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The development and characterization of a high resolution bio-reaction calorimeter for weakly exothermic cultures

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Abstract

A unique bench-scale heat flux calorimeter is described, which is capable of detecting the low heat signal of an animal cell culture (a maximum of 200 mW/l is produced with a batch culture of SF9 insect cells). After the technical modifications that enhanced the sensitivity of a commercial heat flux reaction calorimeter, RC1 from Mettler-Toledo AG. Switzerland, the behavior of this system is analyzed here under biological operation conditions. Not only the heat flows provoked by the bioprocess parameters largely determine the final calorimetric resolution, but they are also more important than the culture heat signal (heat of reaction). Structural modifications aiming at the limitation of the non-biological heat flows are described such as insulating and thermostatic housings, and glass reactors with a different geometry. The current resolution of the RC1 is from ±4 to 12 mW/l compared to the former ±50 mW/l [Biotechnol. Bioeng. 57 (1998) 610], under usual animal cell culture conditions. This resolution is similar to that of microcalorimeters [Biotechnol. Bioeng. 58 (1998) 464; Thermochim. Acta 309 (1998) 63; Thermochim. Acta 332 (1999) 211; J. Biochem. Biophys. Methods 32 (1996) 191] with the advantage of an in situ measurement technique and a well-controlled culture (from the biological point of view). The monitoring sensitivity and control capacity of this instrument shows a clear potentiality for metabolic studies of low heat effect cell cultures and as a metabolic probe for industrial scale bioreactors's control.

Keywords: Bio-calorimetry; High sensitivity calorimetry; Bench-scale calorimetry; Animal cells; Heat production rate

1. Introduction

Calorimetric heat-flow signal, which can be obtained by calorimetric measurements during a bioprocess, gives a general global measurement reflecting all the activities taking place in cells. Although, non-specific heat measurements may provide an important indicator of the nature of the process and the main events occurring during the process. In order to get a quantitative information about the metabolism (underlying chemical changes) additional chemical or thermochemical information is necessary such as the knowledge of the different chemical reactions taking place simultaneously, the number of moles and the heat of reaction of each reaction. This can be done with additional measurements either on-line (FTIR, dielectric spectroscopy, optical density, exhaustion gas analysis, etc.) or off-line (determination of viable and total cell number with a hemacytometer, HPLC, enzymatic analysis of metabolites and products, etc.).

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Table 1
Some biotechnological aspects for batch cultures of cells related to large and low heat effects

	Aerobic growth	Animal cells/Anaerobic growth
Heat production rate	>2 W/l	<2 W/l
Calorimeter resolution needed	100 mW/l	<20 mW/l
Calorimeter stability needed	<24 h	Several days, weeks
Control/monitoring	Different on-line signals available	Very few on-line signals available

Additionally, the calorimetric heat-flow signal can be used as a helpful tool for the monitoring and control of biological processes [6–8]. In terms of production-scale the ultimate consistency of a bioprocess performed in a bioreactor requires adequate continuous monitoring and control means, preferably on-line. The heat generation is often correlated to other process variables such as the concentration of biomass formed, CO_2 production, O_2 consumption, etc. Besides alleviating the shortage of suitable biosensors [9], calorimetric heat-flow rate measurements have a considerable advantage over existing techniques in that it directly measures the time-derivative of heat rather than the total heat produced. Furthermore, calorimetric signal has the particularity of a short-delay acquisition which is essential for metabolic probes especially when they are intended to control industrial processes [10]. Heat measurement could quite easily be used for the on-line measurement and control of industrial bioprocesses, where known characteristics of the calorimetric heat-flow signal determine the moment at which a certain action is to be taken (controlled glucose supply based on the calorimetric heat signal with the aim of avoiding crabtree effect in yeast cultures for instance [11]). This has recently been demonstrated in scaling-up the laboratory scale experiments for a 300 l culture [12].

Heat flux measurements would also be especially useful in animal cell cultures at laboratory scale because of the difficulties of measuring anything on-line in such cultures. For instance, the CO_2 evolution rate is impossible to measure because CO_2 is used to buffer the system [8]. Some aspects related to the application of heat flux measurements to biological processes are shown in Table 1.

Although direct calorimetry has been applied to various investigations concerning medical, pharmacological, toxicological and biotechnological subjects [1,8,13,14], its application to low heat related bioprocesses, such as animal cells cultures, has been hindered by the need to measure very low heat generation rate under tightly controlled culture conditions. The use of microcalorimeters, which achieve the necessary sensitivity to measure such a low heat flows, was hindered by the technical difficulties in fulfilling the biological environmental needs (oxygen and substrates supply, a controlled pH, mixing, etc.) in the small volume of the microcalorimeter measuring cell (typically around 1 ml) [13,15,16]. In contrast, bench-scale calorimeters have the capacity to perform a well-controlled bioprocess from the biological point of view in tightly controlled culture conditions. However, their low sensitivity has hindered their application to low heat related bioprocess [15,17,18].

The aim of this paper is to investigate in detail the factors which limit signal resolution of the bench-scale reaction calorimeter, RC1 from Mettler-Toledo AG, and how these could be overcome by pertinent devices which limit and stabilize heat flow to and from the surroundings. The resolution obtained with the modified RC1 is comparable to that of microcalorimetric systems. This paper also reports the influence of the bioprocess parameters on the final resolution of the RC1 as they show to be determinant for such a high sensitivity measurements.

2. Fundamentals

In dealing with low heat effect cell cultures, such as animal cells cultures, the heat flows arising from operating factors, which have nothing to do with the cell metabolism–heat of reaction, can become more important than the heat of reaction itself. Therefore, a careful examination of all heat flows present is necessary. The heat balance around the bench-scale

RC1 calorimeter is given by Eq. (1):

$$C_{P,r}\frac{dT_r}{dt} = q_{bio} - q_f + q_{cal} + q_s - q_l + q_a \quad (1)$$

The left-hand term in Eq. (1) is the heat accumulation in the reactor's contents. This term can be neglected when working in isothermal mode, provided that the temperature of the reactor broth is tightly kept at the set point.

The variable, q_{bio} is the metabolic heat generation rate, which represents the target heat-flow rate. It can be calculated from Eq. (1) if all the other terms are measured or known. The variable, q_f represents the heat-flow rate transferred to or from the reactor jacket to the culture broth. In heat flux calorimetry, q_f is measured through the temperature difference $(T_r - T_j)$ as

$$q_f = UA(T_r - T_j) \quad (2)$$

where UA is the global heat transfer coefficient. Usually, a calibration heater delivering a well-known amount of heat for a short period of time, q_{cal}, is used to calibrate UA. The variable, q_f may well be negative for an operating temperature (T_r) higher than environment temperature since the heat is supplied to the culture broth in order to reach T_r. If the culture is exothermic the absolute value of q_f will consequently lower, thereby less heat is necessary supplied in order to keep T_r constant.

