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Large-scale calorimetry and biotechnology

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Abstract

Heat dissipation in biotechnical processes is often a very conspicuous phenomenon in large scale bioreactors. These reactors could hence be considered as a form of calorimeter in that the heat dissipation rate could be measured without sophisticated probes or instrumentation. Such measurements are scarce in large scale fermenters, but literature exists on calorimetric measurements carried out in the laboratory under conditions closely resembling those maintained in industrial reactors. The calorimetric techniques used for such experiments are reviewed briefly, followed by a description of a few examples of the results obtained. Published reports are described which illustrate how the knowledge gained by such experiments could be applied to industrial bioprocesses in order to control the reactor, based on monitoring the heat dissipation rate of the culture.

1. INDUSTRIAL BIOREACTORS AS CALORIMETERS

Biological calorimetry works with a whole variety of different calorimeters. The scale of the instruments used ranges from microcalorimeters for samples sizes of just a few millilitres or less to very large calorimeters such as the ones used in whole body calorimetry and includes bench calorimeters of varying sizes and designs for studies on microbes, plants and various types of animals.

What is often overlooked is the fact that large scale bioreactors also represent a form of calorimeter. This is perhaps understandable since heat effects in cellular cultures are not obvious when working with conventional laboratory equipment, most of the heat released by the culture being lost to the environment too quickly to give rise to a noticeable temperature increase. This is completely different at large scale. As opposed to laboratory reactors, industrial size fermenters operate nearly adiabatically due to their much smaller surface to volume ratio. Thus all the heat released by the culture must be removed by appropriate cooling facilities. Since bioprocesses usually operate at temperatures not much higher than the one at which cooling water is available, a very large heat transfer area is often needed to ensure accurate temperature control. It is thus not surprising to find that industrial scale culture vessels are densely packed with cooling

coils. If the temperature increase in the cooling water, its flow rate, and the other relevant energy exchange terms such as agitation and evaporation rates were measured systematically, the heat dissipation rate of the cellular culture could be quite easily and quantitatively monitored on-line.

The heat dissipation rates encountered at large scale may indeed be astounding, and ensuring the corresponding heat transfer rates for proper temperature control sometimes becomes a major technical challenge in the design of bioreactors of very large scales. The process developed in the seventies by Imperial Chemical Industries (ICI) for producing a special form of single cell protein called "Pruteen" is an example. For this process, a gigantic culture vessel of about 2300 m^3 nominal volume [1] was designed and built in order to grow the methylotrophic organism *Methylophilus methylotrophus* monoseptically on methanol as the sole carbon and energy source. The enormous amount of heat generated in this culture, for which an approximate rough figure of 80 MW(!) was given [2], must be dissipated into the environment by means of a cooling tower of considerable size. This figure may be roughly cross checked on the basis of published calorimetric data obtained in laboratory calorimeters [3]. According to this study aerobic growth of *M. methylotropus* on methanol produces 24 kJ per gram of dry biomass formed and thus is one of the most exothermic aerobic microbial growth processes known. On the basis of the process data given in Table 1, the production rate may be estimated as $0.2\ h^{-1} \cdot 30\ g\ l^{-1} \cdot 1.5 \times 10^6$ l, which amounts to 9 t h^{-1} of dry biomass, a figure that is consistent with the published production capacity (see Table 1). A mean methanol

TABLE 1

Compiled process parameter estimates for ICI "Pruteen" process

Process parameter	Value	Reference
Bioreactor type	Airlift with internal downcomers	1 4
Operation	Continuous culture	
Nominal volume (m^3)	2300	1
Working volume (m^3)	1500	4 2
Production capacity (dried product) (t $year^{-1}$)	50000–60000	5
Dry cell mass concentration (g l^{-1})	30	2
Specific Growth rate μ (h^{-1})	0.1–0.2	2
Methanol feed rate (t h^{-1})	14–20	5
Specific power dissipation for agitation and circulation (kW m^{-3})	1.6	4
Total power dissipation (MW)	7	1
Heat dissipation (Mcal h^{-1})	≈ 70000	2

