Continuous Measurement of Microbial Heat Production in Laboratory Fermentors

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The possibility of continuously measuring the heat produced by microorganisms in an ordinary laboratory fermentor was studied. An inventory of the heat flows influencing the temperature of the culture was made. The magnitude and standard deviation in these heat flows were studied from theoretical and practical viewpoints. Calibration procedures were tested, and a model describing the heat flows in steady state and during dynamic conditions was made. Microbial heat production could be calculated accurately with the help of this model, appropriate temperature measurements, and equipment properties measured during the calibration procedures. It was found that the measurement of heat production could be done with an accuracy similar to that in the O2 uptake measurement. © 1993 John Wiley & Sons, Inc.

Key words: microbial calorimetry • heat of growth

INTRODUCTION

Heat production by biological systems has been studied by many investigators. Battley1 has given an overview of the earlier studies done on heat release during microbiological processes. These studies were generally carried out in specially constructed devices that were very different from the fermentors now used in biotechnological research.

In 1968, Cooney et al.3 introduced a method to measure the heat produced by a biological process in a laboratory fermentor. At intervals, the temperature control was switched off and the rate of temperature change in the broth was observed. Comparing this rate to that found during calibration with a known heat source, the heat produced by the process could be calculated. This method had the advantage that no additional equipment was required, and it could thus be used with standard laboratory fermentors. However, the measurements allow the temperature of the broth to change, and the monitoring is not continuous. The dynamics of heat production can only be followed for slowly changing processes.

In 1980, Luong and Volesky6 introduced a method for the continuous measurement of heat generation during fermentation. In this method, a fermentor was cooled at such a rate that an extra supply of heat was needed to keep the temperature of the broth from falling. This extra heat was supplied by a precisely controlled electrical heater. The heat generated during fermentation reduced the need for additional warming by an amount equal to this fermentation heat. From a balance of heat flows around the fermentor, the fermentation heat could be calculated. The cooling rate of the fermentor is the largest component in this heat balance and therefore must be very accurately known. However, wall growth and changing hydrodynamic conditions may seriously influence the heat transfer coefficient and this cooling rate.

In 1985, Marison and von Stockar7 introduced the use of a bench-scale calorimeter to measure fermentation heat continuously. These measurements were done in a jacketed reactor in which the temperature was kept constant by controlling the temperature of the jacket fluid. Heat transfer between jacket and reactor is proportional to the difference in temperature between the two and the product of the heat transfer coefficient (U) and heat transferring surface (A) of the wall between them. As with the method of Luong and Volesky, wall growth and changing hydrodynamic conditions may seriously influence the heat transfer rate, necessitating repeated calibration.

In addition to these in situ methods, studies have been made with instruments that measure heat production outside the fermentor. These methods have the disadvantage that the heat measurements are done under conditions different from those in the fermentor.

A more extensive recent review of both in situ and external heat measuring methods has been given by von Stockar and Marison.10

In summary, the following disadvantages have been found in the published methods:

1. In general, the authors do not measure all the components of the heat balance and do not explain why other components are not measured. A systematic overview of the various heat flows around the fermentor and their standard deviations has not been published to our knowledge.

2. Some of the methods rely on the invariance of a heat transfer coefficient that can be influenced by microbial wall growth or changing hydrodynamic conditions.

3. Little attention has been given to the dynamics of heat production and the accumulation of heat.

Measuring the heat produced by microbes is of interest from four points of view:

1. For purely scientific reasons. Since the law of conservation of energy must hold for biological processes,
heat measurements can provide thermodynamic data on biomass formation.10

2. For the study and control of biological processes, where no other measurements are available. Since heat is practically always produced during biological activity, heat production is an almost universally applicable method.10

3. For redundancy in measurements. Although heat production during aerobic processes closely follows oxygen uptake, the measurement of heat production can provide independent data to be used as a check on the oxygen uptake measurements or to improve the overall quality of measurements using a statistical approach.12

4. For the study of dynamic behavior in microbial cultures. Since microbial heat is directly released into the culture fluid, the effect of changes in behavior can be expected without a significant delay. Heat measurements thus have an advantage over off-gas analysis, which suffers from a delay in response due to tubing and fermentor head space.

