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Biological reaction calorimetry: Development of high sensitivity bio-calorimeters[1]

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Abstract

A review of different types of biological reaction calorimetry systems currently used together with the operating principles is presented. The average resolution of these systems is approximately 20 to 1000 mW l^{-1}, sufficient for studies of a wide range of cell culture processes. Poorly exothermic and endothermic processes require the development of even higher resolution systems. To this end, the Mettler-Toledo RC1 calorimeter has been extensively studied to determine the factors which limit the resolution. By changing both the hardware and software, the resolution has been increased to 2–5 mW l^{-1} for non-aerated processes and to 10–15 mW l^{-1} for aerated systems. The changes include a switchable electrical heater for the oil circulation thermostat, a new higher resolution A/D board, PI controller and a thermostat reactor housing. The on-line measurement of the power input through agitation is proposed to be essential for low heat output biological processes, even under conditions where the rheological properties of the culture are not believed to be changing. The results show that it is possible to develop high-resolution systems capable of operating under standard laboratory bioreactor conditions; however, it is felt that the limits to the instrument resolution have been attained and that the calorimetric signal resolution is limited by the requirement of high agitation, nutrient feeds, gassing, pH control and other external effects which can only be overcome by heat-balancing methods. © 1998 Elsevier Science B.V.

Keywords: Calorimetry; Microcalorimetry; Energy balance; Bioprocess monitoring; Bioprocess control

1. Introduction

Considerable interest has been focused in recent years on the importance of continuous on-line techniques for the monitoring of biological processes [1,2]. This is particularly true for processes producing products of medical or pharmaceutical interest where the process conditions and physico-chemical environment exert an influence on the quality of the product such as glycosylation and activity [3,4]. Remarkably few techniques exist which are suitable for coupling to bioreactors and yet provide reliable data for continuous input to computer acquisition and modelling systems. Of the systems which exist, most are specific, require sophisticated instrumentation and are based on electrode, mass spectrometry or flow-injection analysis, which are generally not applicable to a wide range of biological processes.

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Heat generation and adsorption are a universal feature of biological processes, the measurement of which provides a global measurement of the metabolic activity of the cell system under study. Although non-specific, heat measurements may provide an important indicator of the nature of the process and important events occurring during the process. In order to be more widely applicable, the heat measurements must be rendered quantitative and sensitive enough to detect the small heat signals generated by biological systems. Furthermore, the heat measurements should be possible under conditions which operate in standard bioreactors. This is particularly important for small scale laboratory systems, involving animal cell cultures, anaerobic microbial or weakly endothermic processes in which the heat signal is of the order of 50 to 300 mW l^{-1}, a value which is considerably lower than the power input to the system through effects such as agitation, pH control, gassing effects, etc. This problem is significantly reduced with larger scale bioreactors which operate under essentially adiabatic conditions due to the low surface-to-volume ratio and poor heat-transfer properties of the surrounding air. In these cases, the problem usually is efficient cooling of the system, thus a straightforward heat balance around the system, based upon a knowledge of cooling water temperature, mass flows and other energy exchange terms, would be sufficient for the quantitative determination of biological heat dissipation rates.

Despite the potential importance of calorimetry for on-line measurement and control, very little has been published concerning industrial applications, due to difficulties in undertaking the essential laboratory scale experiments on which scale-up would be based. These difficulties result from the lack of laboratory calorimetric systems which can operate under process conditions, yet at the same time have sufficient resolution to enable precise quantitative measurements of the weak heat dissipation rates, usually associated with biological processes. A number of 'non-microcalorimetric' systems have therefore been developed over the last few years in an attempt to fill this role; however, most systems still suffer from problems of signal resolution. The present paper will briefly review the calorimetric techniques, currently of interest for bioprocess monitoring, and present in detail the factors which limit signal resolution and how these could be overcome with the Mettler-Toledo RC1 reaction calorimeter to achieve a resolution of 5 mW l^{-1}, a value comparable with microcalorimetric systems.

2. Biological reaction calorimeters

Calorimetric systems suitable for biological process studies fall basically in two main categories (1) microcalorimeters and (2) macro- or bench-scale-calorimeters [5,6]. The former generally have measuring cells with small working volumes (1–100 ml) with a volumetric resolution equivalent to 10–200 W l^{-1}. Typical of these systems are the Calvet-type [7] and flow through microcalorimeters [8–10], both of which achieve high resolution by operation under isothermal conditions. While extremely useful for batch studies, the high resolution is obtained at the risk of severe limitations with respect to the bioprocess, monitoring under defined physico-chemical conditions. Thus aerobic processes, such as Baker's yeast production or the study of transients and oscillations in yeast, may rapidly become oxygen limited due to difficulties to aerate the culture especially in the flow channels and measurement cell. Similarly, changes in pH, substrate and product concentrations may affect cell physiology and kinetics, while foaming, changes in rheological properties, wall effects, etc. may cause line blockages, variations in heat-transfer properties and volume resulting in difficulties in the interpretation of data. The principles and theory of different techniques have been extensively described elsewhere [1,11].

Macrocalorimetric systems may be generally defined as those resembling standard laboratory (or larger scale) bioreactors with a working volume of at least 1 l. As a result of the more complex culture requirements these in situ systems generally have a lower resolution than microcalorimetric systems. The principles of operation of the more common macrocalorimetric systems will be described in some detail.

2.1. Dynamic calorimetry

This was probably the first macrocalorimetric technique introduced in 1968 [12,13], involving the development of an energy balance around a well insulated standard 10 l laboratory bioreactor after a brief interruption (5–9 min) of the cooling system. As a result, the temperature of the culture increases over a small

temperature range, typically 1 K, the rate of increase being directly related to the heat dissipation by the following process (Eq. (1)).

$$K_p \frac{dT}{dt} = q_r + q_a + q_l + q_g \quad (1)$$

where K_p is the heat capacity of the system (J K^{-1}); q_r the energy dissipated by the reaction (W); q_a, q_l and q_g are the heat flows due to agitation and losses to the environment and gas stream, respectively (W). The major disadvantages of this system are the physiological changes which may result from a non-constant reaction temperature; the relatively poor resolution (1 W l^{-1}) requiring a high minimum biomass concentration (1–2 g l^{-1}) and relatively large minimum culture volume; unsuitability for weakly exothermic or endothermic bioprocesses, such as anaerobic microbial cultures and animal/plant cell cultures; and difficulties in implementing at larger scale. The major advantage of the system is the requirement of non-specialised equipment and heat sensors, since the terms q_a, q_l and q_g may be readily calibrated before inoculation, although care must be taken to ensure that these parameters remain constant during the process.

2.2. Heat-balance calorimetry

The dynamic calorimetric technique has been developed one stage further to include a complete energy balance around a standard laboratory bioreactor (working volume 1.5 l) without the need for disconnection of the cooling power [14–18]. This method has an obvious advantage of being applicable at almost any scale, without the need for sophisticated equipment, since it involves the development of mathematical models for the calculation of heat flows, although the models themselves are complex and specific for each process, requiring facilities to precisely measure all heat flows.

