

# A Model Based Simulation of Brewing Yeast Propagation

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## ABSTRACT

J. Inst. Brew. 108(2), 248–255, 2002

A comprehensive kinetic model of yeast propagation in breweries is introduced. It represents the basis for a control strategy aimed at the provision of optimal inoculum at the starting time of subsequent industrial beer fermentations. In the metabolic modelling approach presented, respiratory metabolism on sugars and ethanol and fermentative metabolism respectively, are included. Occurring limitations, due to specific nutritional data of the growth medium, were taken into account for sugar, nitrogen, ethanol and oxygen. Correspondingly, inhibitions of the metabolism by ethanol and high sugar concentrations were formulated. The model especially represents the Crabtree-effect. For model validation, literature data were used and selected experiments within the relevant range of manipulated variables (temperature, dissolved oxygen) were conducted. Model based simulations matched these data with a deviation of 5.6% and with standard deviations of 5.6% after an adaptation of three variable parameters. A relationship between the temperature and the variable parameters could be extracted, which allowed predictive simulations of the yeast propagation process using non-isothermal trajectories. It was shown, that with a precise adjustment of trajectories of both, temperature and dissolved oxygen, the crop time of the inoculum could be varied within a period of 7 to 50 h to maintain high fermentation activity for the subsequent anaerobic fermentation.

**Key words:** Growth, kinetic modelling, model-based simulation, *Saccharomyces*.

## INTRODUCTION

The state of the propagated brewing yeast before inoculation exerts a significant influence on the performance of the subsequent fermentation concerning fermentation time and quality of the resulting beer. The inoculum should be taken out of the exponential (log-) state of the propagation with a high proportion of yeast cells in the G2-proliferation state<sup>18</sup>. In practice, it is of essential importance to provide a defined amount of brewery yeast in the desired state at a predefined time. Variations of up to 2-3 days must be possible to compensate for changes in the brewery production plan. For this purpose, active process control is desirable to manipulate the propagation process in order to reach the inoculating time with the best organism

quality. A prerequisite for the corresponding process control strategy is a comprehensive knowledge about the process behavior in the form of a representative process model.

*Saccharomyces cerevisiae* is one of the best-examined microorganisms in biotechnology. To describe the growth and the ethanol formation in the baker's yeast propagation process, many structured and unstructured modelling approaches are known from the literature. Unstructured modelling approaches, based on the Monod equation and its descendants with the assumption of balanced growth and product formation<sup>27</sup>, are often reproached with the fact that they inadequately describe the response of a microorganism population to environmental disturbances. According to Sweere et al.<sup>28</sup> available structured models can be distinguished in different types from approaches based on a structured biochemical pathway<sup>8,26,29</sup> to more segregated modelling approaches regarding different cell ages<sup>2,11</sup>. These approaches consider balanced growth, or are specified to describe particular biochemical pathways inside structured models and therefore represent the basis for the modelling approach introduced in this work.

In most cases the validity of these approaches, however, is limited to defined states of the metabolism (oxidative, oxidoreductive, fermentative). Temperature dependency and maintenance energy are not considered. Also, a lag-time function to describe the adaptation of the microorganisms to the surrounding growth medium, including the time for formation of enzymes, is merely included in single approaches using different equations<sup>12,23,33</sup>. The maintenance energy of the microorganisms is only considered in single approaches, e.g. Pham et al.<sup>23</sup>. However, this approach is only valid if oxygen is available. Most of the reports in the literature used full media as a growth medium to avoid undesired limitation effects during the growth process. This is not possible in this application, because industrial beer wort is used as the growth medium. Combined limitation and inhibition effects, e.g. caused by high ethanol concentrations, are usually not considered. Therefore, the applicability of the mentioned approaches for industrial purposes, in particular considering the usage of nutrient deficient, limiting or inhibiting media, is not guaranteed without qualifications. So, with a comprehensive modelling approach considering the aforementioned inhibition and limitation effects, knowledge can be gained toward a process control tool for industrial applications.

In particular, for the bottom fermenting brewing yeast strain *Saccharomyces uvarum* var. *carlsbergensis* (W34/70) required here, no comprehensive modelling approach

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is available, which takes into account the very limiting composition of the specific growth medium, i.e., brewer's wort. This medium is characterised by high concentrations of sugar (100 g/L), which leads to regulation effects such as the crabtree-effect expressed by reductive, oxidoreductive or oxidative growth of *Saccharomyces* sp<sup>27</sup>. A lack of nitrogen and trace elements, e.g. zinc, causes limitation effects<sup>5,22</sup>. Besides, ethanol production during the growth process causes additional inhibition effects<sup>1</sup>.

In this work the influence of the manipulated variables dissolved oxygen concentration and temperature on the brewing yeast propagation process is examined, in order to adjust the optimal harvesting time. The first objective of this work was to develop a metabolic modelling approach for the brewing yeast propagation process considering limiting and inhibiting effects of industrial media and to validate it with literature and experimental data. The second target was to show the potential of a model based predictive simulation to evaluate process control strategies and to extract the limits of controllability using practice relevant case scenarios.

## MATERIALS AND METHODS

### Technical set-up

A 150 litre (net volume) propagator with an integrated continuous aerator (Frings TRG) and defoamer unit was used. The propagator was equipped with online measurement devices for temperature (PT 100) and dissolved oxygen (Mettler-Toledo, InPro6100). Underlying control loops guaranteed accurate control of the manipulated variables, temperature (+/- 0.1°C) and dissolved oxygen (+/- 0.1 ppm).

