Model-Based Optimization of Equipment and Control for Heat Flux Measurements in a Laboratory Fermentor

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The measurement of microbially-produced heat in standard laboratory fermentors was studied with the help of a mathematical model describing the heat flows. The improvements that were indicated by the modeling work were implemented in the experimental setup. Tests showed that both the standard deviation in the heat measurement and the response time for the experimental setup improved through use of the modeling results.

Introduction

The availability of sensitive and stable temperature probes and the rapid development of powerful personal computers have opened new possibilities for the quantitative determination of microbial heat production as a tool for the analysis of growth and metabolism in small laboratory fermentors (van Kleeff et al., 1993). This heat production can be calculated from an energy balance over the fermentor, and the data can then be used like other independent measurements, to obtain information about the process. A review of the methods used on laboratory scale fermentors has been published (von Stockar and Marison, 1989). The methods described usually rely on a constant heat transfer coefficient from heat exchanger to broth. Since this transfer coefficient can be changed by wall growth, we decided to calculate heat production by making an energy balance over the entire fermentor.

It is obvious that the need to measure heat production places demands on the fermentor and the temperature control system. The system is also restricted by the need to keep the temperature within biological limits. This dual requirement for accurate and rapid heat flux calculations, together with adequate temperature control, must be considered in the design of the fermentor and its control system.

In order to design such a system, an understanding of the various heat flows to and from the fermentor vessel, and of the temperature control equipment, is necessary. This paper describes the laboratory setup and the construction and testing of a dynamic model describing the fermentor and its associated temperature control system, in order to gain such understanding.

Experimental Setup

A schematic drawing of the fermentor and the temperature control system is shown in Figure 1. The fermentor was a well-stirred vessel containing 1.5 L of growth medium. It was aerated via a sparger and equipped with a cooled condenser on the gas outlet to minimize vapor losses. Because of aeration and the cooling of the condenser, the overall energy balance over the fermentor is negative at the beginning of a batch process (extra heat has to be supplied). Near the end of a batch process, however, microbial heat production is high, and heat must be removed to keep the temperature within limits. The temperature control system must therefore be reversible. Earlier workers solved this problem by using a cooling finger that could partly be balanced by an electrical heater (Luong and Volesky, 1980). This approach relies on a constant heat transfer coefficient for the cooling finger. Since it was intended that the current setup would also be used for processes involving heavy wall growth, a different approach was taken.

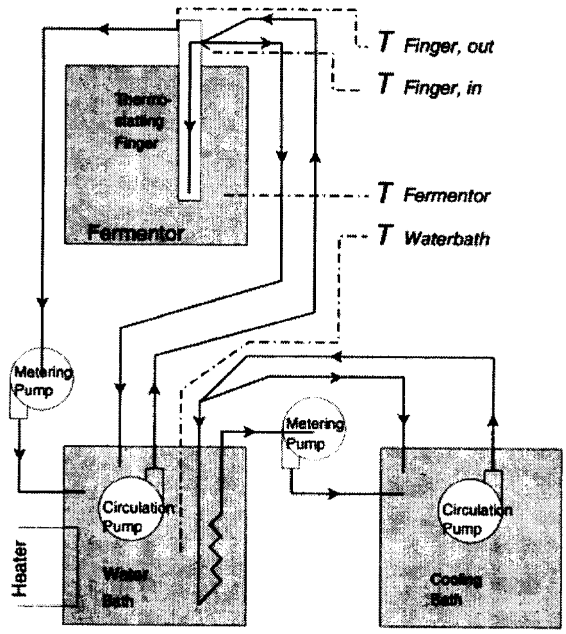

Figure 1. Layout of the experimental system. The water bath and fermentor parts were connected physically by the liquid flow through the finger and logically because the fermentor temperature was used as input for the algorithm that calculates the set point for the water bath temperature.

The temperature of the fermentor was controlled by means of a tubular heat exchanger (thermal finger) fitted in the fermentor lid. A measured and controlled water flow circulated to and from an otherwise closed water

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bath (Haake FE2) through this finger. Since the temperature of the water entering and leaving the finger was also measured, the heat flow to the fermentor could be calculated, regardless of the heat transfer coefficient from the thermal finger to the culture. The water bath (Haake FE2) had reversible heating and constant cooling circuits, thus allowing rapid variation of its temperature and rapid changes in the temperature of the water entering the thermal finger.

To minimize transport time delays, the tubing connecting the water bath and thermal finger was constructed as a full flow bypass circuit wherever possible. The flow *through* the thermal finger was kept constant with a peristaltic pump. The controlled variable was the temperature of the water bath which was set by a control algorithm using the fermentor temperature as the measured variable. For this experimental setup the control algorithm was implemented in a PC which allowed testing of various control algorithms. This PC also carried out other tasks, including the registration of all measurements of flows and temperatures. The measurements were stored at least every 4 s and could be evaluated either on-line or off-line.

Control Problem. The principle aim of these experiments was the accurate and rapid measurement of the biologically-produced heat ($Q_{process}$) as opposed to the strict control of the fermentor temperature. Actually a minimum variance controller is desired, giving a minimum variance in the calculated $Q_{process}$.