A complete energy balance for any bioprocess can be described by Eq. (2).

$$\rho_c c_{p,c} \frac{d(VT)_c}{dt} = \sum(\rho_{in} c_{p,in} F_{in} T_{in}) - \sum(\rho_o c_{p,o} F_o T_o) + V \sum_i (\Delta H_i r_i) + \Delta q + \Delta W \quad (2)$$

The first term in Eq. (2) represents the heat accumulation in the bioreactor, the second and third terms, respectively, the thermal contents of the inflows and outflows from the bioreactor and the 4th term the heat dissipation by the bioprocess. The term ΔW represents the net work performed on the bioreactor while Δq is the net heat flow to the bioreactor. Thus at thermal steady state, in the absence of chemical conversion, the constant values for ΔW and Δq can be determined. It is then possible to determine the reaction rates, r_i, simply by measuring the flows and temperatures of all components, entering or leaving the bioreactor, together with the temperature and flow of the cooling water.

A schematic representation of a simple bioreactor set-up with different components of the heat balance is shown in Fig. 1. From Eq. (2), since the bioprocess is generally operated in the isothermal mode, the accumulation term, $\rho_c c_{p,c} d(VT)_c/dt$, approaches zero and the temperature may be accurately measured using 12 or 16 bit A/D converters coupled to a computer. However, the Δq term is comprised of a number of components described by Eq. (3).

$$\Delta q = q_a + q_l + q_s + q_e + q_c \quad (3)$$

where q_a is the heat flow due to stirring; q_l the heat flow through the reactor walls, head-plate and inserts; q_s the sensible heat flow to the air stream; q_e the heat flow through evaporation and q_c the heat flow through the condenser unit. With the exception of q_a all parameters generally have negative values. Clearly, it is not realistic to assume that these values are always constant as suggested [14,15] and possible to calibrate once at the start of the experiment. However, by the use of suitable temperature sensors and controllers, it is possible to measure all parameters continuously by interfacing with a suitable data acquisition system. This technique has been used for batch and fed-batch Baker's yeast production [14,18] with remarkable resolution (50 mW l^{-1}).

2.3. Continuous calorimetry

A variant of the energy balance technique involves the use of a standard 14 l laboratory bioreactor supplied with a constant cooling power [19] (Fig. 2). An electrical heater, within the reactor, is then used to supply the energy necessary to maintain the set-point reactor temperature. Thus, the power supplied by the

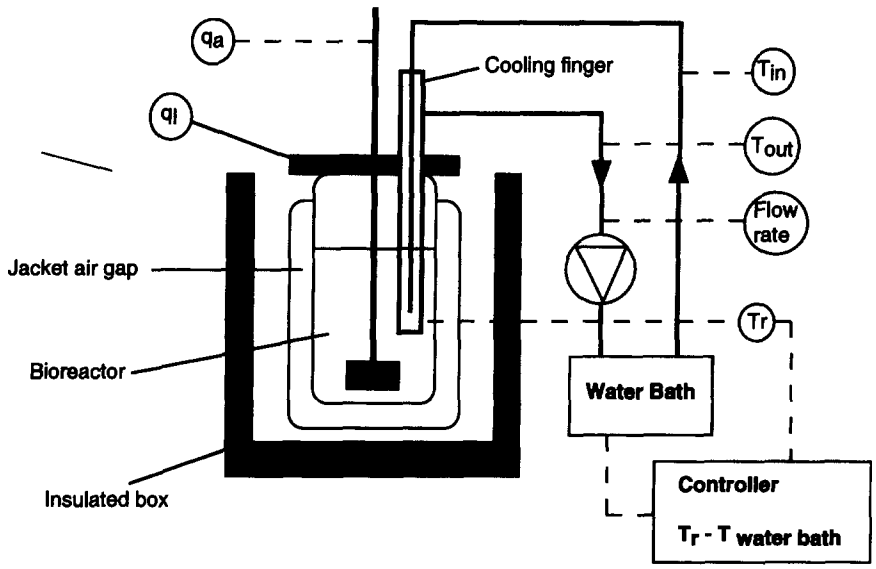

Fig. 1. Schematic representation of a system for heat-balance calorimetry [14,15].

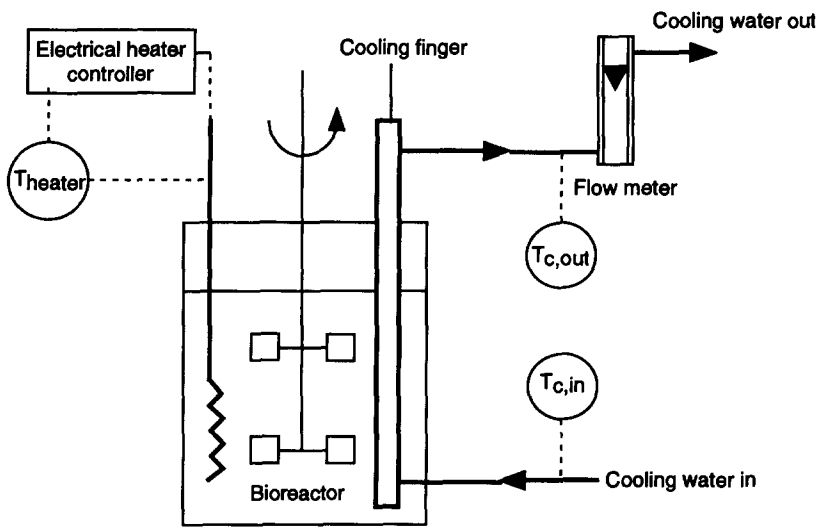

Fig. 2. Schematic representation of a continuous calorimetric system [19].

heater is inversely proportional to the heat dissipation by the culture; however, an energy balance is still required as described by Eq. (4).

$$q_r = q_h + q_c + q_a + q_l + q_g \quad (4)$$

where q_r, q_l, q_a and q_g represent the heat dissipation by the culture, heat flow to the environment, heat flow through agitation and heat flow to the gas stream, respectively (W), while q_h is the power supplied by the electrical calibration heater (W) and q_c the constant cooling power (W). This system is relatively straightforward with a resolution of about 0.5 to 1 W l^{-1}; however it offers few advantages over the energy balance method.

Dedicated commercially available reaction calorimeters, suitable for bioprocess application, primarily

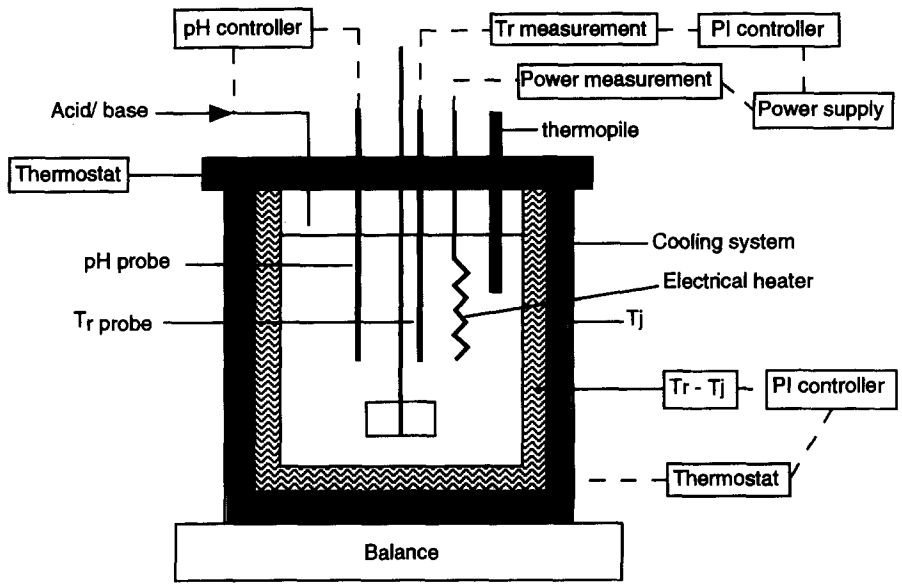

Fig. 3. Schematic representation of the Berghof BFK-I reaction calorimeter [20].

consist of the Berghof Fermentation Calorimeter (BFK) and the Mettler-Toledo Reaction Calorimeter (RC1).

