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Adaptive on-line simulation of bioreactors: fermentation monitoring and modeling system

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SUMMARY

In order to study and control fermentation processes, indirect on-line measurements and mathematical models can be used. Here an on-line model for fermentation processes is presented. The model is based on atom and partial mass balances as well as on stability equations for the protolytes. The model is given an adaptive form by including transport equations for mass transfer and expressions for the fermentation kinetics. The state of the process can be estimated on-line using the balance component of the model completed with measurement equations for the input and the output flows of the process. Adaptivity is realized by means of on-line estimation of the parameters in the transport and kinetic expressions using recursive regression analysis. On-line estimation of the kinetic and mass transfer parameters makes model-based predictions possible and enables intelligent process control while facilitating testing of the validity of the measurement variables. A practical MS-Windows 3.1 model implementation called FMMS—*Fermentation Monitoring and Modeling System* is shown. The system makes it easy to configure the operating conditions for a run. It uses Windows dialogs for all set-ups, model configuration parameters, elemental compositions, on-line measurement devices and signal conditioning. Advanced on-line data analysis makes it possible to plot variables against each other for easy comparison. FMMS keeps track of over 100 variables per run. These variables are either measured or estimated by the model. Assay results can also be entered and plotted during fermentation. Thus the model can be verified almost instantly. Historical fermentation runs can be re-analyzed in simulation mode. This makes it possible to examine different signal conditioning filters as well as the sensitivity of the model. Combined, the data analysis and the simulation mode make it easy to test and develop model theories and new ideas.

INTRODUCTION

When using a process model for on-line simulation, it is favorable to give different roles to the main constituents of the model. The state of the process can be evaluated by means of balance equations, such as total and partial mass balances, atom balances and energy balances in combination with a property database consisting of information about physical and chemical quantities. The balance equations must be completed with as many measurement equations as there are free variables. The choice of these measurement equations defines the set of measurement variables. Such a model interface, constructed to measure the state of the process, can be based on several alternative sets of measurement variables, which can be chosen either from the primary variables of the balance equations or from variables present in the database. This indirect measurement technique creates great potential to obtain information about all the process variables by means of easily measured variables through the model interface. The main principles of this technique have been presented previously [e.g. 3,9].

An adaptive on-line simulation model for bioreactors is applied to aerobic batch and fed-batch *Saccharomyces cerevisiae* fermentation. The model operates as an on-line interface to the process and produces estimates as well as predictions of the state variables. Once the estimates of the state variables are obtained, the state variables can also be controlled. A model-based algorithm, used for maintaining the glucose concentration in the broth at a user-specified level, is presented. The model is implemented into a process interface called *Fermentation Monitoring and Modeling System* (FMMS). The process interface is demonstrated by showing results from industrial fungal and bacterial fermentation runs.

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THE PROCESS STUDIED

In the process studied, *Saccharomyces cerevisiae* (CBS 8066, Delft, Netherlands) is grown on glucose substrate in a fed-batch bioreactor producing ethanol as a metabolic product. Ammonia is used for adjusting the pH level in the broth. A liquid containing glucose, nitrogen and small amounts of sulfur, phosphorus, trace elements and several essential vitamins is used as the initial growth medium. At proper tank conditions (pH, temperature and aeration), the biomass inoculum, is added after which substrate and base are fed as required. Fig. 1 represents the bioreactor set-up to be considered.

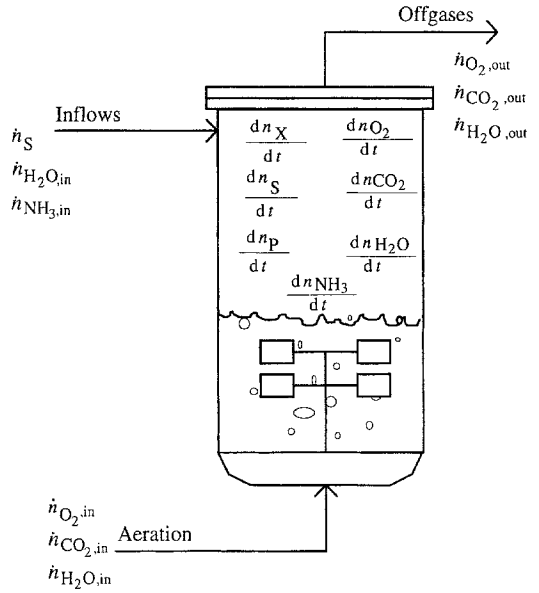

Fig. 1. Flows and accumulation terms for the process model considered.