feed of 18 t h^{-1} would give a biomass yield of 0.5 t per tonne of methanol a typical figure for *M. methylotrophus*. The culture may thus roughly dissipate 24 MJ $kg^{-1} \cdot 9000$ kg h^{-1} or 60 MW. If we add the power needed to agitate and circulate the culture and to provide adequate oxygen transfer (estimated at 7 MW by Schügerl but only at 2.4 MW by Westlake [4]) as well as other energy sources, the total energy dissipation may well be of the order of 70 MW. For this bioreactor, the term "megacalorimeter" is surely appropriate.

Systematic monitoring of heat generation rates by the culture in large scale bioreactors would clearly be of considerable benefit for process optimization and control. Indeed, computer process control schemes in technical fermentations are often limited by the number of relevant process variables that can be measured on-line and used as continuous input for bioprocess computer models. Probes which can be used for such on-line data acquisition have only been developed for a limited number of process variables.

As already discussed, the rate of heat generation by the biological process could be measured quantitatively in industrial size fermenters without resorting to sophisticated on-line probes. This information could then be used along with other on-line data in process control schemes, provided that suitable models are available which relate the heat evolution rate of the cell culture to other relevant process variables, such as substrate consumption, growth rate, or oxygen uptake rate. Unfortunately, the opportunity of using large scale bioreactors as calorimeters in order to collect calorimetric data and to correlate them to other important process parameters has only rarely been exploited. As a matter of fact, published calorimetric data obtained in industrial fermenters is scarce [2,6–11]. Moreover, such data points are unsuited for revealing possible relationships between biological heat release and other relevant process parameters because they have been obtained under a narrow range of operating conditions close to the technical conditions used for production. The large variations in operating conditions, cell metabolism, media composition, and strains needed for elucidating such correlations are impossible to perform in technical bioreactors.

This is why a number of workers have undertaken to study these relationships in calorimetric experiments at the laboratory scale. The purpose of the remainder of this paper will be to review some of the calorimetric techniques used and some of the results obtained in calorimetric experiments specifically aimed at developing quantitative relationships between heat release and other important process parameters for potential use in monitoring and controlling technical bioprocesses.

Due to space limitations, an exhaustive review cannot be attempted, and only a few applications will be discussed by way of typical examples. Part of the subject has already been reviewed elsewhere [12].

2. FROM MICROCALORIMETRY TO MACROCALORIMETRY

2.1. Experimental requirements

In order to obtain results pertinent to biotechnology, the calorimetric experiment must be performed under tightly controlled culture conditions that are realistic for technical bioprocesses. Moreover, all other relevant process parameters and variables must be measured during the culture.

Conventional microcalorimeters do not normally meet these criteria, although modern microcalorimeter designs afford at least some mixing and oxygenation, so that aerobic cultures with modest oxygen uptakes can be studied [13,14]. Yet experimental problems remain severe for the highly aerobic cultures of interest in biotechnology. Furthermore, microcalorimeters suffer from the fact that no parameter other than temperature can be controlled and no variable other than the heat flux can be measured in the culture. Thus even controlling the pH is difficult, let alone measuring the performance of cultures at constant p_{O_2} or under controlled nitrogen limitation. Taking large enough samples for thorough biochemical analyses is often impossible. This usually precludes quantitative interpretation of the observed heat generation rates and their correlation with other rate information.

As a result, most calorimetry work pertinent to biotechnology has been carried out in modified microcalorimeters or in calorimeters at the bench scale.

2.2. Modified and flow microcalorimeters

A number of researchers have designed microcalorimetric measuring cells similar to actual minifermenters by equipping them with miniaturized stirrers and air spargers [15–19]. The calorimetric system used by Ishikawa et al. [15–17] is based on a twin-type compensation calorimeter. Of the two 30 ml working-volume reaction vessels, one contained the growing culture, while the other was filled with medium alone. Sufficient oxygen transfer was attained by aerating with oxygen enriched air that was adequately dispersed by a small turbine stirrer. This system has been successfully operated to study the heat released by *Escherichia coli* under a range of nutrient limitations. Ishikawa and Shoda [17] even succeeded in using these vessels for chemostat cultures, which enabled them to control the pH and to collect samples for analysis.

