Kinetics of Lactose Fermentation Using a Recombinant *Saccharomyces cerevisiae* Strain

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Abstract: This work presents a multi-route, non-structural kinetic model for interpretation of ethanol fermentation of lactose using a recombinant flocculent *Saccharomyces cerevisiae* strain expressing both the *LAC4* (coding for β-galactosidase) and *LAC12* (coding for lactose permease) genes of *Kluyveromyces lactis*. In this model, the values of different metabolic pathways are calculated applying a modified Monod equation rate in which the growth rate is proportional to the concentration of a key enzyme controlling the single metabolic pathway. In this study, three main metabolic routes for *S. cerevisiae* are considered: oxidation of lactose, reduction of lactose (producing ethanol), and oxidation of ethanol. The main bioprocess variables determined experimentally were lactose, ethanol, biomass, and dissolved oxygen concentrations. Parameters of the proposed kinetic model were established by fitting the experimental data obtained in a small lab-scale fermentor with the initial lactose concentrations ranging from 5 g/dm^3 to 50 g/dm^3. A very good agreement between experimental data and simulated profiles of the main variables (lactose, ethanol, biomass, and dissolved oxygen concentrations) was achieved.

Keywords: ethanol fermentation of lactose; recombinant *Saccharomyces cerevisiae*; kinetic model; kinetic parameters estimation

INTRODUCTION

Cheese whey is a collective term referring to the watery part of milk, which is formed as a by-product of the coagulation process during the production of cheese or cottage cheese. Utilization of cheese whey has been a perennial problem of the modern dairy industry for a long time because of its significant environmental problem. Cheese whey represents a high volume of biological oxygen demand (BOD) and chemical oxygen demand (COD) in waste water treatment plants.

Since cheese whey has a high concentration of protein ($\approx 1\%$) and lactose ($\approx 5\%$) (Domingues et al., 2001), it is appropriate to consider this material as a source of added value compounds and not just as a waste effluent. The main part of cheese whey to be utilized as a carbon source is lactose. There are several possibilities of cheese whey (lactose) utilization, production of potable alcohol being one of them.

Saccharomyces cerevisiae is one of the microorganisms of first choice in industrial biotechnology. Unfortunately, this strain is not able to metabolize lactose due to the lack of a lactose permease system, as well as an intracellular enzyme for lactose hydrolysis. If lactose is used as the carbon source, it has to be firstly hydrolyzed to β-D-galactose and α-D-glucose (this hydrolysis is catalyzed by the enzyme β-galactosidase).

One of the effective ways to increase productivity is the development of a genetically modified strain with the ability to express both a lactose permease and an intracellular β-galactosidase. Domingues et al. (1999a) developed a recombinant flocculating *S. cerevisiae* strain, expressing lactose permease and β-galactosidase from *Kluyveromyces lactis*. Continuous ethanol fermentation of lactose using this strain can be operated for a long time in an airlift reactor reaching high productivity (Domingues et al., 1999b; Domingues et al., 2001).

Many structured and unstructured models for microbial growth linked with alcohol fermentation of saccharides can be found in several reviews (Nielsen and Villadsen, 1992; Starzak et al., 1994). There are different approaches for kinetic modelling of bioprocesses. Some authors used a structured unsegregated model to simulate aerobic fermentation of glucose using *S. cerevisiae*, taking into account three metabolic pathways (Di Serio et al., 2001; Jones and Kompala, 1999). An unstructured unsegregated model was

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applied by Dantigny (1995); Sonnleitner and Käppeli (1986). A similar unstructured model was used to simulate growth of *K. marxianus* on cheese whey (Castrillo and Ugalde, 1993; Longhi et al., 2004). An unstructured model for anaerobic fermentation of cheese whey using *Candida pseudotropicalis* including substrate and product inhibition was presented by Ghaly and El-Taweel (1994).

This work presents a multi-route, non-structural kinetic model of fermentation of lactose to ethanol using a recombinant flocculent *S. cerevisiae* strain expressing both the *LAC4* (coding for β-galactosidase) and *LAC12* (coding for lactose permease) genes of *K. lactis*. The kinetic parameters of the developed model were established by fitting the experimental data obtained from four batch fermentations (with respect to the liquid phase) using different initial lactose concentrations in a lab-scale fermentor.

THE THEORY

The Kinetic Model

The presented kinetic model includes three substantial metabolic routes: oxidation of lactose, reduction of lactose (producing ethanol), and oxidation of ethanol. The base of the model was adapted from the work of Sonnleitner and Käppeli (1986), in which the growth of *S. cerevisae* was simulated only on glucose and ethanol. The model was developed on the hypothesis of limited respiratory capacity of the strain. It is based on the fact that carbon source degradation proceeds via two pathways under aerobic ethanol formation conditions (Fig. 1). One part of the substrate is metabolized oxidatively and the second part reductively, with ethanol as the product of reductive energy metabolism. Ethanol can be metabolized only oxidatively.