The research reported here aimed to develop a method for the measurement of the rate of heat production in both steady state and dynamic biological processes, to be implemented with routine laboratory fermentors and without disturbing a running process. The method should be usable in long-term experiments where wall growth could influence heat transfer coefficients. The method should also be usable under dynamic conditions with changes faster than those found with the usual growth rates, where characteristic times are in the order of 10^3 s. If a characteristic time of 10^2 s or better is attained, the response time is comparable to that of outlet gas analysis. Measurement of heat production could then provide an extra tool for the monitoring of changes in the time range found for enzyme induction.5 In principle, the speed of the heat measurements is limited only by the speed and the noise of the temperature measurements.

A systematic approach was taken by first making an inventory of heat flows and then constructing a mathematical heat model of the culture to investigate the possibilities of using heat measurements. This model should then be used on-line to calculate the microbial heat production. This approach is made possible by the recent development of reliable and cheap computers and temperature sensors.

MATERIALS AND METHODS

Fermentation Equipment

Figures 1a and 1b show a schematic layout of the experimental setup.

All experiments and calculations were based on a glass laboratory fermentor (Applikon 31) insulated with 10 cm of polyurethane foam and running under the following conditions: broth volume 1.5 L, aeration rate 1 VVM (1.5 L/min), stirring rate 600 rpm, broth temperature 30°C, condenser temperature 5°C, and room temperature 20°C.

The fermentor vessel was double walled. The outer jacket (intended for temperature control) was filled with air, providing extra insulation. Earlier experiments, in which the jacket was used for thermal control, gave unsatisfactory results due to insufficient mixing and unpredictable accumulation of heat in the jacket fluid. The fermentor temperature was controlled by a water flow through a stainless steel thermal finger. The fermentor was placed in an insulating box and surrounded by metallic bubble foil. This bubble foil was grounded to serve as a Faraday cage, thus improving the quality of the electrical signals. The fermentor was equipped with sensors to measure the following temperatures: fermentor temperature (TFM), temperature of the water flow entering the thermal finger (TIN), temperature of the water flow leaving the thermal finger (TOU), and temperature outside the insulating box (TRM).

All temperature sensors were monitored by placing them in a bridge circuit. The voltage difference between the
measuring and reference points of the bridge was, after amplification, sampled with an analog-to-digital (A/D) converter (Scientific Solutions LabMaster). The temperature sensors were calibrated against a calibrated precision mercury thermometer. Values for sensor offset and slope were stored in an Olivetti M240 computer. All measured values were also stored and evaluated either on-line or off-line with programs written for this purpose.

The stirring speed (RPM) and the airflow through the fermentor (AFL) were monitored in a similar fashion. The water flow through the finger (WFL) was kept at a constant value with a peristaltic pump (ABC electronics TUDelft) and came from a water bath (Haake FE 2) whose temperature was monitored and controlled by the computer using a PI algorithm with the difference between the measured fermentor temperature and its set point as input.

**Microbial Experiment**

*Candida utilis* was batch grown in the experimental fermentor on a mineral medium with 10 g L⁻¹ of glucose. Outlet gas was analyzed for O₂ and CO₂ concentrations.

Glucose, ethanol, acetate, and biomass were determined in samples taken periodically (glucose by the Boehringer Mannheim GOD-Perid method; ethanol and acetate by gas chromatography; biomass by centrifuging in weighed Eppendorff vials, washing once with demineralised water, and drying overnight at 70°C).

**HEAT MODEL FOR A LABORATORY FERMENTOR**

**Inventory of Heat Flows**

The origin of and the magnitude and standard deviation in the heat flows were estimated. This revealed the flows which could be considered to be constant. These constant flows can be lumped to give the background heat flow. All other heat flows must be measured. A heat balance around the fermentor will then give the heat flow produced by the process running in the fermentor.