As mentioned in the introduction, heat production can be calculated from an energy balance (van Kleeff et al., 1993). This dynamic balance was made for the system comprising the fermentor and the thermal finger. For this system, the balance can be written as

$$(dT_{fermentor}/dt)\text{heat capacity}_{fermentor} + (dT_{thermal\ finger}/dt)\text{heat capacity}_{thermal\ finger} = Q_{background} + Q_{disturbance} + Q_{control} + Q_{process} \quad (1)$$

with $Q_{background}$ representing the total of all energy flows that can be considered to be constant during the experiment. $Q_{background}$ is obtained from calibration before the experiment (van Kleeff et al., 1993). The main components are $Q_{condenser}$ and $Q_{stirrer}$ with typical values of -2 and $+1.5$ W (at 600 rpm), respectively. $Q_{disturbance}$ is the total of nonconstant energy flows that can be calculated from temperature and flow measurements (van Kleeff et al., 1993) but cannot be controlled. The main component is the heat exchange with the environment. $Q_{control}$ represents the heat flow that is manipulated to keep the fermentor temperature within limits. $Q_{control}$ is the heat flow exchanged between growth medium and thermal finger. This heat flow is calculated from the temperature difference between finger inlet and outlet and the liquid flow through the finger. $Q_{process}$ is the heat produced by the (biological) process. This is the thermal flux of interest.

The energy balance only contains one element ($Q_{control}$) which can be influenced by the choice of the control system. In general, $Q_{control}$ will counteract deviations of the fermentor temperature from its set point. If the fermentor temperature is very rigidly controlled, large control actions will be needed with large variations (several degrees) in the desired water bath temperature. However, the measured fermentor temperature is contained within process and measurement noise, causing large variations in $Q_{control}$. Since $Q_{control}$ is one of the components of the heat balance, the calculated $Q_{process}$ will have a large standard deviation during a tightly controlled thermal steady state. If, on the other hand, the fermentor temperature is loosely controlled, a change in any of the heat flows might cause a large deviation of the fermentor temperature from its set point. This could result in lower activity or even the death of the microorganisms. It is generally assumed that microorganisms will tolerate temperature variations of up to 0.5 K without significant changes in the overall stoichiometry of their metabolism. From experience it can be predicted that $Q_{process}$ will have short-term variations of up to 4 W.

To sum up, the requirements for the temperature controller are as follows: (1) A change in $Q_{process}$ of up to 4 W should not cause the fermentor temperature to deviate more than 0.5 K from its set point. (2) Within this temperature band, the standard deviation in the calculated $Q_{process}$ must be minimized.

An additional requirement for further research in which $Q_{process}$ could be used in research into microbial processes was the following: The speed with which $Q_{process}$ can be calculated should be at least comparable with the speed of other continuous conversion measurements, such as off-gas analysis.

Modeling the Fermentor and Temperature Control Equipment. The energy models for the fermentor and the thermostat are shown in Figure 2A,B. According to the standard practice in control engineering (Dorf, 1992), Laplace transforms have been used in these block diagrams. The advantage of Laplace transforms is that the differential equations that describe the system can be presented as algebraic equations.

Two of the main components of the system, the fermentor and the water bath were well-stirred and could be modeled as continuously stirred tank reactors. The thermal finger was small and could be modeled as a plug flow reactor. Since the temperature difference between the inlet and outlet of the thermal finger was small, its average temperature, T_{finger}, could be taken to be the mean of inlet and outlet temperatures.

The water flow, F, between the water bath and the thermal finger was equal to the return flow from the finger to the bath. Both volumes could therefore be considered to be constant, connected by a time delay caused by the connecting tubing. Since ϱ and C_p are both also constant, the energy balance for the water bath is then written as:

$$V_{water\ bath}\frac{dT_{water\ bath}}{dt} = \quad (2a)$$

$$\frac{1}{\varrho C_p}Q_{element} + \quad (2b)$$

$$\frac{1}{\varrho C_p}Q_{circulation\ pump} + \quad (2c)$$

$$F(T_{return} - T_{water\ bath}) \quad (2d)$$

Here T_{return} is the temperature of the water returning from the finger to the water bath and $Q_{element}$ is the algebraic sum of the heat flows due to the switched electrical heater and the cooling coil in the water bath, which is constantly cooled by a controlled amount of cooling water. This cooling water serves partly to compensate for the constant generation of heat by the circulation pump $Q_{circulation\ pump}$ of the water bath and partly to reduce the temperature of the water bath if necessary. The numbers of the corresponding equation parts are also given in the block diagram (Figure 2A,B).