The model is based on the following assumptions (Sonnleitner and Käppeli, 1986) applying modifications for the use of lactose as the substrate:

- Growth of strain on lactose and ethanol is described by Monod-type kinetics.
- There are no by-products, the main products are CO_2, H_2O, and under appropriate conditions ethanol.
- The oxygen uptake rate is linearly correlated with the specific lactose uptake rate only under oxidative metabolism (i.e., subcritical lactose flux or ethanol growth). Under oxido-reductive conditions of the supracritical lactose flux, the oxygen uptake rate remains constant at its maximum value and is independent of the growth rate (Fig. 1).
- Lactose can be metabolized both aerobically and anaerobically, with different rates and different efficiencies.
- Ethanol can be metabolized only aerobically.
- Lactose inhibits the uptake of ethanol as a substrate for growth of the strain, if lactose is present in measurable concentration.
- The elemental composition of biomass grown on lactose does not change significantly whether ethanol is produced or not. The same composition of biomass is assumed for biomass grown on ethanol.
- Nitrogen is assumed not to change its oxidation state.

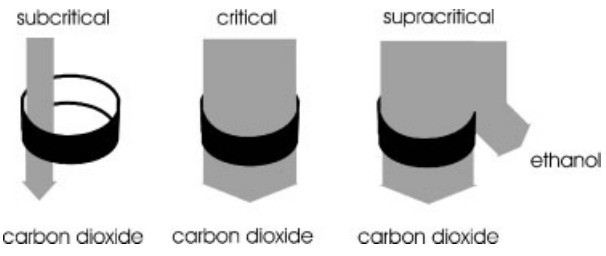

Figure 1. Illustration of the limited respiratory capacity of *Saccharomyces cerevisiae*; the bottleneck of the oxidative metabolism (Sonnleitner and Käppeli, 1986).

The model uses two substrates (lactose and oxygen) and two metabolic products (ethanol and carbon dioxide). The stoichiometric formula of biomass was taken from literature (Sonnleitner and Käppeli, 1986). Three reactions are considered:
Oxidative lactose metabolism:

$$C_{12}H_{22}O_{11} + aO_2 + b[NH_3] \rightarrow c\,CH_{1.79}O_{0.57}N_{0.15} + dCO_2 + eH_2O \quad (R1)$$

Reductive lactose metabolism:

$$C_{12}H_{22}O_{11} + f\,[NH_3] \rightarrow g\,CH_{1.79}O_{0.57}N_{0.15} + h\,CO_2 + i\,H_2O + jC_2H_6O \quad (R2)$$

Oxidative ethanol metabolism:

$$C_2H_6O + kO_2 + l[NH_3] \rightarrow mCH_{1.79}O_{0.57}N_{0.15} + nCO_2 + oH_2O \quad (R3)$$

where a, b, c, d, e, f, g, h, i, j, k, l, m, n, o are the unknown molar stoichiometric coefficients used to define the main mass stoichiometric parameters (yield coefficients on mass basis):

$$\gamma_1 = Y^{oxi}_{X/S} = c\frac{M_X}{M_S} \quad (1)$$

$$\gamma_2 = Y^{red}_{X/S} = g\frac{M_X}{M_S} \quad (2)$$

$$\gamma_3 = Y^{oxi}_{X/P} = m\frac{M_X}{M_P} \quad (3)$$

These mass stoichiometric parameters are reliable, measurable coefficients. From the stoichiometric Equations (R1–R3), three different sets of linear algebraic equations can be derived; each one consists of carbon, oxygen, and hydrogen

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balance. The solutions of the algebraic equations are the other stoichiometric parameters:

$$\alpha_{12} = a\frac{M_{O_2}}{M_S} \tag{4}$$

$$\alpha_{32} = k\frac{M_{O_2}}{M_S} \tag{5}$$

$$\beta_{22} = j\frac{M_P}{M_S} \tag{6}$$

$$\beta_{11} = d\frac{M_{CO_2}}{M_S} \tag{7}$$

$$\beta_{21} = h\frac{M_{CO_2}}{M_S} \tag{8}$$

$$\beta_{31} = n\frac{M_{CO_2}}{M_P} \tag{9}$$

In the model, the rate of lactose uptake is described by Monod-type kinetics with product inhibition:

$$r_S = k_S\frac{c_S}{c_S + K_S}\left(1 - \frac{c_P}{K_{Pi}}\right)c_X \tag{10}$$

Similarly, the maximal possible rate of oxidative metabolism is described by Monod-type kinetics with oxygen concentration (c_{OL}) as the limiting substrate with product inhibition:

$$r_{O,max} = k_O\frac{c_{OL}}{c_{OL} + K_O}\left(1 - \frac{c_P}{K_{Pi}}\right)c_X \tag{11}$$