The variable, q_s is the heat flow produce by the agitation of the broth, $-q_l$ the heat flow lost to the environment, and q_a the heat flow due to liquid or gas addition to the broth. These terms are often assumed to be constant and, therefore, grouped as the base-line heat flow, q_{bl}. In order to determine q_{bl}, q_f is measured under stable operating conditions (T_r, agitation, gas and liquid supplied rates) and before the cells start to grow.

The influence of broth agitation on total heat flux can be monitored using a torque meter. This heat-flow rate, q_s, is then defined by Eq. (3), where R is the stirring speed (rpm) and τ the measured torque (Nm):

$$q_s = 2\pi\tfrac{1}{60}(R)\tau \quad (3)$$

The variable, q_s can vary with foaming, addition of anti-foam, releasing of viscous products, volume changes resulting from sampling/feeding, etc. In the case of animal cells, q_s will frequently remain constant enabling the simpler solution of incorporating q_s in the base-line signal.

The heat losses to the surroundings take place above all through the non-jacketed parts of the reactor. This includes the head-plate and every probe in the head-space which act as heat bridges to the surroundings. The variable, q_l will depend on the surroundings temperature, the temperature inside the reactor, and the heat transfer coefficients of the different parts concerned. Some authors [15,19] have tried to monitor the surroundings temperature and by the means of a proportional model estimate q_l for further correction from q_f. As will be discussed in this work, this solution will add noise to the calorimetric signal (non-negligible in the case of animal cell cultures) mainly due to fast changes in the surroundings temperature. In the present work, insulating or thermostatic housing around the reactor are constructed to limit q_l. Furthermore, the part of the metallic probes and inserts placed in the broth have been constructed, as much as possible, from an insulating material (PEEK) to reduce the heat losses by conduction through a metal bridge. If the variation of the subsisting heat losses is negligible compared to the bioprocess heat flow, q_{bio}, they may be incorporated to the base-line signal.

Gas/liquid additions are usual in bioprocesses even when operating in a batch mode. Cells need to be continuously supplied with gas (commonly O_2, CO_2). Liquid addition is punctually necessary in the case of exhausted metabolites, the addition of a different substrate for a targeted production of a certain compound, the pH control of the broth via acid/base addition, etc. The gas/liquid supply introduces a convective heat flow, which depends on the temperature difference between the fluid and the broth temperatures. Any additional event (chemical reaction heat through neutralization, dilution heat, water saturation of the in-flowing gas, etc.) influences q_a. Liquid additions to a batch animal cell culture are generally limited, hence only the influence of gas supply has been tested here.

A supplementary heat flow should be also taken into account. Water vaporized from a warm broth will condensate in contact with a cold head-plate, thereby the drops will fall into the broth and will provoke a temperature disturbance. This effect is avoided in this work using a thermostatic circuit in the head-plate and/or an insulating housing around the head-space.

Assuming that there is no heat accumulation in the broth, that the influence of q_l and q_a are negligible, and that q_s remains constant, the calorimetric heat flow, q_f, represents the reaction heat, q_{bio}, as a result from Eq. (1):

$$q_f - q_{bl} = UA(T_r - T_j) - q_{bl} = q_{bio} \quad (4)$$

From Eq. (2) it can be seen that the accuracy of the temperature monitoring devices as well as the factor UA determine the sensitivity (short-term noise) of the calorimeter. The option of improving the sensitivity of T_r and T_j measurements reducing UA have been discussed elsewhere [17]. This paper is concerned with the uncertainty (long-term fluctuation) of the calorimeter, which is dependent on the operating conditions.

3. Methods and materials

3.1. Heat-flow calorimeter, RC1

A 2 l commercial reaction calorimeter, RC1 from Mettler-Toledo AG, Switzerland, was used. In order to be able to measure weakly exothermic cell cultures this calorimeter was modified to allow controlled biological reactions and high sensitivity measurements. The former was achieved by modifying the steel head-plate of the glass reactor to enable the introduction of different probes and connections needed for a bioprocess [16,18]. Fig. 1 shows the main modifications achieved in the RC1 calorimeter as well as the housings used in order to minimize the exchange heat to the surroundings. The structural modifications of the RC1 calorimeter, which enable a high sensitivity, were reported elsewhere [17,18].

A torque meter was used in order to allow the measurement of the power input through agitation. This torque meter was a transducer TG-02, processing unit AEG 093 from Vibrometer.

3.2. Housings

Several devices were conceived in order to limit and stabilize heat flow, q_l, to and from the surroundings.

3.2.1. Head-plate

The introduction of a thermostatic circuit inside the steel head-plate of the reactor, as shown in Fig. 1, was intended to avoid the energy exchange provoked by the evaporation of the broth and its condensation on a cold head-plate. Water at a controlled temperature runs in a closed circuit from a water bath into the hollow head-plate. Additionally, this construction would also lower the heat by conduction to surroundings through heat bridges that represent the different probes and connections dipping into the broth. The temperature of the water bath is kept constant and higher than the operating temperature: $T_{hp} = T_r + 2\,^{\circ}C$.

3.2.2. Reactor thermostatic housing

A housing was designed to minimize the heat flux to the surroundings, as shown in Fig. 1. The housing is made essentially of a transparent Plexiglas box with a door on a side that allows a restricted access to the reactor for emptying purposes for instance. Inside the box, a copper heat exchanger is connected to an externally located water bath, N6 from Digitana AG, Switzerland. The temperature of the water bath is dependent on the temperature inside the housing, T_h, by means of a control algorithm relying on the T_h measurement using a Pt-100 probe. Since the cell culture operation is made in isothermal mode, T_h is kept constant (with a resolution of 0.01 K) and equal to the operation temperature, T_r. A small electric foehn inside the housing is also used in order to improve the convection heat transfer and thereby to obtain a homogeneous temperature inside the housing.

3.2.3. Reactor insulating housing

A simpler set up, with the same aim as the preceding one, was also investigated. It consists of an insulate, 50 mm thick, polycarbonate box around the glass reactor, Fig. 1. A door on a side and a smaller one at the bottom allow operations on the reactor (emptying and manipulation of the reactor outlet knob, respectively).