**Heat Balance, Theoretical Aspects**

For any system, a heat balance can be written as

\[ Q_{\text{heat}} = \Delta Q_{\text{heat}} + \Delta Q_{\text{heat}} + \Delta Q_{\text{heat}} + \Delta Q_{\text{heat}} + \Delta Q_{\text{heat}} \]

For the laboratory fermentor, this balance reads

\[
(dT_{\text{fermentor}}/dt) \cdot \text{Heat capacity}_{\text{fermentor}} + \text{Heat capacity}_{\text{finger}} = Q_{\text{background}} + Q_{\text{disturbance}} + Q_{\text{control}} + Q_{\text{process}}
\]

1. \(Q_{\text{background}}\) is the total of all heat flows considered to be constant during the experiment and which can be measured by calibration before the actual experiment.
2. \(Q_{\text{disturbance}}\) is the total of the nonconstant heat flows which can be calculated from measurements (e.g., liquid flow of additions and temperature of the environment). Since additions do not usually have the temperature of the fermentor, they contribute to the heat balance. Their contribution can be calculated from

\[
Q_{\text{additions}} = \Phi_{\text{additions}} \cdot \rho \cdot c_p \cdot (T_{\text{additions}} - T_{\text{fermentor}})
\]

3. \(Q_{\text{control}}\) is the heat flow that is manipulated to keep the process temperature within limits.
4. \(Q_{\text{process}}\) is the heat produced by the process. This is the component of the heat balance that links the biological or chemical processes within the fermentor with the heat balance. It can be calculated if all the other components are known.

During the biological or chemical processes, chemical energy can be converted into thermal energy and/or mechanical or chemical work. For the majority of microbial processes, the work performed is far less than the thermal energy released.

The energy balance for the process thus reads for the whole fermentor

\[
V \cdot (\Sigma \Delta H_i \cdot dc_i/dt) + Q_{\text{process}} = 0
\]

Here \(V \cdot \Sigma \Delta H_i \cdot c_i\) represents the total chemical energy present in the fermentor both as substrate and as biomass or product. If the process is a fed batch or continuous process, the balance is more complicated since substrate is added and biomass, together with products, may be removed. The extension, however, is straightforward.

A method to treat the energy balance together with the chemical or elemental balance was presented by de Kok and Roels.²

**Heat Balance, First Estimation of Contributions**

An overview of the major contributions to the heat balance [Eq. (1)], their magnitudes, and their standard deviations is given in Tables I and II.

**Accumulation Terms**

The accumulation terms can be calculated if the heat capacities of fermentor and thermal finger are known. The heat capacity of the fermentor consists of two parts: the heat capacity of the broth and the heat capacity of the glass vessel and the fermentor lid. The broth term can be found from \(V \cdot \rho \cdot c_p\). Under the experimental conditions this value was 6300 J K⁻¹. The heat capacity of the glass vessel could be calculated from the weight (2.5 kg) and the specific heat (840 J kg⁻¹) to be 2100 J K⁻¹. Approximately half of the glass (the inner wall of the jacket) will follow the broth temperature because it is insulated by the air in the jacket. The outer wall of the jacket will have a temperature between those of the broth and of the environment. The temperature of the stainless steel fermentor lid (with the attached thermal finger) closely follows that of the broth. Its heat capacity could be calculated to be 700 J K⁻¹.
The high multiplication factor, it is clear that the temperature of the fermentor, especially, should be measured accurately.

If the heat accumulation is evaluated over large time intervals, the standard deviation in the accumulation terms will be low, but the dynamic response will be slow. In the experiments reported here, the temperature was averaged every 100 s. A (routinely attainable) standard deviation of 0.6 \times 10^{-3} \text{ K} in TFM then gives a standard deviation of (0.6 \times 10^{-3}/100) \times 8050 = 0.05 \text{ W} in the accumulation term for the fermentor.

For the thermal finger, with its lower heat capacity, the standard deviation was better, although the temperature measurements were worse: (10 \times 10^{-3}/100) \times 120 = 0.012 \text{ W}. Here, too, it is clear that a shorter averaging period leads to a larger standard deviation.

**Flow Terms**

A study of the heat flows to and from the fermentor is necessary to determine whether they can be lumped in the constant $Q_{\text{background}}$, should be calculated from measurements and form part of $Q_{\text{disturbance}}$, or can be manipulated to provide $Q_{\text{control}}$. For practical purposes, the heat flow terms are treated according to their physical origin.