The potential metabolism of ethanol is expressed by Monod-type kinetics with inhibition of lactose and ethanol:

$$r_{P,pot} = k_P\frac{c_P}{c_P + K_P}\frac{1}{1 + c_S/K_{Si}}\left(1 - \frac{c_P}{K_{Pi}}\right)c_X \tag{12}$$

Using these expressions for the uptake rates, the rates of reactions R1–R3 are defined in the following way (Sonnleitner and Käppeli, 1986):

The oxidative lactose metabolism (stoichiometric Equation R1) is determined either by the lactose uptake rate under subcritical conditions or by the maximal possible rate of oxidative metabolism proportional to the mass stoichiometric parameter of α_{12} under critical and supracritical conditions:

$$r_1 = \min\left(r_S, \frac{r_{O,max}}{\alpha_{12}}\right) \tag{13}$$

Thus, the oxidative lactose metabolism is determined by the lactose uptake rate unless this rate reaches the critical rate of oxidative metabolism. If the lactose uptake rate is higher than the rate of oxidative metabolism, the lactose surplus will be metabolized by reductive metabolism (stoichiometric Equation R2). Hence, the rate of this reaction is given by the following relationship:

$$r_2 = r_S - \frac{r_{O,max}}{\alpha_{12}}; \quad r_S > \frac{r_{O,max}}{\alpha_{12}} \tag{14}$$

This means that reaction R2 acts as a sort of overflow reaction for lactose excess, and it is not active if $r_S < \frac{r_{O,max}}{\alpha_{12}}$. If the lactose flux does not take up the whole oxidative capacity of cells, ethanol may be oxidized parallelly with lactose. The excess of the oxidative capacity is given by $r_{O,max} - \alpha_{12}r_S$ or by the rate of oxidative ethanol metabolism:

$$r_3 = \min\left(r_{P,pot}, \frac{r_{O,max} - \alpha_{12}r_S}{\alpha_{32}}\right) \tag{15}$$

Obviously, if there is no excess of the oxidative capacity, ethanol will not be metabolized, even if there exists the potential metabolism of ethanol ($r_{P,pot} > 0$).

MATHEMATICAL MODEL OF A BIOREACTOR

The mass balances in the liquid phase for a batch fermentor can be written on the basis of the mass stoichiometric parameters and reaction kinetics presented above. The fermentor operates in a batch mode with respect to the liquid phase (lactose, ethanol, and biomass), while in a continuous mode with respect to the gas phase (oxygen and carbon dioxide). Hence, the mass balances for the liquid phase are:

$$\frac{dc_S}{dt} = -(r_1 + r_2) \tag{16}$$

$$\frac{dc_X}{dt} = (\gamma_1 r_1 + \gamma_2 r_2 + \gamma_3 r_3) \tag{17}$$

$$\frac{dc_P}{dt} = (\beta_{22} r_2 - r_3) \tag{18}$$

$$\frac{dc_{OL}}{dt} = k_L a(c^*_{OL} - c_{OL}) - (\alpha_{12} r_1 + \alpha_{32} r_3) \tag{19}$$

The initial conditions are:

$$\begin{aligned} t = 0: \quad & c_S = c^0_S, \quad c_X = c^0_X, \quad c_P = c^0_P, \\ & c_{OL} = c^{*0}_{OL}, \quad c^*_{OL} = c^{*0}_{OL}, \end{aligned} \tag{20}$$

The presented mathematical model of the small lab-scale fermentor was developed under the assumption of a negligible effect of the composition of the gas phase (oxygen uptake and carbon dioxide produced). The partial pressure changes resulting from the produced carbon dioxide were assumed to be insignificant and the concentration of oxygen in the gas phase did not change, $c^0_{OG} = c^{in}_{OG} \approx c_{OG} = \text{const}$. Hence, the equilibrium oxygen concentration at the gas–liquid interface on the liquid side, c^*_{OL}, changed only due to changes of the liquid composition. However, the oxygen uptake rate and

production rate of carbon dioxide can be calculated by Equations (21) and (22). Hence, the mathematical model of any bioreactor could be enlarged to simulate the composition of the gas phase.

$$r_O = \alpha_{12} r_1 + \alpha_{32} r_3 \quad (21)$$

$$r_C = \beta_{11} r_1 + \beta_{21} r_2 + \beta_{31} r_3 \quad (22)$$

MATERIALS AND METHODS

Microorganism

A recombinant *S. cerevisiae* flocculent strain with the ability to express both the *LAC4* (coding for β-galactosidase) and *LAC12* (coding for lactose permease) genes of *K. lactis* was used. This yeast was named NCYC869-A3/T1 and its construction has been described elsewhere in detail (Domingues et al., 1999a).