THE ESTIMATION MODEL

When building an on-line simulator, balances provide a useful tool for describing conservation relations. The model for estimation of central state variables, which are not measured on-line, is based on balances for the dominant elements and on an electroneutrality condition. In a system consisting of a given number of substances with known internal composition, the elemental balances can be written in the general matrix form as

$$\mathbf{A} \cdot \dot{\mathbf{n}}_{in} = \mathbf{A} \cdot \frac{d\mathbf{n}}{dt} + \mathbf{A} \cdot \dot{\mathbf{n}}_{out} \tag{1}$$

The vectors, $\dot{\mathbf{n}}_{in}$ and $\dot{\mathbf{n}}_{out}$, describe the molar in- and outflows of the substances. The matrix, **A**, denotes the atom matrix, which describes the composition of the substances included in the model. For a typical fermentation process the atom matrix is:

$$\mathbf{A} = \tag{2}$$

O_2	CO_2	H_2O	NH_3	*S (substrate)* $C_{\alpha''}H_{\beta''}O_{\gamma''}N_{\delta''}$	*X (biomass)* $C_\alpha H_\beta O_\gamma N_\delta$	*P (product)* $C_{\alpha'}H_{\beta'}O_{\gamma'}N_{\delta'}$	
2	2	1	0	γ''	γ	γ'	O
0	1	0	0	α''	α	α'	C
0	0	2	3	β''	β	β'	H
0	0	0	1	δ''	δ	δ'	N

When the bioreactor is run as a fed-batch, several in- and outflows are equal to zero and the equation system can be rewritten as:

Carbon balance:

$$\alpha'' n_{S,in} + \dot{n}_{CO_2,in} = \alpha'' \frac{dn_S}{dt} + \alpha \frac{dn_X}{dt} + \alpha' \frac{dn_P}{dt} + \frac{dn_{CO_2}}{dt} + \dot{n}_{CO_2,out} \tag{3}$$

Hydrogen balance:

$$\begin{aligned} \beta'' \dot{n}_{S,in} + 3\dot{n}_{NH_3,in} + 2\dot{n}_{H_2O,in} = {} & \beta'' \frac{dn_S}{dt} + \beta \frac{dn_X}{dt} + \beta' \frac{dn_P}{dt} \\ & + 3 \frac{dn_{NH_3}}{dt} \\ & + 2 \frac{dn_{H_2O}}{dt} + 3\dot{n}_{NH_3,out} + 2\dot{n}_{H_2O,out} \end{aligned} \tag{4}$$

Oxygen balance:

$$\begin{aligned} & \gamma'' \dot{n}_{S,in} + 2\dot{n}_{O_2,in} + 2\dot{n}_{CO_2,in} + \dot{n}_{H_2O,in} \\ & \quad = \gamma'' \frac{dn_S}{dt} + \gamma \frac{dn_X}{dt} \\ & \quad + \gamma' \frac{dn_P}{dt} + 2 \frac{dn_{O_2}}{dt} \\ & \quad + 2 \frac{dn_{CO_2}}{dt} + \frac{dn_{H_2O}}{dt} \\ & \quad + 2\dot{n}_{O_2,out} + 2\dot{n}_{CO_2,out} + \dot{n}_{H_2O,out} \end{aligned} \tag{5}$$

Nitrogen balance:

$$\delta'' \dot{n}_{S,in} + \dot{n}_{NH_3,in} = \delta'' \frac{dn_S}{dt} + \delta \frac{dn_X}{dt} + \delta' \frac{dn_P}{dt} + \frac{dn_{NH_3}}{dt} + \dot{n}_{NH_3,out} \tag{6}$$

The four elemental balances serve as a base for the model, yielding four conservation equations. If all the in- and outflows are measured or otherwise known, the balances reduce the number of unknown accumulation terms from seven to three. These three terms remain to be measured or calculated using other expressions. In this approach, we have chosen to evaluate the accumulated amounts of oxygen, carbon dioxide and ammonia from measurements and phenomenological expressions, and to solve the elemental balances for the accumulation terms of biomass (yeast), water, product (ethanol) and substrate (glucose).

The accumulation terms of O_2 and CO_2 in the liquid can be calculated from dissolved oxygen measurements in the broth combined with the composition of the exiting gas. This is done using mass transfer theory, resulting in expressions for accumulated oxygen and carbon dioxide. The methods used are presented in [5,10].

The calculation of the accumulated amount of ammonia in the broth is based on an electroneutrality condition for the non-biomass liquid phase of the fermentation medium. The principles used are presented in [10]. Similar approaches have been used in [6,13].

$$\sum_j z_j n_j = K_{el} \tag{7}$$

The expression can, for this defined system, be simplified to

$$V([NH_4^+] + [Na^+] + [H_3O^+] - [OH^-] - [HCO_3^-] - 2[SO_4^{2-}]) = K_{el} \tag{8}$$

K_{el} is introduced in order to include unknown ions in the system. This constant is determined experimentally.

