glucose, but the presence of xylose had a relatively minor effect on uptake of glucose. The model-predicted permeability coefficient of xylitol in cell membrane was consistent with data available for other polyols. Attaining a high xylitol yield in these fermentations required the use of low initial levels of glucose and careful timing of termination of the fermentation. The model developed here enables the prediction of xylitol productivity of the fermentation, for various fed-batch feeding regimens and concentration ratios of the cosubstrates.

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