Culture Media and Cultivation Conditions

The yeast was maintained at 4°C on plates of a SSLactose medium containing (per dm^3) 5 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, 0.4 g $MgSO_4 \cdot 7H_2O$, 1 g yeast extract, and 20 g lactose. Fermentations were performed in yeast-defined mineral medium (Verduyn et al., 1992) containing (per dm^3) 5 g $(NH_4)_2SO_4$, 3 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$. Trace elements were added using 1,000 times concentrated solution. Final trace elements concentrations per liter were: EDTA, 15 mg; $ZnSO_4 \cdot 7H_2O$, 4.5 mg; $CoCl_2 \cdot 6H_2O$, 0.3 mg; $MnCl_2 \cdot 2H_2O$, 0.84 mg; $CuSO_4 \cdot 5H_2O$, 0.3 mg; $CaCl_2 \cdot 2H_2O$, 4.5 mg; $FeSO_4 \cdot 7H_2O$, 3 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.4 mg; H_3BO_3, 1 mg; KI, 0.1 mg. Filter-sterilized vitamins were added after heat sterilization of the medium, using 1,000 times concentrated solution. The final vitamin concentrations per liter were: biotin, 0.05 mg; calcium pantothenate, 1 mg; nicotinic acid, 1 mg; inositol, 25 mg; thiamine HCl, 1 mg; pyridoxine HCl, 1 mg; para-aminobenzoic acid, 0.2 mg. Lactose was autoclaved separately and added after heat sterilization of the medium. Lactose concentrations (g/dm^3) were: 5, 10, 20, and 50. For lactose concentrations above 10 g/dm^3, the concentrations of trace elements and vitamins were doubled. Fermentations were made in a 600 cm^3 bubble column bioreactor with H/D (height/column diameter) ratio of 2.1, assumed as ideally mixed. Fermentors were filled with 400 cm^3 of defined mineral medium. The temperature and pH were maintained at 30°C and 4.0, respectively. Air at a flow rate of 1.0 vvm (adjusted and controlled by a mass flow controller) was supplied through a sintered porous plate (with a diameter of 3 cm) located at the bottom of the bioreactor. A magnetic stirrer was used to keep the entire reactor volume well-mixed. For start-up the fermentations, cells were grown in a 100 cm^3 Erlenmeyer flask filled with 40 cm^3 of culture medium. After cultivation in an incubator at 30°C with agitation (150 rpm) for 20–30 h, the cell suspension was aseptically pumped into the fermentor. Therefore, the initial volume of the medium in the fermentor was 440 cm^3.

Analytical Methods

Biomass growth was monitored by measuring the optical density of the cell suspension at 600 nm (OD600). Before measuring the OD600, the biomass was deflocculated by washing 2–5 times with a 15 g/dm^3 NaCl pH 3.0 solution. A calibration curve of optical density against cellular dry weight (DW) was previously drawn and used to determine the biomass concentration. Biomass DW was determined by filtering 10 cm^3 of yeast culture through a preweighed 0.45 μm filter and washing with 20 cm^3 of water. The filter was dried overnight at 104°C, cooled in a desiccator, and weighed. Lactose and ethanol were analyzed by HPLC, using a Chrompack Organic Acids column. The column was eluted at 60°C with 0.005 M H_2SO_4 at a flow rate of 0.6 cm^3/min. Detection was done by a Jasco 830-RI refractive-index detector.

RESULTS AND DISCUSSION

The presented kinetic model was used to describe the ethanol batch fermentation of lactose using the recombinant *S. cerevisiae* strain. In order to simulate the fermentation performance, the parameters of the model have to be known. The kinetic and stoichiometric parameters used in the model in this study can be divided into three groups:

- Parameters taken from the literature (k_P, γ_3, K_S, K_O, K_P, K_{Si}, K_{Pi}) presented in Table I;
- Parameters estimated by fitting the experimental data (k_S, k_O, γ_1, γ_2);
- Mass stoichiometric parameters determined from γ_1, γ_2, and γ_3 (α_{12}; α_{32}; β_{22}; β_{11}; β_{21}; β_{31}).

Some values of the parameters listed in Table I, k_P, γ_3, K_{Si}, were originally proposed for simulation of the oxidative metabolism of ethanol by *S. cerevisiae*, reaction R3. The K_{Pi} parameter is related to ethanol inhibition of the bioprocess. The value of the K_S parameter was originally used for simulation of ethanol fermentation on glucose using *S. cerevisiae*.

Table I. Parameters used in the model taken from the literature.