2.4. Berghof fermentation calorimeter (BFK)

The BFK, marketed by Berghof Labor- und Automationstechnik GmbH, Eningen, Germany and developed by Meiers-Schneiders et al. [24], is an isothermal compensation calorimeter specifically developed for bioprocess applications [20–25]. The BFK type 1 instrument is based on a principle similar to the continuous calorimetric method [19,24,25]. The standard 2 l working volume glass reactor is double jacketed, as is the reactor top-plate (Fig. 3). A thermopile wall is contained within the inner air jacket and a constant temperature difference is maintained across this thermopile wall, between the reactor contents and external jacket, through which a silicone oil circulates. The reactor temperature is monitored by a high-resolution thermistor (5×10^{-4} K) and maintained at the desired set-point by a variable output electrical compensation heater. The temperature difference between the reactor contents and cooling oil is thus measured via the heat flow through the thermopile wall.

An advantage of the BFK-1 is that no corrections for heat losses through the reactor wall are required; however, this system could not provide sufficient cooling capacity for all applications, the thermopile wall is extremely fragile and the reactor design is impractical. For these reasons, a BFK type 2 was developed in which a 2 l working volume reactor was housed in a jacketed glass vessel with an air gap between [21,25] (Fig. 4). Silicone oil is passed through the jacket of the housing in order to maintain the temperature at precisely that of the reactor contents, thus acting as an adiabatic shield. The temperature of the reactor is maintained by a compensation principle with constant cooling power and variable output electrical heater as for the BFK-1. Thus in the absence of a bioreaction, a basal heat signal is obtained which is only proportional to the cooling power q_c, heater power q_h^0 and agitation power q_s (W), according to Eq. (5).

$$q_c = q_s + q_h^0 \tag{5}$$

When a bioreaction takes place, the heat balance may be described by Eq. (6).

$$q_c = q_s + q_r + q_h \tag{6}$$

where q_r is the heat dissipated by the bioprocess

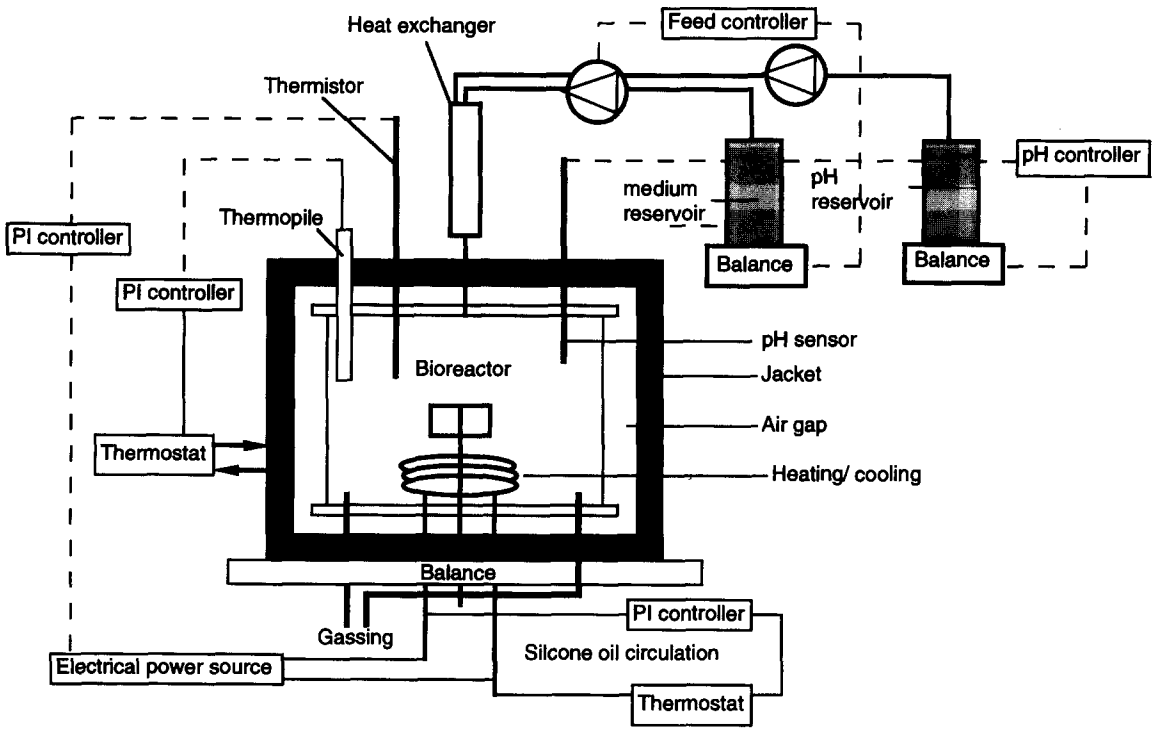

Fig. 4. Schematic representation of the Berghof BFK-II reaction calorimeter [24].

and q_h the 'new' power output of the heater (W). Combining Eq. (5) with Eq. (6), since the cooling power q_c remains constant, and solving for q_r yields:

$$q_r = q_h^0 - q_h = \Delta q_h \tag{7}$$

Eq. (7) shows that the changes in heater power output are directly proportional to the heat dissipation by the bioprocess. This system has been widely used for studying PHB (poly-B-hydroxybutyrate) production by *Alcaligenes eutrophus* [21], batch and fed-batch cultures of *Saccharomyces cerevisiae*, *Zymomonas mobilis* and *Bacillus licheniformis* with a resolution of 20 mW l^{-1} [20,23,25].

2.5. Mettler-Toledo RC1

The RC1 was developed by Mettler-Toledo AG, Schwerzenbach, Switzerland for chemical processes and modified for bioprocess monitoring and control [26–39]. The BioRC1 is essentially a standard 2 l jacketed glass reactor, capable of operating in isothermal, adiabatic or isoperibol modes [11,26] (Fig. 5). In the isothermal mode silicone oil is pumped at a high rate through the reactor jacket (2 l s^{-1}). The jacket temperature, T_j, is controlled by blending oils from a 'hot' with that from a 'cold' oil circuit, via an electronically controlled metering valve, in order to maintain a constant reactor temperature, T_r. Thus a bioprocess dissipating or taking up heat will result in an increase or decrease in T_j, leading to a temperature gradient across the reactor wall which is directly proportional to the energy liberated or taken up by the bioprocess according to:

$$q = UA(T_r - T_j) \tag{8}$$

where U represents the global heat-transfer coefficient (W m^{-2} K^{-1}), A the heat-transfer area (m^2) and (T_r–T_j) the temperature difference between the reactor contents and jacket oil (°C).