1. $Q_{\text{stirr}}$: The stirrer delivers energy into the broth. This energy is dissipated as heat. According to the literature\(^6\), this energy is proportional to the third power of the stirrer speed. The speed of a modern stirring motor will not vary by more than 1 rpm. The variation in $Q_{\text{stirr}}$ at 600 rpm is then less than 0.5%. Since $Q_{\text{stirr}}$ at 600 rpm was later found to be approximately 1.5 W, this variation is less than

\[
(6300 + 1050 + 700) \cdot dT_{\text{fermentor}}/dt + (120) \cdot dT_{\text{finger}}/dt = 8050 \cdot dT_{\text{fermentor}}/dt + 120 \cdot dT_{\text{finger}}/dt
\]
0.01 W. At constant stirrer speeds, $Q_{\text{stirrer}}$ can be regarded to be constant and thus part of $Q_{\text{background}}$.

If the stirring speed is varied (e.g., to control the dissolved oxygen tension), the speed must be monitored and $Q_{\text{stirrer}}$ calculated. In this case, $Q_{\text{stirrer}}$ must be treated as part of $Q_{\text{disturbance}}$. Since $Q_{\text{stirrer}}$ also depends on gas holdup and turbulence, its calculation is difficult if the gas flow is also changed. Preferably the operating conditions should be kept in a region where power uptake is only marginally influenced by changing gas flow and hydrodynamic conditions.

2. $Q_{\text{environment}}$: The fermentor exchanges heat with its environment by means of radiation, convection, and conduction. Radiation can be minimized by reflective layers. Convection and conduction can be minimized by thermal insulation.

Insulation with a thickness of 10 cm and thermal conductivity of 0.3 mW cm$^{-2}$ cm K$^{-1}$ around the surface of the fermentor (1500 cm$^2$) will permit a heat flow of 0.05 W K$^{-1}$. In practice, $Q_{\text{environment}}$ will be greater than expected because the sensors penetrate the insulation, providing thermal bridges. The temperature difference between environment and fermentor determines $Q_{\text{environment}}$. It must be calculated on the basis of measurements and calibration data. Here $Q_{\text{environment}}$ can be included in $Q_{\text{background}}$ if the temperatures of the fermentor and the environment are constant.

3. $Q_{\text{broth-finger}}$: The broth exchanges heat with the thermal finger through the wall of the finger. This exchange between finger and broth represents $Q_{\text{control}}$ since it was the only manipulated flow. It can be calculated from an energy balance over the finger:

$$Q_{\text{broth-finger}} = (T_{\text{IN}} - T_{\text{OU}}) \cdot W_{\text{FL}} \cdot 4.1819 \quad (5)$$

Although the heat transfer coefficient between broth and finger may vary, this does not influence the measured heat flow. Actually one could calculate this heat transfer coefficient from the measurements.

The inlet temperature of the thermal finger is controlled by controlling the temperature of the water bath from which it is taken. Inlet and outlet temperatures of the finger are measured, and the finger temperature is assumed to be the mean of the two. The measured temperatures therefore provide not only the heat flow term but also the accumulation term.

The standard deviation in the heat flow is dependent on both the standard deviation in the liquid flow and the temperature measurements. Simple calculations show that the standard deviation in $Q_{\text{control}}$ is different for different heat flows. The value of this standard deviation can theoretically be minimized if the liquid flow is varied.

4. $Q_{\text{cooldrop}}$ and $Q_{\text{gasflow}}$: The attachment point of the condenser on the lid of the fermentor makes a cold spot. Even when no gas is flowing through the fermentor, diffusion and convective transport of water vapor from the broth to this cold spot, and to the condenser itself, causes the loss of heat from the system. This effect is influenced by the mixing conditions in the fermentor head space (e.g., the presence of a foam paddle and the gas flow rate). If the gas flow rate, the mixing conditions, and the temperatures of the fermentor and the condenser are constant, the effect is constant and can be included in $Q_{\text{background}}$.