Parameter	Values	Source	
k_P	0.17	g/g·h	Sonnleitner and Käppeli (1986)
γ_3	0.72	g/g	Sonnleitner and Käppeli (1986)
K_S	0.1	g/dm^3	Sonnleitner and Käppeli (1986)
K_O	1×10^{-4}	g/dm^3	Longhi et al. (2004); Sonnleitner and Käppeli (1986)
K_P	0.1	g/dm^3	Sonnleitner and Käppeli (1986)
K_{Si}	0.1	g/dm^3	Sonnleitner and Käppeli (1986)
K_{Pi}	80	g/dm^3	Ghose and Tyagi (1979)

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The equilibrium dissolved oxygen concentration, c^*_{OL}, in Equation (19) was determined with respect to composition of the liquid phase, in terms of lactose and ethanol concentration (Rischbieter et al., 1996). Based on two independent process conditions the gas hold-up and the air flow rate the volumetric mass transfer coefficient was set to the value of 20 per h (Bello et al., 1985; El-Temtamy et al., 1984).

In this study, four kinetic parameters, k_S, k_O, γ_1, γ_2, were fitted using four sets of experimental data from four different experimental runs. The experimental data consist of off-line analyzed lactose, ethanol, and dry biomass concentrations and on-line values of oxygen saturation in percentage. Afterwards, the percentages of oxygen saturation were recalculated to dissolved oxygen concentration with respect to the composition of the liquid phase (Rischbieter et al., 1996). The experimental runs are marked according to the initial substrate concentration as: run A: $c_S = 5$ g/dm^3, run B: $c_S = 10$ g/dm^3, run C: $c_S = 20$ g/dm^3, run D: $c_S = 50$ g/dm^3.

The estimated parameters are presented in Table II, as well as in the form of plots in Figures 2–5. Only the parameters related to oxidative lactose metabolism and reductive lactose metabolism were estimated. Estimation of the parameters related to oxidative ethanol metabolism was omitted because none of the fermentation runs reached the state of fully metabolized ethanol, and only few experimental data were available from this part of fermentation. Moreover, from the practical point of view, oxidation of ethanol is usually an undesirable phenomenon decreasing ethanol yield. As earlier mentioned, when the mass stoichiometric parameters, γ_1,γ_2, andγ_3, are known, it is possible to calculate the other mass stoichiometric parameters, α_{12}, α_{32}, and β_{22} (Table III). The α_{32} mass stoichiometric parameter in Table III was calculated using the non-fitted γ_3 parameter.

Figure 2A shows the concentration profiles of lactose, ethanol, and biomass for fermentation with the initial substrate concentration of 5 g/dm^3. Figure 3 shows percentages of dissolved oxygen saturation against time. From the experimental values of dissolved oxygen, the diauxic lag phase between the two growth phases can be noted. The percentages of dissolved oxygen started to rise as a consequence of lower oxygen demand. The diauxic lag phase lasted approximately about 1 h. The kinetic model used in this study does not include description of the diauxic lag phase. Hence, sharp changes appearing on the time curves of the predicted lactose, ethanol, and biomass concentrations are presented in all plots in this study. In the original study of Sonnleitner and Käppeli (1986) the simulated lines were presented discontinuously with a break in the time axis

Table II. Parameters established by fitting the experimental data.

Run	A	B	C	D	Averaged values
k_S (g/g·h)	0.834	0.880	0.781	0.778	0.818
k_O (g/g·h)	0.125	0.113	0.099	0.089	0.106
γ_1 (g/g)	0.545	0.562	0.582	0.573	0.566
γ_2 (g/g)	0.051	0.0525	0.060	0.063	0.057