Inserting the measurements as well as the calculated expressions for accumulated oxygen, carbon dioxide and ammonia, the equation system (3–6) can be solved for accumulated substrate (glucose), biomass (yeast), product (ethanol) and water. This results in model-based on-line estimates of glucose, yeast and ethanol concentrations in the fermenter. The methods for obtaining flows and accumulations are summarized in Table 1.

For the *Saccharomyces cerevisiae* fermentation studied the elemental compositions are defined as follows [4,12]:

	S (Glucose)	*X* (*S. cerevisiae*)	*P* (Ethanol)
C	$\alpha'' = 6$	$\alpha = 1.00$	$\alpha' = 2$
H	$\beta'' = 12$	$\beta = 1.66$	$\beta' = 6$
O	$\gamma'' = 6$	$\gamma = 0.50$	$\gamma' = 1$
N	$\delta'' = 0$	$\delta = 0.16$	$\delta' = 0$

It should be pointed out that the terms of NH_3 and CO_2 in Eqn (1) represent total amounts. Ammonia occurs as NH_4^+ and NH_3, whereas carbon dioxide occurs in the form of H_2CO_3, HCO_3^-, CO_3^{2-} and $CO_{2,aq}$. The distribution between the dissociated forms depends on the pH level in the broth.

ADAPTATION OF KINETIC PARAMETERS

Based on the estimates achieved from the elemental balances, a kinetic model for biomass growth and product formation is continuously adapted to the process. The volumetric production rates are obtained from partial mass balances

$$R_i = \frac{c_i \dot{V}_{out}}{V} + \frac{dc_i}{dt} + \frac{c_i}{V}\frac{dV}{dt} - \frac{c_{i,in}\dot{V}_{in}}{V}, \; i = X, S, P \tag{9}$$

where c_i is achieved from the estimation model presented in the previous section. The feed concentration is denoted $c_{i,in}$. Kinetic expressions, such as

$$R_i^* = f(S,X,\Psi) \tag{10}$$

are used to describe the volumetric production rates of substrate, biomasss and product. The expressions include correction parameters (Ψ), which are adapted on-line by minimizing the differences betweeen R_i and R_i^*. The method for parameter adaptation is presented in [10]. When a kinetic description of the process is included in the model, it can be used for both prediction and process control purposes.

TABLE 1

Flows and accumulation terms used in the estimation model

Term	Source
Inflow	
O_2	Obtained from air flow measurement and known air composition
CO_2	Obtained from air flow measurement and known air composition
NH_3	Obtained from ammonia flow measurement and known ammonia concentration
H_2O	Obtained from air, glucose and ammonia flow measurements and known air composition, glucose and ammonia concentration
S	Obtained from glucose flow measurement and known glucose concentration
Accumulation	
O_2	Broth: obtained from dissolved O_2 measurement Head-gas: obtained from off-gas measurements
CO_2	Broth: obtained from phenomenological expressions Head-gas: obtained from off-gas measurements
H_2O	Obtained from the elemental balances
NH_3	Obtained from the electroneutrality condition
S	Obtained from the elemental balances
X	Obtained from the elemental balances
P	Obtained from the elemental balances
Outflow	
O_2	Obtained from air flow and off-gas measurements
CO_2	Obtained from air flow and off-gas measurements
H_2O	Obtained from gas–liquid equilibrium and Raoult's Law

Set-point calculation

The desired change in substrate concentration with respect to time is calculated as the difference between the substrate set-point and the estimated substrate concentration divided by a parameter specifying the time for achievement of the set-point [7],

$$\frac{dS}{dt} = \frac{S_{set} - S}{t_{control}} \tag{11}$$

To avoid overshoot in the controlled variable caused by overly powerful control actions, the value of $t_{control}$ is normally chosen as three to five times the sampling interval. For calculating the required feed of substrate, a partial mass balance for the substrate is used. The balance which is originally in the form

$$S_{in}\dot{V}_{in} + R_S^* V = \frac{d(SV)}{dt} + S\dot{V}_{out} \tag{12}$$

is expanded to

$$\frac{S_{in}\dot{V}_{in}}{V} + R_S^* = \frac{dS}{dt} + \frac{S}{V}\frac{dV}{dt} + \frac{S\dot{V}_{out}}{V} \quad (13)$$

which is solved for the substrate flow

$$\dot{V}_{in} = \frac{R_S^* V - \frac{dS}{dt} V}{S - S_{in}} \quad (14)$$

where dS/dt is achieved from Eqn (11) and R_S^* is the volumetric production rate for the substrate achieved from the adapted kinetic expression describing glucose consumption. In the fed-batch runs performed, the kinetic expression for R_S^* is chosen as

$$R_S^* = \Psi \frac{\mu_{max} S}{K_S + S} X \quad (15)$$

which is the Monod equation for biomass growth extended with a correction parameter, Ψ. Another approach where the fixed kinetic structure is substituted with a neural network is tested in [8]. The constant values in the Monod expression are chosen to be $\mu_{max} = 0.45\ h^{-1}$ and $K_s = 0.5\ g\ L^{-1}$. The adaptation of Ψ is realized by recursive minimization at every sampling instance according to

$$\min_{\Psi} \left\{ \sum_{i=1}^{n} [R_S - R_S^*(\Psi)]^2\ \lambda^{n-i} \right\} \quad (16)$$

where R_S is achieved from Eqn (9), λ is a memory factor (chosen as 0.95) and n is the actual sampling.