If gas is passed through the fermentor, the gas flow exchanges energy and matter with the broth. As pointed out in the literature, no heat effect due to the isothermal work performed by the gas stream is to be expected. During mixing with the broth, the gas assumes the temperature of the broth and becomes saturated with water vapor. The gas leaves the fermentor through the condenser, where it is cooled and where some of the water vapor condenses and returns to the fermentor as cold water.

The energy to warm the gas from room temperature to broth temperature can be found from $\rho$, $c_p$, and the gas flow to be 0.3 W. The evaporation energy needed to saturate the gas with water vapor is independent on the humidity of the inlet gas. Since the heat flow connected with the evaporation process is large, it must be known accurately. The inlet gas must therefore have a known humidity. This can be done by drying or saturating the gas completely or, as was done here, conditioning the humidity by passing the gas flow through a "dummy" fermentor with stirrer and condenser before it enters the fermentor used for the heat measurements.

From the partial pressures of water vapor in the conditioned air and at broth temperature, it can be calculated that 0.625 mg s$^{-1}$ of water evaporates from the broth. This evaporation requires a heat flow of 1.52 W. In the condenser, excess water vapor condenses and returns to the broth at a temperature of 5°C. To reheat this flow, 0.065 W is needed.

The total heat loss to the condenser and the cold spot will be greater than the calculated evaporation enthalpy, because of radiation losses and backmixing of partially dried gas from the cold spot into the broth. At constant mixing conditions in the head space and constant temperature and humidity of the gas flow, the standard deviation in these effects is only caused by variations in the gas flow rate. Using a mass flow controller for the gas flow, a standard deviation better than 0.05 L min$^{-1}$ can be expected in the flow rate of 1.50 L min$^{-1}$, giving a standard deviation in $Q_{\text{gasflow}}$ better than 0.06 W.

If temperature, humidity, gas flow, and mixing conditions can be kept constant, $Q_{\text{gasflow}}$ can be included in $Q_{\text{background}}$; otherwise $Q_{\text{gasflow}}$ is part of $Q_{\text{disturbance}}$.

4. $Q_{\text{process}}$: If a biological or chemical process is taking place, the heat balance consists of the terms described above and the term $Q_{\text{process}}$.

The heat balance for the standard fermentor then reads

$$\text{Accumulation rate} = Q_{\text{stirrer}} + Q_{\text{environment}} + Q_{\text{cooldrop}} + \Phi_{\text{fing}} \cdot c_p \cdot (T_{\text{IN}} - T_{\text{OU}}) + Q_{\text{gasflow}} + Q_{\text{process}} \quad (6)$$

From Table I, it follows that all of the terms in the heat balance can be calculated if a limited number of measure-
ments are made: the temperature of the fermentor (TFM), the temperature of the inlet of the thermal finger (TIN), the temperature of the outlet of the thermal finger (TOU), the temperature of the environment (TRM), the stirrer speed (RPM), the flow through the thermal finger (WFL), and the gas flow through the fermentor (AFL). In addition, a number of calibrations must be done linked to the $U \cdot A_t$ for the heat transport from fermentor to environment, the heat capacities of the fermentor and the thermal finger, the stirrer characteristics, and the effect of the cold spot.

CALIBRATION EXPERIMENTS

In these experiments, the thermal properties of the equipment were determined. The fermentor was stirred slowly and equipped with a controlled electrical immersion heater that could deliver an amount of heat $Q_{heater}$ into the broth.

Heat Capacity

The heat capacity of the fermentor could be estimated from the initial rate of temperature increase $dT/dt$ after switching on the heater: Heat capacity$_{fermentor} \cdot dT/dt = Q_{heater}$. The value found for the heat capacity (6500 J K$^{-1}$) was lower than the calculated value (8050 J K$^{-1}$), indicating that mainly the heat capacity of the broth was measured. Since this experiment closely mimics a transition in $Q_{process}$, the lower value for the heat capacity was accepted.

For the thermal finger, the calculated value of 120 J K$^{-1}$ was accepted.