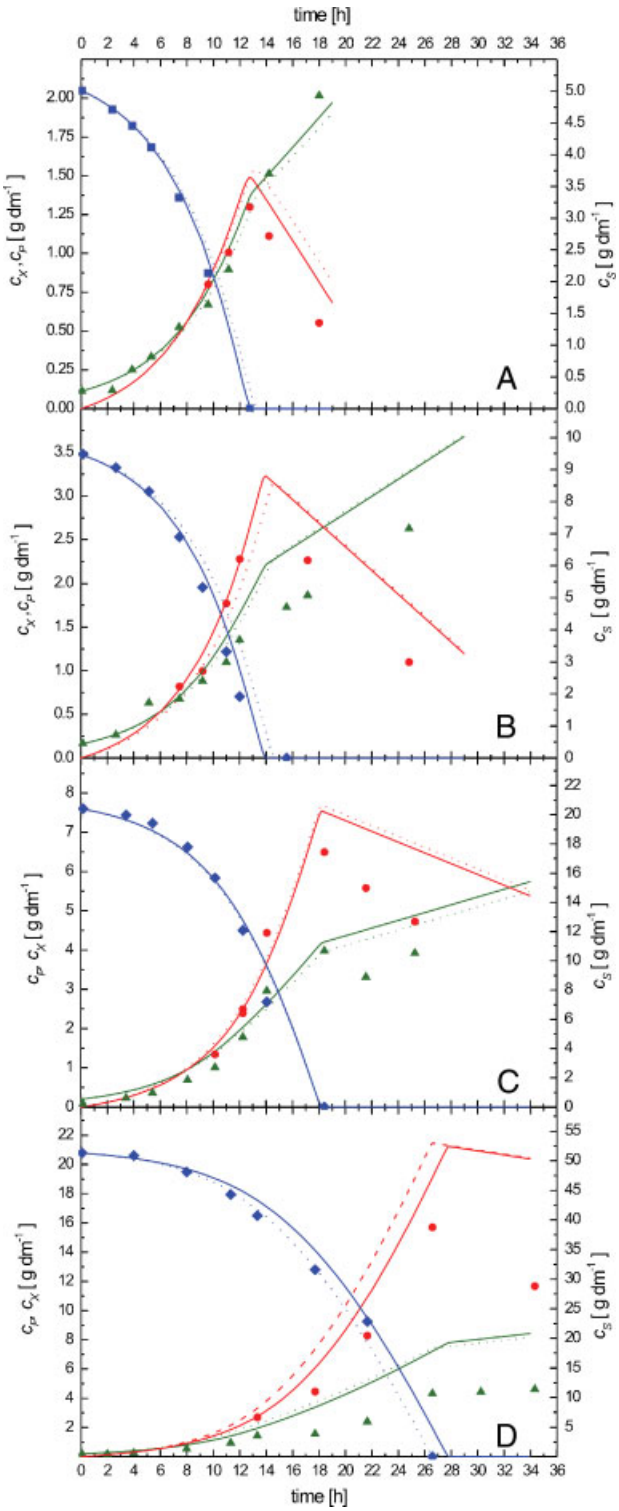

Figure 2. Experimental (symbols) and simulated (line) time profiles of lactose (■), biomass (▲), and ethanol (●) concentrations. Solid lines represent simulation using the fitted parameters established for experimental data of each run separately. Dashed lines represent the simulation using the averaged kinetic parameters of all run fittings (Tables II and III). **A**: run A, (**B**) run B, (**C**) run C, (**D**) run D. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

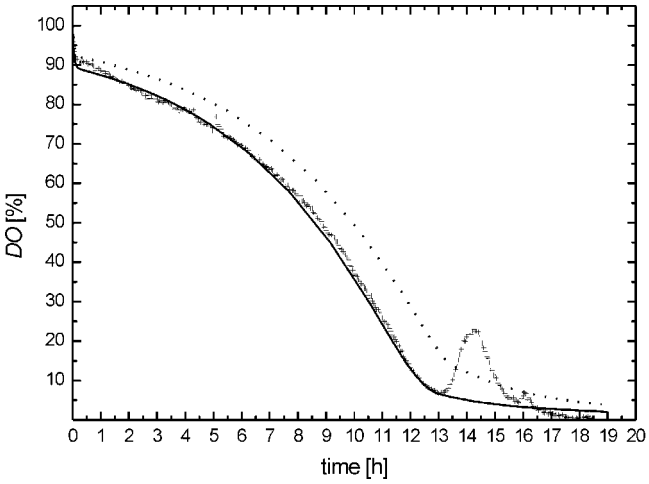

Figure 3. Experimental (symbol +) and simulated (line) time profiles of dissolved oxygen percentages. Solid line represents simulation using the fitted parameters established for experimental data of run A. The dashed line represents the simulation using the averaged kinetic parameters of all run fittings (Tables II and III).

approximately corresponding to the diauxic lag phase. In this work, the simulation lines are presented continuously just to demonstrate possible trends of kinetic parameters after switching lactose to ethanol metabolisms, when lactose was totally exhausted. Unfortunately, all fermentation runs have been stopped before the ethanol concentration reached zero. Therefore, kinetic parameters related to the ethanol oxidative metabolism, k_P and γ_3, were not fitted.

Figure 2B–D present a comparison of the experimental and calculated time profiles of all the remaining fermentations, runs B, C, and D. As can be seen, the model describes well the experimental values of lactose and ethanol concentrations for all runs. Description of biomass concentration was also satisfactory, except for the fermentation run D (Fig. 2D). The model line using the fitted parameters (Table II) overpredicts the experimental biomass concentration. However, during fermentation of run D, a significant biomass film on the fermentor wall was observed. Hence, the

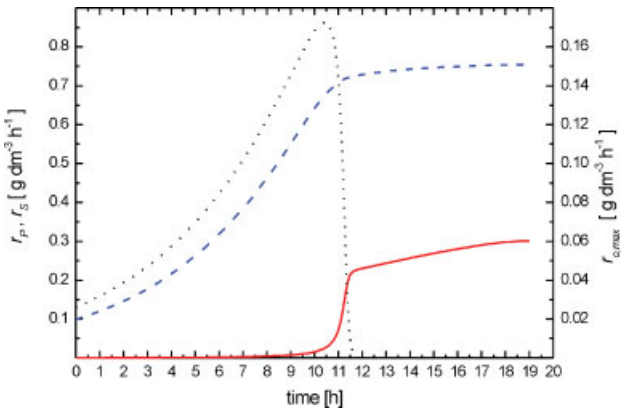

Figure 4. Time profiles of the lactose uptake rate (doted line), the maximum possible oxygen uptake rate (dashed line), and the potential rate of ethanol uptake (full line) calculated for run A. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