EXPERIMENTS

The theory outlined above is applied to a laboratory-scale Chemap CF 3000 fermenter (Chemap AG, Volketswil, Switzerland), with a total volume of 3.5 L. The vessel is in-situ sterilizable and equipped with a Rushton-type impeller. The fermenter is controlled by the CBC-10 digital control unit. The fermentation unit is presented in Fig. 2. The organism used is a strain of *Saccharomyces cerevisiae* (CBS 8066, Delft, Netherlands). Bacto-yeast nitrogen base (Difco Laboratories, Detroit, MI, USA) is used as growth medium and D(+)-glucose (Fluka, Buchs, Switzerland) is used as a substrate providing carbon and energy for the organism.

The fermentation with *Saccharomyces cerevisiae* is carried out either in batch or fed-batch mode using the developed fermentation modeling and monitoring system described in a later section. In an aerobic batch fermentation with significant initial glucose concentration, biphasic growth normally occurs. Initially, glucose is consumed forming ethanol and biomass together with carbon dioxide and some minor by-products such as acetic acid and glycerol. This respiro-fermentative phase continues until the broth is exhausted of glucose. After a lag-phase, during which the yeast adapts to new conditions, the ethanol formed together with the glycerol formed are consumed in the respirative growth phase producing mainly biomass and carbon dioxide. The aerobic fed-batch fermentation is initiated with a significant initial yeast concentration and no initial glucose. Glucose concentration is controlled to 2.5 g L^{-1} during the fermentation.

Control strategy

Normally, when the aim of aerobic fed-batch fermentation is to produce baker's yeast by respirative growth without ethanol formation, the glucose is kept at low concentration, typically below 0.1 g L^{-1} [1,2,11]. The results presented in this paper are based on a different control strategy. The aim is to maintain both biomass growth and product formation under aerobic conditions, in line with many commercially important industrial processes using other organisms.

On-line measurements

The vessel is equipped with a temperature sensor, a pressure sensor and an Ingold probe (Ingold Messtechnik AG, Urdorf, Switzerland) for pH measurements. The concentration of dissolved oxygen is measured with an Ingold pO_2 probe giving the corresponding partial pressure of the gas. The air inflow is measured with a Hi-Tec thermal mass flow controller (Bronkhorst High-Tech B.V., Ruurlo, The Netherlands) and the added volumes of acid and base are obtained from the operating times of the fixed-speed peristaltic pumps. Off-gas composition (O_2 and CO_2) is determined by a Datex Multicap gas monitoring system (Datex OY, Helsinki, Finland) where the O_2 content is measured by a paramagnetic oxygen sensor and the CO_2 content is measured using infrared absorption.

Data transfer

Signals from the CBC-10 control unit (Chemap AG) and the Multicap gas monitoring system are digitally transferred to the personal computer using RS-232 serial communication. The data transfer is bi-directional allowing set-points for the control loops in the CBC-10 to be changed from the personal computer.

Preparation of the inoculum

Cells from a culture maintained on bacto agar (Difco Laboratories) are added to a 1000-ml Erlenmeyer flask containing 300 ml sterile solution of glucose, yeast nitrogen base and water. The cells are cultivated for approx. 72 h in the stirred flask tempered to 30 °C. Due to a drop in pH caused by consumption of NH_3 the growth ceases when the cell concentration is approximately 1.5 g L^{-1} (dry weight). Alternatively, fermentation broth gained from a previous run with a yeast concentration of approx. 3 g L^{-1} is used as inoculum.