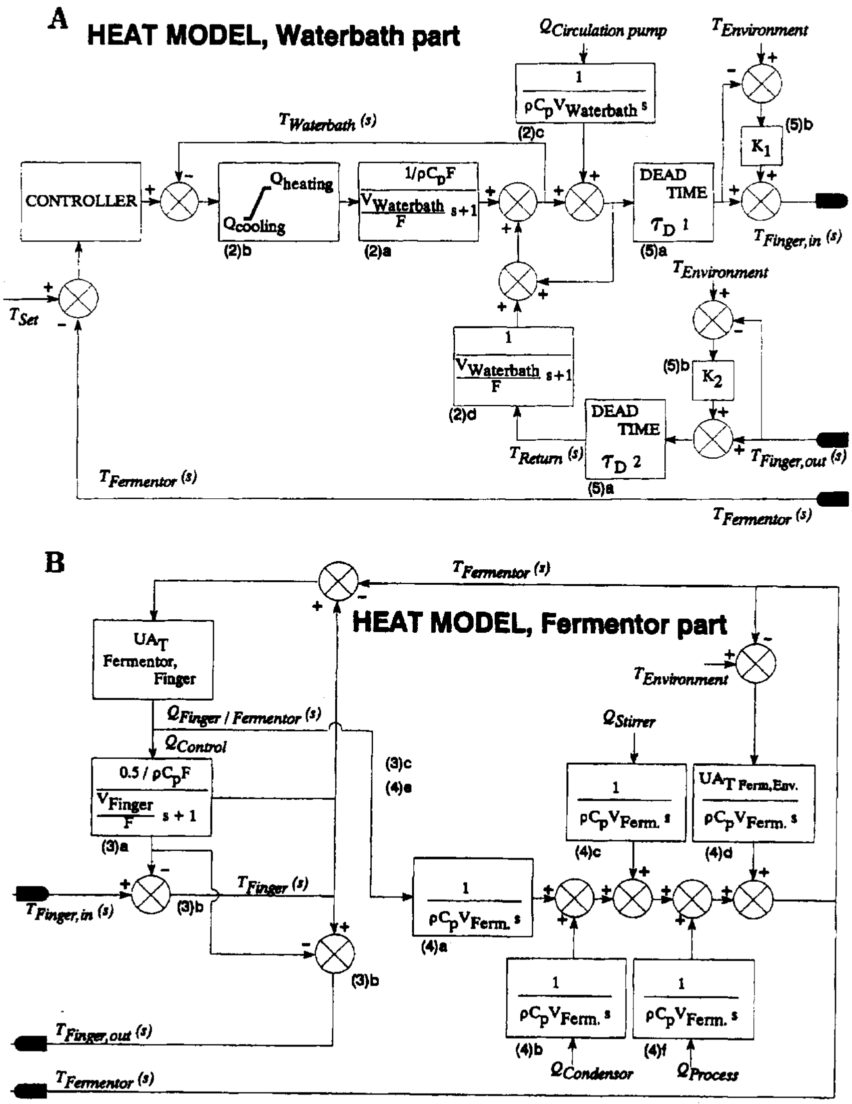

Figure 2. Heat model shown as a block diagram with Laplace transforms. The connections between the water bath (A) and the fermentor (B) are indicated by the tabs on the left and right of the figure, respectively.

In a similar way, the energy balance for the thermal finger is written as

$$V_{\text{finger}}\frac{dT_{\text{finger}}}{dt} = \qquad (3a)$$

$$F(T_{\text{in}} - T_{\text{out}}) + \qquad (3b)$$

$$\frac{UA_{\text{T,fermentor,finger}}}{\varrho C_{\text{P}}}(T_{\text{fermentor}} - T_{\text{finger}}) \qquad (3c)$$

Here the last term represents the heat exchange between fermentor and finger, which is also Q_{control}. This term can be calculated on-line because the inlet and outlet temperatures of the finger and the liquid flow through the finger are measured. This calculation is independent of the value of UA_{T}, (heat transfer coefficient multiplied by heat transferring surface), and therefore not influenced by wall growth.

The energy balance for the fermentor is written as

$$V_{\text{fermentor}}\frac{dT_{\text{fermentor}}}{dt} = \qquad (4a)$$

$$\frac{1}{\varrho C_{\text{P}}}Q_{\text{condensor}} + \qquad (4b)$$

$$\frac{1}{\varrho C_{\text{P}}}Q_{\text{stirrer}} + \qquad (4c)$$

$$\frac{UA_{\text{T,fermentor,environment}}}{\varrho C_{\text{P}}}(T_{\text{environment}} - T_{\text{fermentor}}) + \qquad (4d)$$

$$\frac{UA_{\text{T,fermentor,finger}}}{\varrho C_{\text{P}}}(T_{\text{finger}} - T_{\text{fermentor}}) + \qquad (4e)$$

$$\frac{1}{\varrho C_{\text{P}}}Q_{\text{process}} \qquad (4f)$$

Since all the terms in this equation are either constant

($Q_{condenser}$ and $Q_{stirrer}$) or can be measured on-line, $Q_{process}$ can be calculated on-line.

In the model, the interconnecting tubes are represented as a time delay, τ_D (5a), with a typical value of 10 s.

The interconnecting tubes also cause some heat loss to the environment. Since they are short and well insulated, this effect is small and can be calculated by the simplified equation

$$\Delta T_{tube} = K(T_{water\ in\ tube} - T_{environment}) \qquad (5b)$$

where K is a lumped thermal transfer parameter. The typical value is very small (<0.01). It could only be estimated from experiments with long (10 m) lengths of insulated tubing.

Verification of the Model Describing Heat Exchanges for the Fermentor. The relationships derived in the preceding section could be used to compose a flow diagram of the fermentor setup, showing each term as a separate component. This model was also translated into the simulation language, PSI/e (BoZa, P.O. Box 264, 5670 AG Nuenen, The Netherlands). The various parameters in the model were measured and entered in the computer model for use in the simulations. Relevant parameters are shown in Table 1.

The characteristic times of the model and the real system were found to be very similar. For small signals, the response of the model was also similar to the response of the real system. For larger signals, however, small modifications to the model were necessary.