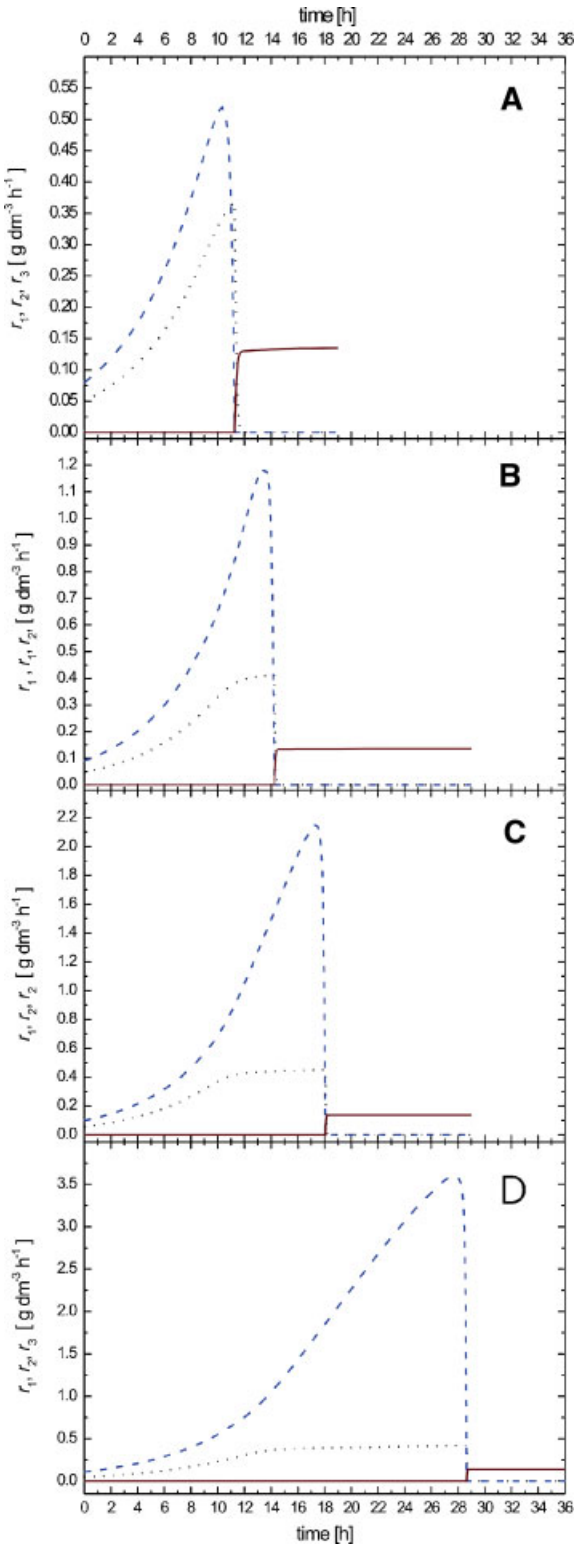

Figure 5. Time profiles of calculated reaction rates R1, R2, R3 for each run: the rate of oxidative lactose metabolism r_1 (doted line), the rate of reductive lactose metabolism r_2 (dashed line), the rate of oxidative ethanol metabolism r_3 (full line). **A**: run A, (**B**) run B, (**C**) run C, (**D**) run D.

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Table III. Mass stoichiometric parameters determined from γ_1, γ_2, and γ_3.

Run	A	B	C	D	Calculated from averaged values
α_{12}	0.39	0.37	0.34	0.35	0.36
α_{32}	1.12	1.12	1.12	1.12	1.12
β_{22}	0.505	0.504	0.499	0.497	0.501

measured biomass concentration for this fermentation might be lower than the overall biomass grown inside the fermentor.

The experimental and calculated values of dissolved oxygen concentrations for the remaining runs (B, C, and D) are not presented in this study. Nevertheless, the predicted values of dissolved oxygen described the experimental values comparably well, as in the case of the lowest initial lactose concentration (run A, Fig. 3).

The time profiles of the lactose uptake rate, Equation (10), the maximum possible oxygen uptake rate, Equation (11), and the potential rate of ethanol uptake, Equation (12), for run A are presented in Figure 4. The lactose uptake rate, r_S, is immediately rising from the beginning and reached the maximum values just before all lactose is exhausted. Then it quickly drops to zero values.