### Analytical methods

*Saccharomyces uvarum* var. *carlsbergensis* yeast strain W34/70 was used for the experiments. For the propagator experiments, yeast suspension was taken from the aforementioned propagator system. The yeast dry weight was measured gravimetrically. Separated yeast slurry (4 g) was dried for 3 h at 105°C. The dry weight was calculated using the differential weight and the cell count of a defined amount of the yeast slurry. A mean value for the cell dry weight of 4E-11 g/cell confirmed literature data<sup>21,22</sup>. This value remained constant during the propagation process. For the required experiments industrial wheat beer wort (OG 12.5 w/w%) was used as growth medium. Zinc (0.2

ppm) was added to avoid limitation effects. The initial concentration of free amino acids was nearly constant over all trials at about 220 ppm. Yeast fermentable extract was determined using the SCABA measurement system.

Cell counts were made with a haemocytometer. Three areas were counted for each sample to avoid outliers. For the simulation software, the biomass concentration was calculated using the dry weight of the yeast, the yeast count, and the molecular weight of the biomass was set to 25.01 g/mol, according to the literature<sup>27</sup>.

### Computational methods

Modelling and simulations were carried out using the software package AQUASIM 2.0<sup>25</sup>. AQUASIM allows the identification and simulation of aqueous systems. For each simulation the starting conditions including concentrations of biomass, gravity, nitrogen, ethanol and dissolved oxygen in the growth medium as well as the progression of the manipulated variables were required. During the parameter estimation procedure, in a first step  $f_{temp}$  (temperature coefficient for the substrate uptake rate) and  $t_{lag}$  (lag time coefficient) were fitted to the reference data of substrate concentrations. In a second step, the maximum specific oxygen uptake rate  $q_{O_2,max}$  was fitted to all reference data (substrate-, ethanol- and biomass concentrations).

## RESULTS

### Mathematical modelling

In this work a new metabolic modelling approach is introduced, that describes the states of metabolism dependent on the surrounding medium, following the approaches of Heijnen<sup>16</sup>, van Gulik et al.<sup>29</sup> and Krzystek et al<sup>20</sup>. Considered substances and rates for this modelling approach are shown in Table I. For each substance a rate of metabolic turnover was defined. Here, biomass composition was adopted from Sonnleitner and Käppeli<sup>27</sup>.

As the metabolic pathways were known, stoichiometric relationships were formulated with eight reactions as shown in Table II. The oxidative degradation of glucose is following the glycolysis, an oxidative decarboxylation step and the Tricarboxylic Acid Cycle. Glucose is fully

TABLE I. Considered substances and rates.

Substance	Formula	Rate
Glucose	$C_6H_{12}O_6$	$r_s$
Biomass	$CH_{1.79}O_{0.57}N_{0.15}$	$r_x$
Nitrogen source	$NH_3$	$r_n$
Dissolved oxygen	$O_2$	$r_o$
Carbon dioxide	$CO_2$	$r_c$
Water	$H_2O$	$r_w$
Ethanol	$C_2H_5OH$	$r_e$
Glycerol	$C_3H_8O_3$	$r_{gly}$
NADH/H <sup>+</sup>	$NADH_2$	$r_{NADH_2}$
ATP	$ATP$	$r_{ATP}$

TABLE II. Description of the relevant metabolic pathways for the yeast propagation process.

- (1) Oxidative degradation out of glucose  $r_1$   
 $C_6H_{12}O_6 + 6 H_2O \rightarrow 6 CO_2 + 12 NADH_2 + 2 ATP$
- (2) Respiratory chain  $r_2$   
 $NADH_2 + \frac{1}{2} O_2 \rightarrow \delta ATP + H_2O$
- (3) Formation of biomass out of glucose  $r_3$   
 $G C_6H_{12}O_6 + 0.15 NH_3 + K ATP \rightarrow CH_{1.79}O_{0.57}N_{0.15} + c CO_2 + n NADH_2 + w H_2O$
- (4) Formation of ethanol out of glucose  $r_4$   
 $C_6H_{12}O_6 \rightarrow 2 C_2H_5OH + 2 CO_2 + 2 ATP$
- (5) Formation of glycerol out of glucose  $r_5$   
 $C_6H_{12}O_6 + 2 NADH_2 + 2 ATP \rightarrow 2 C_3H_8O_3$
- (6) Maintenance  $r_6$   
 $ATP \rightarrow ADP$
- (7) Oxidative degradation of ethanol  $r_7$   
 $C_2H_5OH + 3 H_2O \rightarrow 2 CO_2 + 6 NADH_2$
- (8) Biomass formation out of ethanol  $r_8$   
 $E C_2H_5OH + 0.15 NH_3 + K_e ATP + w_e H_2O \rightarrow CH_{1.79}O_{0.57}N_{0.15} + c_e CO_2 + n_e NADH_2$