(1) The cooling of the water bath by the flow from the cooling bath could not be modeled as a constant cooling but (as in the real system) had to be modeled as proportional to the temperature difference between the (thermal control) water bath, which had a varying temperature, and the cooling flow from the cooling bath, which had a constant low temperature. The cooling could be modeled as

$$Q_{cooling} = 2(T_{water\ bath} - T_{cooling\ bath})$$

Here the factor 2 is a lumped thermal transfer parameter for the heat exchange between the bulk of the water bath and the constant cooling coil. This approximation held in practice for a water bath temperature from 15 to 35 °C.

For a temperature difference of 25 K, this gave a cooling effect of 50 W. This was in agreement with the experimentally-derived value.

(2) The heating of the water bath could not be modeled at the value that was found from voltage and current measurements but had to be modeled somewhat lower, probably because of heat losses from the electrical heater to the environment.

The responses of the model and the experimental system to a large heat pulse are shown in Figure 3A,B.

Table 1. Relevant Parameters Used in the Model

parameter	symbol	value	units
broth volume	$V_{fermentor}$	1.5	L
finger volume	V_{finger}	30	mL
water bath volume	$V_{waterbath}$	2.5	L
water bath cooling	$-q_{cooling}$	$-2(\Delta T)$	W
water bath heating	$q_{heating}$	166	W
heat transfer coefficient fermentor-finger	$UA_{T,fermentor,finger}$	2.36	W K^{-1}
time delay in tubing	τ_D	10	s
liquid flow through finger	F	variable	L s^{-1}

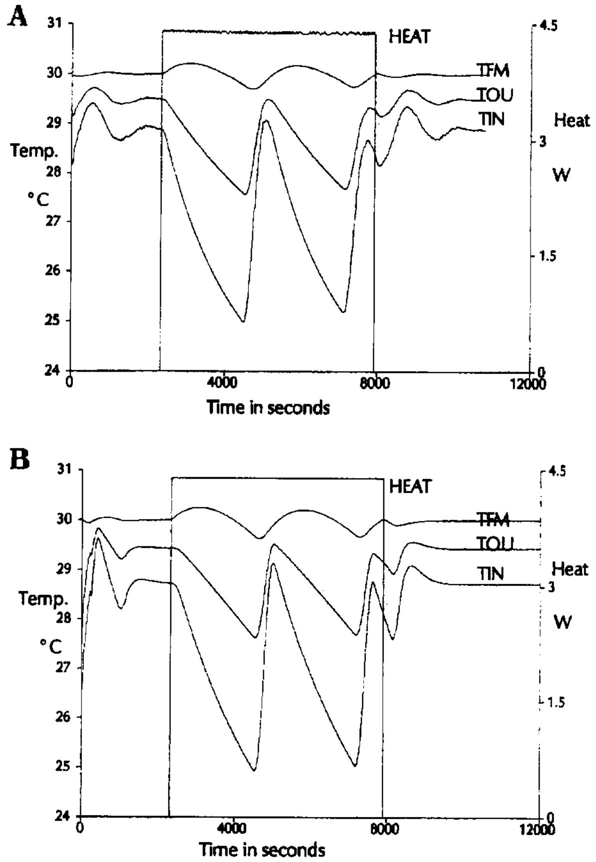

Figure 3. Response of the experimental system (A) and the mathematical model (B) to a disturbance. A heat pulse of the same magnitude and duration was used for both cases (TFM = broth temperature, TIN = temperature of liquid flow into the finger, TOU = temperature of liquid flowing out of the finger, HEAT = extra heat).

Simulations

Noise in the Calculated $Q_{process}$. During the simulations, proportional plus integral (PI) or proportional plus integral plus derivative (PID) control algorithms were used. Since $T_{fermentor}$, $\int(dT_{fermentor}/dt)$, and $(dT_{fermentor}/dt)$ all contain measurement and process errors, this algorithm introduces noise terms related to these errors into the temperature of the water bath and thus into $Q_{control}$.

From the energy balance, it follows that the standard deviation in the calculated $Q_{process}$ is dependent on the standard deviation in the measured fermentor temperature, as well as on the standard deviation in $Q_{control}$. Moreover, the measured liquid flow through the thermal finger was not constant, but also contained noise. Since $Q_{control}$ is calculated by multiplying the liquid flow through the finger by the temperature difference over the thermal finger, the standard deviation in the calculated $Q_{process}$ is also influenced by the noise in these temperature measurements.

Experimental measurements showed that the noise in the temperature measurements was not influenced by the actual temperature (the standard deviation had a constant absolute value). In contrast, the standard deviation in the liquid flow had both a relative component (roughly 0.5% of the liquid flow) and an absolute component (roughly 0.01 mL s^{-1}).

