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Improvement of continuous calibration based on temperature oscillation and application to biochemical reaction calorimetry

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Abstract

This paper describes an improvement of a method for continuous calibration of the global heat transfer coefficient, recently described in literature [A. Tietze, Möglichkeiten und Grenzen der Temperaturschwingungskalorimetrie, Doctoral Thesis, Technishe Universität Berlin, 1998; Chem. Eng. Sci. 51 (1996) 3131; Chem. Ing. Tech. 68 (1996) 97]. The continuous calibration method is based on induced sinusoidal jacket temperature oscillations, allowing the uncoupling of the chemical heat production from the heat transfer parameters during the reaction. A mathematical computation procedure based on two-anchors, before and after the reaction, has been developed which gives better results as compared to the one reported in literature, using one-anchor only, either before or after the reaction. The applicability of this method to biotechnology has been explored with respect to different culture parameters, which affect the global heat transfer coefficient, i.e. stirring speed, reactive volume, and medium viscosity. This oscillating reaction calorimetry method (ORC) has been successfully applied to a fed-batch culture of *Saccharomyces cerevisiae* with a non-linear increase of reactive volume.

Keywords: Biochemical reaction calorimetry; On-line calibration; Calibration by temperature oscillations

1. Introduction

Like any chemical reaction, biological events related to living organisms involve changes in the chemical potential of substrates, products and cells. This variation of the Gibbs energy is mostly expressed as heat production [2]. Hence, the monitoring of this heat production provides information on the metabolism and the biological activity present. Since there are not many other signals that can be acquired on-line, in biotechnology, the heat signal also represents an invaluable tool for control purposes.

A heat-flow reaction calorimeter, in isothermal mode, determines the heat flow rate (in W) from the contents of the reaction vessel to the thermosetting fluid in the jacket by monitoring the temperature difference across the reactor wall. The heat flux is evaluated as shown by Eq. (1). In this equation T_r is the temperature of the reactor contents, T_j the temperature of the thermosetting oil in the jacket, and UA is the global heat transfer coefficient of the system. This heat flow rate, Q_f, corresponds to the heat of reaction as long as no other heat fluxes are present.

$$Q_f = UA(T_r - T_j) \tag{1}$$

A complete energy balance around the calorimeter reactor can be described by Eq. (2)

$$Q_{acc} = Q_f + Q_R + Q_d + Q_l + Q_A + Q_e + Q_c + Q_{cal} \tag{2}$$

where Q_{acc} is the accumulated power in the reactor contents, Q_f the heat flow rate through the reactor wall to the oil, Q_R the heat flow rate resulting from the bioprocess or the reaction (often the target measurement variable), Q_d a convective heat flow rate due to any addition to the reactor at a different temperature than T_r, Q_l the power lost to the environment, Q_A the power input through agitation, Q_e the heat flow rate due to evaporation and gas evolution, Q_c the power removal in any condenser device, and Q_{cal} the calibration power. Measuring these different heat fluxes will be required to determine the reaction power output whenever concerned intensities are of comparable value.

A simple electric calibration — a power input of known value Q_{cal}, see Eq. (3) — allows the determination of UA

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Nomenclature

A	heat transfer area (m^2)
c	concentration ($mol\,l^{-1}$)
c_{Pd}	specific heat capacity of the feed stream ($J\,g^{-1}\,K^{-1}$)
C_{Pr}	integral heat capacity of the reactor contents ($J\,K^{-1}$)
E	activation energy ($J\,mol^{-1}$)
F	substrate feed rate ($l\,s^{-1}$)
k_0	pre-exponential factor (s)
$\dot{m}_d$	flow rate of feed stream ($g\,s^{-1}$)
p	period (min)
Q_A	heat flow rate due to agitation (W)
Q_{acc}	power accumulated in the reactor (W)
Q_c	power removed through a condenser device (W)
Q_{cal}	calibration power (W)
Q_d	convective heat flow rate due to additions (W)
Q_e	heat flow rate due to evaporation and gas evolution (W)
Q_f	heat flow rate through the reactor wall (W)
Q_l	power lost to the environment (W)
Q_R	heat production or consumption rate by the reaction (W)
r	reaction speed ($mol\,l^{-1}\,s^{-1}$)
R	gas constant ($J\,K^{-1}\,mol^{-1}$)
S	substrate concentration ($g\,l^{-1}$)
t	time (s)
T_d	temperature of the feed stream (K)
T_j	jacket temperature (K)
T_r	reactor temperature (K)
UA	global heat transfer coefficient ($W\,K^{-1}$)
U^{-1}	heat transfer resistance ($W^{-1}\,m^2\,K^{-1}$)
V	volume of the reactional mass (m^3)
X	biomass concentration ($g\,l^{-1}$)
$Y_{X/S}$	biomass yield ($cmol\,(cmol)^{-1}$)

Greek letters

δT_j	oscillation amplitude of the jacket temperature (K)
δT_r	oscillation amplitude of the reactor temperature (K)
ΔH_R	reaction enthalpy ($J\,mol^{-1}$)
μ	specific growth factor (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})
ρ	temperature oscillation phase shift (rad)
τ	time constant ($\tau = f(\delta T_r/\delta T_j)$) (s)
τ_R	reaction time constant (s)
ω	temperature oscillation frequency (s^{-1})

Sign

-	non-oscillating component
$\sim$	oscillating component

Indices

i	initial
f	final
stat	stationary value determined by electrical calibration
ref	reference value, determined by a stationary electrical calibration in absence of temperature oscillations
osc	stationary value, determined by electrical calibration in presence of temperature oscillations
one-anchor	modeled value calculated with the one-anchor method
two-anchor	modeled value calculated with the two-anchor method

in Eq. (1). This procedure gives a static calibration of the global heat transfer coefficient UA_{stat}

$$UA_{stat} = \frac{\int Q_{cal}\,dt}{\int (\bar{T}_r - \bar{T}_j)\,dt} \quad (3)$$

In general, the system is calibrated before and after the reaction, while at steady state [3]. These two static calibrations can be joined in different possible ways, the most common and simple one being a linear interpolation. However, in cases of strong UA variations, especially non-linear variations, the continuous determination — throughout the reaction — of the global heat transfer coefficient becomes necessary.

Strong variations of UA are frequently observed in chemical reactions like polymerization [4], due to an increase of the reaction mass viscosity. In biotechnology, UA variations arise from different factors. Many fermentation processes are accompanied by a change in broth viscosity with time. This can result from an increased cell concentration within the bioreactor, after changes in the microbial morphology (growth of filamentous microorganisms [5–8]), or because of the accumulation of extracellular products, which alter the rheological properties of the fermentation medium [8]. Variations in UA also result from changes in the heat transfer area A due to volume increase/decrease which results from sampling during the culture, addition of fluids for controlled feeding (fed-batch culture) or for pH control purposes. Finally, microbial adhesion to the reactor wall may also affect the overall heat transfer coefficient in a non-negligible way. Although UA variations in biotechnology are relatively small compared to those accompanying polymerization reactions, the low levels of heat signals in the former require a high sensitivity and therefore an accurate calibration of the calorimeter.

In simple cases, for instance variation of working volume, UA can be corrected by monitoring on-line the changing

parameter and multiplying this variation by a factor (which is determined in a separate run). This solution implies the need for multiple on-line monitoring devices, provided that the changing parameter can be monitored and that the dependence factor of UA on the given parameter is constant. Another way to determine a changing heat transfer coefficient is to perform a heat balance calorimetry [4], which is based on the combination of complete individual energy balances over the calorimeter reactor and the thermosetting circuit. However, the choice of the heat balance boundaries is complex, requiring the accurate determination of all parameters. As a result, the sensitivity of heat balance calorimeters is considerably poorer than that of heat flow calorimeters, particularly on a small scale (<5 l). Attempts have also been made to mathematically estimate the heat transfer coefficient, Carloff et al. [9] mention dimensionless Nusselt equations and Kalman filtering techniques.

A simpler and more direct solution is to continuously determine the global heat transfer coefficient throughout the reaction. The idea of analyzing induced temperature oscillations to determine heat transfer coefficients was introduced already in the 1930s [10]. Theories based on this technique were first developed and applied to thermal countercurrent flow regenerators and other heat exchangers, fixed beds, and turbulent flow in tubing.

The oscillating reaction calorimetry method (ORC), as presented in this paper, is based on the mathematical development made by Tietze [1] for the continuous determination of UA. This model was further improved and its applicability explored for situations with large changes in viscosity and volume. The application of the method was then explored with a heat flow calorimeter, Bio-RC1, from Mettler-Toledo AG, Greifensee, Switzerland, which has specially been modified for measuring the weak heat dissipation rates encountered in biological reactions [12].