oxidised to carbon dioxide and 12 NADH/H<sup>+</sup> are formed (r<sub>1</sub>). In the Respiratory Chain (r<sub>2</sub>), δ ATP are formed out of NADH/H<sup>+</sup> by oxidative phosphorylation. This relation ATP/NADH/H<sup>+</sup> is named P/O-Relation<sup>20,29</sup>. From the biomass synthesis (r<sub>3</sub>) from glucose c = 0.095-0.22 mol CO<sub>2</sub>/C-mol biomass result<sup>20,29</sup>. The other stoichiometric coefficients were computed from the balance of the single elements. The coefficient K characterises the amount of ATP for biomass synthesis. In (r<sub>4</sub>) the NAD<sup>+</sup> is regenerated by the hydrogenisation of pyruvate. Under fully anaerobic conditions an additional regeneration of NAD<sup>+</sup> is necessary (r<sub>5</sub>), because NADH/H<sup>+</sup> is formed during biomass synthesis. The reduction-equivalent is transferred to glycerol-phosphate and finally to glycerol<sup>20</sup>. ATP is needed for the maintenance of the yeast cells (r<sub>6</sub>). For non-growth associated maintenance quantities of m<sub>ATP</sub> = 0.007-0.018 mol ATP/C-mol biomass and hour are given in the literature<sup>23,29</sup>. Under aerobic conditions, ethanol can be degraded (r<sub>7</sub>), if the glucose concentration in the medium is very low<sup>27</sup>. In this case, ethanol is transformed to acetaldehyde, acetate and acetyl-CoA. The latter is used in the Tricarboxylic Acid Cycle. The biomass formation from ethanol (r<sub>8</sub>) works analogous to (r<sub>3</sub>). Here c<sub>e</sub> = 0.266 mol CO<sub>2</sub>/C-mol biomass are formed and K<sub>e</sub> = 5.1 ATP/mol biomass are demanded.

The rate of metabolic turnover of one substance can be formulated as the sum of the turnover in the single reactions. So, it is possible to define linear equations for the relation between the turnover of all substances and the rates of all reactions.

$$r = A \cdot v \quad (1)$$

with r representing the vector for the turnover of the single substances (see Table I). A the matrix of stoichiometry and v the vector for reaction rates (see Table II).

It was assumed, that NADH/H<sup>+</sup> and ATP are neither accumulated in the cell nor excreted (r<sub>NADH2</sub> = 0 and r<sub>ATP</sub> = 0). Therefore for this modelling approach a linear equation system with 10 equations and 16 unknown rates (r<sub>s</sub>, r<sub>x</sub>, r<sub>n</sub>, r<sub>o</sub>, r<sub>c</sub>, r<sub>w</sub>, r<sub>e</sub>, r<sub>gly</sub>, r<sub>1</sub>, r<sub>2</sub>, r<sub>3</sub>, r<sub>4</sub>, r<sub>5</sub>, r<sub>6</sub>, r<sub>7</sub>, r<sub>8</sub>) was formulated:

$$r = \begin{bmatrix} r_s \\ r_x \\ r_n \\ r_o \\ r_c \\ r_w \\ r_e \\ r_{gly} \\ r_{NAHDH2} \\ r_{ATP} \end{bmatrix} A = \begin{bmatrix} -1 & 0 & -g & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & -0.15 & 0 & 0 & 0 & 0 & -0.15 \\ 0 & -0.5 & 0 & 0 & 0 & 0 & 0 & 0 \\ 6 & 0 & c & 2 & 0 & 0 & 2 & c_e \\ -6 & 1 & w & 0 & 0 & 0 & -3 & -w_c \\ 0 & 0 & 0 & 2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 2 & 0 & 0 & 0 \\ 12 & -1 & n & 0 & -2 & 0 & 6 & n_c \\ 2 & \delta & -K & 2 & -2 & -1 & 0 & -K_c \end{bmatrix} v = \begin{bmatrix} r_1 \\ r_2 \\ r_3 \\ r_4 \\ r_5 \\ r_6 \\ r_7 \\ r_8 \end{bmatrix} \quad (2)$$

The coefficients δ, K and K<sub>e</sub> were calculated using the known yield coefficients for purely aerobic growth on glucose, purely anaerobic growth on glucose and aerobic growth on ethanol (δ = 1.5, K = 2.2 and K<sub>e</sub> = 5.1). Coefficients in the same order of magnitude are presented in literature<sup>16,20,29</sup>. The yield coefficients were assumed to be constant. Table III shows the final applied parameters taken from the literature. So, the stoichiometry of this modeling approach is fixed.

In order to solve the equation system (equation 2) six rates have to be determined first. Kinetic equations for the characterisation of the different metabolic states have to be taken into account. Known are rates for oxidative growth OG (r<sub>c</sub>=r<sub>gly</sub>=r<sub>7</sub>=r<sub>8</sub>=0), oxidoreductive growth ORG (r<sub>gly</sub>=r<sub>7</sub>=r<sub>8</sub>=0) and fermentative growth FG (r<sub>o</sub>=r<sub>1</sub>=r<sub>7</sub>=r<sub>8</sub>=0) on sugar and for oxidative growth on ethanol OGE (r<sub>1</sub>=r<sub>3</sub>=r<sub>4</sub>=r<sub>5</sub>=0), respectively. Merely four different rates remain unknown including r<sub>s</sub>, r<sub>6</sub>, r<sub>o</sub> (for oxidative growth on glucose) and r<sub>c</sub> (for oxidative growth on ethanol). The missing rates for OG (r<sub>s</sub>, r<sub>6</sub>), ORG (r<sub>s</sub>, r<sub>o</sub>, r<sub>6</sub>), FG (r<sub>s</sub>, r<sub>6</sub>) and OGE (r<sub>s</sub>, r<sub>6</sub>) must be calculated for the different metabolic states using kinetic equations describing rates for substrate turnover and maintenance.