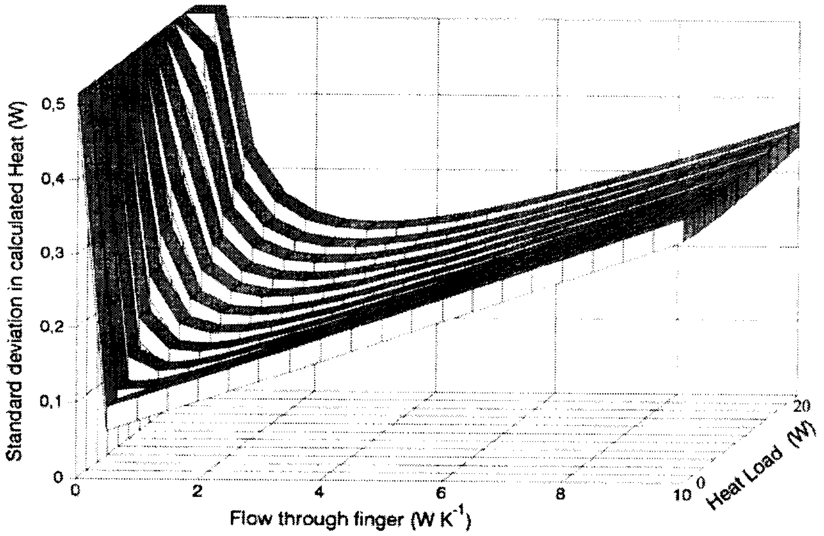

Figure 4. Influence of the flow through the finger on the standard deviation in the calculated heat for various heat loads in the fermentor. The flow is expressed as the product of volume flow, specific density, and specific heat, to make the figure independent from the nature of the liquid (water or thermal oil).

Minimizing the Noise. The simulation model was used to investigate various possibilities of minimizing the noise in the calculated $Q_{process}$. In addition, equipment modifications could be analyzed. The experimental design allowed the manipulation of both the liquid flow through the thermal finger and the controller design.

To investigate the noise in the calculated $Q_{process}$, the model was supplemented with blocks that generated noncorrelated normal distributed noise. For temperature measurements, the noise was added to the calculated values; with the liquid flow measurements, the relative component was multiplied by the liquid flow. The absolute component was added to the liquid flow.

Initially, the worst noise values found for the individual measurements in typical experiments were taken as values for the noise blocks in order to provide a "worst case" prediction. (The mean values used for the noise were the following: fermentor temperature 0.0001 K; finger temperature, 0.005 K; liquid flow, 0.5% relative plus 0.01 mL s^{-1} absolute. It should be remembered that the noise values given do not indicate that the absolute temperatures could be measured with this accuracy, but rather that a the measurements of a constant temperature were contaminated with this noise.) Simulations were done in which $Q_{process}$ was calculated from the noisy "measurements", and the standard deviation in the calculated value was determined.

Effect of Varying the Thermostatic Liquid Flow. The influence of varying the liquid flow through the thermal finger is shown in Figure 4 for different values of the total heat amount generated in the fermentor. Three points should be noted with regard to these results:

(1) As mentioned before, the stirrer will dissipate energy into the fermentor, leading to a high heat load. This heat must be compensated for by $Q_{control}$, which will cause an additional ΔT between the finger inlet and outlet, influencing the relative value of the standard deviation in ΔT. Since $Q_{stirrer}$ is large at higher stirring rates, the total heat load may be much larger than $Q_{process}$. $Q_{stirrer}$ may contain noise, but as this noise cannot be influenced by the temperature control system, it was not separately included in the model.

(2) PI controllers were used for these modeling experiments. These had settings that had been found by the methods described by Ziegler and Nichols (1942) in which the (simulated) system was brought to the border of instability using only a proportional controller (see below).

(3) The liquid flow through the finger is given in this figure as the product of the volume per second, the specific density, and the thermal properties of the liquid. This makes the results independent of the nature of the liquid (either thermal oil, brine, or water). For water, 4.1819 W K^{-1} equals 1 mL s^{-1}.

From Figure 4, it is clear that there is an optimal liquid flow through the finger for different thermal loads. Very slow liquid flows through the finger will increase the standard deviation in the calculated value for $Q_{process}$. This is easily understood, since with very small flows the absolute noise component in the flow becomes relatively large. When much faster liquid flows were used, the calculated standard deviation rose again. This is also easily understood because the temperature difference between finger inlet and outlet then becomes very small. The relative increase in the standard deviation in this temperature difference then increases the standard deviation in the calculated $Q_{process}$.

From the simulations, it was predicted that a liquid flow value around 4.2 W K^{-1} could be expected to work well, even with high heat loads from excessive stirring speeds. In practice, this value (1 mL of water s^{-1}) had already been used with good results.

Controller. The next stage was to investigate whether the form and setting of the controller could be used to minimize the standard deviation in the calculated $Q_{process}$ by appropriately selecting the P, I, and D parameters of the controller in the model.

In order to maintain the link with the experiments, the control properties of the equipment were determined by the methods of Ziegler and Nichols (1942). When the experimental system was brought to the border of instability using only proportional control action, a characteristic time T_{char} of 2800 s and a maximum amplification factor K_{max} of 150 were found.

According to the rules of Ziegler and Nichols (1942), this would give the following:

For a PID controller:

$$K = 90 \quad \text{(amplification)}$$

$$\tau_i = 1400 \text{ s} \quad \text{(integration time constant)}$$

$$\tau_d = 350 \text{ s} \quad \text{(derivative time constant)}$$

resulting in

$$\Delta T_{\text{water bath}} = 90(\epsilon + 0.064\int \epsilon \, dt + 350(d\epsilon/dt))$$

For a PI controller:

$$K = 67.5 \quad \text{(amplification)}$$

$$\tau_i = 2240 \text{ s} \quad \text{(integration time constant)}$$

resulting in

$$\Delta T_{\text{water bath}} = 67.5(\epsilon + 0.03\int \epsilon \, dt)$$

with ϵ representing the difference between fermentor temperature and its set point and $\Delta T_{\text{water bath}}$ representing the change in set temperature for the water bath calculated by the controller to counteract this difference.