As with all heat-flux calorimeters, a complete energy balance, similar to that described by Eq. (2), must be made. This can be expressed as:

$$q_r + q_{add} + q_f + q_{acc} + q_l + q_a + q_s + q_e + q_c = 0 \tag{9}$$

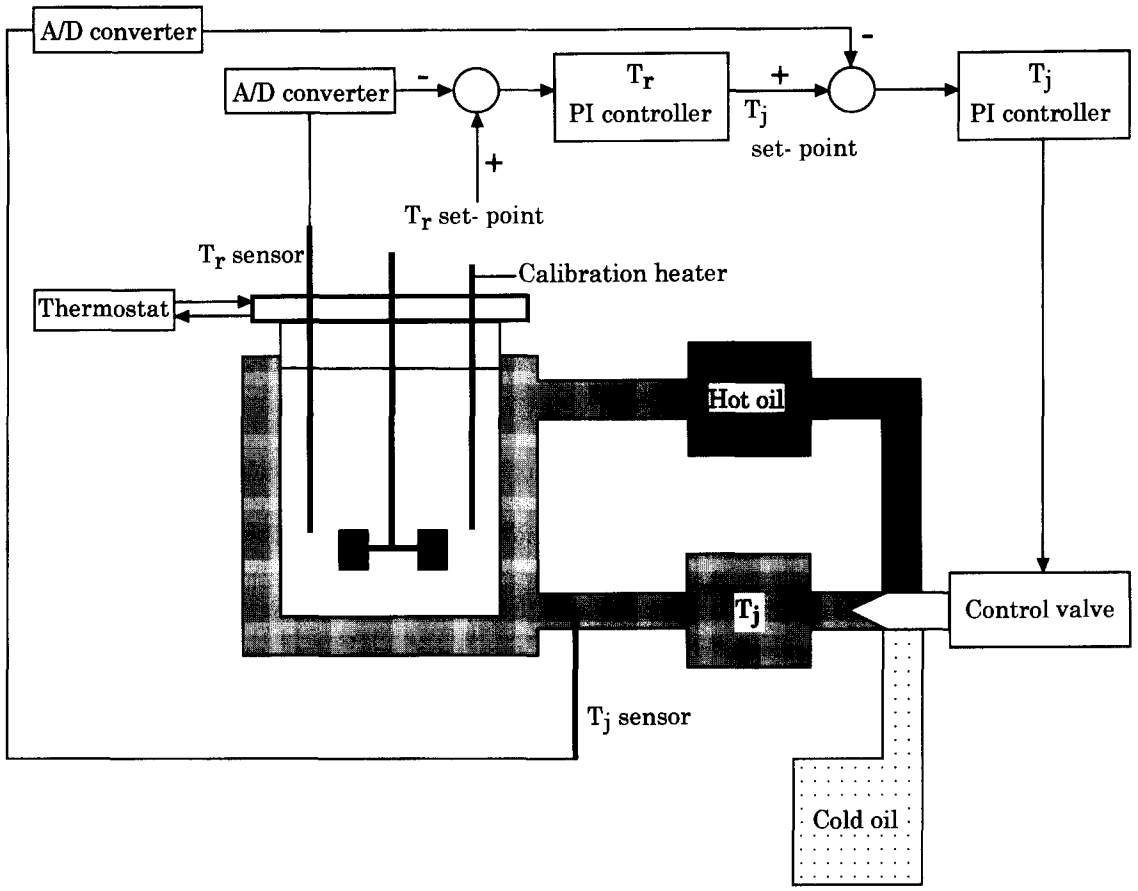

Fig. 5. Schematic representation of the Mettler-Toledo/ EPFL BioRC1 [6].

where q_r is the heat flow resulting from the bioprocess, q_{add} the heat flow due to additions to the reactor, q_f the heat flow through the reactor wall to the oil, q_{accu} the accumulated heat flow in the reation mass, q_l the heat flows to the environment, q_a the heat flow through agitation, q_s the sensible flow to the air stream, q_e the heat flow due to evaporation and q_c the heat flow across any condenser device (W). It is possible to measure all these parameters, as for the energy balance method, or to simply maintain the parameters (except q_r) as constant as possible. In this case Eq. (9) reduces to Eq. (8) and the base-line signal for q_r before inoculation represents the sum of all the heat losses and gain terms. Providing that these terms remain constant, the value of q_r, during the bioprocess, can simply be determined by subtracting the measured q_r (from Eq. (8)) from that of the base-line signal:

$$q_b = U_b A_b (T_r - T_j) \tag{10}$$

$$q - q_b = UA(T_r - T_j) - U_b A_b (T_r - T_j) \tag{11}$$

In batch processes, the heat-transfer area (A) is constant and, provided that mixing ensures homogeneous conditions and no wall growth occurs, the heat-transfer coefficient, U, also remains constant, therefore Eqs. (10) and (11) reduce to:

$$q - q_b = \Delta T - \Delta T_b = q_r \tag{12}$$

where q_r is the heat dissipation due to the bioprocess (W). It is not essential that the term UA remains constant, since a calibration heater can be employed at any time for its determination and subsequently used for correcting the base-line.

The BioRC1 has been extensively used for studying a wide range of microbial and animal cell biopro-

cesses, under batch, fed-batch and continuous operation with a sensitivity of 50 mW l^{-1} [26–39]. In addition to monitoring the bioprocess, this system has also been used for the calorimetric control of a fed-batch Baker's yeast process [28].

3. Calorimetric resolution

Before further consideration, it is important to define the meaning of the terms resolution and sensitivity as applied to calorimetric systems. Sensitivity is the limit of detection of a signal and is determined by the ability of temperature sensors, flow meters and other equipment to detect a change in the measured parameter. Thus, the sensitivity of the temperature sensors in the RC1 is 2×10^{-4} K (T_r) and 4×10^{-3} K (T_j), while those for the BFK-II are 5×10^{-4} and 10^{-3} K, respectively. Assuming a value of UA of 10 W K^{-1} this equates to a maximum potential sensitivity of the heat-flow measurement for these machines of between 2 and 40 mW.

However, the measured calorimetric signal is a combination of the sensitivity and the signal noise, and it is the latter which determines the ability of a calorimetric system to detect the changes in temperature or heat flow, and thus the calorimetric resolution. Clearly, the resolution may be improved by improvements in A/D conversion, frequent data sampling combined with mathematical smoothing procedures, such as Kalman filtering or floating point averaging which act by reducing noise. Therefore, care must be taken while comparing calorimetric techniques since the stated resolution (most frequently called 'sensitivity') will be governed by the number of data points and filtering technique used. Thus, a high resolution is usually obtained at the expense of the response time and may seriously affect kinetic measurements.

4. Towards high-resolution calorimetry

All reaction calorimetry techniques involve a heat balance in one or other form, in order to obtain the desired resolution and to facilitate the quantification of data. As a result, the following discussion will focus primarily on the ways to improve the resolution of the Mettler-Toledo RC1, although the principles can be generally applied.

There are two basic ways to obtain high resolution: by measuring all relevant parameters continuously with the highest sensitivity equipment available or by essentially maintaining the values of most parameters as constants that they may be removed from the heat-balance equations. In addition, it is important to differentiate between resolution limits of the calorimetric measurement principle from the limits of the resolution resulting from the heat flows associated with the bioprocess.

4.1. Resolution due to process variables

In laboratory scale calorimeters for bioprocess applications, it is important to culture cells under conditions which resemble as closely as possible to those found in a standard bioreactor, such as a stirred tank reactor (STR). In this case it is essential to provide: the ability to operate under sterile conditions; suitable mixing and agitation to ensure sufficient mass and heat transfer; mass flows, to and from the bioreactor, for controlling pH and for fed-batch and continuous operation; the supply of gaseous substrates, such as O_2, CO_2, H_2, etc; and the measurement of rheological properties to correct the changes in heat-transfer coefficients.