Another way adopted by a number of researchers to alleviate problems associated with microcalorimeters is to grow the culture in a standard bench scale fermenter but to pump a continuous sample stream through a flow microcalorimeter (Fig. 1). The first flow calorimeters for precise biological measurements have been developed by Monk and Wadsö [20].

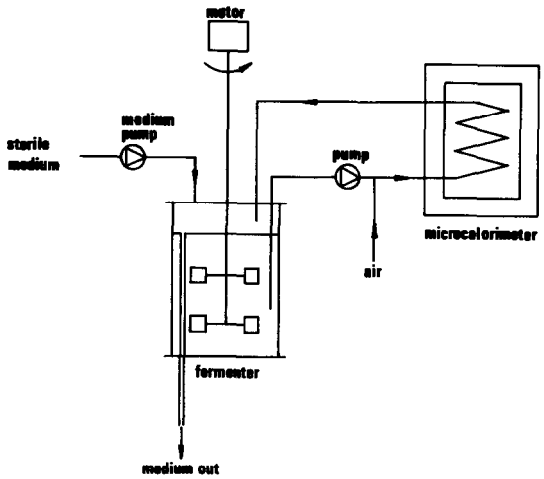

Fig. 1. Principles of flow microcalorimeters. Taken from ref. 12.

This design has been improved upon and forms the basis of the "Biological Activity Monitor" commercialized by LKB Produkter, Sweden [21]. This type of experimental calorimetry set-up can therefore be realised on the basis of commercially available equipment such as the Biological Activity Monitor or a similar instrument [22].

Coupling a flow-through microcalorimeter to a bench-scale fermenter enables the cultures to be grown under full control and all relevant process parameters to be monitored, if necessary, under strongly aerobic conditions. The technique, however, is plagued by new experimental difficulties, such as nutrient and oxygen exhaustion in the sample line and wall growth in the measuring cell [23]. An excellent account of all experimental pitfalls and technical difficulties encountered with microcalorimeters up to the late 1970s is given by Wadsö [24]. Recent experience with this calorimetric technique has been reported by Gustafsson and co-workers [22], who studied the energy balance during growth of the yeast *Saccharomyces cerevisiae* for different periods during batch growth.

2.3. Bench-scale calorimeters

Other efforts over the last two decades have resulted in three different types of calorimeter at the bench scale. These can be operated like standard laboratory fermenters but enable measurement of the heat dissipation rate in situ. Consequently, they are especially well suited for quantitative research under process-relevant conditions and for the highly aerobic cultures of interest in biotechnology. The first development of this kind was due to Cooney et al. [25] and became known as "Dynamic Calorimetry". They used a carefully insulated 14 l (10 l working volume)

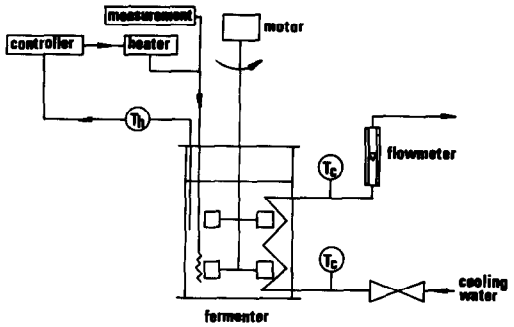

Fig. 2. Principles of continuous calorimetry. Taken from ref. 12.

fermenter, and based their measurements on a heat balance. After inoculation, the cooling system was switched off for periods of 5–9 min and the rate of temperature increase of the culture measured over a temperature change of approximately 1 °C. This basic method has been improved by Cooney's group and a number of other workers [26–29]. However, a detailed knowledge of each of the heat loss and gain terms on the energy balance is required, because each of these terms shows up directly as an error in the measured signal. The sensitivity is thus low and has to be compensated for by relatively high biomass concentrations and/or culture volumes. The system also suffers from the discontinuous nature of the measurement. Therefore, it is difficult to measure sudden changes in the heat generation signal such as those obtained during the late exponential phase of batch growth. Since the system is not rigorously isothermal, the temperature changes involved in the measurement may result in significant changes in the growth kinetics of the culture.