3.2.4. Head-space thermostatic housing

As mentioned earlier, the probes and connections which go through the head-plate into the broth act as heat bridges to the surroundings. A similar set up as the one used for the reactor thermostatic housing is used around the head-space of the reactor. The box was designed to allow the permanent passage of the different connections needed (electric, gas and liquid

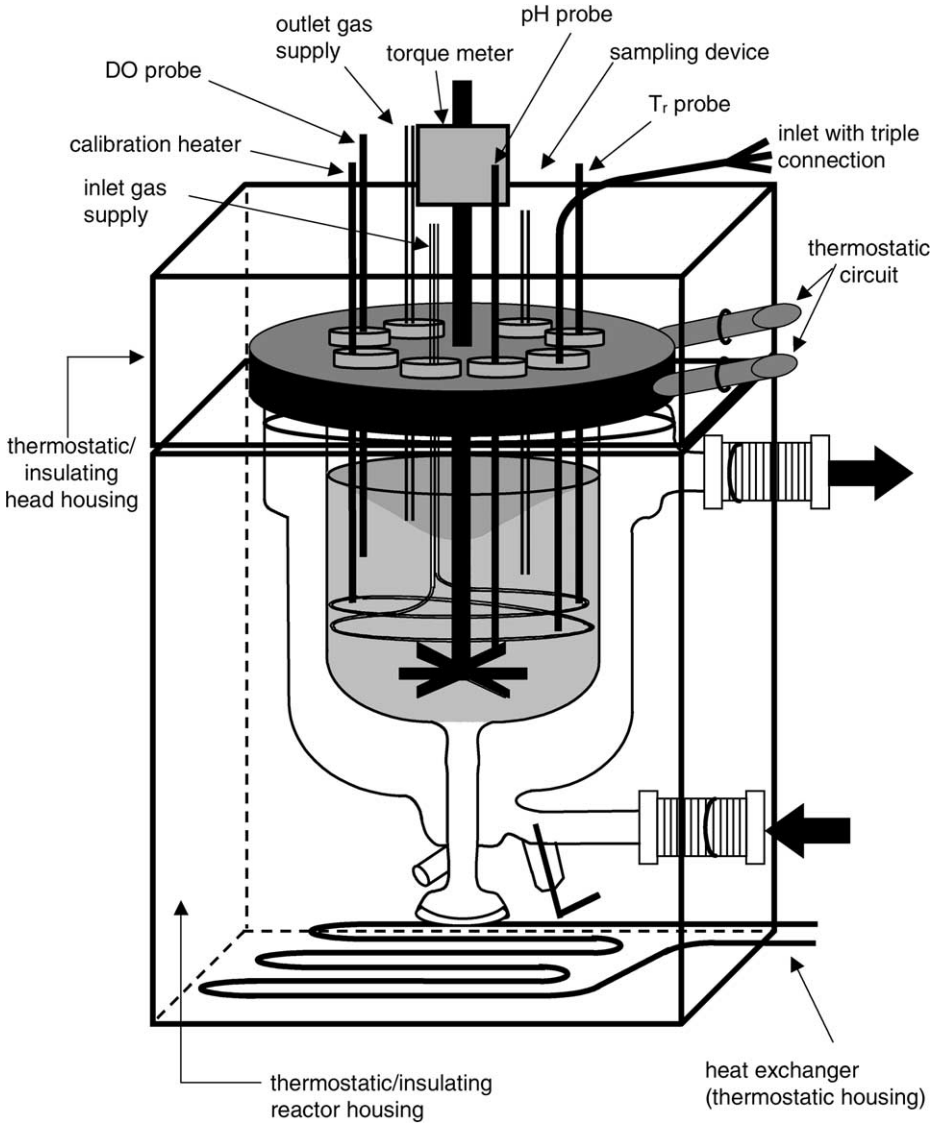

Fig. 1. Schematic representation of RC1 as a bioreactor with the thermostatic circuit in the head-plate, the thermostatic/insulated housings of the reactor and the head-space, and all the probes and connections necessary in the bioprocess.

supply, gas and broth sampling, etc.). A door on a side allows diverse manipulations necessary to the bioprocess (inoculation, supply of metabolites, acid–base for pH control, anti foam, etc.), Fig. 1.

3.2.5. Head-space insulating housing

A Plexiglas insulating housing was placed around the head-plate of the reactor as shown in Fig. 1.

3.3. Identification of non-homogeneous jacket oil flow-pathways in glass reactors of different geometry

The hydrodynamics of the oil inside the reactor jacket was suspected to be non-homogeneous, and this would affect to the calorimetric heat flux measurement. Indeed, it was observed that small air bubbles in the oil follow preferential pathways indicating

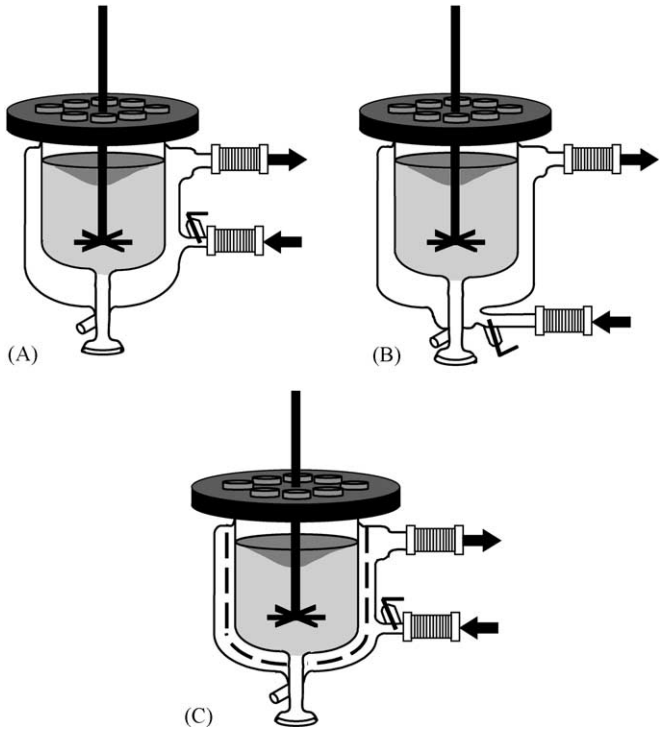

Fig. 2. RC1 glass reactors: (A) version I, (B) version II, and (C) ''Emmental'' version.

stagnant zones in the jacket. One way to confirm this behavior was to measure the internal wall temperature of the jacketed glass reactor at different places of the broth side surface.

Three glass reactor models provided by Glass Keller AG, Switzerland, were tested. A schematic representation of the three glass reactors used is shown in Fig. 2. Reactors A and B are the modified versions of the standard RC1 glass reactor, and the reactor C is an ''Emmental'' reactor. Compared to the standard RC1 reactor, the reactor A has a larger jacket at the bottom of the reactor. In this way, the pressure of the oil in this region decreases allowing a homogeneous rise of oil all around the reactor. A second modification in reactor A is the presence of a baffle (not shown in Fig. 2) in order to create turbulences in the oil before it rises along the reactor wall. The reactor B is a further modification of the reactor A where the inlet of the thermostatic oil is placed directly at the bottom of the reactor instead that on the side, accentuating the homogeneity of the oil up-flow around the reactor. The ''Emmental'' version (reactor C) is based on the standard RC1 reactor and consists of a double jacket where the two jackets are interconnected by a series of randomly placed holes all around the reactor. In this last version, the thermostatic oil enters into the outer jacket. From there and through the holes the oil passes into the inner jacket from where it finally reaches the exit. The ''Emmental'' glass reactor was kindly developed by Glass Keller AG, Switzerland.