4.1.1. Sterile operation

Usually this is not a problem for reaction calorimeters, since they are generally based on the design of laboratory bioreactors. However, whether sterilizable in situ or autoclavable, all components must support relatively high temperatures. This may influence the resolution since the temperature measurement is digitized over the full range of temperatures employed. Thus for the standard RC1, the operational range from −246 to +474°C which is digitized over 40 000 points, while for the BioRC1, the temperature range has been reduced from 0 to 150°C for the same number of points with a resultant increase in resolution. Ideally, this could be increased further by a switching mechanism, allowing a reduced range – say from 20 to 40°C – during the cultivation process.

4.1.2. Mixing and agitation

In general, mixing and agitation are achieved through mechanical devices using suitable impellers. It is necessary to have sufficient power input to the

culture (q_a) in order to ensure homogeneity, low temperature and concentration gradients, a constant liquid film at the heat-transfer surface, constant heat-transfer area, and break up of gas bubbles resulting in a high rate of transfer of the gas to the liquid phase. As a result, q_a, at least for laboratory scale bioreactors, is usually much greater than q_r, the heat dissipated by the process. It is therefore essential to control q_a as precisely as possible. However, bioprocesses are frequently accompanied by changes in the rheological properties of the culture medium due to the increase in biomass concentration, particularly for filamentous organisms, and the liberation of products such as polysaccharides, proteins, DNA, etc. into the medium. An increase in viscosity will automatically result in an increased q_a in order to maintain a constant agitation rate. The change in q_a can be approximated by measuring the power supplied to the stirrer motor in order to maintain the constant agitation rate, by measuring the integral component of the stirrer motor controller or by the incorporation of a torque meter [27]. In a recent article [27] it was shown that the power–time curves, from the RC1 of batch cultures of the erythromycin-producing strain *Saccharopolyspora erythraea*, exhibited distinct phases which could be correlated with the torque-meter signal. One of these phases corresponded to a decrease in q_a due to foam formation and the second to antifoam addition with concomitant foam breakage. The third phase, however, could not be correlated with changes in viscosity, morphology or some other biological event such as the limitation of an essential nutrient. Correction of the base-line calorimetric signal for these changes in q_a, however, led to important changes in the power–time curves.

Similar effects have been observed recently with batch cultures of insecticidal protein (ICP), producing *Bacillus thuringiensis* str. galleriae grown on an yeast extract/glucose/salts medium in the RC1 (unpublished data). During growth, q_a remained relatively constant, however, approximately 15 h after cessation of growth q_a rose by approximately 0.5 W (Fig. 6). This increase did not parallel sporulation or ICP formation but correlated with the appearance of other proteins, presumably resulting from cell lysis.

Thus care must be taken when making heat balances, not to assume a constant q_a, even when there is no apparent evidence of viscosity problems, particularly where morphological or foaming problems may occur.

4.1.3. Mass flows

The term q_{add} must be determined in non-batch processes, or in conditions in which mass is added to the reactor, such as for the control of pH. In these cases

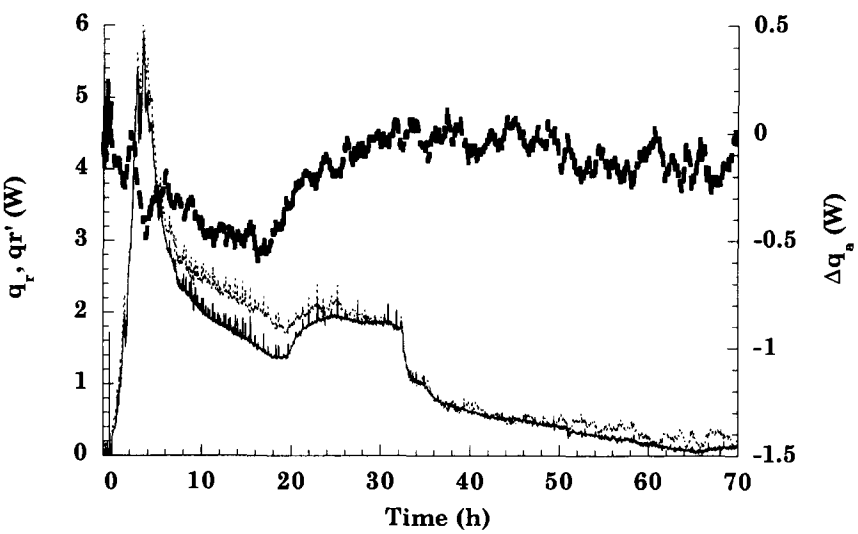

Fig. 6. Batch cultures of insecticidal protein (ICP)- producing *Bacillus thuringiensis* str. galleriae grown on a yeast extract/glucose/ salts medium in the BioRC1.

it is imperative to measure the mass-flow rate, temperature, pH and c_p of the added material. Pre-thermostatting of the feed stream is possible if the temperature controller is sufficiently precise. Due to the relatively complex nature of culture media, care must be taken to account for effects, such as the heat of solution and neutralization. This latter case was shown for hybridoma cell cultures grown on RPMI medium containing 10% foetal calf serum in the RC1 [38] in which the buffering of the medium was achieved by a combination of sodium bicarbonate and CO_2 (5% v/v). This combination resulted in poor signal resolution due to both CO_2 solubilization and neutralization effects being larger than the biological heat dissipation rate of approximately 150 W.

4.1.4. Gassing

One of the major difficulties with biological reaction calorimetry is the requirement for oxygen or other gasses as growth substrates. Since the biologically useful form of these gases is the dissolved state and, since most gaseous substrates have relatively poor solubilities in aqueous solutions (for example the solubility of oxygen in water at 25°C and 1 atm is 5 mg l^{-1}) it is important to achieve a high gas-transfer rate in order to supply the necessary biological demand. This is achieved by high gassing rates (typically of the order of 1 vvm (volume of gas per volume of culture per minute)) and vigorous agitation (typically 500 to 1000 rpm) with a Rushton turbine impeller. There are at least 3 heat flows in Eq. (9) which are related to gassing; q_s, q_e and q_c. The three terms can be maintained constant and close to zero by pre-saturation and thermostatting of the inlet gas stream by passage through a suitable gas–liquid contacter operating at the reactor temperature T_r. Under these conditions, there is no need for a condenser unit, unless it is desired to trap volatile components, since the water content of the inlet and outlet gasses will be the same. In the same way, evaporation effects, q_e, are negligible and q_c can be reduced further by the use of a thermostat reactor top-plate. In certain cases, such as animal and plant cell cultures [38], the cells are shear sensitive with the result that high agitation rates and gas sparging must be avoided. For the latter, bubble-free membrane systems exist, while for the former care must be taken to avoid concentration and thermal gradients.

4.1.5. Heat losses

The heat losses to the environment, q_l, are particularly difficult to avoid or precisely quantify with systems which are not perfectly adiabatic. Even the latter cannot really exist due to the need for pH, pO_2, redox, temperature and other sensors and reactor inserts which effectively act as heat bridges to the surroundings. Some authors [14,15] attempt to quantify q_l by calibration before inoculation. However, since bioprocesses are inherently relatively slow, lasting from a few hours to several weeks or months in the case of continuous processes, the heat losses must be regularly estimated, for example by the use of an internal electrical calibration heater or by controlling the environment temperature.

4.2. Towards a high-resolution RC1

The standard Mettler-Toledo RC1 has a resolution of 100 to 200 mW l^{-1}, while the BioRC1, with agitation, aeration, pH control, etc., has a resolution of 50 mW l^{-1}, making it suitable for most aerobic biological applications (q_r, 10–30 W l^{-1}) as well as high yielding anaerobic processes (1–3 W l^{-1}) [33,35,36]. However, interest in animal and plant cell cultures, in addition to weakly exothermic or endothermic processes, requires an even higher resolution [38,40,41]. This has been achieved by improvements in both the hardware and in the analysis of the factors causing signal noise.