$U \cdot A_t$

The $U \cdot A_t$ for the heat flow to the environment could be estimated from a series of experiments in which $Q_{heater}$ was stepwise increased. The difference between TFM and TRM was observed until a steady state was reached with the fermentor at the working temperature (30°C). An extra step in $Q_{heater}$ will then produce an extra step in TFM - TRM:

$$U \cdot A_t \cdot \Delta(TFM - TRM) = \Delta Q_{heater}$$

A value around 0.3 W K$^{-1}$ was found for $U \cdot A_t$. This value is far higher than expected, probably because of the thermal bridges formed by the sensors. This value was difficult to reproduce if the fermentor was taken from the insulation and replaced. Since the fermentor has to be taken from the insulation to be sterilized, a further calibration should precede each experiment. This calibration can be done during the period before inoculation as described under Zero Offset Calibration.

$Q_{coldspot}$ and $Q_{gasflow}$

The loss of heat through the condenser and its cold attachment point on the fermentor lid can be estimated by switching on the condenser cooling, observing TFM, and increasing $Q_{heater}$ until steady state is reached at the same TFM as before the condenser is cooled. A value of 1.8 W was found for $Q_{coldspot}$ without gas flow.

To determine the influence of gas flow, the experiment was repeated with gas flow. The gas flow was conditioned by sparging the gas through a "dummy" fermentor with medium, stirrer, and cooled condenser. During previous weight loss experiments without cooling the condenser of the "real" fermentor, only 90% of the theoretical evaporation losses were found. The cause of this is probably the condensation of a small part of the water vapor on the slightly colder glass wall in the head space of the fermentor vessel. Since condensation on the glass wall will return the enthalpy to the system, the same correction factor of 90% was adopted for the heat loss, giving a value of 0.9 $\times$ 1.52 = 1.37 W. Only the total heat loss, the combination of $Q_{coldspot}$ and $Q_{gasflow}$, could be measured by controlling $Q_{heater}$ as described above. A total effect of 2.4 W was measured, indicating a $Q_{coldspot}$ of 1 W with gas flow. If the gas flow is kept constant, the total effect of $Q_{coldspot}$ and $Q_{gasflow}$ can be determined during the period before inoculation as described under Zero Offset Calibration.

$Q_{control}$

The temperature control was switched on for this measurement, the temperatures TIN and TOU were measured, and the water flow WFL was kept at 1 mL s$^{-1}$. Also, $Q_{heater}$ was given different values. Theoretically, at steady state $\Delta Q_{heater} = \Delta(TIN - TOU) \times 4.1819$. The values found for TOU - TIN closely (within 5%) matched the theoretically expected values.

$Q_{stirrer}$

Using the values found for $Q_{control}$, the heat input from the stirrer was studied at several stirring speeds. Without air flow and for stirring speeds between 400 and 1000 rpm,

$$Q_{stirrer} = 6.0 \cdot (RPM)^3 \cdot 10^{-9} \text{ W}$$

With air flowing (1 VVM) through the fermentor, it was found that

$$Q_{stirrer} = 5.3 \cdot (RPM)^3 \cdot 10^{-9} \text{ W}$$

Zero Offset Calibration

After calibration of the various heat flows, the total heat model was calibrated. The fermentor was allowed to run for several days with the temperature control switched on and constant stirrer speed. If no gas flow is present, the heat balance reads

$$\text{Accumulation rate} = Q_{coldspot} + Q_{environment} + Q_{stirrer} + Q_{control} + Q_{process}$$

(7)
During this calibration \( Q_{\text{process}} = 0 \). Furthermore, \( Q_{\text{coldspot}} \) and \( Q_{\text{air}} \) are constant:

\[
Q_{\text{environment}} = U \cdot A_t \cdot (\text{TFM} - \text{TRM}) \\
Q_{\text{control}} = 4.1819 \cdot (\text{TIN} - \text{TOU})
\]

Measurements were taken every 100 s, and the results were evaluated off-line. During this evaluation, \( U \cdot A_t \) was varied around the value found during the setup calibration to obtain a constant value for the heat balance, thus minimizing the influence of the environmental temperature TRM. After this, the heat balance could be shifted to zero by varying \( Q_{\text{coldspot}} \). Best results were obtained with \( U \cdot A_t = 0.25 \text{ W K}^{-1} \) and \( Q_{\text{coldspot}} = 2.1 \text{ W} \) (compared to \( 0.3 \text{ W K}^{-1} \) and \( 1.8 \text{ W} \) during the setup calibration).