The temperature mapping was performed with RTD probes, Pt-100 FKG, from Omega, USA, with a four-cable type connection. The RTD probes were interfaced with isolated RTD input modules, 5B34, from National Instruments with an accuracy of 0.05% full range (0–100 °C). The acquisition was performed with a user developed LabView program. The measurement system was calibrated against a quartz probe.

The RTD probes are small flat surfaces (10.2 mm × 3.2 mm × 1 mm) with only one sensitive side. They were randomly placed to the inner reactor surface with a thin layer of a thermoconductive paste and covered

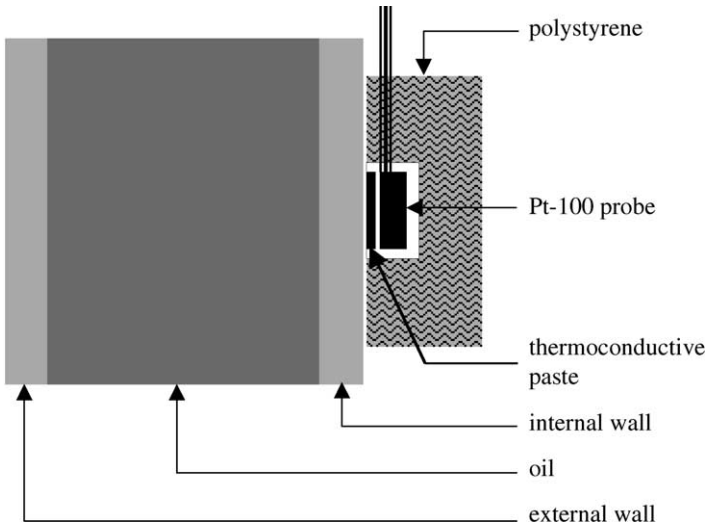

Fig. 3. Temperature probe set up used to check the oil flow-pathways in the jacket of glass reactors.

with a piece of insulated polystyrene, as shown in Fig. 3. The empty reactor was then covered with a polystyrene lid to avoid heat convection from the surroundings directly inside the reactor. The RC1 is operated in T_j mode, which sets constant the temperature of the thermostatic oil. In this way, if there are no stagnant zones the temperature of the internal reactor wall should be homogeneous. Usual animal cell culture temperatures were tested: 28, 37 and 50 °C.

3.4. Uncertainty of base-line measurements

A series of blank experiments were performed to determine the resolution of the calorimeter. The glass reactor B was used in these experiments. The resolution of the system is determined both by the intrinsic sensitivity of the RC1 and by the uncertainty introduced through the experimental set up. Two factors affecting the intrinsic sensitivity of the RC1 are studied: the electronic noise and the temperature control algorithm. Several experiments are also performed to study the influence of the different housing set ups on the heat losses. Experimental parameters relevant to the bioprocess are also tested in terms of uncertainty that they introduce to the calorimetric measurement: operating temperatures, stirring speeds, and gas supply flow rates.

The influence of different parameters of the temperature control algorithm, i.e. the sensitivity of the RC1, was assessed by the standard deviation, σ, determined over a time interval of 200 s (100 data points since RC1 acquires data every 2 s). During the evaluation of electronic noise assessment, heat-loss experiments and the uncertainty introduced by the process parameters, an average value was calculated for each of the respective time intervals. The resolution of the system, expressed as ±mW/l, was assessed via the half of the maximum peak-to-peak difference of the q_f averaged data measured during a given period. In the case of electronic noise assessment and heat-loss experiments an averaging interval of 600 s (300 data points) was used and these values were observed over a period greater than 24 h. During the evaluation of the uncertainty introduced by the process parameters the averaging interval used was of 60 s (30 data points).

3.4.1. Electronic noise

The monitoring of relevant temperatures of the RC1 introduces a significant noise to the calorimetric signal due to electronic signal transport and conversion from analogs to digital data. In order to assess the basic-noise of the system both temperature probes, T_r and T_j, were replaced by highly stable 100 Ω resistors. Each resistor has a constant resistance value, which is equal

to the resistance of a Pt-100 RTD probe at 0 °C. The resistors have a low temperature dependence of 5 mK/K. Furthermore, both resistors were kept at 0 °C by immersion in a gently stirred ice-water bath in a Dewar flask during the measurement in order to limit their temperature variation. During the experiments the temperature of the ice-water bath was also measured with an extra Pt-100 probe.

3.4.2. Heat-loss experiments

Blank experiments were performed to assess the influence of the different housing set ups. The reactor was set up with all the probes and connections necessary for a bioprocess but it was only filled with water (volume of 1.6 l). The RC1 was operated in isothermal mode, T_r remained constant, at 28 °C. Agitation rate was 150 rpm and no gas was introduced during these experiments. The different housing devices were used alone and in combination. The housing thermostat was set to $T_h = T_r = 28$ °C. The temperature of the circulating water in the head-plate was set to 30 °C. The temperature of the head-space thermostat was also set to 30 °C. The temperature inside the housings and of the surroundings was simultaneously monitored by different Pt-100 probes. Each measurement lasted over 24 h to evaluate the behavior of the different housings under significant changes of ambient temperature (day and night).

3.4.3. Uncertainty introduced by process parameters

Usual parameters in animal cell cultures were tested. In most cases, a mild agitation is used to avoid damaging the fragile animal cells. Here, 150 and 500 rpm were tested; 1000 rpm was also tested for comparison means. The operation temperatures tested were 28, 37 and 50 °C. Animal cells need a gas supply (the most common: O_2, CO_2 or N_2) usually at low flow rates. A supply of 160 ml/min of air was provided through a tubular porous membrane placed inside the reactor and regulated using a mass flow meter (Fig. 1). A higher air flow rate was also tested, 300 ml/min, sparged into the reactor after passing through a bubble column whose temperature was set equal to T_r. These series of experiments were carried out using a thermostatic housing at $T_h = T_r$ (except for $T_r = 50$ °C, where $T_h = 40$ °C for technical reasons), and a thermostatic circuit in the head-plate at $T_{hp} = T_r + 2$ °C. The experimental results of this section are presented using an averaging interval of 30 data points (60 s).

4. Results

4.1. Sensitivity of RC1 influenced by the temperature control algorithm

The sensitivity of the system is determined by the electronic noise as well as by the *PI* parameters of the temperature control algorithm for the thermostatic oil [18]. A good tuning of these *PI* parameters means a compromise between a higher sensitivity (reduced noise) and a short response time, i.e. a short settling time after a dynamic event.

Table 2 shows the influence of *PI* parameters on the sensitivity of the RC1. A detailed expression of these measurements has been published elsewhere [18]. It is worth noting that the standard deviation of T_r remains fairly constant and close to the resolution of the temperature probes A/D converters (0.2 mK), which is not the case for T_j. This result suggests that in any case the control of the temperature broth is excellent and, therefore, heat accumulation in the reactor can be neglected.