2. Oscillating reaction calorimetry

2.1. Principle of ORC

The application of a periodic oscillation to the temperature on either side of the calorimeter reactor wall will result in a periodic oscillation of the temperature on the other side of the wall. However, the amplitude and the phase of the resulting oscillation will differ from those of the original oscillation in a way defined by the UA of the system, Eq. (1). Hence, the evaluation of the resulting temperature oscillation in comparison to the induced one allows the determination of UA. During a reaction, the heat flow rate signal can be divided in two parts: a slowly changing one, which corresponds to the reaction power output, and a fast changing one but with a much smaller intensity, which corresponds to the induced temperature oscillations. The temperature oscillations should not influence significantly the different heat flow rates and especially the one of the reaction under investigation.

Sinusoidal oscillations are chosen since any periodic function can be described by a combination of different sinusoidal functions. Oscillations are generated at the jacket side, Fig. 1, to avoid any possible local temperature gradient in the reactor provoked by generating oscillations at the reactor side.

The mathematical development proposed in the following paragraphs, based on the work of Tietze [1], defines a relationship between temperature oscillation amplitudes and UA. This model can be applied to reactions where the set

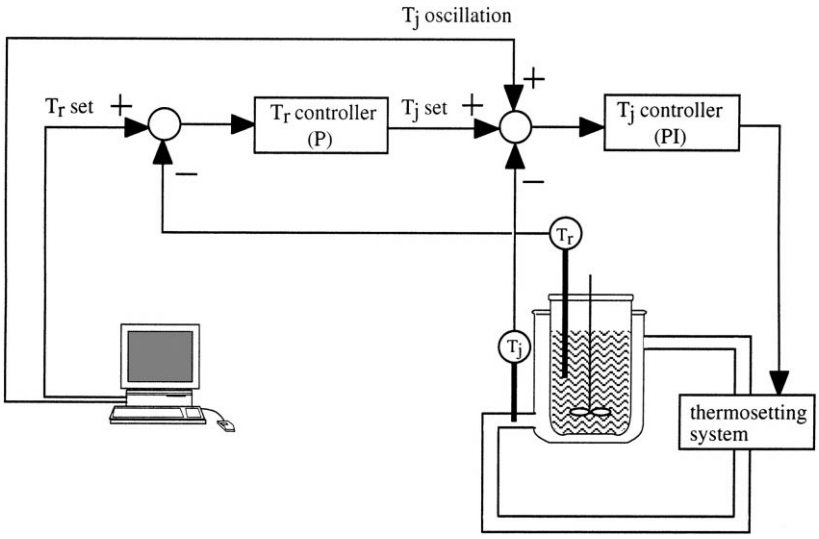

Fig. 1. Schema of the reaction biocalorimeter Bio-RC1 from Mettler-Toledo AG. The control system offers the possibility of applying temperature oscillations to the jacket fluid.

point of the reactor temperature T_r is constant (isothermal mode). It is based on a constant wall resistance hypothesis, i.e. a temperature distribution in the reactor wall is not accounted for.

By introducing a sinusoidal oscillation in the jacket temperature, T_j, and the resulting reactor temperature, T_r, can be defined as

$$T_j = \bar{T}_j + \tilde{T}_j = \bar{T}_j + \delta T_j \exp(i\omega t) \quad (4)$$

$$T_r = \bar{T}_r + \tilde{T}_r = \bar{T}_r + \delta T_r \exp(i\omega t + i\rho) \quad (5)$$

where the sign $\bar{T}$ indicates the unchanged component of temperature, that is the non-oscillating part of temperature, $\tilde{T}$ the oscillating component of temperature, δT the amplitude, ω the frequency of temperature oscillations and t the time. T_r includes a phase shift ρ, resulting from a delay in the response of the reactor temperature oscillation to the temperature oscillation applied in the jacket. The principle of the method is to extract UA from the oscillating component of both temperatures and, to evaluate the heat of reaction from the non-oscillating component of both temperatures.

In the presence of temperature oscillations, each term of Eq. (2) is described by the sum of a constant term $\bar{Q}$ which represents the heat flow rate without oscillations and an oscillating term $\tilde{Q}$ which represents the influence of the temperature oscillation on the considered heat flow rate. Each term will be described in the following paragraphs.

In the presence of temperature oscillations, the heat flow rate, Eq. (1), through the reactor wall becomes

$$Q_f = UA(\bar{T}_j - \bar{T}_r) + UA[\delta T_j \exp(i\omega t) - \delta T_r \exp(i\omega t + i\rho)] = \bar{Q}_f + \tilde{Q}_f \quad (6)$$

In the present approach only two temperatures T_r and T_j, on either side of the reactor wall are defined. The temperature distribution in the reactor wall is considered to be constant.

Although working in isothermal conditions the determination of UA by this method requires the evaluation of the energy accumulation in the reactor contents. In the present work, T_r is constant within ± 0.01 K in the presence of temperature oscillations, see Fig. 2.

$$Q_{acc} = C_{Pr} \frac{dT_r}{dt} \quad (7)$$

The energy accumulated in the reactor, in the presence of temperature oscillations, is given by

$$Q_{acc} = C_{Pr} \frac{d\bar{T}_r}{dt} + C_{Pr} \frac{d(\delta T_r \exp(i\omega t + i\rho))}{dt} \quad (8)$$

$$Q_{acc} = C_{Pr} \frac{d\bar{T}_r}{dt} + C_{Pr}\, i\omega\, \delta T_r \exp(i\omega t + i\rho) = \bar{Q}_{acc} + \tilde{Q}_{acc} \quad (9)$$

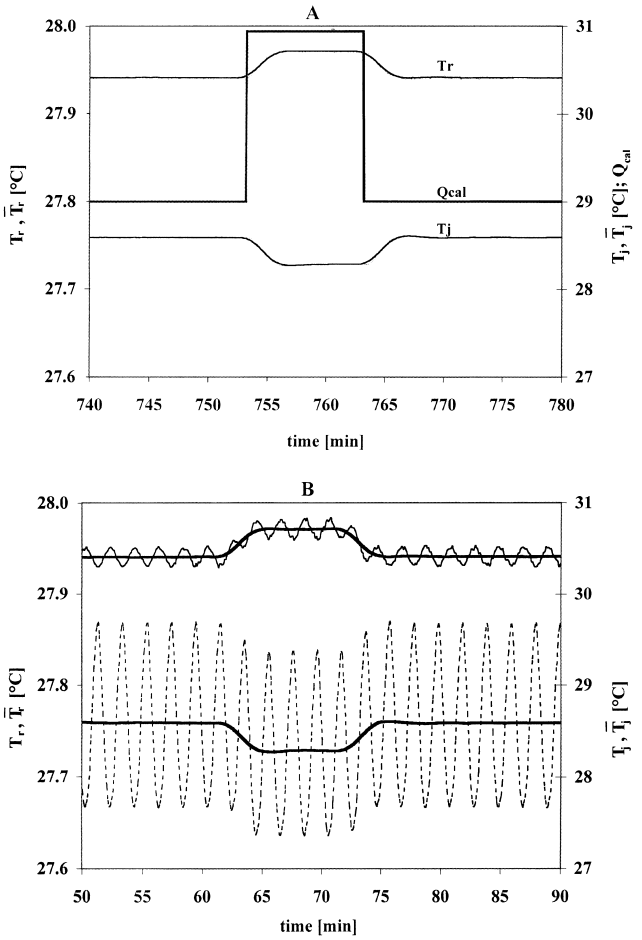

Fig. 2. Bio-RC1 behavior during an electrical calibration. (A) Stationary calibration in absence of temperature oscillations. Electrical power input Q_{cal} (presented with a zero offset). T_r and T_j are presented as raw data (thin lines) and as an average computed via Eqs. (22) and (23) (bold lines). (B) Stationary calibration under temperature oscillations. The raw data show temperature oscillations: T_r (upper signal) and T_j (lower signal). Bold lines show the average values computed via Eqs. (22) and (23).

In the present case, the reaction heat production rate Q_R, and the heat flow rate due to feeding Q_d, can be considered to have no influence on temperature oscillations, and therefore no influence on the determination of UA. This point is discussed in detail in Appendix A.

The terms corresponding to the energy input due to stirring, Q_A, and those corresponding to heat losses, Q_l, Q_e and Q_c, and to calibration, Q_{cal}, do not contribute to temperature oscillations, therefore they are not taken into account in the determination of UA.