Operating conditions

The test runs are operated at 30 °C, 0.3 bar over pressure and pH 4.5. The pH is controlled by the addition of 0.05 M H_2SO_4 and 0.25 M NaOH in batch run Y9307 or 0.5 M NH_3 in fed-batch run Y9408. The glucose feed concentration in the fed-batch run Y9408 is 100 g L^{-1}. The inflow of virtually dry air is normally kept at 1 L min^{-1}. This fairly low air flow is maintained to enhance exit gas measurement accuracy. The

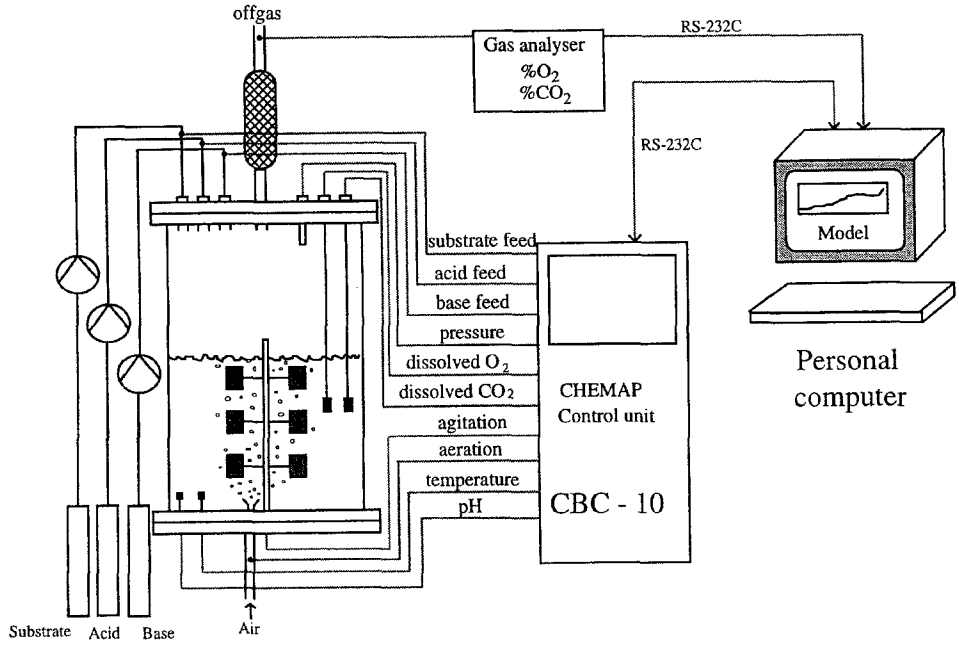

Fig. 2. The experimental set-up of the fermentation unit.

agitation is kept at 400 r.p.m. In order to prevent foaming, a small amount of silicon anti-foam emulsion (Serva M-30 from Serva Feinbiochemica GmbH & Co, Heidelberg, Germany) is added to the fermentation broth.

RESULTS

Among several similar test runs, one typical run (Y9307), consisting of the two phases mentioned above, is chosen for presentation of the on-line estimation model and verification of a batch run. A second run (Y9408) shows the performance of the model when keeping a fixed glucose concentration in a fed-batch run.

The most important measured variables are presented in Fig. 3. The sampling time used in the estimation model is 60 s. In Fig. 4, curves showing on-line estimations of yeast (X), glucose (S) and ethanol (P) for the Y9307 batch run are presented. Figure 5 shows the same set of state variables for the Y9408 fed-batch run as well as the manipulated glucose feed rate.

In order to verify the model-based estimations, the broth is frequently analyzed. The yeast dry-cell weight is analyzed gravimetrically using 0.45-μm nitro-cellulose filters. An HPLC equipped with a FAM-Pak column (Waters, Millipore Corporation, Milford, MA, USA) is used for analyzing glucose, ethanol and compounds present in minor amounts, e.g. acetic acid and glycerol. The analyzed concentrations of yeast, glucose and ethanol are shown in Figs 4 and 5. It should be noted that the HPLC analyses are *not* used in the estimation model, i.e. they are only used for verification of the estimates.

The estimation model fits the analyzed values well during both growth phases. At the end of the batch fermentation (Y9307) the estimation model becomes slightly inaccurate. This is because acetic acid is formed and consumed, but not at this stage included as a species in the model. Neither is

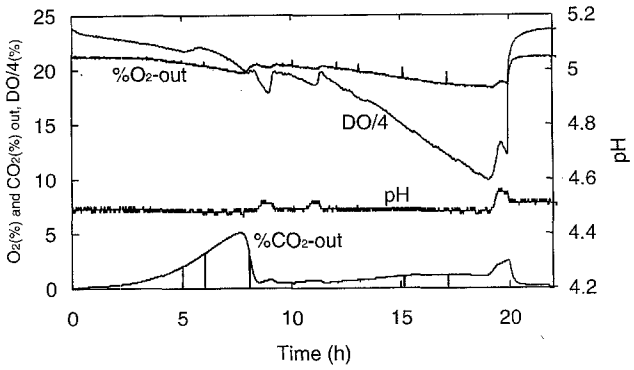

Fig. 3. On-line measurements for run Y9307.