4.2.1. Effect of high-resolution A/D conversion and improved controllers

As described by Eqs. (10)–(12), the RC1 operates by measurement of the temperature difference between the reactor contents and jacket oil. The resolution of T_r in the standard RC1 is 0.2 mK, whereas that for T_j is 10 mK. This was initially chosen since the aim was to reduce the accumulation term, q_{accu}, while working under isothermal conditions, with the heat-transfer resistance in the glass reactor wall (thickness 5 mm) and liquid and oil films as modelled by Zaldivar et al. [42] This system allows for kinetic investigations of rapid chemical processes. For slower biological processes, a thicker walled glass reactor (9 mm) was employed to increase the heat-transfer coefficient of the glass wall and, thus, reduce the effect of other resistances. Clearly this reduces the response

time, although this is unlikely to be a problem for biological reactions. The resolution of the A/D conversion of the T_j signal was increased to 0.2 mK, equivalent to that for T_r, by the incorporation of a second A/D board to work in tandem with the original one. A model-based predictive controller was then developed to fully exploit the increase in T_j resolution while permitting a more precise yet dynamic control. The standard RC1 'hot' oil circuit has an electrical heater unit incorporated, with a maximum power output of 2 kW and minimum of 400 W, in order to allow the extended temperature range of operation (−246 to +374°C). However, since the full 2 kW is only required during in situ sterilization of the BioRC1 and, in order to maximize the T_j resolution, a switchable transformer has been incorporated to allow a reduced maximum heating power of 500 W during bioprocess operation with a modified EPROM permitting a 100 W minimum heating power. The q_{accu} term has also been reduced to insignificant levels, as well as decreasing the response time during isothermal operation by the implementation of a PI (proportional-integral) controller for T_r, in place of the P (proportional only) controller of the standard RC1. Optimization of the time constants for the PI controller allowed for standard deviations between measured and calculated q_b values of less than 2 mW [30] (Tables 1 and 2).

4.2.2. *Short-term signal noise analysis of BioRC1*

If the variations in the baseline signal, q_b, are distributed in a Gausian fashion, the peak-to-peak noise at the 99% confidence interval can be measured by:

$$\text{Peak-to-peak noise} = 2 \times 2.58 \times \text{SD} \qquad (13)$$

Table 1
The effect of the proportional controller (P) settings on T_r, T_j and (T_r-T_j) for the RC1 (constant integral component I of 0.05)

	Standard deviation of value			
P	T_r (mK)	T_j (mK)	(T_r-T_j) (mK)	q_b [a] (mW)
2	0.27	0.50	0.49	2.9
4	0.21	0.64	0.65	3.9
6	0.22	0.66	0.69	4.2
8	0.23	1.00	1.00	6.2
10	0.22	1.20	1.30	7.8
12	0.23	1.80	1.70	10.0

[a] Calculated from Eq. (8)

Table 2
The effect of the integral controller (I) settings on T_r, T_j and (T_r-T_j) for the RC1 (constant proportional component P of 4)

	Standard deviation of value			
I	T_r (mK)	T_j (mK)	(T_r-T_j) (mK)	q_b [a] (mW)
0.01	0.21	0.71	0.73	4.37
0.03	0.20	0.70	0.67	4.02
0.05	0.18	0.65	0.60	3.61
0.07	0.21	0.83	0.83	4.97
0.09	0.21	0.84	0.94	5.64

[a] Calculated from Eq. (8)

Table 3
BioRC1 short-time noise (P=4, I=0.05)

Short-time noise	T_r (mK)	T_j (mK)	q_b (mW)
Calculated	0.9	3.3	18.6
Measured	0.8	3.0	19

where 2.58 is the t-value for a 99% confidence interval and SD designates the standard deviation. With P=4 and I=0.05, the peak-to-peak noise was calculated, and compared with the measured values in Table 3. The measured values are shown in Fig. 7(a–c).

Short-time noise can be reduced by averaging over a suitable number of data points, n, and the resolution calculated from:

$$\text{resolution} = \pm\frac{\text{peak-to-peak noise}}{2\sqrt{n}} = \pm\frac{2.58 \times \text{SD}(q_b)}{\sqrt{n}} \qquad (14)$$

Thus, by taking n points for averaging, the short-time resolution can be improved by $\sqrt{n}$, however, the sampling interval will increase by n times. Table 4 shows calculations for n between 1 to 300.

Table 4
Short-time resolution of the improved BioRC1. Experimental conditions: 1.6 l water, T_r=28°C, R=500 rpm, P=4, I=0.05, n, number of data sampling points

n	Sampling interval	Short-time resolution (± mW)	Volumetric resolution (±mW l^{-1})
1	2 s	9.3	5.8
15	30 s	2.4	1.5
30	1 min	1.7	1.1
150	5 min	0.76	0.48
300	10 min	0.54	0.34

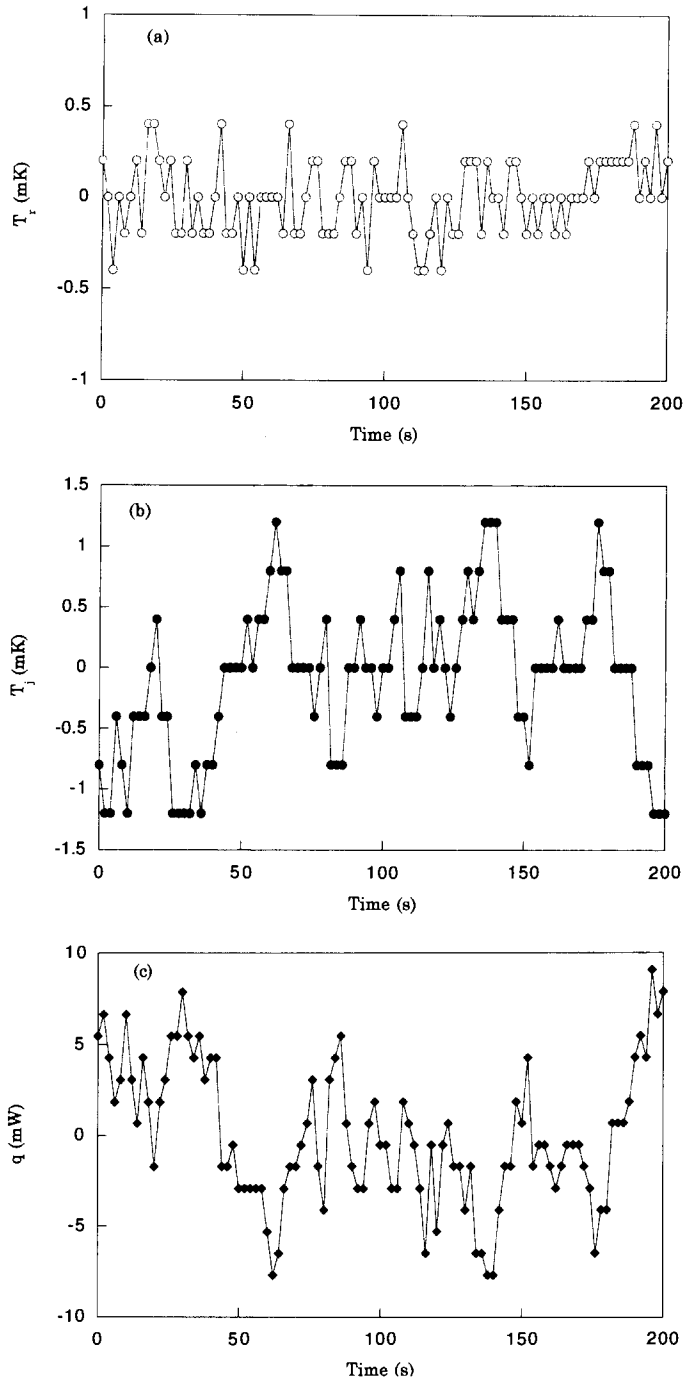

Fig. 7. RC1 short-time signal noise of (a) reactor temperature (T_r), (b) jacket temperature (T_j) and (c) heat flux (q).