As a result, the energy balance, see Eq. (2), of those terms contributing to temperature oscillation becomes

$$\tilde{Q}_{acc} = \tilde{Q}_f \quad (10)$$

$$C_{Pr}\, i\omega\, \delta T_r \exp(i\omega t + i\rho) = UA[\delta T_j \exp(i\omega t) - \delta T_r \exp(i\omega t + i\rho)] \quad (11)$$

Simplification of exp(iωt) and replacement of exp(iρ) by Euler's formula: $\cos\rho + \mathrm{i}\sin\rho$ in Eq. (11) gives

$$\mathrm{UA}\,\delta T_\mathrm{j} - \mathrm{UA}\,\delta T_\mathrm{r}(\cos\rho + \mathrm{i}\sin\rho) = C_\mathrm{Pr}\,i\omega\,\delta T_\mathrm{r}(\cos\rho + \mathrm{i}\sin\rho) \tag{12}$$

The real part of Eq. (12) is given by

$$\mathrm{UA} = -C_\mathrm{Pr}\omega\frac{\delta T_\mathrm{r}\sin\rho}{\delta T_\mathrm{j} - \delta T_\mathrm{r}\cos\rho} \tag{13}$$

and the imaginary part is given by

$$\mathrm{UA} = \frac{\omega C_\mathrm{Pr}}{\tan\rho} \tag{14}$$

Combination of Eqs. (13) and (14), and a further algebraic simplification gives

$$\frac{\delta T_\mathrm{j} - \delta T_\mathrm{r}\cos\rho}{\delta T_\mathrm{r}\sin\rho} = \frac{\sin\rho}{\cos\rho} \tag{15}$$

$$\cos\rho = \frac{\delta T_\mathrm{r}}{\delta T_\mathrm{j}} \tag{16}$$

Introducing Eq. (16) in Eq. (14) gives

$$\mathrm{UA} = \frac{\omega C_\mathrm{Pr}}{\tan[\arccos(\delta T_\mathrm{r}/\delta T_\mathrm{j})]} = \frac{\omega C_\mathrm{Pr}}{\sqrt{1-(\delta T_\mathrm{r}/\delta T_\mathrm{j})^2}}\frac{\delta T_\mathrm{r}}{\delta T_\mathrm{j}} \tag{17}$$

which can be written as

$$\mathrm{UA} = \frac{C_\mathrm{Pr}}{\tau} \tag{18}$$

where the time constant τ is defined as

$$\tau = \frac{1}{\omega}\left(\frac{\delta T_\mathrm{r}}{\delta T_\mathrm{j}}\right)^{-1}\sqrt{1-\left(\frac{\delta T_\mathrm{r}}{\delta T_\mathrm{j}}\right)^2} \tag{19}$$

Hence, this model allows the continuous determination of UA based on the computation of the ratio of temperature oscillation amplitudes, δT_r and δT_j, and the knowledge of C_Pr.

2.2. Amplitude computation of temperature oscillations in the jacket and in the reactor

The non-oscillating part of the temperature, see Eqs. (4) and (5), can be defined as

$$\bar{T} = \frac{1}{p}\int_{-p/2}^{p/2} T\,\mathrm{d}t \tag{20}$$

This corresponds to the mean value of the oscillating temperature signal over one period p. Also from Eqs. (4) and (5) the oscillating part of the temperature can be defined as

$$\tilde{T} = T - \bar{T} \tag{21}$$

with $\bar{T}$ as defined by Eq. (20).

Among different possible methods to calculate the temperature oscillation amplitude, the following has been chosen here

$$\delta T_\mathrm{j} = \frac{\sqrt{2}}{p}\int_{-p/2}^{p/2}[\tilde{T}_\mathrm{j}(t)]^2\,\mathrm{d}t \tag{22}$$

$$\delta T_\mathrm{r} = \frac{\sqrt{2}}{p}\int_{-p/2}^{p/2}[\tilde{T}_\mathrm{r}(t)]^2\,\mathrm{d}t \tag{23}$$

2.3. Determination of UA

UA can be easily calculated via Eqs. (18) and (19). In order to do so, the ratio of temperature amplitudes, τ, needs to be evaluated. Introducing harmonic temperature oscillations can readily do this. The second factor necessary to the determination of UA is C_Pr. Although the present model accounts for a C_Pr defined as the sum of the heat capacity of the reaction mass plus the heat capacity of every object present inside the reactor, attempts to use theoretical values gave unsatisfactory results [1]. Tietze observed that the UA determined by a static electrical calibration gives higher values than the one determined via τ and a theoretical C_Pr. In fact, heat is also accumulated in the heat-conducting reactor wall, heat is partially used to heat or cool this wall and therefore it is not completely transmitted to the reaction mass. Tietze found that including in the theoretical value of C_Pr half of the heat capacity of the reactor wall gave better results. This was confirmed in our own studies.

2.3.1. One-anchor method

A way to solve this problem is to actually measure a value for C_Pr. Literature [1] reports the use of a static calibration, before or after the reaction, to obtain C_Pr. From Eq. (18)

$$C_\mathrm{Pr} = \mathrm{UA}_\mathrm{stat}\,\tau \tag{24}$$

where $\mathrm{UA}_\mathrm{stat}$ is obtained by an electrical calibration via Eq. (3), in presence of temperature oscillations, see also Fig. 2. This type of calibration is operated while the system is in a steady state, i.e. no reaction and all other heat flow rates — besides Q_f — being optimally constant. Thus the evaluated C_Pr value is then used in Eq. (18) to continuously determine UA. Furthermore, if C_Pr has been evaluated before the reaction, UA can be determined on-line during the reaction, with a delay of one period needed to compute τ.

However, the value of C_Pr obtained via this "one-anchor" method is correct only at the anchor point (UA and τ) from where it is evaluated. As a matter of fact, experiments show that values of C_Pr obtained via a static calibration before the reaction are different from those obtained after the end of the reaction. The reason for this is the fact that most factors which affect UA also affect the temperature distribution in the reactor wall. Since C_Pr contains some of the heat capacity of the wall, it will also change.

2.3.2. Two-anchor method

A new method is proposed here, where UA is determined as a linear function of UA versus τ^{-1}, over two extreme anchor points, see Fig. 3. At each one of the two-anchor points an electrical calibration, Eq. (3), enables the determination of a static value of UA for a given τ^{-1}. Thus, the continuous determination of UA — throughout the reaction and between the two-anchor points — is performed according to the following equation:

$$\mathrm{UA} = \frac{\mathrm{UA_f} - \mathrm{UA_i}}{1/\tau_\mathrm{f} - 1/\tau_\mathrm{i}} \left(\frac{1}{\tau} - \frac{1}{\tau_\mathrm{f}} \right) + \mathrm{UA_f} \tag{25}$$

where indices f and i refer to the initial and final anchor points, respectively, for instance before and after a reaction.

With this technique UA values are not only correct at the two limiting anchor points but are also close to the real values in the whole determined range, see Fig. 3. The force of this method is that UA follows tightly the evolution of the system throughout the reaction, in the range delimited by the two-anchor points, without having to monitor on-line the changing parameters. This largely outweighs the disadvantage introduced by the necessity for a calibration at the end of the experiment, whose results will thus not be known on-line. The determination of UA via the two-anchor method is valid in most generally encountered situations, as will be illustrated in the experimental chapter.

2.4. Choice of the amplitude and period of temperature oscillations

For evaluation purposes the amplitude of the reactor temperature oscillation, δT_r, needs to be clearly distinguishable from signal noise, even under the poorest heat transfer conditions during the reaction. This is obtained with a large enough δT_j, as the temperature oscillation in the reactor is generated from the temperature oscillation in the jacket. On the other hand, δT_r is aimed as small as possible to avoid influencing the kinetics of the reaction under study. This last aspect has been discussed in Appendix A.

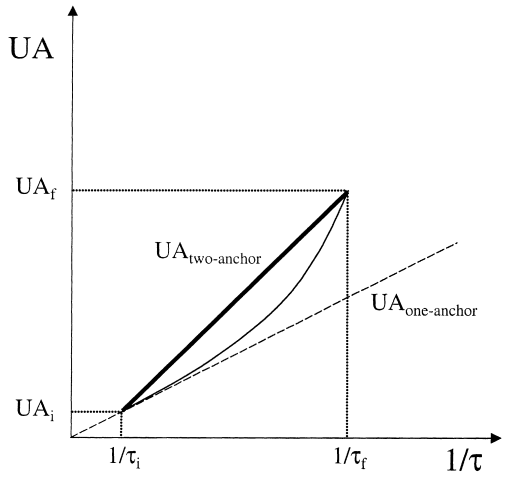

Fig. 3. Representation of UA modeling as a function of $1/\tau$. Real UA values are represented as the curved line. The dotted line represents $\mathrm{UA_{one\text{-}anchor}}$ modeled values, where the anchor point is at $(\mathrm{UA_i}, 1/\tau_\mathrm{i})$. The bold line represents $\mathrm{UA_{two\text{-}anchor}}$ modeled values, where the anchor points are at $(\mathrm{UA_i}, 1/\tau_\mathrm{i})$ and $(\mathrm{UA_f}, 1/\tau_\mathrm{f})$.