Kinetics for substrate uptake and oxygen uptake are expressed by Monod-Terms. The specific substrate uptake rate q<sub>s</sub> is limited by the available substrate concentration (S), formulated as glucose equivalents. Additionally, a dependency on ethanol concentration (E), nitrogen concentration (N) and temperature (T) exists. Ethanol is toxic for yeast cells and causes an inhibition of growth in higher

TABLE III. Stoichiometric and kinetic parameters used in the modelling approach for the brewery yeast propagation process.

Parameter	Value	Unit	Reference
<b>Stoichiometric parameters</b>			
Y <sub>x/sox</sub>	3.527	mol/mol	Sonnleitner and Käppli <sup>27</sup>
Y <sub>x/sf</sub>	0.72	mol/mol	Heijnen <sup>16</sup>
Y <sub>x/e</sub>	1.12	mol/mol	Heijnen <sup>16</sup>
HX	1.79	mol/mol	Sonnleitner and Käppli <sup>27</sup>
NX	0.15	mol/mol	Sonnleitner and Käppli <sup>27</sup>
OX	0.57	mol/mol	Sonnleitner and Käppli <sup>27</sup>
C	0.095	mol/mol	Krzystek and Ledakowicz <sup>20</sup> , Heijnen <sup>16</sup>
C <sub>e</sub>	0.266	mol/mol	Heijnen <sup>16</sup>
<b>Kinetic parameters</b>			
M <sub>ATP</sub>	0.013	mol/mol/h	Heijnen <sup>16</sup>
q <sub>s,max</sub>	0.4863	mol/mol/h	Sonnleitner and Käppli <sup>27</sup>
K <sub>S</sub>	2.8	mmol/L	Sonnleitner and Käppli <sup>27</sup>
K <sub>O</sub>	0.00121	mmol/L	Hartmeier <sup>15</sup>
K <sub>i,eth</sub>	500	mmol/L	Hutter and Oliver <sup>17</sup>
K <sub>i,eth,o</sub>	1000	mmol/L	Sonnleitner and Käppli <sup>27</sup>
K <sub>eth</sub>	2.2	mmol/L	Sonnleitner and Käppli <sup>27</sup>
K <sub>N</sub>	2	mmol/L	Cartwright et al. <sup>6</sup>

concentrations. The reduction of the growth rate can be traced back to a reduction of the substrate and oxygen uptake. The inhibition is formulated in this model by a term for non-competitive inhibition<sup>1</sup> with a half saturation constant  $K_{i,eth} = 500$  mmol/L<sup>17</sup>. Without a usable nitrogen source (FAN), no protein synthesis and biomass synthesis can occur. Additionally, due to an inactivation of glucose transport systems less glucose can be taken in by the yeast cells<sup>5</sup>. The limitation effect is formulated by an additional Monod term in the expression for the substrate uptake rate, with a half saturation constant  $K_n = 2$  mmol/L<sup>6</sup>. To consider the temperature-dependency of the substrate uptake rate a coefficient  $f_{temp}$  is introduced, which is multiplied by the specific substrate uptake rate.

Monod kinetics are only suitable to describe the exponential and the stationary state of the propagation. After inoculation, the yeast cells need a certain time (lag-time  $t_{lag}$ ) to get adapted to the surrounding medium. The duration of this adaptation is dependent on substrate and yeast activity. For a description a lag-time coefficient  $L_t$  is introduced as a function of time  $t$ , and multiplied by the specific substrate uptake rate  $q_s$ . In this approach a sigmoid function is used for  $L_t$ .

$$L_t = \frac{1}{1 + e^{-(t-t_{lag})}} \quad (3)$$

Summarising, the specific substrate uptake rate can be formulated as (valid for growth on glucose during OG, ORG, FG)

$$q_s = q_{s,max} \cdot \frac{S}{S + K_s} \cdot \frac{K_{i,eth}}{K_{i,eth} + E} \cdot \frac{N}{N + K_n} \cdot f_{temp} \cdot L_t \quad (4)$$

The specific oxygen uptake rate,  $q_{O_2}$ , is limited by the oxygen concentration and inhibited by the ethanol concentration in the growth medium with  $K_{i,eth} = 1000$  mmol/L<sup>27</sup> and  $K_o = 0.00121$  mmol/L<sup>15</sup>. The value of  $q_{O_2}$  is limited by a maximum specific oxygen uptake rate  $q_{O_2,max}$ , which is a measure for the oxidative capacity of the yeast cell.

$$q_{O_2} \leq q_{O_2,max} \cdot \frac{O}{O + K_o} \cdot \frac{K_{i,eth,o}}{K_{i,eth,o} + E} = q_{O_2,lim} \quad (5)$$

At a critical specific substrate uptake rate  $q_s$ , the oxidative capacity of the cell is reached. With an increase of  $q_s$  the metabolism changes from pure oxidative to oxidoreductive metabolism, that means that the glucose is no longer degraded using only the oxidative pathway, but a portion is degraded fermentatively as well. At the critical substrate uptake rate,  $q_{O_2}$  reaches its limit  $q_{O_2,lim}$ . This phenomenon is known as the 'Crabtree effect'.