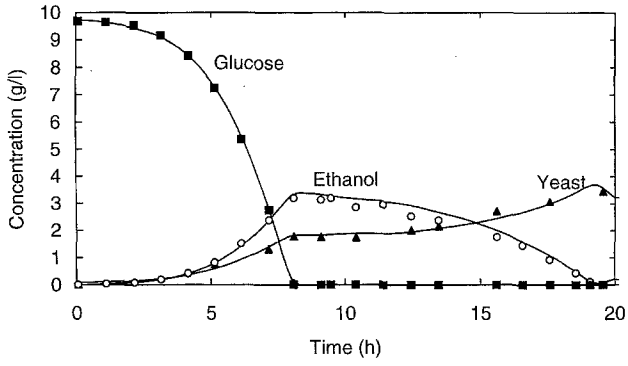

Fig. 4. On-line estimated variables (solid lines) and assay results (dots), run Y9307.

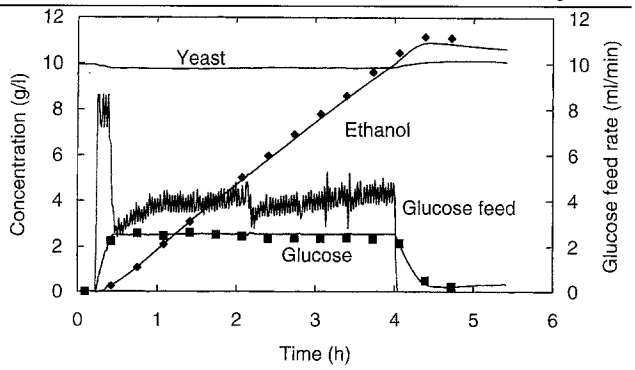

Fig. 5. On-line estimated and manipulated variables (solid lines) as well as assay results (dots), run Y9408. At $t = 2.15$ h a 500-ml sample is removed.

glycerol, which can explain the slightly high estimation of ethanol concentration during the entire fermentation process. In Fig. 5 the estimated glucose concentration follows the set-point (2.5 g L^{-1}) closely during the fed-batch phases, which is an indication of successful model-based control.

FERMENTATION MONITORING AND MODELING SYSTEM*

The model equations presented above are implemented in a system called FMMS—*Fermentation Monitoring and Modeling System*. FMMS runs on an IBM-compatible personal computer under Microsoft Windows 3.1. It is a product of the BioSim project. BioSim is a joint research project between Genencor International and Åbo Akademi University. The complete name and objective of the project is the creation of 'Adaptive On-Line Simulation and Neural Nets for Chemical and Bioengineering Processes'. FMMS was created and developed by the authors of this paper. All data acquisition, on-line modeling and on-line model-based process control of runs presented in this paper were made with FMMS.

Figure 6 shows the FMMS main display. The display shows all continuous on-line measurements and important standard calculations, like transfer rates, respiratory quotient and mass transfer coefficients during a fermentation run. The units of measure displayed are user definable. In addition, the user can define what is actively displayed and what is not. Inactive values are grayed out according to Windows standards. At the top general information about the run, including run number, the project name, and supervisor is displayed. Other information displayed includes the current fermenter, alarms and messages indicator and mode (on-line, off-line), status (running, measuring, simulating, stopped), run-time and measurement indicators.

In the upper left corner information about the three feeds, substrate, base, and acid is displayed. This information is shown as a feed rate as well as a totaled value. Below that are the current fermenter conditions and further below is the Gas In display of rate and total amounts. The fermenter and the level indicator are shown in the center. You can 'open up' the fermenter by simply double-clicking the main fermenter graphic. When you do this, an agitator and several flow indicators will be shown. During an on-line or simulated run, the graphics are animated, indicating movement of the agitator or flow in the inlet or outlet streams. Below the fermenter graphic is a display that shows the current values of the agitation speed.

FMMS makes it easy to configure the operating conditions for a run. It uses Windows dialogs for all set-ups, model configuration parameters, elemental compositions, on-line measurement devices and signal conditioning.

A powerful charting tool makes it possible to compare a variable with any other variable. A variable can be plotted as an ordinary function of time or as a function of any other available variable. The FMMS charting tool can compare trends of up to twelve different variables (measured, calculated, modeled and entered assay results) on a single page. Up to ten different pages can be defined, all pages and plotted variables are user definable and can be changed any time during the fermentation. The variables to be plotted are defined by 'dragging and dropping' variables from the selection lists on the right hand side to a tag label or a chart scale. The current version of FMMS tracks over 100 variables per run. Figure 7 shows the FMMS chart display as the on-line modeling of a bacterial enzyme fermentation is completed.

All of the FMMS variables displayed in the charts are color coded. This means that for each chart, the same color is used for the variable's axis and numbers, its trend (curve or points), and its tag. The color of the plot can be changed by double-clicking the chart area.