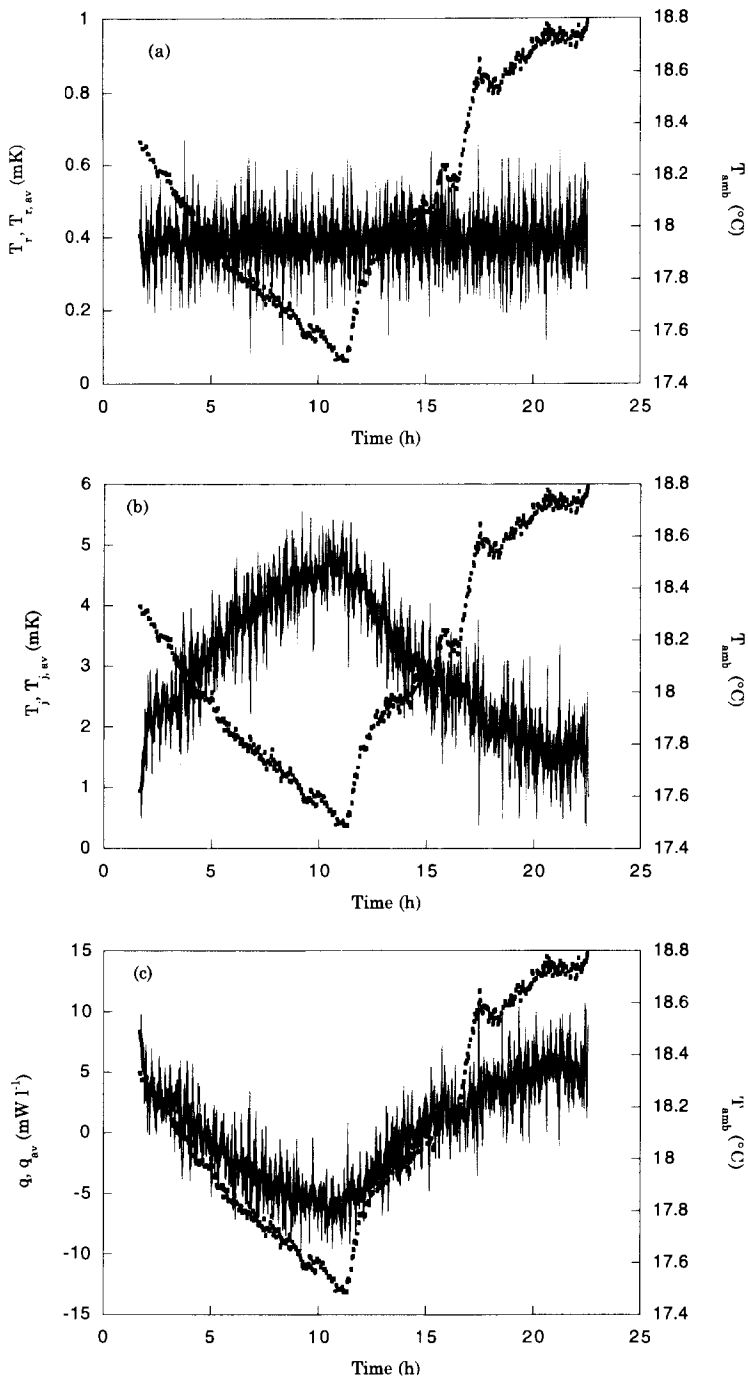

Fig. 8. Influence of ambient temperature, T_{amb}, on (a) T_r, (b) T_j and (c) q_b. Tr 28°C; no aeration; agitation rate 150 rpm.

As shown in the above analysis, the modified BioRC1 has improved measurement and control for both T_r and T_j, and therefore has a much higher short-time resolution.

4.2.3. Long-term signal noise analysis

In addition to the 'short-term' noise of the baseline calorimetric signal there is, in addition, a *long-term noise* resulting in baseline drift over the course of an experiment. If long-term noise is generally higher than the *short-term noise*, which is normally the case for the BioRC1, then the resolution depends on the former.

Long-term noise is more troublesome than short-time noise since it cannot be easily eliminated by averaging or correction, especially when a high resolution is required. This is particularly important in the study of processes which accompany weak heat effects and extend over long periods.

4.2.4. Long-term signal noise: Influence of ambient temperature

Due to fluctuations in the ambient temperature, T_{amb}, the heat losses, q_l, from the reactor to the environment will vary. This will result in variations in the baseline heat signal, q_b, and is perhaps characterized approximately by the factor dq_b/dT_{amb}.

Fig. 8(a) shows that T_j changes in an inverse way with respect to variations in the ambient temperature, while T_r remains very stable (Fig. 8(b)). As a result, q_b also changes with ambient temperature (Fig. 8(c)). At 150 rpm the value of dq_b/dT_{amb} is 18 mW K^{-1} while at 500 rpm (results not shown), the value of dq_b/dT_{amb} is 30 mW K^{-1}. The increased baseline drift at higher agitation rates may partly be due to minor variations in the higher power input through stirring, q_a, thus showing the importance of this parameter is on q_b.

In the laboratory in which the experiments reported here were performed, the variation of the ambient temperature is approximately 1°C, thus the resulting baseline drift due to T_{amb} is the most significant factor, limiting the resolution of the BioRC1. In order to minimize this influence, a thermostat housing was built around the reactor to control T_{amb}.

Using the thermostat housing and an agitation rate of 150 rpm, the fluctuations in T_{amb} were dramatically reduced and resulted in no obvious correlation between fluctuations in T_{amb} and the baseline drift (Fig. 9(a)). This resulted in a very high signal resolution of ±2 mW l^{-1}. The remaining low level baseline drift is probably due to electronics noise, which was found to be ±3 mW l^{-1} for a change in the ambient temperature of 1.8°C. At a higher agitation rate (500 rpm), the baseline drift increased to 10 mW K^{-1} with a signal resolution of ±5 mW l^{-1} (Fig. 9(b)).

4.2.5. Effect of operating temperature on resolution

The operating temperature might be expected to have an influence on the resolution of the BioRC1 through effects on q_l. To investigate this, q_b was measured at different operating temperatures (Table 5).

As expected, the magnitude of the detection limit increased as the operating temperature increased. This could be overcome by further insulation or thermostatting of the region above the reactor top-plate, since the higher the ambient temperature the greater is the heat loss through reactor inserts, such as calibration heater, temperature, pH and pO_2 electrodes.