For yeast growth on ethanol (OGE) the specific oxygen uptake rate is different from equation 5. As no metabolism regulation effects such as the 'Crabtree effect' have to be considered, a sufficient description of the metabolism can be achieved with one specific uptake rate. Therefore, additionally to equation 5, the specific oxygen uptake rate is limited by available ethanol and nitrogen sources. In addition, as glucose is the preferred substrate, growth on etha-

nol is only possible if the glucose concentration is very low. According to Sonnleitner and Käppeli<sup>27</sup>, this aspect was expressed by an extra inhibition term ( $K_{is} = K_s$ ). During growth on ethanol equation 6 describes the kinetics for the specific oxygen uptake rate  $q_{O_2,e,lim}$ .

$$q_{O_2,e,lim} = q_{O_2,e,max} \cdot \frac{O}{O + K_o} \cdot \frac{E}{E + K_e} \cdot \frac{N}{N + K_n} \cdot \frac{K_{i,eth,o}}{K_{i,eth,o} + E} \cdot \frac{K_s}{K_s + S} \quad (6)$$

It is assumed that the demand on maintenance energy of yeast cells is constant. The maintenance energy of *Saccharomyces sp.* is very low, therefore it is often neglected in modelling approaches. If the energy demand  $m_{ATP}$  is known, the rate  $r_6$  can be calculated using equation 7.

$$r_6 = m_{ATP} \cdot X \quad (7)$$

The kinetic is herewith determined for every metabolic state. Reaction rates  $r_i$  are calculated by means of  $r_i = q_i \cdot x_i$ . The switch between the different metabolic states is realised at the critical specific substrate uptake rates for oxidative, oxidoreductive and fermentative metabolism. Critical rates are calculated by combining conditions of two states and solving the equations for  $q_s$ .

#### Model identification and sensitivity analysis

A first rough validity of the developed modelling approach could be verified with literature data from baker's yeast batch propagations<sup>3,4,27</sup>. Reference data on biomass, substrate and ethanol concentrations were compared to model based simulation runs. For the required propagations yield coefficients, maximum specific oxygen uptake rate  $q_{O_2,max}$ , temperature coefficient  $f_{temp}$  and lag-time  $t_{lag}$  were extracted as parameters with a high influence on the progression of the considered concentrations in a sensitivity analysis. In this work, only the latter three parameters were intended for the parameter estimation procedure in order to fit model based simulation runs on experimental data. On the other hand, yield coefficients and parameters

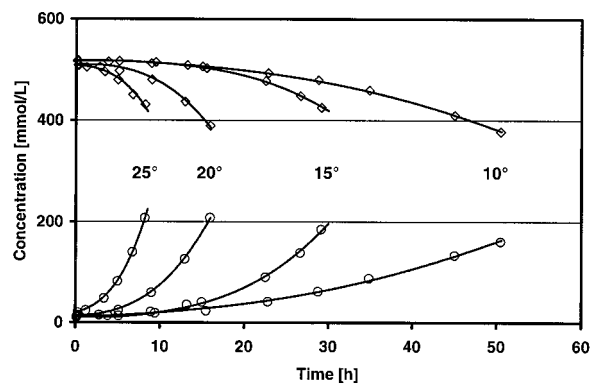


FIG. 1. Simulation runs (lines) and experimental data (dotted) of propagations at 10, 15, 20 and 25°C. Plotted are the progressions of substrate (glucose equivalents) ( $\diamond$ ) and biomass ( $\circ$ ) concentrations. Experiments were made with a 150 L propagator with continuous aeration with a dissolved oxygen concentration of 0.5 ppm.

with low sensitivity were assumed to be constant (see Table III).

In Fig.1 progressions of brewery propagations with temperatures of 10, 15, 20 and 25°C with a constant concentration of dissolved oxygen (0.5 ppm) are presented. Mean values and standard deviations of the estimation procedures considering the estimated parameters  $q_{O_2,max}$  and  $f_{temp}$  for four experiments at each temperature are given in Table IV.

The accuracy of the simulation concerning the biomass concentration was validated with the deviation between simulation and reference values. A mean value for the deviation of 5.7% could be reached with a standard deviation of 5.6%.

As single measurement values were not plausible, the median was calculated with 3.7%. Considering the measurement error of the yeast count of  $\pm 5\%$ , it can be said that the simulation represents the reference values accurately, which could be reproduced also with different propagation plants with a conventional discontinuous aeration system (not shown). So, the validity of the modelling approach for the required industrial applications could be proven.

### Process management

Aiming at the development of a process management tool, which is able to predict and manipulate the propagation process, it was necessary to describe its dependency on the common manipulated variables temperature and dissolved oxygen concentration. This knowledge had to be transferred into the process model by formulating

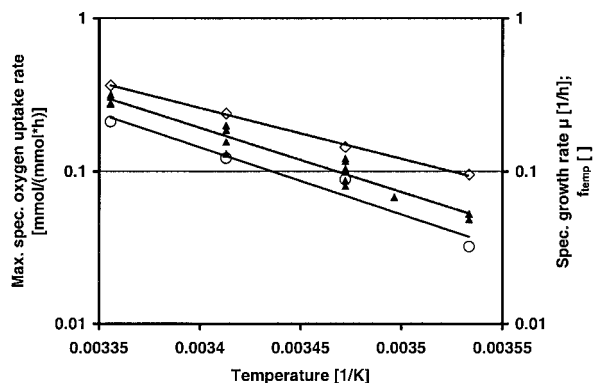


FIG. 2. Half logarithmic plot of maximum oxygen uptake rate  $q_{O_2,max}$  ( $\circ$ ), specific growth rate  $\mu$  ( $\blacktriangle$ ) and temperature coefficient ( $\diamond$ ) versus the inverse temperature. The data points are significantly matched by an Arrhenius approach.

estimated parameters as functions of the manipulated variables. In this regard, the analysis of the experimental data revealed important specific information about the relationship between temperature and oxygen content during the yeast propagation, substrate uptake and biomass formation respectively.