Historical fermentations can be re-analyzed in simulation mode. This feature makes it possible to test and develop new models and also serves as an excellent process follow-up tool. FMMS can be used to advance process development technology and to systematize projects. Higher quality processes and shorter development times are direct benefits of its continued use. Simply put, FMMS allows you to get more mileage from your data. Figure 8 shows an example of on-line modeling results from a complex large scale (reactor size >100 m^3) fungal enzyme fermentation. As can be seen, modeling results are fair even in this more complex environment, although not as accurate as the results from the defined media yeast experiments presented above.

DISCUSSION

The atom and mass balance based on-line model is shown to be a useful tool in monitoring, estimating and controlling fermentation processes. The on-line model estimates in real time all important state variables of interest. Hence the model could be considered as a soft sensor for biomass, substrate and product concentration. In this way variables, which cannot be measured on-line, might serve as control variables. The control is here realized by recursively estimating parameters for the fermentation kinetics. In this study we have applied the on-line model to batch and fed-batch processes. The presented methods could also be utilized for process optimization resulting in higher product yields.

Implementing the modeling theories into a practical and

*FMMS is not currently a commercial product.

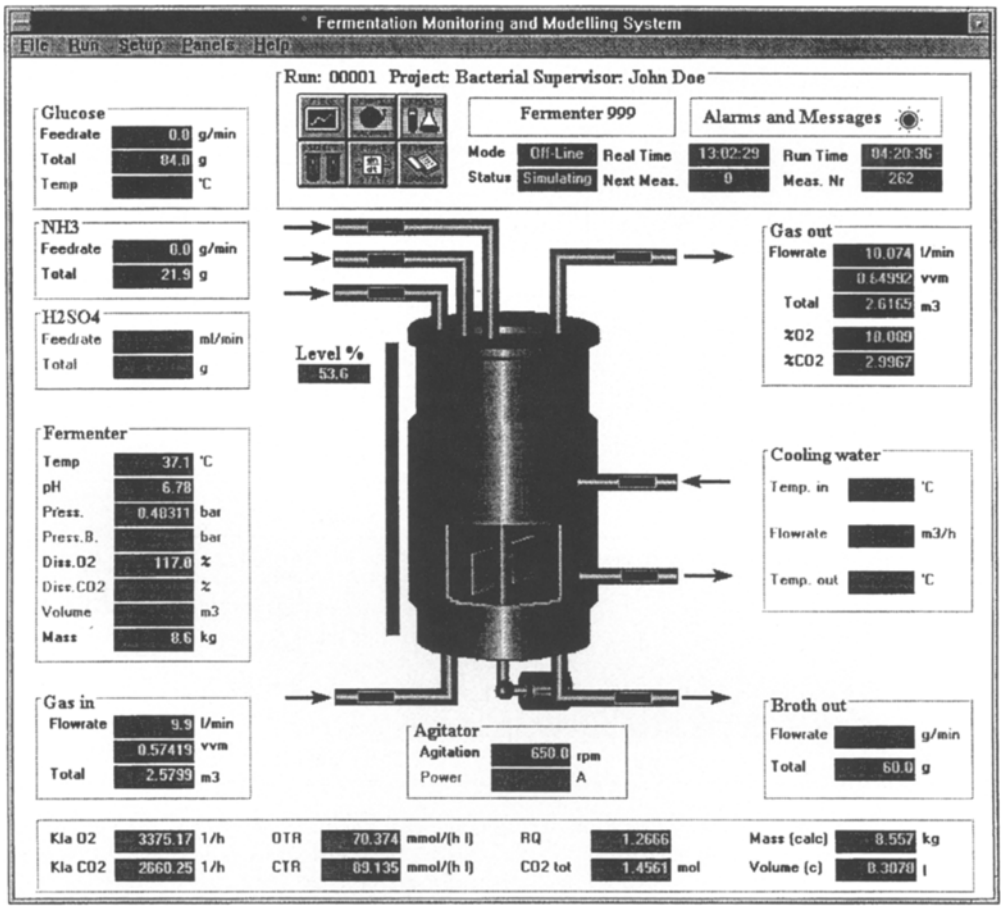

Fig. 6. The main FMMS screen, currently displaying data from an industrial bacterial fermentation run that is being re-analyzed in simulation mode.

easy to use Windows program has enabled us to apply and test the model in industrial environments. Development of FMMS continues to make it even more suitable for industrial environments and large research and development facilities.

To study more complicated fermentation systems, additional information using other on-line analyses as well as neural network methods would be useful. This concept is the subject of on-going studies on applying adaptive on-line simulation to fed-batch fermentation processes in both laboratory and industrial fermenters.