In order to permit continuous measurements of the heat generated during bench-scale bioreactions in situ, a group of workers [30–32] developed a so-called Continuous Calorimetry system (Fig. 2).

This system is composed of an insulated 14 l standard laboratory bioreactor. Cooling water, of a precisely controlled temperature, is supplied through a cooling coil such that heat has to be supplied through a variable output electrical heater in order to maintain the reactor temperature constant. The power supplied to the heater is thus inversely related to the heat generated by the culture. An energy balance around the bioreactor is still required in order to determine the heat generated by the culture, and consequently this measuring system suffers from problems similar to those of dynamic calorimetry. However, the measurement is continuous and has a higher degree of sensitivity than the dynamic method [28].

The third type a bench-scale calorimetry used for biotechnology is based on a measuring principle known as "Isothermal Reaction Calorimetry". It was first developed by Ciba-Geigy AG, Basle, Switzerland [33–35] for

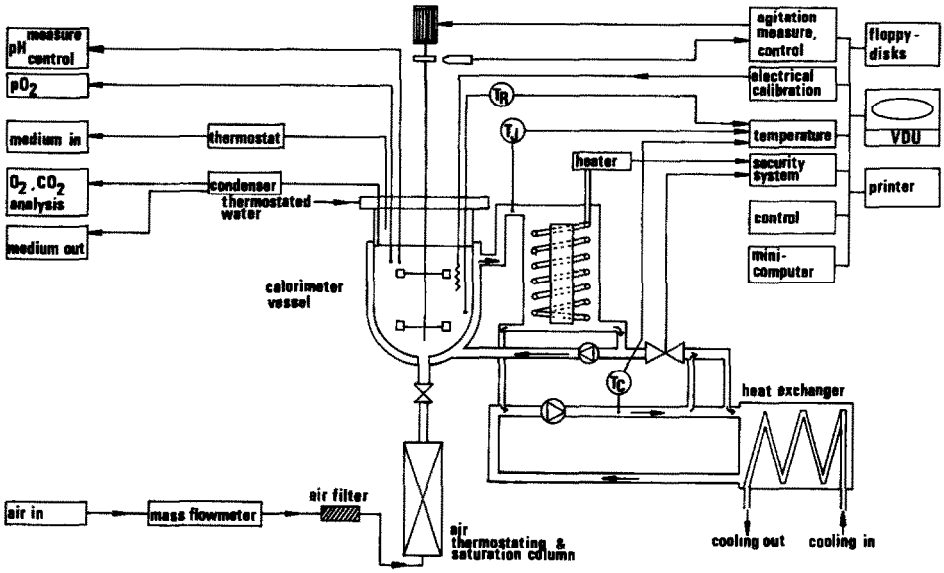

Fig. 3. Principles of isothermal reaction calorimetry [12].

chemical work and later commercialized by Mettler Instruments AG, Greifensee, Switzerland as a "Reaction Calorimeter" or RC1.

The RC1 is composed of a 2 l jacketed glass reactor, through the jacket of which is pumped a silicone oil at the rate of 2 l s^{-1} (see Fig. 3). The temperature of this oil T_J is controlled by a special thermostat in the circulatory system and can be modified quite rapidly. This is achieved by mixing warm and cool oil in different proportions using an electronic valve operated automatically by an IBM PS/2 computer. The temperature T_R in the reaction vessel itself is measured very accurately and the control algorithm adapts the temperature T_J continuously in such a way that T_R remains at its set point value. The difference $T_R - T_J$ is a measure for the heat flow rate ϕ_F transferred to the jacket (W) and may be used to determine the latter