The choice of the period of temperature oscillations is also limited by opposite constraints. Since the non-oscillating term of temperature is obtained by averaging the temperature signal, Eq. (20), the temperature oscillation period should be as small as possible, to avoid distortion of the heat signal and an averaging out of dynamic events. On the other hand, under a certain limit defined by the heat transfer parameters and the calorimeter temperature control system, too short a period of imposed temperature oscillations will only generate signal noise on the temperature of the other side of the reactor wall.

As can be foreseen from the discussion above, the choice of the period and the amplitude of the imposed temperature oscillations are related to each other and strongly dependent on the sensitivity of temperature sensors and on the quality of the temperature control system of the calorimeter.

In the case of the reaction calorimeter tested, the Bio-RC1, imposed values for amplitude and period of jacket temperature oscillations of 1 K and 2 min, respectively, generate a δT_r of 0.01 K and a period of 2 min in the reactor temperature oscillations. Oscillations in T_r, Fig. 2, are well defined and clearly distinguishable from signal noise. Furthermore, it is important to emphasize that δT_r is well below the 1 K limit necessary to obtain a measurable disturbance of the reaction kinetics. On the other hand, the period used here to achieve this was considerably shorter than the 6–10 min recommended in the literature [1]. This was made possible by the powerful thermostat of the RC-1 and by the high resolution of the temperature sensors.

3. Materials and methods

3.1. Reaction calorimeter

Calorimetric measurements were performed in a 2 l reactor calorimeter, RC1, (Mettler-Toledo AG. Greiffensee, Switzerland) modified for biological work [11,12], see Fig. 1. The Bio-RC1 calorimeter is operated with the WinRC software supplied by the manufacturer, which runs on QNX system. Calorimetric data was acquired every 2 s. An electrical calibration heater of 1.96 W was used to perform the determination of the stationary values of UA: in absence and in presence of temperature oscillations, $\mathrm{UA_{ref}}$ and $\mathrm{UA_{osc}}$, respectively. A second computer was used to control and acquire on-line different biological parameters. Thus, a NB-MIO-16H I/O board from National Instruments is used to acquire analog and digital data, i.e. pH, dissolved oxygen (DO), torque meter, exhaust O_2 and CO_2, and ambient temperature. A LabView program was developed to perform data acquisition of all calorimetric and biological data and the control of the glucose feed rate. RC1 operation

on windows system, as foreseen by the manufacturer, will allow the use of a single computer to operate the calorimeter, and to control and to monitor it as a bioreactor.

3.2. Cells

The yeast strain *Saccharomyces cerevisiae* GIV 2009 kindly provided by Givaudan Roure Aromen AG (Dübendorf, Switzerland) was cultured in a defined complex medium. Cells were thawed from −20°C and incubated in Erlenmeyer flasks at 30°C and 600 rpm. With this culture the calorimeter reactor was inoculated and a batch culture was performed. The batch culture was necessary to obtain a high cell concentration as a starting culture for the fed-batch.

3.3. Medium

The medium composition is as following: 20 g l^{-1} glucose; 5 g l^{-1} $(NH_4)_2SO_4$, 3 g l^{-1} KH_2PO_4, 0.5 g l^{-1} $MgSO_4{\cdot}7H_2O$, 50 μl l^{-1} PPG2000, 1 ml of trace element solution, and 1 ml of vitamin solution.

Composition of trace element solution (per liter of solution): 40 g Titriplex III, 12 g $FeSO_4{\cdot}7H_2O$, 2.7 g $MnCl_2{\cdot}4H_2O$, 0.8 g $CoCl_2$(II)$\cdot 6H_2O$, 0.8 g $CuSO_4{\cdot}5H_2O$, 1.1 g $Na_2MoO_4{\cdot}2H_2O$, 12 g $CaCl_2{\cdot}2H_2O$, 8 g $FeSO_4{\cdot}7H_2O$, 2.7 g H_3BO_3 and 0.3 g KI.

Composition of vitamin solution (per liter of solution): 0.13 g vitamin H, 2.67 g Ca D(+) panthotenate, 2.67 g niacin, 66.67 g myo-inositol, 2.67 g vitamin B6 and 0.53 g $C_7H_6O_2$.

3.4. Fed-batch

Saccharomyces cerevisiae yeast has the particularity of producing ethanol in the presence of an excess of glucose. This so called Crabtree effect lowers substantially the biomass yield and hence has a major implication on industrial production of baker's yeast. This phenomenon is caused by a limitation of the oxidative metabolism rather by a limitation of the oxygen supply [13]. Therefore, to avoid ethanol accumulation, glucose excess should be avoided, yet a maximum possible growth rate should be aimed for. A continuous supply of glucose with an exponential feeding rate, correlated to the growth rate, has been performed to avoid ethanol accumulation. In this way a faster growth rate can be obtained while avoiding ethanol formation.

The following exponential equation was used to control glucose feeding rate

$$F = \frac{\mu X_0 V_0}{S Y_{X/S}} \exp(\mu t) = F_0 \exp(\mu t) \tag{26}$$

where F is the glucose feeding rate, μ the specific growth rate (= 0.15 h^{-1}), X_0 the initial biomass concentration of the fed-batch (= 17 g l^{-1}), V_0 the initial volume of the fed-batch (= 1.3 l), S the substrate concentration (= 80 g l^{-1} of glucose), and $Y_{X/S}$ the biomass yield (= 0.55 cmol (cmol)$^{-1}$). This equation is based on a mass balance. Typical values of *Saccharomyces cerevisiae* found in literature [13] are applied here.

Cultures in the calorimeter reactor were performed at 30°C with a stirring rate of 600 rpm. Aeration was supplied at a constant rate of 100 l h^{-1} of air using a mass flow controller (Model 5850 TR, Brookes Instrument, BV, Veenendal, NL). Air entering the reactor was sterilized with a filter of 0.2 μm pore size (Gelman Sciences, Michigan, USA). A pH of 4 was maintained constant by the automatic addition of 4 M NaOH using a pH controller (Bioengineering AG, Wald, Switzerland).

3.5. Analysis

Dissolved oxygen was monitored using a polarometric probe (Ingold, Urdorf, Switzerland). Exhaust-gas was analyzed for carbondioxide and oxygen using a gas analyzer (Bioengineering AG), with infrared (Binos100, Fischer-Rosemount, Baar, Switzerland) and paramagnetic (Servomex, Esslingen, Switzerland) detectors.

Dry weight was determined by filtration of 5 μl culture samples through 0.8 μm pore size pre-dried filters (Gelman Sciences, Michigan, USA), followed by incubation of the filters in a microwave oven (MioStar, Switzerland) at 150 W for 15 min, and subsequent cooling in a dessicator, before re-weighting. Cell-free samples were analyzed for ethanol and glucose by HPLC.

4. Results and discussion

A series of preliminary experiments are performed with the aim of investigating the behavior of the ORC method under different working conditions, i.e. the viscosity and the volume of the reactive mass.

In these series of experiments the viscosity of the reaction mass and the reactive volume are varied step-wise. For each set of parameters, an electrical calibration is performed to determine the stationary value of UA, Eq. (3), both in presence and in absence of temperature oscillations. The stationary value of UA, determined by electrical calibration, in absence of temperature oscillations is the reference value and it is denoted by "UA_{ref}". The stationary value of UA, determined by electrical calibration, in presence of temperature oscillations is denoted by "UA_{osc}". Modeled, non-stationary values of UA are calculated based on determination of τ and either via the one-anchor or the two-anchor model developed here. For the one-anchor method, "$UA_{one\text{-}anchor}$", the initial pair of values (UA_{osc}, $1/\tau$) is used. In the case of changing viscosity, for instance, this means the pair of values (UA_{osc}, $1/\tau$) evaluated at the lowest viscosity. For the two-anchor method, "$UA_{two\text{-}anchor}$", the initial and final pairs of values (UA_{osc}, $1/\tau$) are used. In the case of changing viscosity this means the pairs (UA_{osc}, $1/\tau$) evaluated at the lowest and at the highest viscosity.