Concerning the *influence of temperature*, in the field of baker's yeast propagation work was done on a comparably small scale. In contrast to this, several authors described the temperature dependence of microbial growth in general<sup>9,32</sup> or for specific bacteria. The most common approaches were exponential following the Arrhenius equation<sup>10,14,19</sup> or parabolic Belehradek-type equations<sup>24,31</sup>. The latter relation, however, is better suited to the description of microorganism growth at sub-optimal temperatures. Reliable data considering an Arrhenius approach could not be found in the literature for industrial propagations of *Saccharomyces sp.* in the required temperature range. Solely, Manger et al.<sup>22</sup> described the influence of temperature especially for brewing yeast propagations. However, fixed generation times were used to predict the resulting yeast amount after a specific time. The doubling times varied in a wide range between the different trials even for one temperature (e.g. between 7.2 and 8.5 h at 15°C). So, for an active process control applying variable temperature profiles, this approach could not be used.

Fig. 2 shows a half logarithmic plot of the specific growth rate for all experiments versus the inverse absolute temperature. It can be seen, that the measured specific growth rates of all experiments at different temperatures are represented significantly by an exponential function of the inverse temperature. Following the principle of a master reaction for the biomass growth, an Arrhenius approach with an activation energy  $E_a = 84.801 \text{ kJ mol}^{-1}$  was used to describe the temperature dependency of the specific growth rate at the required temperatures. The activation energy  $E_a$  lies in a reasonable range for microbial specific growth rates<sup>10,14,19</sup>. A comparison of these results to experimental data of baker's yeast propagations and industrial trials with top and bottom fermenting brewing yeasts from Manger et al.<sup>22</sup> and Lehmann<sup>21</sup> showed, that the activation energy for the growth of *Saccharomyces* type yeasts did not seem to be dependent on the growth medium or the specific species.

In a similar manner to the growth rate in Fig. 2, the estimated parameters  $f_{temp}$  and  $q_{O_2,max}$  (see Table IV) versus the inverse temperature were plotted. The coefficient of variation for these parameters estimated from data of experiments at different temperatures were between 12 and 21% for  $f_{temp}$  and between 2.6 and 10.7% for  $q_{O_2,max}$ . Similar to the specific growth rate, the temperature depen-

TABLE IV. Mean values and standard deviations for the specific growth rate  $\mu$  and the estimated parameters  $q_{O_2,max}$  and  $f_{temp}$  for experiments between 10 and 25°C.

Temperature T [K]	n	Mean value			Standard deviation		
		$\mu$ [h <sup>-1</sup> ]	$f_{temp}$	$q_{O_2,max}$ [mol/mol h <sup>-1</sup> ]	s( $\mu$ ) [h <sup>-1</sup> ]	s( $f_{temp}$ )	s( $q_{O_2,max}$ ) [mol/mol h <sup>-1</sup> ]
298	4	0.294	0.387	0.226	0.021	0.048	0.006
293	4	0.168	0.243	0.125	0.031	0.041	0.008
288	4	0.106	0.143	0.075	0.014	0.031	0.008
283	4	0.047	0.094	0.035	0.009	0.016	0.002

dency of the two mentioned parameters is abstracted by master reactions following an Arrhenius approach with an activation energy of  $E_a = 66.85 \text{ kJ mol}^{-1}$  for  $f_{temp}$  and  $85.79 \text{ kJ mol}^{-1}$  for  $q_{O_2,max}$ , respectively. This approach is supported by Hartmeier<sup>15</sup>, who described the temperature dependency of the maximum specific oxygen uptake for *Saccharomyces cerevisiae*. The two parameters are represented very well by the exponential approach with correlation coefficients of 0.92 and 0.96 respectively.

For the required experiments already adapted yeast, inoculated in an exponential state, was used for the propagation. Less than one hour for the lag time resulted by the parameter estimation for all experiments. Therefore the parameter lag time was fixed to zero. Experiments with dried yeast (not presented here) yielded a temperature dependency of the lag-time similar to trials described in Wijtzes et al.<sup>31</sup> or Gianuzzi et al.<sup>14</sup>. However the results could not be confirmed statistically.

Practice relevant concentrations of *dissolved oxygen* in the required range of 0.1 to 0.8 ppm for experiments at 15°C exerted only a minor influence on the biomass growth compared to the temperature. However, lower dissolved oxygen concentrations than 0.1 ppm decreased the specific growth rate due to oxygen limitation effects, e.g. to 70% at 0.05 ppm dissolved oxygen concentration. This confirmed results of Manger et al.<sup>22</sup> and kinetic experiments of Hartmeier<sup>15</sup> or Cho et al.<sup>7</sup> and therefore is not presented in more detail. A sensitivity analysis based on data with an occurring dissolved oxygen limitation, showed a relevant influence of  $K_o$  on the biomass growth. However, an *implementation of the dissolved oxygen dependency* on the specific growth rate was not necessary, because the latter was represented by the temperature dependent maximum specific oxygen uptake rate  $q_{O_2,max}$  and the fixed half saturation constant  $K_o$  without an individual adaptation.