The model was run with the noise generators as described above and with a simulated liquid flow of 4.2 W K^{-1} and a PID or PI controller. During these runs, the standard deviation in Q_{process} was calculated. Additional simulation runs were made using a step disturbance of 4 W to check whether the control algorithm was able to keep the temperature of the fermentor within the limits of ±0.5 K even with such strong disturbances.

These simulations confirmed that a PID controller is not suitable for the calculation of Q_{process} from the temperature and flow measurements with a reasonable standard deviation. The system (containing delay times) becomes unstable, demanding large control actions and thus giving a high standard deviation in the calculated Q_{process}. A PI controller proved to be more suitable, giving a reasonable standard deviation. The standard deviation in the calculated Q_{process} will not change significantly, even if the setting of the controller is changed from that derived using Ziegler and Nichols' rules of thumb.

Equipment Modifications. The mathematical model was also used as a tool to study the influence of hardware modifications of the equipment. Even hardware modifications that would not be readily feasible in practice can be tested in this way. Changing the thermal transfer surface of the thermostat finger by between 25% and 400% of its original value did not improve the standard deviation in the calculated heat and only slightly influenced the temperature overshoot. Alteration of the properties of the water bath only adversely influenced the standard deviation in the calculated heat and the temperature overshoot when unrealistic values (higher than 1000 W or lower than 10 W) of cooling capacity and heating capacity per liter were used. (The actual values are shown in Table 1.) Modifying the standard deviations in the simulated measurements indicated that a better measurement of the fermentor temperature would significantly improve the standard deviation in the calculated heat. On the basis of these results, it was decided to measure the effects of variations in the thermostat liquid flow and improved fermentor temperature measurements on the noise in the Q_{process} calculated from the measurements.

Comparing Simulation Results with the Real System. The influence of the liquid flow through the thermal finger was determined for a constant heat load of 2 W. The results of the actual experiments and the simulations are compared in Figure 5. It can be seen that the experimental setup performed better than the "worst case" simulation. Further simulations using noise values of 0.66 times the "worst case" were in excellent agreement with the experimental values. The actual experiments (Figure 5) also indicated a rather flat optimum for the liquid flow through the finger at about 1–4 W K^{-1} (4.2 W K^{-1} equals 1 mL s^{-1} if the liquid is water). It was clear from the simulations that the temperature measurements of the fermentor had to be improved because the noise in this measurement is multiplied by the large heat capacity of the fermentor. Close examination of the temperature measurements revealed that the noise was partly due to the conversion of the analog temperature signal to a digital value. The noise increased as the analog signal passed the steps of the analog to digital converter. To minimize this effect, the fermentor was equipped with four independent temperature sensors at different levels in the broth and the independent readings were averaged. Measurements indicated an improvement by a factor 2 (standard deviation = 0.000 05 K).

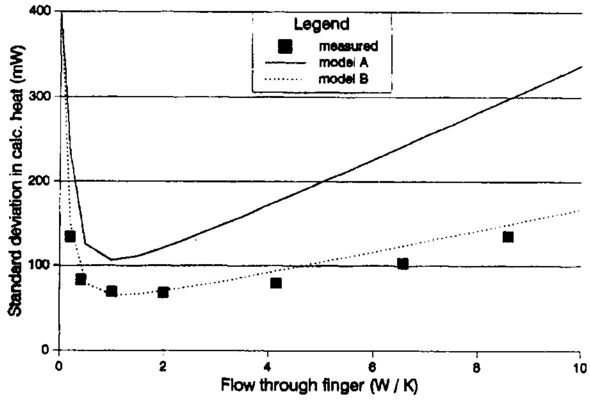

Figure 5. Comparison of the results of experiments and simulations. Model A: model with "worst case" noise. Model B: model with noise = 0.66 × "worst case" noise. "Worst case" noise = fermentor temperature 0.0001 K, finger temperature 0.005 K, liquid flow 0.5% relative plus 0.01 mL s^{-1} absolute.

Since all measurements were stored at least every 4 s, Q_{process} could be calculated from measurements that were averaged using a moving window. With this technique the average of measurements is taken during a time interval. This time interval moves along with the experiment time. Off-line it is possible to take the average of readings that were taken before and after the experiment time and so include readings that were not yet known at this time of evaluation. On-line, it is only possible to use a moving window which holds only the measurements done before the time of evaluation. Therefore, if this averaging technique is used on-line, it will introduce a time delay in the readings.

Figure 6 shows the results of an off-line calculation where a moving window of 20 s was used, centered around the time of evaluation. In this experiment, an electrical heat source of 4.134 W was switched on at time = 0. A PI control algorithm was used. As predicted by the model, the temperature of the fermentor was kept well within the bounds of ±0.5 K from the set temperature, even with this strong disturbance.