The optimum values found for the purpose of this study were $P = 4$ and $I = 0.05$. Under these conditions the response time to an excitation of 1.2 W/l was found to be less than 20 min [17]. The response time, which is dependent on the amplitude of the excitation power, is expected to be shorter in case of dynamic events related to animal cells. Indeed, animal cell cultures are generally low-enthalpy bioprocesses. Approximately, 0.2 W/l are liberated at a maximum

Table 2
Sensitivity expressed for as standard deviation for different *PI* parameters of the temperature control algorithm of the thermostatic oil[a]

P	*I*	$\sigma(T_r)$/mK	$\sigma(T_r)$/mK	$\sigma(q_f)$/mW/l
4	0.05	0.21	0.64	2.4
12	0.05	0.23	1.80	6.3
4	0.01	0.21	0.71	2.8
4	0.09	0.21	0.84	3.5

[a] Experiments were carried out with 1.6 l of water at $T_r = 28$ °C, stirring speed = 500 rpm and no aeration. Data are collected as an average of 100 data points (200 s) to avoid long-term deviations, which are not due to *PI* parameters.

cell concentration (8×10^6 cells/ml) for SF9 insect cell cultures, and approximately 0.05 W/l are liberated at a maximum cell concentration (3×10^6 cells/ml) for SP2/0-52 murine cell culture. Furthermore, in the case of a batch mode the time scale of an animal cell culture is of the order of several days to weeks, therefore, a calorimetric response time of a few minutes is perfectly reasonable. The P value will be increased to 14 for the sterilization sequence and whenever a shorter response time is required.

4.2. Influence of electronic noise on the RC1 resolution

During a 20 h run the ambient temperature signal presented a maximum peak-to-peak difference of 1.8 °C, whereas the volumetric heat-flow rate, q_f, calculated from the $T_r - T_j$ measurements, presented a maximum peak-to-peak difference of ±3 mW/l, as shown in Fig. 4(A). Assuming that the electronic noise is linearly related to ΔT_{amb}, this means that for every degree of change in the surroundings temperature the electronic noise varies by ±1.6 mW/l. This is a limiting value that corresponds to a system in a strict steady state. During actual operation, the uncertainty will be further affected by the working conditions.

4.3. Influence of heat-losses (different housing set ups) on the RC1 resolution

During blank experiments the RC1 is programmed to keep the reactor temperature, T_r, constant. Since there is no biological process and additional heat flows are kept constant (agitation rate = 150 rpm, no gas supply, no liquid supply) only heat losses will affect the calorimetric measurement. In Fig. 4(B) the calorimetric heat-flow signal follows carefully the ambient temperature signal and it is equal to minus the heat flow lost to the surroundings.

Limiting heat losses to surroundings clearly enhances the calorimetric resolution of the system as shown in Fig. 4. The resulting resolution of the different set ups appears to be dependent on the changes in T_{amb} during the measurement, therefore, a normalized resolution is used in order to compare the effect of the different housings. The normalized resolution is the resolution value normalized for a change in ambient temperature of 1 °C:

$$\text{resolution}_{nrm} = \frac{\text{resolution}}{\Delta T_{amb}}$$

The resolution and normalized resolution values for the different housing configurations are listed in Table 3. The normalized resolution value for the RC1 without housings was found to be ±42.6 mW/l K. An improvement of the calorimetric normalized resolution was observed in Fig. 4(C) and (D) when heat losses at the reactor side or at the head-plate, respectively, are lowered individually. The normalized resolution values decreased to ±9.8 mW/l K when the reactor insulating housing was used and to ±15.0 mW/l K when the thermostatic circuit in the head-plate was used. The simultaneous limitation of heat losses at the reactor and at the head-space

Table 3
Characteristics of different housing configurations[a]

Reactor housing	Head-plate thermostat	Head-space housing	ΔT_{amb}/K	Resolution in mW/l	Resolution_{nrm} in mW/l K
Electronic noise			1.7	±2.8	±1.6
–	–	–	1.1	±46.9	±42.6
Insulating	–	–	2.3	±22.5	±9.8
–	Thermostatic	–	2.0	±30.0	±15.0
Thermostatic	Thermostatic	–	1.5	±5.6	±3.8
Insulating	Thermostatic	–	2.0	±10.5	±5.2
Insulating	–	Insulating	2.5	±28.1	±11.3
Insulating	Thermostatic	Insulating	2.0	±6.0	±3.0
Insulating	–	Thermostatic	3.2	±9.38	±2.9

[a] Base-lines are taken as an average of 300 points (600 s). T_r = 28 °C. Reactor housing thermostat = 28 °C. Head-plate thermostat = 30 °C. Head-space housing thermostat = 30 °C. Stirring speed = 150 rpm. No aeration. The "Resolution_{nrm}" are resolution values that have been normalized to a ΔT_{amb} = 1 °C for comparison means.

results in an optimized resolution close to that of the electronic noise. Fig. 4(E) and (F) show the base-line signal when a combination of different housings was used with a thermostatic circuit in the head-plate in both cases. In both figures, a fairly flat base-line signal similar to that obtained in Fig. 4(A) is observed. The normalized resolution values were ±3.8 and ±3.0 mW/l K, respectively. This proves that the insulation/thermostatic of the entire system, i.e. the glass reactor and the reactor head-space, is needed for a good resolution.

Regarding the heat losses from the glass reactor, it is interesting to note that the simple insulating housing used here gives comparable results as the more complicated thermostatic one (±5.2 and ±3.8 mW/l K, respectively, as listed in Table 3). In the case of the heat losses at the reactor head-space side, the addition of an insulating device around the head-space