The zero offset calibration was repeated with gas flowing through the fermentor at \( 1 \text{ VVM} \) \( (Q_{\text{gasflow}} = 1.37 \text{ W}) \). Best results for this calibration were obtained with \( U \cdot A_t = 0.25 \text{ W K}^{-1} \) and \( Q_{\text{gasflow}} + Q_{\text{coldspot}} = 2.40 \text{ W} \).

The zero offset calibration is comparable to the calibration that should be done for any analyzing method. Preferably it should be done immediately before each experiment.

**RESULTS**

After calibration, the setup was steady state and dynamically tested and a growth experiment was carried out. Since the calibration experiments had shown that the insulation of the fermentor was difficult to reproduce, each set of experiments was preceded by a zero offset calibration. The parameters that were found during this preceding period could well be used for the steady state that followed the experiments, even after several days, if the insulation material was left in place.

**Steady State Tests**

The fermentor was allowed to run for several days with the temperature control switched on. Known electrical heat sources were used to simulate \( Q_{\text{process}} \). Results are shown in Table II.

**Dynamic Tests**

The dynamic behavior of the system was tested by switching a known electrical heat source on and off, simulating \( Q_{\text{process}} \). The \( Q_{\text{process}} \) was then calculated from measurements taken every 100 s. If all parameters in the mathematical model are correct, the calculated \( Q_{\text{process}} \) should be equal to the imposed \( Q_{\text{process}} \), both in magnitude and form (square wave). The results shown in Figure 2 were calculated using the before-mentioned calibration terms.

**Growth Experiment**

Inoculation was preceded by an equilibration period of several hours to allow the fermentor to reach the thermal steady state, during which calibration factors, the zero offset, and standard deviation for all on-line measurements (CO₂, O₂, and heat) were determined. The model parameters that were found during the period before growth could be used to describe the steady state that followed after the growth had stopped. Results of the on-line measurements are shown in Figures 3a–c. Final results were corrected when necessary for sampling volume and additions such as inoculum, glucose solution, and pH control.

**DISCUSSION**

**Steady State Tests**

From Table III it can be concluded that steady state heat production can be measured with a standard deviation of 0.2 W if the measuring experiment is preceded by a calibration period to measure zero offset in the heat measurements.

**Dynamic Tests**

As can be seen in Figure 2, a square wave change in \( Q_{\text{process}} \) can be observed with a characteristic time between 100 and 200 s. A faster response would be possible if shorter evaluation periods for the accumulation terms were used. However, this faster response would then be reached at the expense of a higher standard deviation, making it more difficult to distinguish small signals with certainty.

**Growth Experiment**

The results of the growth experiment can be seen in Figures 3a–c. The two initial negative peaks in the heat production (Fig. 3c) were caused by the addition of glucose solution and the inoculum. The fall and subsequent rise in the curves just before the end of the batch indicate diauxic growth. However, no ethanol or acetate was found in the broth. Visual inspection of the curves indicates that the standard deviation in the O₂ measurement during the growth phase was worse than that of the other on-line measurements. This also followed from the standard deviation.
calculated during the preceding calibration period. Combustion enthalpy value for biomass (560 kJ Cmol⁻¹) was taken from Roels² and for glucose and NH₄⁺ (468.7 and 296.2 kJ mol⁻¹, respectively) from Larsson.² The enthalpy taken from the nitrogen source was calculated from the amount of biomass formed. The ash content (6.02%) and the elemental composition of Candida utilis CH₁₆₇O₃₉₉N₀₉₅ were calculated from the values given by Verduyn et al.¹¹

Figure 3. (a) CO₂ production during batch growth of C. utilis on glucose. (b) O₂ consumption during batch growth of C. utilis on glucose. (c) Heat production calculated from temperature and flow measurements during batch growth of C. utilis on glucose.