Figure 7 shows the trade-off between rise time (speed of response) and standard deviation for a number of different averaging window sizes. During this experi-

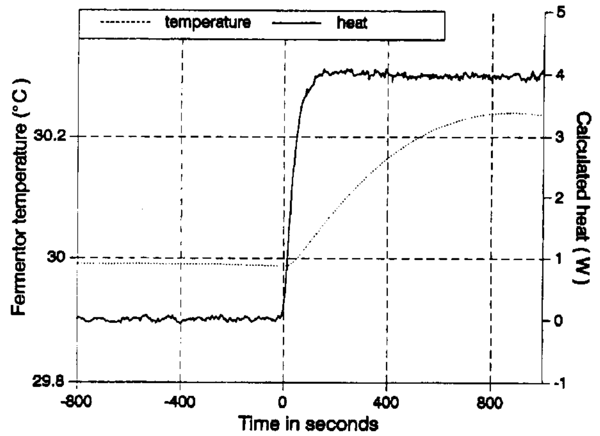

Figure 6. Results of an experiment where a known electrical heat source of 4.134 W was switched on at time = 0. The heat was calculated from measurements taken every 4 s and averaged in a 20 s moving window, centered around the time of evaluation. This experiment was done using the improvements in software and hardware that were suggested from the modeling work.

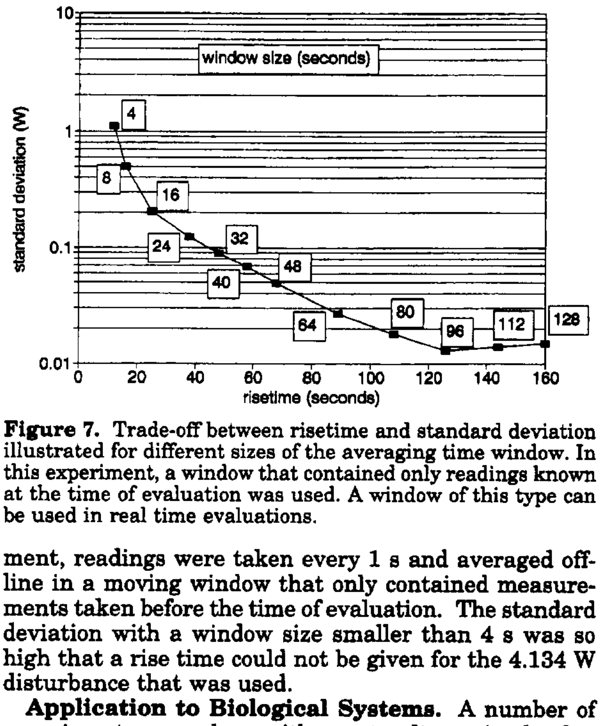

Figure 7. Trade-off between risetime and standard deviation illustrated for different sizes of the averaging time window. In this experiment, a window that contained only readings known at the time of evaluation was used. A window of this type can be used in real time evaluations.

ment, readings were taken every 1 s and averaged off-line in a moving window that only contained measurements taken before the time of evaluation. The standard deviation with a window size smaller than 4 s was so high that a rise time could not be given for the 4.134 W disturbance that was used.

Application to Biological Systems. A number of experiments were done with yeast cultures in the fermenter/calorimeter. A continuous culture of *Saccharomyces cerevisiae*, running in steady state at a dilution rate of 0.05 h^{-1} to which a glucose pulse was added, is given as an example. Figure 8 shows the response of the oxygen uptake and the calculated heat production of the culture to the glucose pulse. Both values were corrected for the steady state values; thus only the extra effects were measured. From Figure 8 it is clear that both curves have roughly the same shape. The first phase, extending to 1500 s after the glucose pulse, is the period where the excess glucose is metabolized partly through fermentation with ethanol formation and partly oxidized. The second phase is the period where the ethanol formed is further oxidized to water, carbon dioxide, and acetate. The acetate formed is further oxidized to water and carbon dioxide during this same period. This reaction

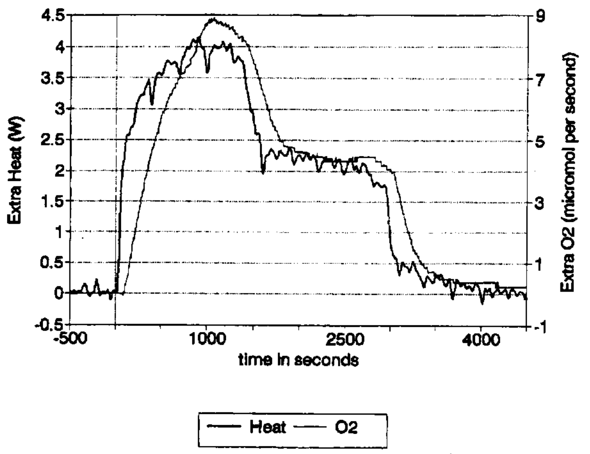

Figure 8. Responses of the heat signal and oxygen uptake signal after addition of a glucose pulse to a continuous culture of *Saccaromyces cerevisiae*. The shape of the curves is similar, but the oxygen signal lags behind.

sequence is well-known for *Saccharomyces cerevisiae* and was checked during this experiment in samples that were taken every 300 s (analyses not shown). It is also clear in Figure 8 that the response of the heat signal is much faster than that of the oxygen signal. This effect is shown even better in Figure 9, where only the first 350 s of the experiment are used. The lag time of the oxygen signal is roughly 60 s longer than that of the heat signal. This delay is due to liquid–gas exchange phenomena, mixing in the head space of the fermentor, and transport of the gas to the analyzers. Since there is no way to correct for lag times in real-time situations, it is clear that the heat signal is the preferable signal in a control system. Moreover, due to mixing in the head space of the fermenter, the initial slope of the oxygen signal is low, suggesting—erroneously—a gradual rather than an immediate response of the yeast.