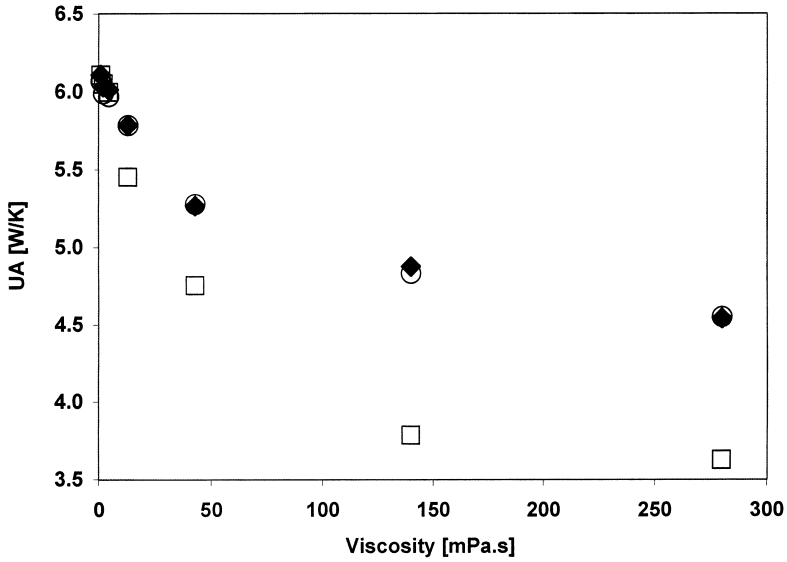

Fig. 4. Global heat transfer coefficient, UA, as a function of reaction mass viscosity. Stationary calibration values: in absence of temperature oscillations UA_{ref} (○) and in presence of temperature oscillations UA_{osc} (◆). Modeled values $UA_{one\text{-}anchor}$ (□), where the anchor point is at 1 mPa s. Experimental conditions are the following: a reaction volume of 1.5 l (different concentrations of polyethylene glycol 4000 in water), a working temperature of 28°C and a stirring speed of 500 rpm.

4.1. ORC under different viscosity conditions

Different values for the reaction mass viscosity are obtained by different concentrations of polyethylene glycol 4000 (Fluka No. 81240) in water.

The stationary values, evaluated in presence and in absence of temperature oscillations, correspond well, Fig. 4. UA_{osc} deviates randomly from UA_{ref} within 1.5%. This deviation range is only slightly higher than the repeatability of UA_{ref} stationary calibration, which is within 1%.

As expected $UA_{one\text{-}anchor}$ values increasingly deviate from UA_{osc} as the points are further away from the anchor point, which is at 1 mPa s, Fig. 4. The same trend is observed for $UA_{one\text{-}anchor}$ evaluated with the anchor at 280 mPa s (results not shown). This can be perfectly understood from Fig. 5. In this figure, C_{Pr} has been calculated from the stationary values of UA_{osc} at each point with Eqs. (24) and (3). It is clear that C_{Pr} is not constant, it increasingly deviates from the anchor point (1 mPa s).

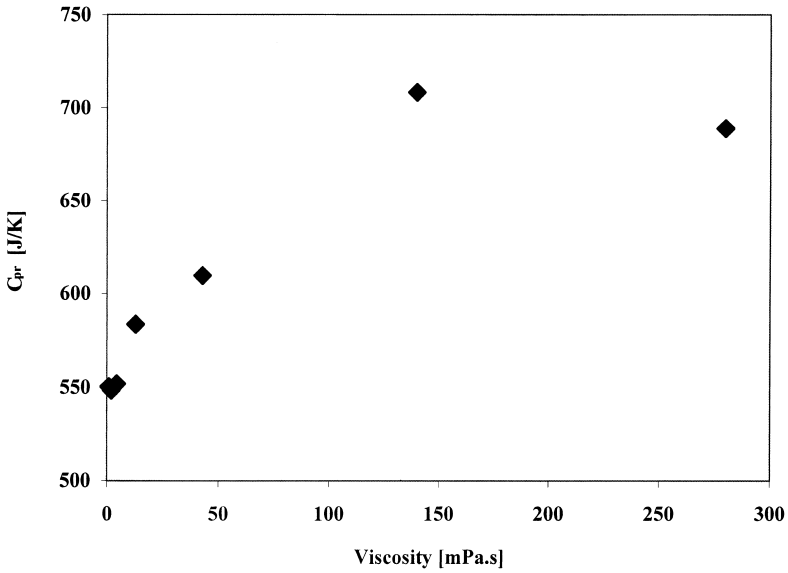

Fig. 5. Integral heat capacity of reactor contents, C_{Pr}, as a function of reaction mass viscosity. C_{Pr} values are calculated via Eqs. (24) and (3). Experimental conditions are the following: a reaction volume of 1.5 l (different concentrations of polyethylene glycol 4000 in water), a working temperature of 28°C, and a stirring speed of 500 rpm.

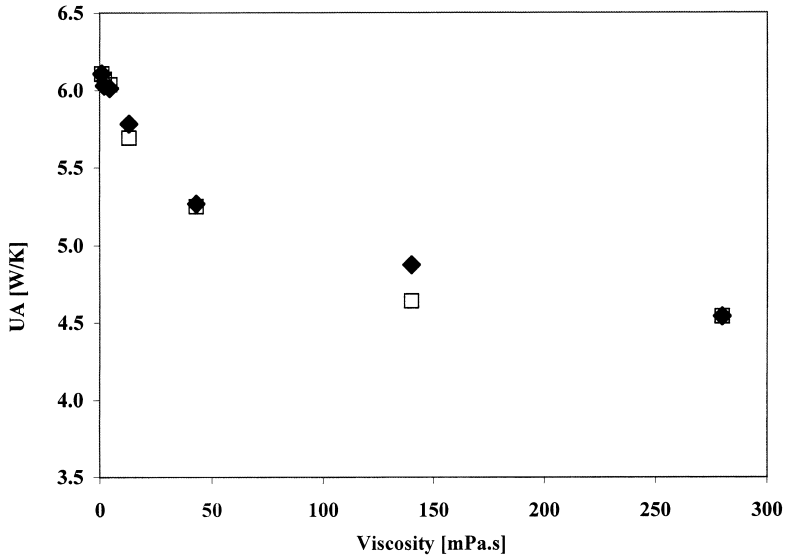

Fig. 6. Global heat transfer coefficient, UA, as a function of reaction mass viscosity. Stationary calibration values in presence of temperature oscillations UA_{osc} (◆). Modeled values $UA_{two\text{-}anchor}$ (□), where the initial and final anchor points are at 1 and 280 mPa s, respectively. Experimental conditions are the following: a reaction volume of 1.5 l (different concentrations of polyethylene glycol 4000 in water), a working temperature of 28°C, and a stirring speed of 500 rpm.

In contrast, $UA_{two\text{-}anchor}$ values correspond better to UA_{osc} (within 2.5% deviation), Fig. 6, over the whole range of viscosity studied. This can be explained by a fairly linear relationship between UA_{osc} and $1/\tau$, Fig. 7, which enables the application of Eq. (25) for the continuous determination of $UA_{two\text{-}anchor}$. Although, the need of a "final" anchor point preludes the use of this model as an on-line method for the determination of UA, the two-anchor method can be successfully applied for a post-reaction continuous determination of UA, throughout the reaction, in the range of viscosity studied.

Repeating the measurements at different stirring speeds reveals similar trends, Fig. 8. By increasing the viscosity of the reaction mass the value of UA decreases, Figs. 4, 6 and 8, as predicted from the film theory. With increasing viscosity the mass thickness of the boundary layer, at the reactor wall, increases resulting in an increase of the resistance to the heat transfer and hence in a decrease of UA. For the same

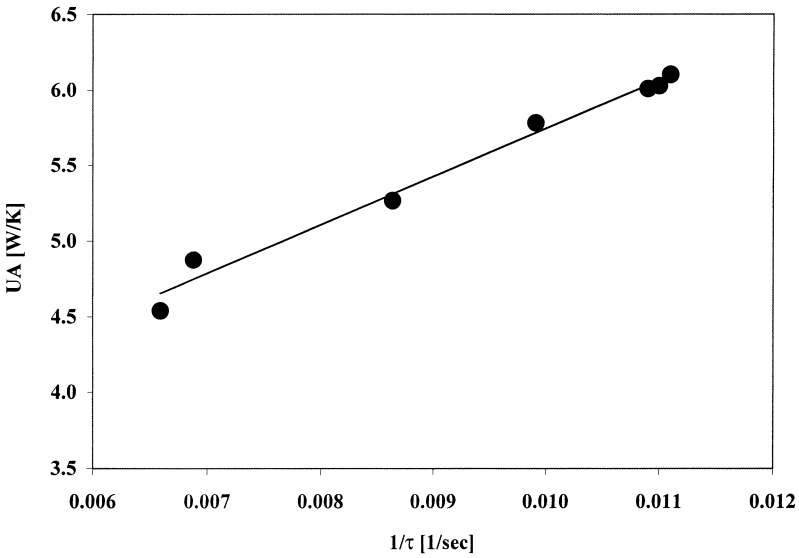

Fig. 7. Global heat transfer coefficient, UA, as a function of the inverse of the time constant ($1/\tau$). Experimental conditions are the following: a reaction volume of 1.5 l (different concentrations of polyethylene glycol 4000 in water), a working temperature of 28°C, and a stirring speed of 500 rpm.