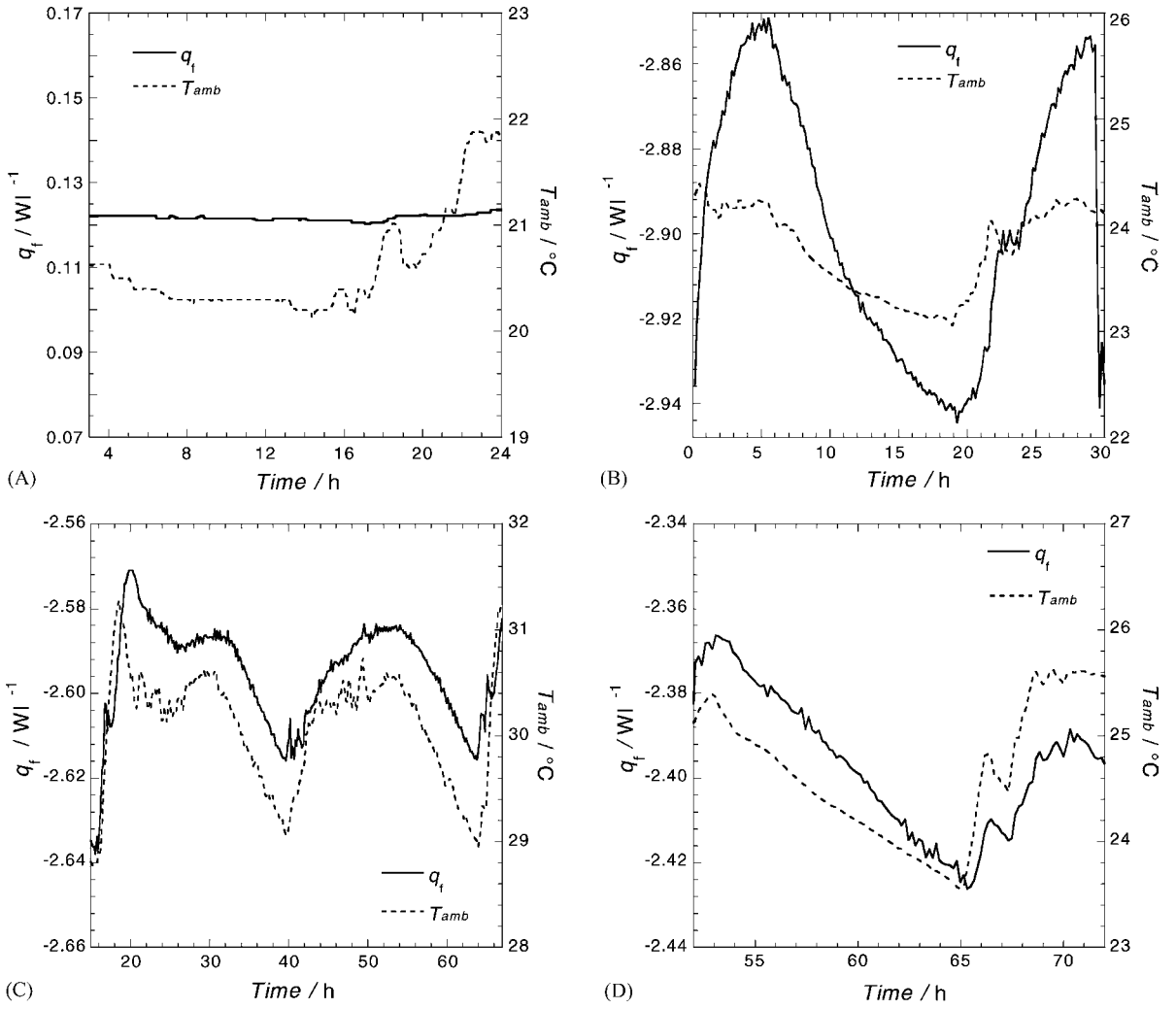

Fig. 4. Bio-RC1 base-lines taken for an averaging interval of 600 s (300 points) during a period greater than 24 h in all the cases. Experiments were carried out with 1.6 l of water at T_r = 28 °C, stirring speed = 150 rpm, and no aeration. Solid lines represent the heat flux transferred to the culture broth and dashed lines represent the ambient temperature. (A) Electronic noise only, (B) without housings, (C) with reactor insulating housing, (D) with head-plate thermostat = 30 °C, (E) with reactor housing thermostat = 28 °C and head-plate thermostat = 30 °C, and (F) with reactor insulating housing, head-space insulating housing, and head-plate thermostat = 30 °C. The resolution$_{nrm}$ are resolution values that have been normalized to a ΔT_{amb} = 1 °C for comparison means.

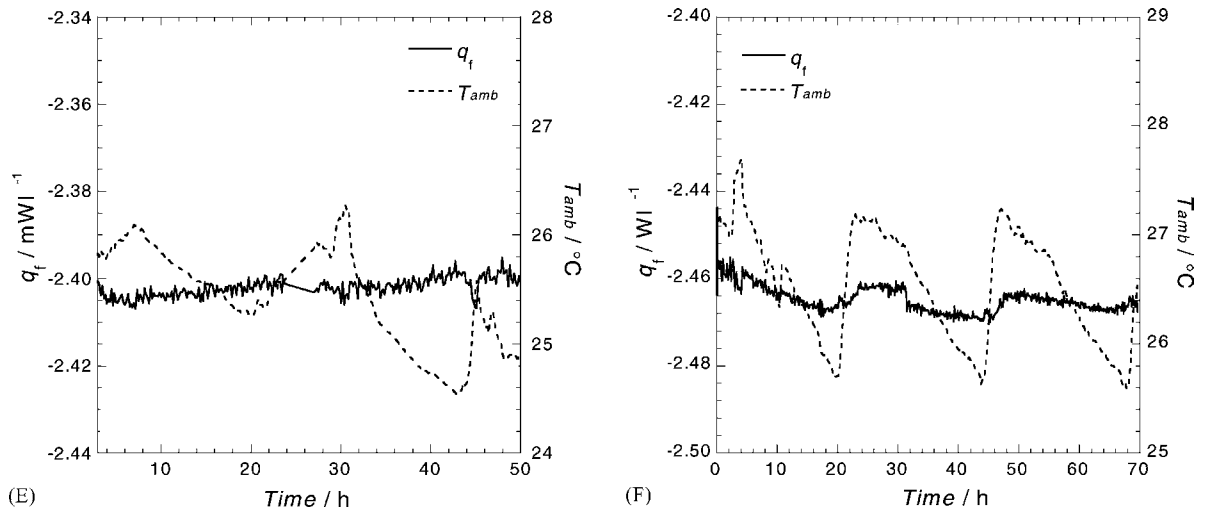

Fig. 4. (*Continued*).

improves the normalized resolution decreasing from ±5.2 to 3.0 mW/l K, as listed in Table 3. In contrast, the use of a thermostatic device gives better results than the insulating housing only, ±2.9 and ±11.3 mW/l K, respectively, Table 3. This result is probably due to that the insulating housing of the reactor is made of 50 mm thick polycarbonate while the insulating housing of the head-space is made of thinner Plexiglas. The normalized resolution is equal when a thermostatic housing of the head-space or a combination of insulating housing and thermostatic circuit in the head-plate are used (compare ±2.9 to 3.0 mW/l K in Table 3, respectively).

It must be pointed out that even in an ideal situation where the calorimeter would be placed in a thermally isolated room, care should be taken every time the operator enters and exits the room for the bioprocess usual manipulations (inoculation, sampling, etc.). From this point of view the devices developed here are perfectly suitable for an easy operation with an enhanced normalized resolution of ±3.0 mW/l K (in absence of other operating influences). The use of a proportional model to correct the heat losses to the surroundings by multiplying an on-line monitored ambient temperature by a correcting factor has been reported in the literature [15]. This solution would introduce additional uncertainty to the measurement since T_{amb} is not perfectly proportional to the measured calorimetric heat flux, as can be seen in Fig. 4(B). Furthermore, small peaks are observed in the calorimetric data whenever changes in ambient temperature are sudden and important.