The maximal potential oxygen uptake rate, $r_{O,max}$, is connected with the biomass concentration. However, it significantly follows the increase of biomass concentration only until the concentration of dissolved oxygen dropped to the values close to the value of K_O. Then, the term of $(c_{OL}/(c_{OL}+K_O))$ in Equation (11) is the limitation term and any further increase of biomass concentration does not cause any significant increase of the maximum possible oxygen uptake rate. It is worth noting that the lactose uptake rate is from the beginning higher than the maximal potential oxygen uptake rate.

Finally, the potential rate of ethanol uptake rate, $r_{P,pot}$, is kept almost at zero values during the lactose metabolism due to lactose inhibition expressed by the term of $1/(1+c_S/K_i)$ in Equation (12). When the lactose is exhausted, it starts immediately rising. For the other runs (B, C, and D), these profiles exhibit the same trends. However, they are not presented in this study.

From the time profiles of reaction rates R1–R3 the following observation can be done (Fig. 5): In all runs, reactions R1 and R2 are active from the beginning of fermentation. That means that the oxidative capacity of the strain is fully utilized from the start. Then, the rates of reaction R1 are according to expression (13), described by the term of $(r_{O,max}/\alpha_{12})$. In all fermentation runs, the dissolved oxygen concentrations dropped to values closed to zero. As mentioned above, the maximal possible oxygen metabolism, $r_{O,max}$, is then strongly dependent on the dissolved oxygen concentration. Hence, the limiting value of the oxidative lactose rate was about 0.35 g/dm^3/h for all runs.

The rate of reaction R2 reached its maximal value just before all lactose was exhausted. The maximal values of reductive lactose rate increased from about 0.52 g/dm^3/h for run A up to about 3.6 g/dm^3/h for run D.

The oxidative ethanol metabolism was not active at the beginning of the fermentations and was activated just as soon as lactose was exhausted.

CONCLUSIONS

A kinetic model was developed to describe the metabolic behavior of the recombinant flocculating *S. cerevisae* strain grown on lactose. The model basis was derived from the concept of a limited respiratory capacity originally proposed by Sonnleitner and Käppeli (1986) for growth of baker's yeast on glucose. A proper description of three main metabolic fluxes oxidation of lactose, reduction of lactose (producing ethanol), and oxidation of ethanol allows to simulate satisfactorily the kinetic behavior of the recombinant yeast in batch cultures with initial lactose concentrations ranging from 5 g/dm^3 to 50 g/dm^3.

Parameters for the simulation of fermentations come from three different types of sources; parameters from the literature, fitted parameters using the experimental data from four experimental runs with different initial lactose concentrations (from 5 g/dm^3 to 50 g/dm^3), and calculated stoichiometric parameters. Parameters related to ethanol oxidative metabolism were not fitted in this work due to the fact that none of the fermentations last until all the ethanol was exhausted. The presented model with the established parameters describes the experimental data in a satisfactory way.

When the averaged parameters of fitting are used for simulations of fermentation runs, the model described the experimental data well in the range of the initial lactose concentrations used.

In the theoretical part of this study a possibility of implementing carbon dioxide balance is presented. In a large-scale fermentor, carbon dioxide balance has to be included because of an increased amount of produced carbon dioxide would have a significant effect not only on oxygen transfer due to lowered partial pressure of oxygen in the gas phase but also on the hydrodynamics of the bioprocess (gas flow, gas hold-up).

NOMENCLATURE

c	concentration (g/dm^3)
DO	saturation percentage of dissolved oxygen (%)
k_La	volumetric mass transfer coefficient (per h)
k_O	maximal specific oxygen uptake rate (g/g·h)
K_O	saturation parameter for oxygen uptake (g/dm^3)
k_P	maximal specific growth rate on ethanol (g/g·h)
K_P	saturation parameter for growth on ethanol uptake (g/dm^3)
K_{Pi}	ethanol inhibition paramater (g/dm^3)
k_S	maximal specific lactose uptake rate (g/g·h)
K_S	saturation parameter for lactose uptake (g/dm^3)
K_{Si}	inhibition parameter: free lactose inhibits ethanol uptake (g/dm^3)
M	molar weight (g/mol)
r_1, r_2, r_3	rate of reactions R1, R2, and R3 (g/dm^3/h)
$r_{O,max}$	the maximal possible rate of oxidative metabolism (g/g·h)

$r_{P,pot}$	potential rate of ethanol uptake (g/g·h)
r_O	oxygen uptake rate (g/g·h)
r_C	carbon dioxide production (g/g·h)
r_S	rate of lactose uptake (g/g·h)
t	time (h)
Y	yield coefficient on mass basis (g/g)
$a, b, c, d, e, f, g, h, i, j, k, l, m, n, o$	molar stoichiometric coefficients

Greek Letter

$\alpha\ \beta\ \gamma$	mass stoichiometric parameters (g/g)

Subscripts

L	liquid phase
O	oxygen
P	product (ethanol)
S	substrate (lactose)
X	biomass

Superscripts

in	in coming
*	equilibrium
0	initial condition

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