ACKNOWLEDGEMENTS

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NOTATION

Roman letters

$\mathbf{A}$	atom matrix
c	mass-based concentration (g L^{-1})
K_{el}	electroneutrality balance constant (mole)
K_S	saturation constant (g L^{-1})
n	amount of substance (mole)
P	mass-based concentration of product (g L^{-1})
R	volumetric production rate (g L^{-1} h^{-1})
S	mass-based concentration of substrate (g L^{-1})
t	time (h)
V	volume (L)
X	mass-based concentration of biomass (g L^{-1})
z	ion charge

Greek letters

α	coefficient for carbon in molecular formula
β	coefficient for hydrogen in molecular formula
γ	coefficient for oxygen in molecular formula
δ	coefficient for nitrogen in molecular formula
λ	memory factor
μ_{max}	maximum specific growth rate (h^{-1})
Ψ	correction parameter

Subscripts

in	feed
out	outflow
P	product
S	substrate
X	biomass
set	set-point for substrate concentration

Other notations

dot ($\dot{}$) above a symbol denotes flow

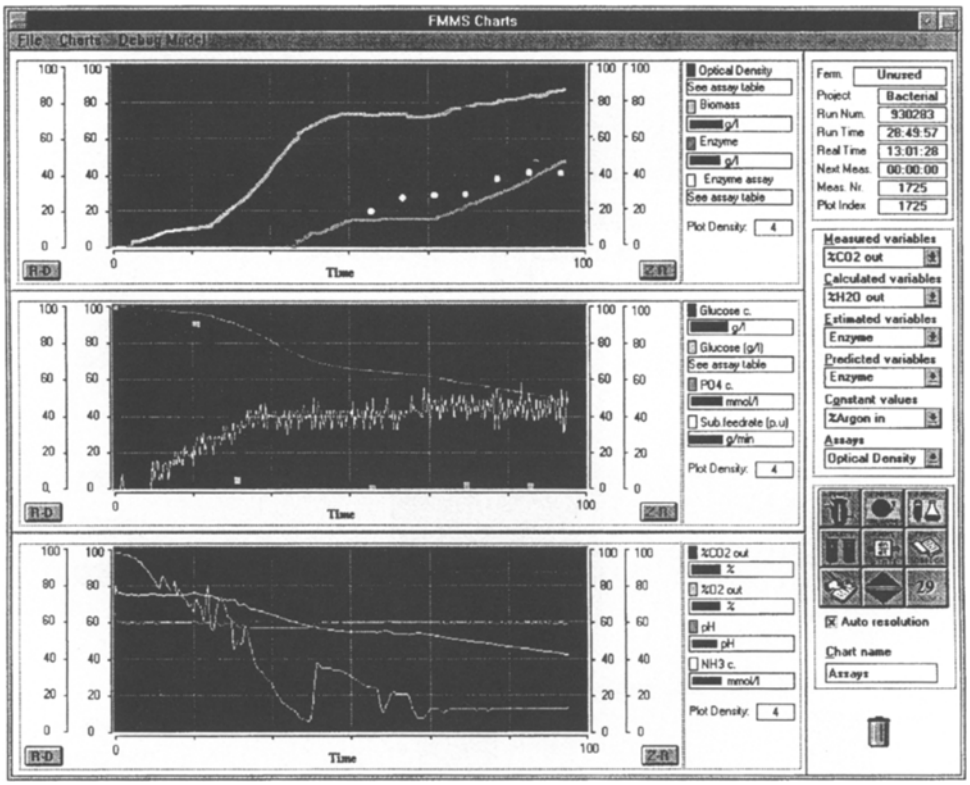

Fig. 7. FMMS charts displaying on-line state estimations of an industrial bacterial enzyme fermentation. The topmost graph shows on-line estimations (solid lines) and assay results (dots) of biomass and enzyme. (NOTE: Chart scales have been normalized to 0–100% in accordance with a non-disclosure agreement.)

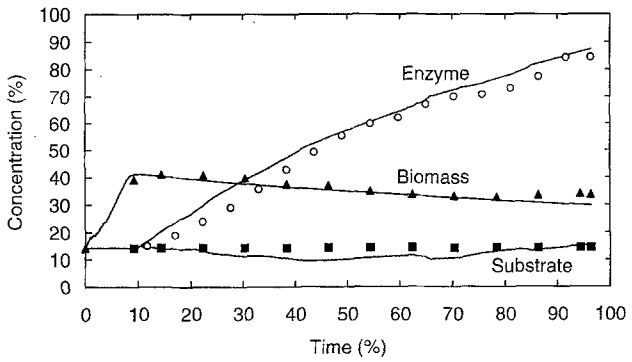

Fig. 8. On-line estimated variables (solid lines) and assay results (dots) for a large-scale industrial fungal enzyme fermentation. (NOTE: Chart scales have been normalized to 0–100% in accordance with a non-disclosure agreement.)

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