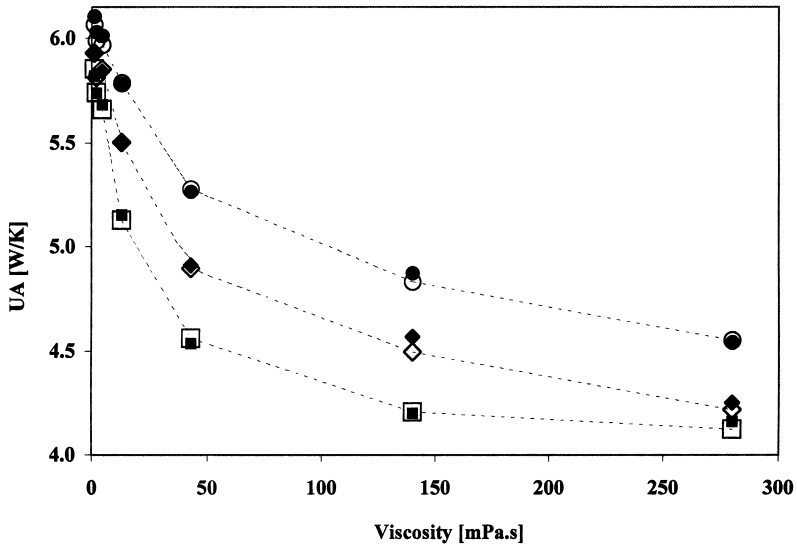

Fig. 8. Global heat transfer coefficient, UA, as a function of reaction mass viscosity at different stirring speeds. 500 rpm (○ and ●), 300 rpm (◇ and ◆) and 150 rpm (□ and ■). Stationary calibration values in presence of temperature oscillations UA_{osc} (plain symbols). Modeled values $UA_{two\text{-}anchor}$ (open symbols), where the initial and final anchor points are at 1 and 280 mPa s, respectively. Experimental conditions are the following: a reaction volume of 1.5 l (different concentrations of polyethylene glycol 4000 in water), and a working temperature of 28°C.

viscosity, on the other hand, UA increases with increasing stirring speed as expected.

4.2. ORC under changing reactive mass volume

This experiment is carried out with water at 28°C. Reactive volume is increased from 1380 to 1530 ml by step-wise addition of water at 30°C with 30 ml per step.

$UA_{two\text{-}anchor}$ is continuously evaluated with the initial and final anchor points, respectively, at 1380 and 1530 ml. $UA_{two\text{-}anchor}$ follows the step-wise change of volume, Fig. 9, showing a linear relationship between them. In fact, as expected in the present case, a change in volume only causes a change in the area of heat transfer. Therefore, UA changes linearly with a reactive volume change, provided that *U* remains constant.

With the exception of the big peaks at the beginning of every step, the differences between the continuously modeled $UA_{two\text{-}anchor}$ and the stationary reference values, UA_{ref}, are clearly below the 1.5% standard deviation

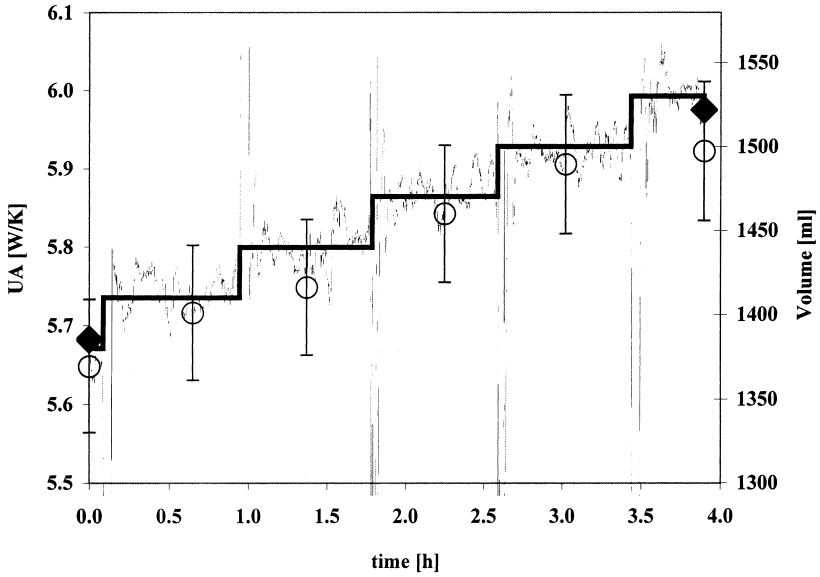

Fig. 9. Global heat transfer coefficient UA under varying reaction volume conditions. Modeled values $UA_{two\text{-}anchor}$ (thin line), where the initial and final anchor points are at 1380 and 1530 ml, respectively. Stationary calibration values are shown: in absence of temperature oscillations UA_{ref} (○) and in presence of temperature oscillations UA_{osc} (◆). Error bars on UA_{ref} represent 1.5% error. The two UA_{osc} values are the anchor points for $UA_{two\text{-}anchor}$. Reaction volume (bold line).

of the reference calibration (see error bars in Fig. 9). The system needs a certain time to stabilize after an abrupt change in volume; especially if the liquid added has a different temperature than the reactive mass. Indeed, by injecting water at a different temperature, the reactor temperature is disturbed and consequently the amplitude of the reactor temperature oscillation. As a result, τ is disturbed, as it is continuously calculated from the amplitudes of the temperature oscillation in the jacket and in the reactor, and therefore, $UA_{two\text{-}anchor}$ is also disturbed.

It is interesting to note that the "two-anchor" method is sensitive even to small volume variations, i.e. 30 ml. This method can be successfully applied to the continuous determination of UA under changes in the reactive mass viscosity and volume, with a deviation from the stationary values, UA_{ref}, below 2.5%.

4.3. ORC during a fed-batch culture of Saccharomyces cerevisiae

In this section the ORC method, as developed here, is applied to a fed-batch culture of *Saccharomyces cesevisiae* in order to determine its applicability to complex biological reactions. The fed-batch culture is performed with a non-linear change in the reaction mass, as a result of which UA changes in a non-linear fashion during the culture. This type of experiment was chosen to enable comparing the continuous UA values measured by the ORC method with values obtained by stationary calibration after correcting the latter approximately for the volume change.

UA_{osc} stationary values are determined at the beginning and at the end of the fed-batch culture. These two-anchor points are situated before and after the non-linear addition of glucose, i.e. at the initial volume of 1300 ml and, after adding 300 ml, Fig. 10. After the supply of glucose with a feeding rate given by Eq. (26) some time is allowed to the system to stabilize, before performing the final stationary calibration. Hence, the two-anchor points are situated at an experimental time of 0 and 18 h, respectively.

The $UA_{two\text{-}anchor}$, continuously modeled, follows the trend of reactive volume, Fig. 10, with only slight deviations. The peaks bigger than the 1.5% error bars are disturbances due to sampling. From the preceding sections, a variation of UA of about 0.6 W/K can be expected from a variation of 300 ml of reactive volume. The variation of UA observed here is around 0.45 W/K. By sampling throughout the fed-batch the reactive volume decreased by some 65 ml. Furthermore, small volume variations are expected from NaOH addition (for pH control purposes) and medium evaporation. These uncontrolled volume variations together with the controlled feeding account for the increase of UA observed of 0.45 W/K instead of 0.6 W/K. It appears that the variation of UA accompanying the fed-batch is mostly due to changes in volume and therefore in the heat transfer area. This is not surprising since a cell concentration of less than $20\,g\,l^{-1}$ of *Saccharomyces cerevisiae* is not expected to change significantly the viscosity of the medium.

Fig. 11 shows a detail of the heat production rate obtained during the fed-batch. Because the reaction volume increases during the reaction, Q_R calculated with the modeled $UA_{two\text{-}anchor}$ yields as expected higher values (by about 4%) than the one calculated with the conventional stationary method (where UA_{osc} is obtained via the initial electrical calibration), bold and dotted lines in Fig. 11, respectively.