$$\phi_F = UA(T_R - T_J) \tag{1}$$

where U and A stand for the heat transmission coefficient (W m^{-2} K^{-1}) and the effective heat transfer area (m^2), respectively. Provided T_R is really constant, ϕ_F is related to the heat flow rate generated by the reaction ϕ_R as

$$\phi_R = \phi_F - \phi_A + \phi_I + \phi_L \tag{2}$$

where ϕ_A, ϕ_I and ϕ_L represent, respectively, the heat dissipation rate due to agitation, the heat flow rate introduced or removed by the aeration, and the residual losses through the top plate. The first of these three terms can be measured independently using a torque meter. If it is constant during an

experiment, it generates no more than a constant offset of the base line. The latter two terms are minimized by appropriate experimental precautions. The term UA is calibrated by an internal electrical heater.

This measuring principle has been adapted for use in biotechnology [36–38]. It has the considerable advantage over other bench-scale calorimeters that the actual measurement is based on eqn. (1) and not on a heat balance. Heat losses through the jacket are irrelevant and insulating the glass reactor is thus unnecessary. The only heat losses showing up in the signal are those through the top plate (ϕ_L). As a result, this form of bench-scale calorimetry is much more sensitive than previous designs. Working with a modified RC1 equipped with a thick reactor wall (9 mm) and with a mathematical model able to estimate non-steady state heat conduction in the glass wall, heat dissipation rates of the order of 50 mW may be measured in a 1.5 l culture with adequate accuracy [39].

In the remainder of this paper, typical results obtained with the calorimetric techniques just discussed will briefly be reviewed. A few examples of research into more fundamental aspects of interest to biotechnology will be followed by two demonstrations of how calorimetric measurements in bioreactors might be applied in order to control and to characterize bioreactors.

3. FUNDAMENTAL INVESTIGATIONS

3.1. Calorimetric investigations of aerobic microbial growth efficiency

A typical heat release curve obtained during aerobic growth of the yeast *Kluyveromyces fragilis* is shown in Fig. 4 [3]. As can be seen, the heat dissipation rate ϕ_R as a function of time may be integrated to obtain a curve showing the total heat (Q) generated up to a certain point in time which parallels the biomass concentration X (g l^{-1} dry mass) quite nicely. It is therefore not surprising to find a straight correlation when plotting Q as a function of X (Fig. 5 (left)). The slope of this correlation indicates the amount of heat generated per unit dry biomass formed and is often called a "heat yield" ($Y_{Q/X}$). As can be seen from Fig. 5 (right), the cumulative amount of heat released also correlates linearly with the total amount of oxygen consumed, thereby giving rise to another type of heat yield ($Y_{Q/O}$), sometimes called "calo-respirometric quotient".

It is obvious that these heat yields correspond to enthalpy changes during growth and could therefore be called $\Delta_G H_X$ or $\Delta_G H_O$. However, it seems awkward to use such terms for such a complex "reaction" as microbial growth, which is the result of an enormous network of biochemical reactions and whose stoichiometry is ill-defined and often even highly variable. In order to emphasize the experimental nature of these quantities, the term heat yield ($Y_{Q/i}$) will be preserved.