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<th>Table III. Overview of the results of test experiments.</th>
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<td>Heat produced</td>
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Integrals of the values measured on-line were taken between 15,000 s (just after inoculation) and 60,000 s.

Table IV gives an overview of the results, and the balances that were made.

The energy balance could also be evaluated by comparing the measured heat effect with the expected heat effect calculated from the combustion enthalpy: calculated 80 ± 11 kJ, measured 80 ± 4 kJ.

Using the total O₂ uptake and the measured heat effect, it was possible to calculate the heat effect per mole of O₂. The value found was 410 ± 75 kJ (mol O₂)⁻¹, which is not in good agreement with the value of 460 kJ (mol O₂)⁻¹ usually mentioned in the literature.³

However, the O₂ consumption shows a large standard deviation. Since there were redundant measurements in this experiment, it was possible to use the statistical methods described by Wang and Stephanopoulos¹² to calculate an estimate for the heat production and the O₂ consumption.
on the basis of all measurements. The estimates were

Heat production: 81 ± 2 kJ and O₂ uptake 0.177
± 0.004 mol
Division yields: 457 ± 12 kJ (mol O₂)⁻¹

which is in close agreement with the value of 460 kJ
(mol O₂)⁻¹ for aerobic processes.

Finally the heat transfer coefficient between finger and
broth was calculated before, during, and after growth. The
average value was 2.36 ± 0.14 W K⁻¹. As expected, no
significant change due to wall growth was observed during
the batch growth of this organism.

CONCLUSIONS

Measurement of heat production by microorganisms
is possible in routine laboratory fermentors if some
precautions are taken.

1. Even with well-insulated equipment, the influence of
the environment is highly significant; the tempera-
ture of the environment should therefore also be
monitored,

2. The accumulation of heat in the fermentor is very
important, even in apparent steady state situations
where the rate of temperature change is well below
0.01 × 10⁻³ K s⁻¹. The temperature of the fer-
tor must therefore be monitored precisely.

If these precautions are taken, heat production can be mea-
sured with a standard deviation better than 0.2 W. Given
that the heat production in aerobic processes is usually
several watts,⁷ the relative standard deviation is better
than 10%. This is comparable with that of oxygen uptake
measurements. Measurement of heat production could well
be used to complement oxygen uptake measurements, since
the two are closely related.

For anaerobic processes, heat production is usually small.
However, the measurement of heat production is one of the
very few on-line measurements that are available.

The simplified models used above serve well for the
calculation of heat effects. A more detailed model will be
used in further research to optimize the fermentor setup
in order to obtain the smallest standard deviation in the
calculated Qprocess at different values for Qprocess.

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NOMENCLATURE

Measurements

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFM</td>
<td>temperature of fermentor</td>
</tr>
<tr>
<td>TIN</td>
<td>temperature of finger inlet</td>
</tr>
<tr>
<td>TOU</td>
<td>temperature of finger outlet</td>
</tr>
<tr>
<td>TRM</td>
<td>temperature of environment</td>
</tr>
<tr>
<td>AFL</td>
<td>airflow through fermentor</td>
</tr>
<tr>
<td>WFL</td>
<td>water flow through thermal finger</td>
</tr>
<tr>
<td>RPM</td>
<td>stirring speed, revolutions per minute</td>
</tr>
</tbody>
</table>

General

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>heat transfer coefficient</td>
</tr>
<tr>
<td>A₁</td>
<td>heat transferring surface</td>
</tr>
<tr>
<td>Qsub</td>
<td>energy flow related to &quot;subscript&quot;</td>
</tr>
<tr>
<td>Φ</td>
<td>flow of matter</td>
</tr>
<tr>
<td>ρ</td>
<td>specific density</td>
</tr>
<tr>
<td>rₚ</td>
<td>specific heat at constant pressure</td>
</tr>
<tr>
<td>ΔHᵢ</td>
<td>enthalpy of component i</td>
</tr>
<tr>
<td>VVM</td>
<td>gas flow in volume (gas) per volume (broth) per minute</td>
</tr>
</tbody>
</table>

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