The use of a linear regression between both stationary values of UA (initial and final electrical calibrations) would give a straight line between these two points, which does not correspond to the real evolution of UA, Fig. 10. The

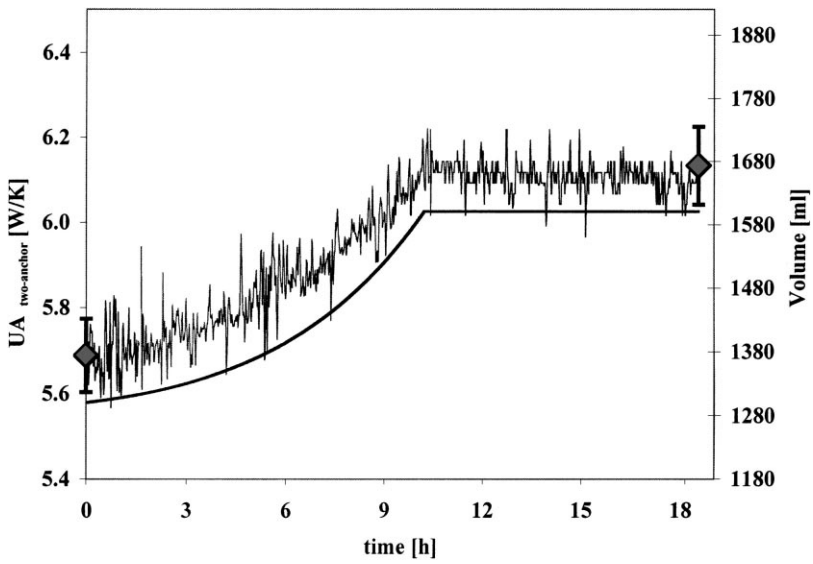

Fig. 10. Modeled global heat transfer coefficient $UA_{two\text{-}anchor}$ (normal line) and reaction volume (bold line) during a fed-batch culture of *Saccharomyces cerevisiae*. Stationary calibration values in presence of temperature oscillations, UA_{osc} (◆), are shown with error bars representing 1.5% error.

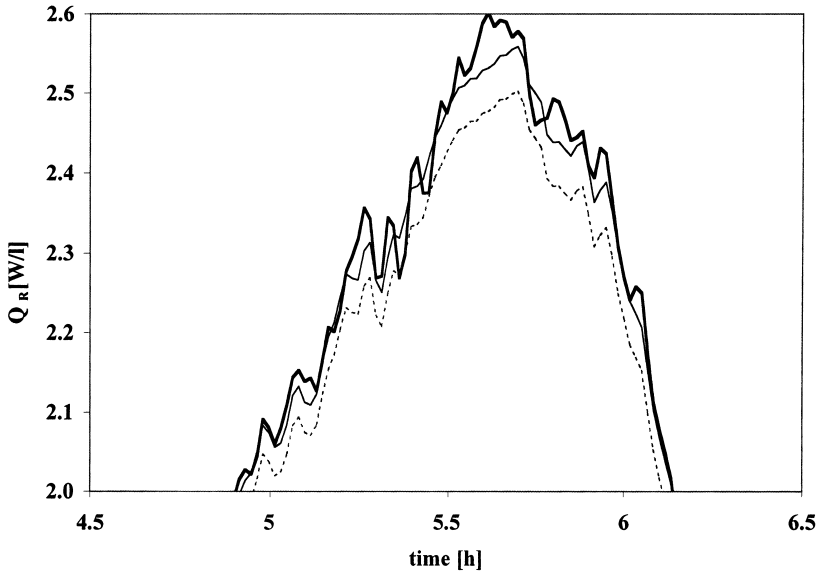

Fig. 11. Comparison of reaction power output during a fed-batch culture of *Saccharomyces cerevisiae*: Q_R calculated with the modeled value $UA_{two\text{-}anchor}$ (bold line). Q_R calculated with the conventional technique using the initial stationary calibration (doted line). And Q_R calculated with an UA (initial stationary calibration) corrected for a modeled change in volume throughout the reaction (normal line).

monitoring of all volume changes, which is technically cumbersome, would be necessary in order to obtain a correct description of UA throughout the culture. The knowledge of the dependence factor of UA on volume would also be necessary in this last case.

For comparison, a modified UA has been calculated by correcting the initial UA_{osc} by an approximate volume change (the exact volume change with time is unknown because of minor factors that were only determined at the end of the culture like evaporation and base addition). The Q_R calculated with this modified UA matches well the Q_R obtained with the modeled $UA_{two\text{-}anchor}$, thus validating this technique.

For about the first 4 h of the fed-batch, Fig. 12, ethanol accumulation could be avoided by a controlled supply of glucose. In fact, during this period glucose concentration is below the detection limit, indicating that all supplied glucose is consumed. After this point, glucose starts accumulating resulting in the immediate production of ethanol, which in turn reduces the growth rate of yeast. As a result, the dry weight shows hardly any increase. Finally, just before 6 h of fed-batch culture all metabolic activity drops dramatically, (see Q_R curve in Fig. 12), and dry weight decreases. These last events are probably due to a limitation of nutrients, different from glucose, since after the batch culture the reactive volume was lowered from 1600 to 1300 ml and

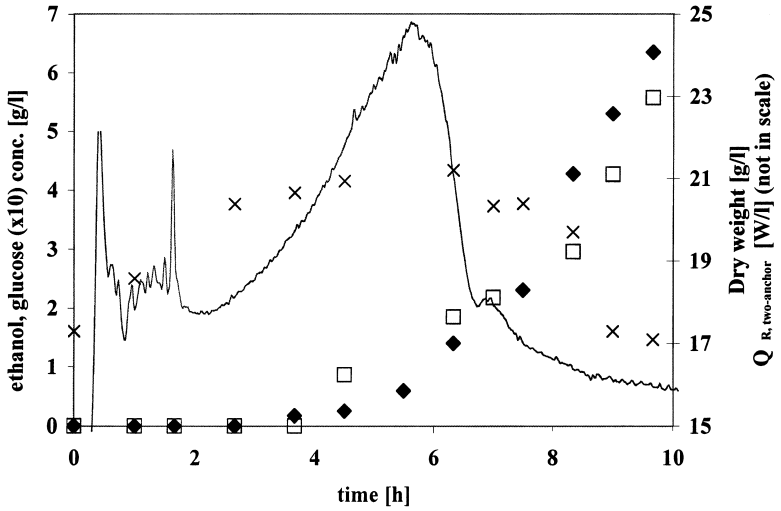

Fig. 12. Fed-batch culture of *Saccharomyces cerevisiae*. Reaction power output Q_R (line). Q_R is calculated with the modeled value $UA_{two\text{-}anchor}$. Although Q_R is given in W/l it is not in scale. Dry weight (×). Concentration of ethanol (◆) and of glucose (□). This last one is shown multiplied by 10.

only glucose was supplied during the fed-batch. Nevertheless, the effect of volume variation on UA and consequently on Q_R could be observed. Furthermore, the applicability of the ORC method, as described here, to a complex biological reaction could be demonstrated.

5. Conclusion

Calibration in calorimetry represents a crucial and unavoidable step in measuring the heat power output of reactions and the further thermodynamic considerations. The modeling ORC method as presented here is powerful in that it allows the continuous determination of UA without making use of any physical or chemical constant of the system, nor requiring the monitoring of influencing parameters, i.e. viscosity, working volume, stirring, etc. To our knowledge, the ORC method is unique in this respect; all other conventional methods either make an intelligent guess of UA outside the stationary calibration values, or require the cumbersome monitoring of all influencing parameters so as to subsequently correct the stationary calibrated value of UA.

A new version of ORC was developed which eliminates the error arising from measuring the relevant heat capacity term only once and assuming it to stay constant as the reaction proceeds (so-called "one-anchor" method). The new so-called "two-anchor" method is based on measuring the relevant heat capacity twice, at the beginning and at the end of the run, and on adjusting the on-line calibration of the overall heat transfer coefficient accordingly after the experiments. In order to avoid any disturbances of the reaction kinetics by the oscillating reactor temperature, oscillation periods as short as 2 min were used in this work. This was possible owing to the highly efficient temperature control system of the RC-1.

Test runs in which the heat transfer coefficient was modified by changing the viscosity of the reactor contents from 1 to 280 mPa s, by changing the stirrer speed and the by modifying the reaction volume in steps by a total of about 10% showed that the overall heat transfer coefficients obtained by the two-anchor method agreed with the standard electrical calibrations to within the standard measurement error of the latter.

The new method was also successfully applied to a weakly exothermic process such as is typical in biotechnology. During the fed-batch culture of yeast, which was chosen as a test system for this work, the increase in reaction volume by about 18% resulted in a non-negligible modification of the overall heat transfer coefficient. Although the factors affecting the reaction volume during the culture were complex, it was still possible to come up with an approximate estimation of the culture volume as a function of time and to correct the classically calibrated heat transfer coefficient for the volume change as a basis for comparison. These corrected heat transfer coefficients agreed indeed quite well with the ones obtained by the new ORC method.

In conclusion, the new ORC method has been shown to be sensitive enough for use in biotechnology. It should prove highly valuable in cases where the factors affecting the overall heat transfer coefficient cannot be monitored, such as in fed-batch cultures with changing viscosity and/or with a tendency for wall cell growth, or as in chemical polymerization reactions.