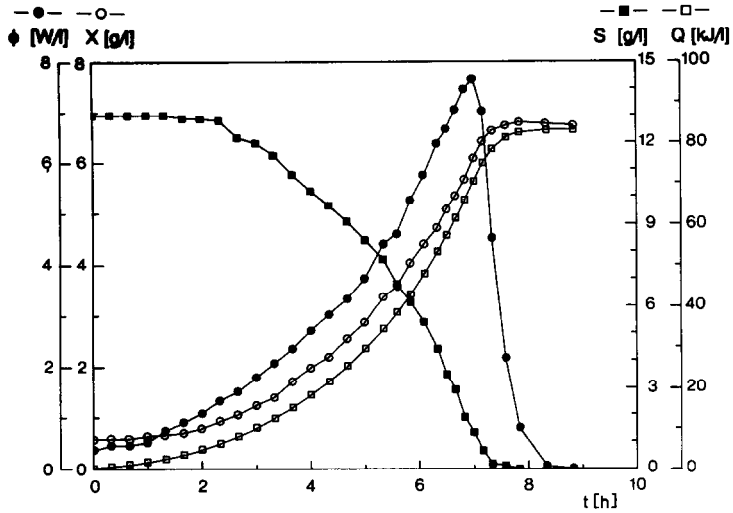

Fig. 4. Volumetric heat flow rate curves for aerobic growth of *Kluyveromyces fragilis* NRRL1109 on glucose: ■, substrate concentration (g l^{-1}); ○, biomass (g l^{-1}); ●, volumetric heat generation rate (W l^{-1}); □, total heat produced per unit volume (kJ l^{-1}). Taken from ref. 3.

The heat yields were measured for a large number of aerobic microbial cultures by Birou and von Stockar [3] (see Table 2). Strong aeration was maintained in order to avoid as much as possible the formation of fermentation products. Based on simultaneous analyses of carbon dioxide evolution rates, substrate consumption rates as well as on quantitative characterization of the biomass by elemental analysis and bomb calorimetry, the carbon and energy balances were checked for each experiment. The errors were typically of the order of 2–8% and rarely exceeded 10%.

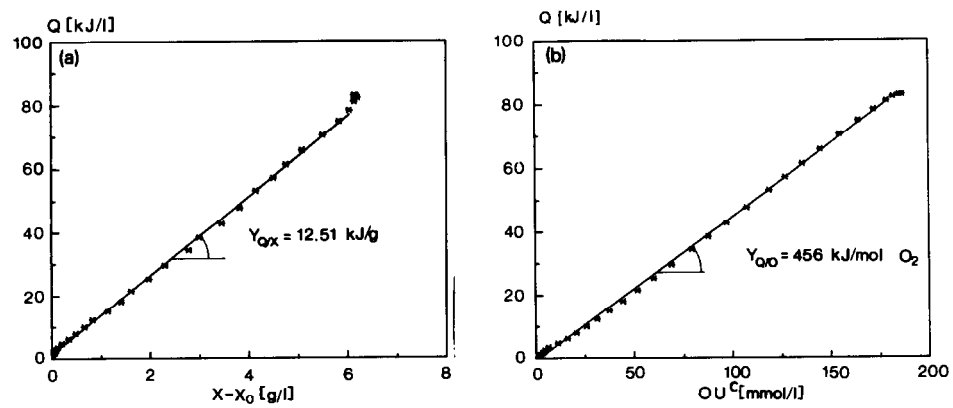

Fig. 5. Correlation between total heat released and (left) biomass formed, (right) oxygen consumed (ref. 3).

TABLE 2

Heat yields as a function of reductance degree of substrate γ_s^0 according to ref. 3

Organism	Substrate	γ_s^0	$Y_{X/S}$ ($g g^{-1}$)	$Y_{Q/X}$ ($kJ\ g^{-1}$)	$Y_{Q/O}$ ($kJ\ mol^{-1}$)
Candida lipolytica	Citrate	3	0.290	14.99	423
Candida lipolytica	Succinate	3.5	0.344	16.76	492
Kluyveromyces fragilis	Glucose	4	0.520	12.51	447
Kluyveromyces fragilis [a]	Glucose	4	0.465	12.51	456
Kluyveromyces fragilis [a]	Glucose (3)	4	0.388	15.16	–
Candida pseudotropicalis [a]	Glucose	4	0.456	13.50	408
Candida utilis	Glucose	4	0.481	11.66	421
Escherichia coli [a]	Glucose	4	0.416	8.70	–
Enterobacter cloacae [a]	Glucose	4	0.345	9.35	–
Kluyveromyces fragilis	Galactose (2)	4	0.494	13.63	418
Kluyveromyces fragilis	Lactose (2)	4	0.485	14.58	495
Candida utilis	Acetate	4	0.406	17.78	385
Candida utilis	Glycerol	4.67	0.562	10.79	474
Candida boidinii	Ethanol	6	0.790	16.70	–
Candida utilis [a]	Ethanol	6	0.557	19.91	421
Methylophilus methylotrophus	Methanol (1)	6	0.406	23.70	–
Candida lipolytica	Hexadecane(1)	6.13	0.986	25.63	437