### Case scenarios

With the formulation of the variable parameters  $f_{temp}$  and  $q_{O_2,max}$  as  $f_{temp}(T)$  and  $q_{O_2,max}(T)$ , respectively, and a fixed set of stoichiometric and kinetic parameters, in particular the half saturation constant  $K_o$  for oxygen limitation (see Table III), a predictive simulation of the yeast propagation process was possible even for the application of temperature and dissolved oxygen profiles.

Herewith a prerequisite for the realisation of an active process control and evaluation tool for control strategies could be established. In order to show this potential, case scenarios were carried out, which simulate a delay in a preliminary production step. The yeast propagation has to be decelerated by manipulating the temperature or the dissolved oxygen concentration. Additionally, in scenarios a disturbance in temperature control and effects on the propagation process could be evaluated.

Fig. 3 presents scenarios with two different temperature profiles and a dissolved oxygen profile. Progressions of biomass (○) and substrate (◆) during propagations are shown in each case. Profiles of the manipulated variables were applied for an active control of the yeast propagation process. In scenario A the temperature was decreased from 20 to 10°C very quickly. After a holding time the temperature was increased slowly back to 20°C. The experiments

were run with a dissolved oxygen concentration of 0.5 ppm. Similar to the latter scenario, in scenario B) the dissolved oxygen concentration was decreased from 0.55 ppm to 0.04 ppm. After a holding time of 4 h it was increased back to the initial concentration. These experiments were run at a temperature of 20°C.

It can be seen, that the model based simulation runs (lines) match the experimental data points very well in both cases without any specific parameter estimation. The mean deviation of the simulation data from the reference data was 8% with a standard deviation of 3.9%. The potential of an active control can be pointed out with these scenarios, in particular by the deceleration of biomass growth and substrate uptake at a process time of 7.5 h and the acceleration at process time 15 h during the temperature scenario. With the required temperature profile a delay of the yeast propagation process of about 6 h could be achieved compared to an isothermal 20°C propagation run (cf. specific growth rates from Fig. 2) and the pitching could be harvested out of the exponential state of the propagation. With the presented scenario for dissolved oxygen a reduction of the specific growth rate to 70% could be achieved. A further deceleration of the growth

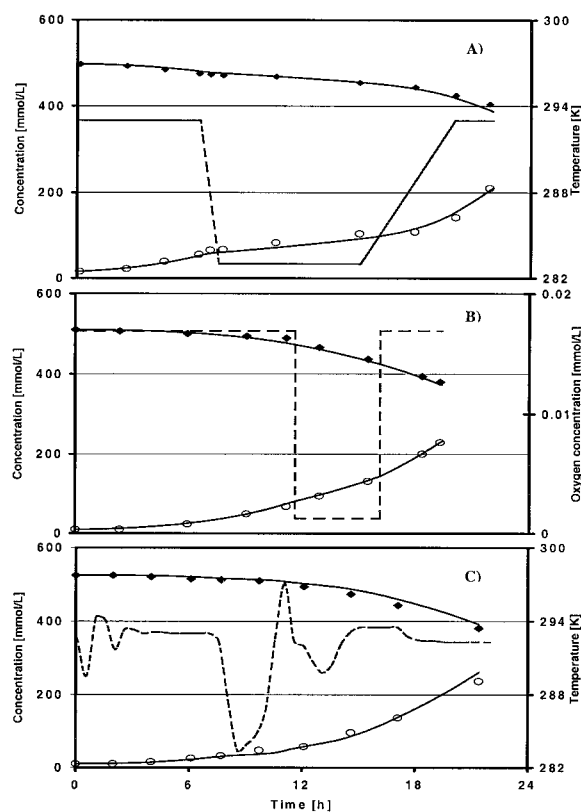


FIG. 3. Scenarios for temperature profiles A) and C) and a dissolved oxygen profile B). Experimental data: shown are progressions of concentrations of biomass (○) and substrate (◆). The right axis represents the temperature or the dissolved oxygen concentration. It can be seen, that the profiles of these manipulated variables have a relevant influence on the actual biomass growth and substrate uptake, respectively. The dotted lines represent the temperature or dissolved oxygen curves. Solid lines represent the simulation.

rate should be possible using nitrogen or carbon dioxide for aeration. But the desired deceleration of biomass growth would cause a higher ethanol production and potentially a lower activity during following fermentations.

The failure of the temperature control is a practice relevant disturbance, which allows one to prove the validity of the temperature functions in the modelling approach in particular. In such a case it is important to evaluate the effect of the "unplanned" temperature profile on the propagation process in order to create a new propagation strategy. Scenario C in Fig. 3 shows the progressions of the simulation runs and the reference data for a temperature profile resulting from a simulated failure of the temperature control. After 8 h the temperature was decreasing to 10 °C followed by a fast increase to 25°C and finally a temperature of 19°C was reached. The deceleration of the biomass growth within the cooling phases and the acceleration during the heating phases of the scenario is obvious. The mean deviation of the simulation results was 9.3% with a standard deviation of 6.4%.