4.4. Oil flow pathways in the jacket of glass reactors of different geometry

The temperature mapping was performed to visualise the oil flow pathways for three jackets with different geometry. The internal wall temperature of the jacketed glass reactor was measured at different places of the internal side surface. Fig. 5 shows the temperature mappings obtained for the three jackets studied here. A clear preferential pathway of the thermostatic oil is observed with the RC1 glass reactor A (see Fig. 5(A)). The oil enters the jacket through the inlet connection located at the side of the reactor (lower position) and preferentially takes the shortest way to reach the exit at the outlet connection (higher position). In this way, the side opposite to the connections has a lower oil flow rate, i.e. lower temperature, with a clear stagnant zone directly opposite to the outlet (top level of the jacket). A maximum temperature difference of 1.5 °C ± 0.1 °C has been observed at the internal surface of the reactor when operating at a jacket constant temperature of $T_j = 50$ °C.

If the inlet connection is placed at the bottom of the reactor, reactor B, the above observed preferential oil pathway is avoided. This reactor shows a very

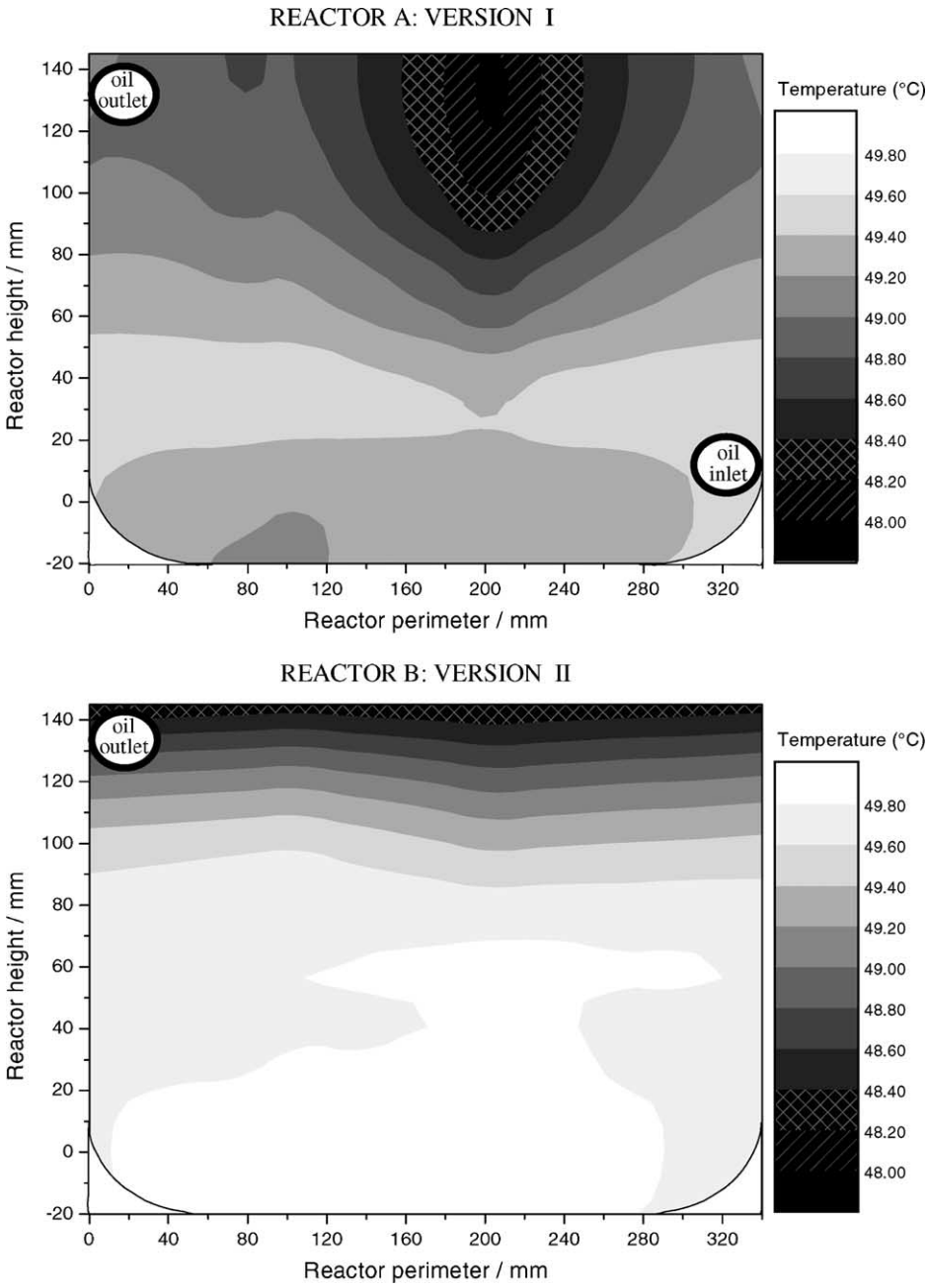

Fig. 5. Oil flow-pathways in the jacket of different reactors. The panels depict a flat representation of the cylindrical reactor internal wall. Different shadings indicated different temperatures.

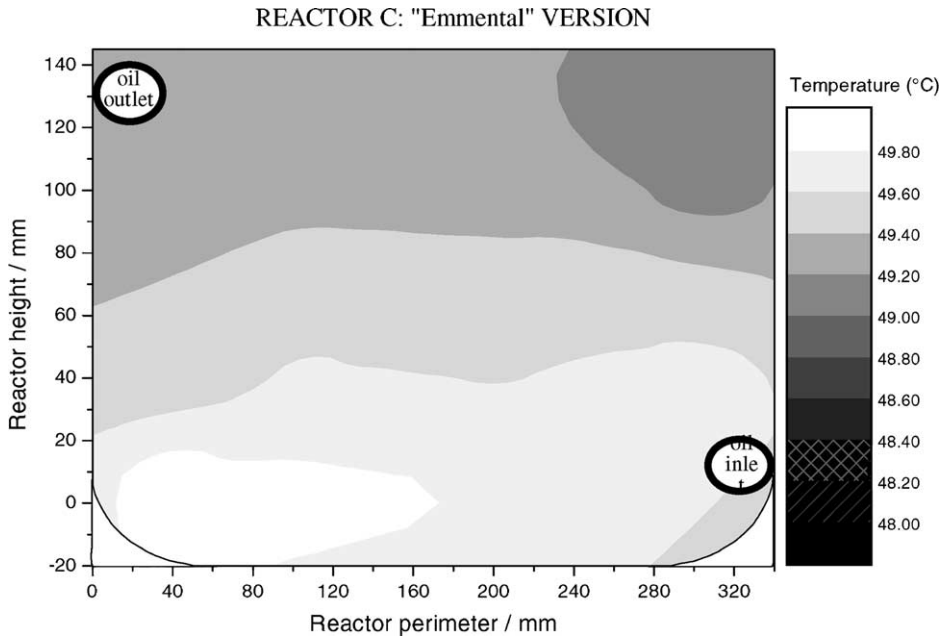

Fig. 5. (*Continued*).

homogeneous internal reactor surface temperature (bottom and side wall) of 49.8 °C ± 0.1 °C with the exception of the top ring zone of the jacket. A maximum temperature difference of 1.6 °C ± 0.1 °C has been observed at the internal surface of this reactor when operating at a jacket constant temperature of $T_j = 50$ °C.