[a] Indicates product formation. Initial substrate concentration was 3 g l^{-1} for *E. coli* and *Ent. cloacae*, and 10 g l^{-1} for all other cases, except those labelled, (1) 5 g l^{-1}, (2) 15 g l^{-1}, (3) 40 g l^{-1}.

The biomass yield $Y_{X/S}$ and the heat yield $Y_{Q/X}$ reflect in some way the efficiency of growth and thus must be interrelated. From Table 2 it appears that they both correlate in specific ways with the energy content of the carbon and energy substrate. In Table 2, the energy content $\Delta_C H_S$ is indicated by its degree of reductance γ_S^0. For any compound i of the generalized chemical C-molar formula $CH_{e_{i1}}O_{e_{i2}}N_{e_{i3}}$ the degree of reductance is defined by

$$\gamma_i^0 = 4 + e_{i1} - 2_{e_{i2}} \tag{3}$$

This definition makes γ_i^0 four times the number of moles of oxygen required to oxidize one C-mole of compound i to CO_2, H_2O and N_2. As already shown by a large number of researchers [40–46], the heat released during such a combustion per mol of O_2 consumed is very nearly the same for most organic compounds. This also means that the standard C-molar enthalpy of combustion $\Delta_c H_i'$ may be estimated quite accurately as

$$\Delta_c H_i' \approx Q_0 \gamma_i^0 \tag{4}$$

The best estimate is obtained by assuming $Q_0 = -115$ kJ deg^{-1} of reductance [43–46].

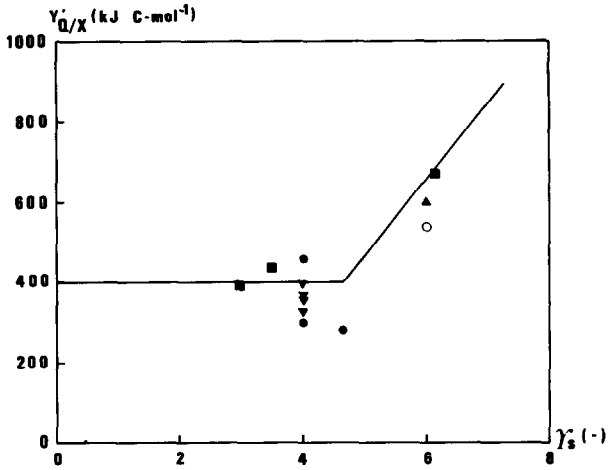

Fig. 6. Relationship between the heat yield $Y'_{Q/X}$ and the reductance degree of the substrate γ_S (ref. 3).

For characterizing the energy content of carbon and energy substrates in microbial growth processes, a "generalized" degree of reductance is often used [9,47,48]

$$\gamma_i = \gamma_i^0 - 3e_{i3} \tag{5}$$

which measures the number of moles of O_2 consumed in combustion processes yielding NH_3 rather than N_2 and is more appropriate in our context for reasons explained in detail elsewhere [12].

The C-molar biomass yield $Y'_{X/S}$ and the C-molar heat yield $Y'_{Q/X}$ are shown as a function of the energy content of the substrate, as expressed by γ_S, in Figs. 6 and 7. It has been pointed out by many authors that the biomass yield in general appears to increase with increasing energy content