## CONCLUSIONS

A reasonable modelling approach for the industrial yeast propagation was developed and validated with literature data and experiments. Model based simulations matched experimental data very well and the validity of the modelling approach for the required brewery propagations could be proven. Therefore, an automatic adaptation of the parameters of the model, as it is proposed in other works<sup>13</sup> to reach a higher accuracy of the model-based simulation, was not indicated in the required application. With only three variable parameters an easy manageable simulation tool is provided for the engineer.

Experiments on the influence of dissolved oxygen on biomass growth could be reproduced for the case of oxygen limitation below 0.05 ppm dissolved oxygen concentration. Here, further examinations are necessary concerning the influence of dissolved oxygen limitation on the yeast activity. Not included in the modelling approach are additional factors concerning the growth process of *Saccharomyces uvarum* var. *carlsbergensis*, e.g. trace elements such as zinc. In further work it must be investigated whether this or other quantities have to be taken into account in the modelling approach to represent limitation and inhibition effects occurring in industrial practices. Also the yeast growth at low temperatures was not examined in detail in this work, because temperatures lower than 10°C were not relevant for the required brewing yeast propagations in practice. On the other hand, these temperatures are used for yeast storage and to bridge longer interruptions in production. In further trials, it is planned to determine, whether an exponential approach is suitable to describe the biomass growth in a temperature range from 4 to 10°C or whether an alternative model is necessary, e.g. a Belehradek type model, which is often suitable for sub-optimal temperatures<sup>9</sup>. Similar experiments at higher temperatures (>25°C) must be carried out to allow a description of the transfer of the growth related exponential function to a decay related function. With these trials the limits of controllability of the process and the limits of the modelling approach could be extracted. Addi-

tionally, examinations on the temperature dependency of the lag time and the inaccuracies caused by the formulation of the substrate as glucose equivalents instead of different sugars with different uptake mechanisms must be carried out, in order to improve the sufficiency of the approach. In a next step, development of a model based active process control tool for the integration of information about yeast vitality in a modelling approach is intended. In particular work concerning the actual proliferation state and enzymatic activities of key enzymes of different metabolic pathways is in progress. In order to describe non-stirred propagator systems as well, a model extension considering flocculation is in preparation.

The benefit for the brewer from this work, lies in the gain of process knowledge about both the influence of manipulated variables and the growth medium on biomass growth and resulting biomass activity. Through using an industrial growth medium, process control strategies can be simulated and evaluated. The knowledge gained about the process behaviour allows the employment of optimisation algorithms, e.g. genetic algorithms, in order to plan propagation strategies more efficient than before. Mistakes in process strategies can be avoided and active yeast can be provided for inoculation even considering practice relevant scenarios). The applicability of the developed system is not limited to the considered case. Conceivable are applications for other industrial fermentations, where economical or traditional aspects prohibit the usage of customised growth media, such as for the production of vinegar, wine, single cell proteins or secondary metabolites.

## ACKNOWLEDGEMENTS

This work was supported by the "Wissenschaftsförderung der Deutschen Brauwirtschaft e.V.", grant No. B68.

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(Manuscript accepted for publication May 2002)

## NOMENCLATURE APPENDIX

E, N, O, S, X	Concentrations of ethanol, nitrogen, oxygen, substrate (glucose equivalent) and biomass [mmol/L]
CH <sub>HX</sub> O <sub>OX</sub> N <sub>NX</sub>	Composition of biomass equivalent
HX	Hydrogen portion of biomass composition
NX	Nitrogen portion of biomass composition
OX	Oxygen portion of biomass composition
C, c <sub>e</sub>	Stoichiometric coefficient for CO <sub>2</sub> formation during biomass formation for growth on glucose/ethanol [mmol/mmol]
m <sub>ATP</sub>	ATP demand for non growth associated maintenance [mmol/mmol]
K, Ke	ATP demand for growth associated maintenance (growth on glucose/ethanol) [mmol/mmol]
δ	P/O relation (ATP/NADH/H <sup>+</sup> ) [mmol/mmol]
c, g, n, n <sub>e</sub> , w, w <sub>e</sub>	Stoichiometric coefficients
Y <sub>x/sox</sub>	Yield for oxidative growth on glucose [mmol/mmol]
Y <sub>x/sf</sub>	Yield for fermentative growth on glucose [mmol/mmol]
Y <sub>x/e</sub>	Yield for oxidative growth on ethanol [mmol/mmol]
q <sub>s,max</sub> , q <sub>s</sub>	(maximum) specific substrate uptake rate [mmol/(mmol*h)]
q <sub>O2,max</sub> , q <sub>O2</sub>	(maximum) specific oxygen uptake rate [mmol/(mmol*h)]
K <sub>S</sub> , K <sub>O</sub> , K <sub>N</sub> , K <sub>eth</sub>	Half saturation constants for glucose, oxygen, nitrogen and ethanol [mmol/L]
K <sub>i,eth</sub>	Inhibition constant for ethanol inhibition of glucose uptake [mmol/L]
K <sub>i,eth,o</sub>	Inhibition constant for ethanol inhibition of oxygen uptake [mmol/L]
f <sub>temp</sub>	Coefficient for temperature dependence of q <sub>s</sub>
L <sub>t</sub> , t <sub>lag</sub>	Lag time coefficient, lag time [h]
μ	Specific growth rate [1/h]
r <sub>i</sub>	Reaction rate of reactant i [mmol/(L*h)]
r <sub>1-r8</sub>	Reaction rate of biochemical reaction 1-8 [mmol/(L*h)]