4.2.6. Effect of gas sparging on resolution

As described earlier, gassing may have important effects on at least three components of q_b. The resolution of the BioRC1 was consequently determined by passing the air through a bubble column, operating at the same temperature as T_r, before sparging into the reactor at a flow rate of 300 ml min^{-1} and compared with a non-aerated system. The result was that, at 37°C and an agitation rate of 500 rpm, the resolution decreased from 12 mW l^{-1}, in the absence of aeration, to 15 mW l^{-1} with gas sparging. Thus an effective control of the air-flow rate and pre-thermostatting/pre-saturation of the air stream can result in very low signal noise. Indeed in cases, such as poorly exothermic plant and animal cell cultures, where gas sparging

Table 5
Resolution of BioRC1 at different operating temperatures (500 rpm; no aeration; $T_{housing}$, temperature of thermostatted housing)

T_r (°C)	$T_{housing}$ (°C)	Resolution (±mW l^{-1})
28	28	5
37	37	12
50	40	15

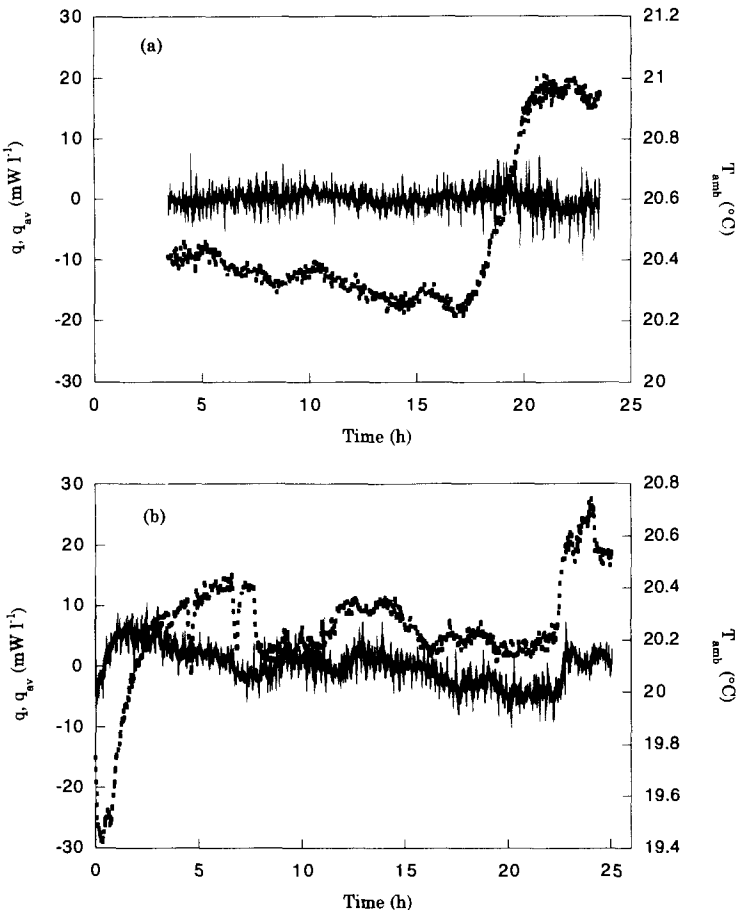

Fig. 9. Baseline measurements, q_b, with thermostatting housing operating at 28°C surrounding the reactor. Tr, 28°C; no aeration (a) agitation rate, 150 rpm and (b) 500 rpm.

may have a negative effect on cell viability, the signal noise may be reduced even further by the use of bubble-free membrane aeration systems.

4.3. Comparison of resolution of BioRC1 and other reaction calorimetric systems

The results of the investigation determining the ways of improving the resolution of the BioRC1 have resulted in an improved BioRC1 with a resolution, under gas sparging conditions, of 5 mW l^{-1}, or 2 mW l^{-1} in the absence of sparging. This compares very favourably with other calorimetric systems, and approaches the resolution level of microcalorimeters (Table 6).

5. Conclusions

The work presented here has shown that reaction calorimetry can play a very major role in future bioprocess development and control at both laboratory and even production scale. However, in order to achieve this, it is important that laboratory scale calorimetric systems are developed which can be used to model the process under standard bioprocess conditions and be generally applicable to microbial, plant and animal cell systems. For this, such calorimeters must be flexible in design and operation, and have a high resolution. The results show that this is entirely feasible with modern reaction calorimeters, such as the BioRC1 which can have a resolution as low as 2–5 mW l^{-1} with the potential to achieve 1 mW l^{-1} in

Table 6
Comparison of the maximum resolution of biological reaction calorimeters

Methods	Working volume	Resolution (± mW l^{-1})	Reference
Microcalorimetry	1–200 ml	10	[2]
Microcalorimetry	30 ml	200	[10]
Flow microcalorimetry	0.5–2 ml	20	[9]
Dynamic calorimetry	14 l	1000	[13]
Continuous calorimetry	14 l	1000	[19]
Anaerobic Calorimeter (heat-flux sensor)	1 l	>70	[43]
Standard fermentor [a] (heat balance)	1.5 l	50	[14,15]
BFK [a]	2 l	20	[20,24]
BioRC1 [a]	1.6–1.8 l	50	[6]
Improved BioRC1 [a]	1.6 l	5	Present work

[a] Sampling time: 60 s

the near future by improving the short term noise of the calorimetric signal. The results of the optimization of the BioRC1 have also shown that the resolution is limited by factors which are independent of the calorimeter itself but which are necessary for bioprocesses, such as agitation, gassing and environmental effects.

6. List of symbols

A	heat-transfer area (m^2)
A/D	analog-digital converter or conversion
BFK	Berghof Fermentation Calorimeter (models I and II)
BioRC1	Reaction Calorimeter of Mettler-Toledo AG modified for biological process operation by EPFL
c_p	specific heat capacity (J kg^{-1} K^{-1})
dq_b/dT_{amb}	change in base-line of power-time curve as a function of the change in ambient temperature (W K^{-1})
F	flow into or out of reactor (l h^{-1})
ΔH_i	specific enthalpy of component i (kJ mol^{-1} or kJ g^{-1})
I	integral component of controller
ICP	insecticidal crystal protein (of *B. thuringiensis*)
K_p	heat capacity of the system (J K^{-1})
n	number of data sampling points
P	proportional component of controller
PI	proportional-integral controller, combination of P and I
q_a	heat flow through agitation (W)
q_{accu}	accumulated heat flow in the reation mass (W)
q_{add}	heat flow due to additions to the reactor (W)
q_b	base-line heat flow, i.e. the sum of all of the heat loss and gain terms in the absence of a reaction (W)
q_c	heat flow across any condenser device (W).
q_e	heat flow due to evaporation (W)
q_f	heat flow through the reactor wall to the oil (W)
q_g	heat flow to the gas stream (W)
q_h	power supplied by the electrical calibration heater (W)
q_l	heat flow to the environment (W)
q_r	heat flow due to the reaction (W)
q_s	sensible heat flow to the air stream (W)
r_i	rate of conversion of component i (g l^{-1} h^{-1})
RC1	Reaction Calorimeter of Mettler-Toledo AG
SD	standard deviation
STR	stirred tank reactor
T_{amb}	ambient temperature (°C)
T_j	temperature of the jacket oil (°C)
T_r	temperature of the reactor contents (°C)
U	global heat-transfer coefficient (W m^{-2} K^{-1})
V	volume (l)
vvm	volume of gas per volume of liquid per minute (1 l^{-1} min^{-1})

ΔW net work performed on the bioreactor ($kJ\,l^{-1}$)

ρ_x specific mass of component$\times$($g\,l^{-1}$)

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