Acknowledgements

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Appendix A. Rate of heat production or consumption by the reaction under temperature oscillations

For a first order reaction we have

$$r = k_0 \exp\left(\frac{-E}{R\bar{T}_r}\right) c \tag{A.1}$$

where r is the reaction rate, c the concentration, k_0 the pre-exponential factor and E the activation energy.

The rate of heat production (or consumption) by the reaction is given by

$$Q_R = rV(-\Delta H_R) \tag{A.2}$$

where V is the volume of the reaction mass and ΔH_R is the reaction enthalpy.

The development of the Arrhenius relation as a first order Taylor polynomial gives

$$r = k_0 \left\{ \exp\left(\frac{-E}{R\bar{T}_r}\right) + \frac{E}{R\bar{T}_r^2} \exp\left(\frac{-E}{R\bar{T}_r}\right) (T_r - \bar{T}_r) \right\} c \tag{A.3}$$

Therefore, the equation describing the rate of production or consumption of heat by the reaction, in presence of temperature oscillations, is obtained by multiplying Eq. (A.3) by $V(-\Delta H_R)$. This equation is the sum of a constant term $\bar{Q}_R$ and an oscillating term $\tilde{Q}_R$

$$Q_R(\bar{T}_r) = Q_R(\bar{T}_r) + \frac{E}{R\bar{T}_r^2} Q_R(\bar{T}_r)\tilde{T}_r = \bar{Q}_R + \tilde{Q}_R \tag{A.4}$$

Hence

$$\tilde{Q}_R = \frac{E}{R\bar{T}_r^2} Q_R(\bar{T}_r)\tilde{T}_r = \frac{E}{R\bar{T}_r^2} Q_R(\bar{T}_r)\delta T_r \exp(i\omega t + i\rho) \tag{A.5}$$

A.1. Heat flow rate due to feeding under temperature oscillations

Many chemical reactions require the supply of reactants — substrate, acid or base — to the process. This is also

true in biotechnology where the use of a fed-batch type process is very common. Indeed, metabolites are often added in a controlled way so as to avoid inhibition by substrates, or to avoid the production of undesired byproducts due to an over-concentration of a substrate (Crabtree effect).

A heat flow rate due to feeding is caused by the heating or cooling of the feed stream while getting in contact with the reaction mass.

$$Q_\mathrm{d} = \dot{m}_\mathrm{d} c_\mathrm{Pd} (T_\mathrm{d} - T_\mathrm{r}) \tag{A.6}$$

where $\dot{m}_\mathrm{d}$ is the mass flow rate of the feed stream, c_Pd the specific heat capacity of the feed stream and T_d the temperature of the feed stream.

Therefore, in presence of temperature oscillations

$$\begin{aligned} Q_\mathrm{d} &= \dot{m}_\mathrm{d} c_\mathrm{Pd} (T_\mathrm{d} - \bar{T}_\mathrm{r}) - \dot{m}_\mathrm{d} c_\mathrm{Pd}\, \delta T_\mathrm{t} \exp(\mathrm{i}\omega t + \mathrm{i}\rho) \\ &= \bar{Q}_\mathrm{d} + \tilde{Q}_\mathrm{d} \end{aligned} \tag{A.7}$$

A.1.1. General energy balance equation under temperature oscillations

The energy balance of those terms contributing to temperature oscillation is

$$\tilde{Q}_\mathrm{acc} = \tilde{Q}_\mathrm{f} + \tilde{Q}_\mathrm{R} + \tilde{Q}_\mathrm{d} \tag{A.8}$$

$$\begin{aligned} C_\mathrm{Pr}\, \mathrm{i}\omega\, \delta T_\mathrm{r} \exp(\mathrm{i}\omega t + \mathrm{i}\rho) &= \mathrm{UA}[\delta T_\mathrm{j} \exp(\mathrm{i}\omega t) \\ &\quad - \delta T_\mathrm{r} \exp(\mathrm{i}\omega t + \mathrm{i}\rho)] + \frac{E}{R\bar{T}_\mathrm{r}^2} Q_\mathrm{R}(\bar{T}_\mathrm{r})\, \delta T_\mathrm{r} \exp(\mathrm{i}\omega t + \mathrm{i}\rho) \\ &\quad - \dot{m}_\mathrm{d} c_\mathrm{Pd}\, \delta T_\mathrm{r} \exp(\mathrm{i}\omega t + \mathrm{i}\rho) \end{aligned} \tag{A.9}$$

Simplifying $\exp(\mathrm{i}\omega t)$ in Eq. (A.9) and replacing $\exp(\mathrm{i}\rho)$ by Euler's formulae: $\cos\rho + \mathrm{i}\sin\rho$ gives

$$\begin{aligned} &\mathrm{UA}\, \delta T_\mathrm{j} - \mathrm{UA}\, \delta T_\mathrm{r}(\cos\rho + \mathrm{i}\sin\rho) \\ &\quad = C_\mathrm{Pr}\, \mathrm{i}\omega\, \delta T_\mathrm{r}(\cos\rho + \mathrm{i}\sin\rho) - \beta \bar{Q}_\mathrm{R}\, \delta T_\mathrm{r}(\cos\rho + \mathrm{i}\sin\rho) \\ &\qquad + \dot{m}_\mathrm{d} c_\mathrm{Pd}\, \delta T_\mathrm{r}(\cos\rho + \mathrm{i}\sin\rho) \end{aligned} \tag{A.10}$$

The real part of Eq. (A.10) is given by

$$\mathrm{UA} = \frac{-C_\mathrm{Pr}\omega\, \delta T_\mathrm{r} \sin\rho - \beta \bar{Q}_\mathrm{R}\, \delta T_\mathrm{r} \cos\rho + \dot{m}_\mathrm{d} c_\mathrm{Pd} \delta T_\mathrm{r} \cos\rho}{\delta T_\mathrm{j} - \delta T_\mathrm{r} \cos\rho} \tag{A.11}$$

and the corresponding imaginary part is given by

$$\mathrm{UA} = \frac{\omega C_\mathrm{Pr}}{\tan\rho} - \dot{m}_\mathrm{d} c_\mathrm{Pd} + \beta \bar{Q}_\mathrm{R} \tag{A.12}$$

A.1.1.1. Importance of the heat flow rate due to the reaction and to the feeding related to temperature oscillations. The heat flow rate of reaction can be considered to have no influence on the temperature oscillation, and therefore on the determination of UA, under the following conditions, determined from Eq. (A.12)

$$\frac{\beta \bar{Q}_\mathrm{R}}{\omega C_\mathrm{Pr} - \beta \bar{Q}_\mathrm{R}} < 0.05 \tag{A.13}$$

where $\beta = E/R\bar{T}_\mathrm{r}^2$.

The influence of Q_d on temperature oscillations, and hence on UA determination, can be analyzed in the same way. The heat flow rate related to feeding can be considered to have no influence on the temperature oscillation, and therefore on the determination of UA, under the following conditions:

$$\frac{\dot{m}_\mathrm{d} c_\mathrm{Pd}}{\omega C_\mathrm{Pr} - \dot{m}_\mathrm{d} c_\mathrm{Pd}} < 0.05 \tag{A.14}$$

These conditions are generally met in biotechnology where feed flow rates are typically of the order of $0.3\,\mathrm{h}^{-1}$ per liter of culture volume or less, while normal values of C_Pr are in the order of $\mathrm{kJ\,K}^{-1}$.

Therefore, the influence of the feeding and the reaction power on the determination of UA can be neglected.

On the other hand, the influence of temperature oscillation on the reaction under study has been explored by Tietze [1]. An average radical polymerization reaction, at 50°C, has an activation energy of $84\,\mathrm{kJ\,mol}^{-1}$ and a reaction time constant τ_R, Eq. (A.15), of 5 min

$$\tau_\mathrm{R}^{-1} = k_0 \exp\left(\frac{-E}{RT_\mathrm{r}}\right) \tag{A.15}$$

with these parameters and for a range in the amplitude and period of temperature oscillations of up to ±1 K and up to 20 min, respectively, the deviation in the time necessary to achieve a reaction turnover of 95% was found to be less than 1% [1]. This dependence is even smaller for higher reaction time constants. In biotechnology, typical reaction time constants are in the order of hours or even days: τ_R is around 2.8 h for *Saccharomyces cerevisiae* yeast and around 33.3 h for *Sf9* insect cells (calculated from a specific growth rate, μ_max, of 0.35 and $0.03\,\mathrm{h}^{-1}$, respectively).

Since the oscillations amplitude and period of T_r obtained in this work are 0.01 K and 2 min, respectively, see Fig. 2, and because of the high reaction time constants under consideration, the effect of temperature oscillation on the reaction is negligible.

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