In the case of the ''Emmental'' version, reactor C, preferential pathways are more difficult to determine because of the double jacket. The top ring of the jacket presents, as in the preceding cases, a lower temperature. This is not astonishing since the heat losses between the jacket end and the heat-plate of the reactor to the surroundings is larger in all three reactor versions. A maximum temperature difference of 0.8 °C ± 0.1 °C has been observed at the internal surface of the reactor when operating at a jacket constant temperature of $T_j = 50$ °C.

Best set up seems to be the reactor B since it shows the most homogeneous surface temperature of the reactor although the maximum temperature difference was greater than that for the reactor C. It is worth noting that the usual working volume is 1.6 l, which corresponds to two-thirds of the reactor volume. Similar behaviors were observed for the three versions at lower operating temperatures with smaller of maximum temperature differences (results not shown in this work).

4.5. Influence of process parameters on the calorimetric resolution

In these series of experiments a thermostatic housing reactor as well as a thermostatic circuit of the head-plate were used. Although the sensitivity of the calorimeter has been greatly enhanced so far, the uncertainty of measurements will essentially depend on the heat-flow fluctuations brought about by the bioprocess operation parameters.

In the range of animal cells working temperature, between 28 and 50 °C, the resolution of the system varies between ±5 and ±15 mW/l (with a stirring speed of 500 rpm), as listed in Table 4. As can be seen in Table 5, mild stirring speeds, up to 500 rpm, have a limited influence on the calorimetric resolution, from ± 2 to ± 5 mW/l for stirring speeds of 150–500 rpm, respectively, at $T_r = 28$ °C. Above 800 rpm, bubbles are formed in the medium, which break on the walls around and a vortex is also formed. These events explain the considerable lower resolution at 1000 rpm

Table 4
Calorimetric resolution for different operating temperatures[a]

T_r/°C	Resolution in mW/l
28	±5
37	±12
50	±15

[a] No gas supply, stirring speed = 500 rpm. Data are collected as an average of 30 data points (60 s).

Table 5
Calorimetric resolution for different stirring speeds, R[a]

R in rpm	Resolution in mW/l
150	±2
500	±5
1000	±35

[a] $T_r = 28$ °C and no gas supply. Data are collected as an average of 30 data points (60 s).

(±35 mW/l). A similar analysis can be made on the influence of gas supply. The low gas flow rates tested, 160 and 300 ml/min, introduce a limited noise to the resolution of the system (see Table 6).

Cultures of SF9 insect cells are typically performed at 28 °C, 150 rpm of stirring speed and 160 ml/min of aeration gas flow rate. Under these conditions and for a batch culture (no substrate supply) a calorimetric resolution of ±4 mW l^{-1} can be expected. Murine SP2/0-52 cell cultures are generally performed at 37 °C, 150 rpm of stirring speed and 160 ml/min of aeration gas flow rate. Under these conditions and for a batch culture (no substrate supply) a calorimetric resolution around ±9 mW/l can be expected. Calorimetric results of different animal cell cultures performed with this instrument will be published elsewhere.

Table 6
Calorimetric resolution for different gas supply flow rates[a]

G in ml air/min	Resolution in mW/l	
	$T_r = 28$ °C, $R = 150$ rpm	$T_r = 37$ °C, $R = 500$ rpm
0	±2	±12
160	±4	nd.
300	nd.	±15

nd.: no data available.
[a] Data are collected as an average of 30 data points (60 s).

4.6. Uncertainty against delay in data processing

Although already reported before [15,18], the importance of this point when the resolution of different systems is studied and especially when it is compared is such that the following paragraph shows to be relevant here. The resolution of measured signal can be considerably improved by averaging out the data. This should be taken into account when the performances of various calorimeters are compared. Although, with a large averaging interval the uncertainty will be consequently reduced, as can be seen in Table 7, data will only be available with some delay, and, therefore, relevant dynamic events might also be averaged out. On the other hand, a small averaging window will provide with a more recent but also greater uncertainty.

A compromise is necessary depending on the particular bioprocess under study. In the case of yeasts, these microorganisms present a short response time to changes in environmental conditions (seconds rather than minutes). The use of a short averaging interval (a few seconds) allows a calorimetric detection of dynamic events (for instance metabolic reaction to pulses of metabolites) which can not be detected by other means like gas analyzers [15]. Among all on-line monitoring means available for bioprocess, mainly for animal cells, calorimetry has the particularity of a high measurement speed (2 s for the RC1). As a comparison, analysis of exhaustion gas has a response time in terms of minutes. The delay caused by the gas hold up in the broth and in the head-space of the reactor plus the gas transport to the analyzing device represents a built-in averaging mechanism.

Table 7
Calorimetric sensitivity expressed as standard deviation for different averaging intervals, data is acquired every 2 s[a]

Number of points	Averaging interval (s)	Sensitivity in mW/l
1	2	±5.8
15	30	±1.5
300	600	±0.34

[a] $T_r = 28$ °C, stirring speed = 500 rpm and no gas supply.

5. Conclusion

The actual resolution of the bench-scale reaction calorimeter, RC1, is largely determined by the heat flows produced by the bioprocesses parameters. By limiting heat losses to the surroundings and under gentle broth agitation (below 500 rpm) and mild gas supply (below 300 ml/min) a resolution from ±4 to 15 mW/l is observed. These are usual conditions of animal cell cultures, however it is necessary to take special care when liquid supply is required because of the possible large heat side-effects like the heat through neutralization for pH control. In most animal cell cases, such additions are made drop-wise and with a low concentration. Therefore, in these cases they are visible on the calorimetric signal as small punctual peaks in the same way as sudden and strong variations of surroundings temperature.

The use of thermostatic or insulating housings both at the reactor side and at the head-space side have shown to be necessary and adequate in limiting the heat losses to the environment. The glass reactor B offers the most homogeneous temperature control through the whole contact surface, provided that the reactor is full at two-third and, therefore, the upper ring of the jacket is not in contact with the broth.

The novel resolution of this instrument is comparable to the one reported from microcalorimeters in the literature [2–5]. In contrast to the microcalorimeter, in a bench-scale calorimeter the environmental needs of the bioprocess are perfectly controlled and monitored. Furthermore, the modified RC1 calorimeter is an in situ on-line tool suitable as metabolic-probe in industrial bioprocess. Its application, in combination with additional analytical means, offers for instance the potentiality to study metabolic pathways, the rational optimization of medium, and the productivity enhancement of target proteins.

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