A check on the consistency of the two signals could be made by integrating them over the entire pulse. (This was also done for biomass, products, carbon dioxide, and oxygen to check on the carbon and oxygen balances; they were found to close within 5%.)

The result of this integration is given in Figure 10. Again this figure shows that the heat signal is running ahead of the oxygen signal. The quotient of the two final values was 481 kJ/mol of oxygen uptake (heat quotient). According to the rule of thumb given by Roels (Roels, 1983) the quotient should be 460 kJ/mol of oxygen uptake. Both values agree within 5%. The agreement was even better when it was taken into account that some of the heat formation was due to alcoholic fermentation which has an infinite heat quotient, while part of the ethanol, which has a low heat quotient, was washed out during the experiment because the dilution rate of the continuous culture was maintained.

Discussion and Conclusions

A mathematical model describing heat flows around a fermentor is a useful tool for understanding the various factors that influence the dynamics and accuracy of the heat flux measurement. The results of the modeling indicated the most suitable settings for the water flow through the thermal finger and the selection of the controller and its setting. The model also pinpointed instrumental modifications that would give significant improvement in the results of the experiments. Predictions, resulting from simulations in which the model was used, agreed well with the experimental results.

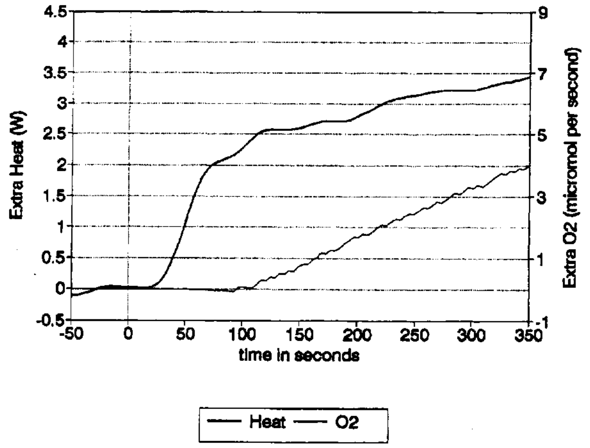

Figure 9. As in Figure 8, but the first 350 s of the experiment are shown. This gives an even clearer view of the high speed of the heat signal.

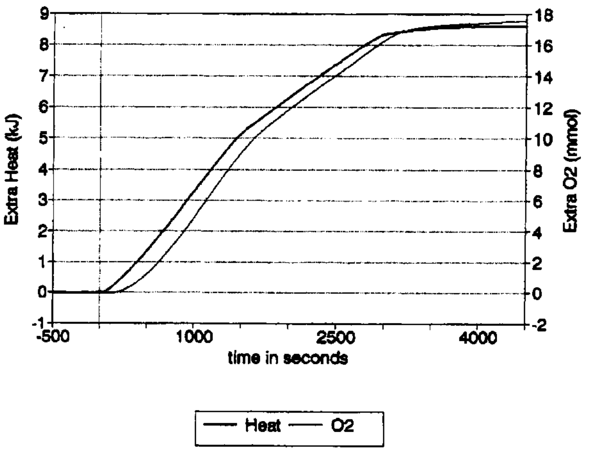

Figure 10. Both signals of Figure 8, integrated over the entire duration of the experiment. The influence of the lag times in the signals is eliminated for the end values. Division of the end values yields the heat quotient. The bend in both curves is due to the different forms of glucose metabolism in *Saccaromyces cerevisiae*.

Using the model allowed an improvement in the standard deviation of the results with this equipment from 0.2 W with an averaging time of 100 s and a characteristic time for a step response of >100 s published before (van Kleeff et al., 1993) compared to the final results shown in Table 2. The step response can be made faster if a shorter averaging time is used. However, process noise will then be more visible, the standard deviation in a thermal steady state will increase, and small heat signals will go unnoticed. In this respect, it should be noted that one single drop (0.05 mL) of cold (5 °C) water falling from the condenser into 1.5 L of warm (35 °C) broth (heat capacity = 6300 J K^{-1}) will give an almost instant temperature drop of 10^{-3} K. This effect, evaluated over 1 s, would equal the effect of a −6.3 W step change in process heat. A large averaging time is therefore necessary to diminish process noise. A similar effect can be expected in fed batch or continous cultures, if the growth medium is added as drops, which is usually the case in small fermentors. Conventional gas analyses cannot be expected to show the effect of this discontinuous feed regime because of mixing in the head space. Thermal measurements are fast enough to provide the choice of either averaging over short periods to get a fast, albeit noisy, response or using a longer averaging period to gain a more stable impression of the process.

Table 2. Characteristics of the Fermentor/Calorimeter before and after the Improvements Suggested by the Modeling Work

	before	after
averaging time needed (s)	100	20
standard deviation in calculated heat (steady state) (W)	0.2	0.05
characteristic time for step response (4 W) (s)	>100	20
temperature overshoot on 4 W step (K)	<0.3	<0.3

Finally, it can be concluded that the measurement of heat is well possible in routine laboratory fermentors. The speed and precision of the measurements compares favorably with that of conventional off-gas analysis. Heat measurement does not make extreme demands on the temperature control of the fermentor. It is, in fact, sufficient to control the temperature within biological bounds, using a simple PI controller.

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