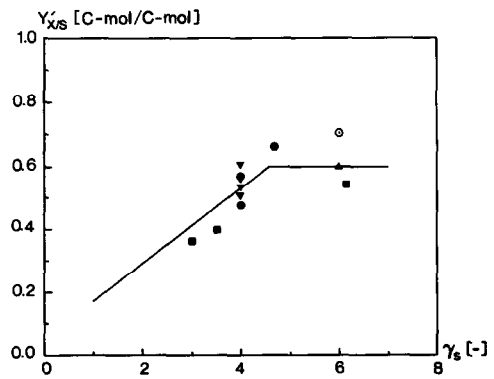

Fig. 7. Relationship between the carbon assimilation efficiency (C-molar biomass yield) $Y'_{X/S}$ and the reductance degree of the substrate γ_S (ref. 3).

of the substrate up to a reductance degree of about 4.5 and then seems to stay approximately constant [45,49,50]. Several equations have been proposed to describe this correlation. The solid lines appearing in Fig. 6 represent the correlation suggested by Roels [45]

$$Y'_{X/S} \approx 0.13\gamma_S \qquad \text{for } \gamma_S \leqslant 4.67 \tag{6a}$$

$$Y'_{X/S} \approx 0.6 \qquad \text{for } \gamma_S > 4.67 \tag{6b}$$

As can be seen, these expressions predict the experimental data [3] quite well, although this simple model cannot reproduce the scatter found in $Y'_{X/S}$ that is due to biological differences from one strain to another.

On the basis of this model, the heat yield $Y_{Q/X}$ may also roughly be predicted. An enthalpy balance for a simple aerobic growth process converting substrate (S), oxygen and NH_3 (N) into one C-mol of biomass (X), CO_2 and H_2O yields

$$\frac{\Delta_c H'_S}{Y_{X/S}} + Y'_{N/X}\Delta_c H'_N + \Delta_G H'_X = \Delta_c H'_X \tag{7}$$

where $Y'_{N/X}$ stands for the number of moles of NH_3 necessary for the synthesis of one C-mol of biomass. Assuming that the substrate does not contain nitrogen, $Y'_{N/X} = e_{x3}$. $\Delta_G H'_X$ is the enthalpy change of growth per C-mol biomass formed. Since produced heat is counted as a positive form of energy in this study, $\Delta_G H'_X = -Y'_{Q/X}$. $\Delta_c H'_i$ are the C-molar enthalpies of combustion for substrate ($i = S$), NH_3 ($i = N$), and biomass ($i = X$).

The energy balance may be further simplified by approximating all energy contents by means of eqn. (4). Accounting for the facts that $\gamma_S^0 = \gamma_S$ (no N) and $\gamma_N^\circ = 3$ one obtains

$$\frac{Q_0\gamma_S}{Y'_{X/S}} + 3Q_0 e_{x3} = Q_0\gamma_x^0 + Y'_{Q/X} \tag{8}$$

This simplified energy balance can be solved for $Y'_{Q/X}$. If in addition $Y'_{X/S}$ is expressed by the correlation (6), γ_X^0 is replaced by γ_X (eqn. (5)) and an average value of 4.3 is used for γ_X (Cordier et al., [46], the heat yield may approximately be estimated on the sole basis of γ_S as

$$Y'_{Q/X} \approx 400 \left(\text{kJ (C-mol)}^{-1}\right) \qquad \text{for } \gamma_S \leqslant 4.67 \tag{9a}$$

$$Y'_{Q/X} \approx 192\gamma_S - 497 \left(\text{kJ (C-mol)}^{-1}\right) \qquad \text{for } \gamma_S > 4.67 \tag{9b}$$

As may be seen in Fig. 6, this correlation is roughly confirmed by the data.

The heat yield and its relationship with the degree of reduction shown in Fig. 6 may be interpreted in terms of the efficiency of microbial growth energetics. Since the heat yield is a direct measure of the chemical energy initially available in the substrate which has been dissipated as heat, it must be possible to deduce from it the fraction of the available energy not

dissipated, i.e. retained in the biomass. This energy fraction can be calculated from an energy balance. Dividing eqn. (8) by the energy initially available, i.e. by its left hand side, gives

$$1 = \frac{\gamma_X}{\gamma_S/Y'_{X/S} + 3e_{x3}} + \frac{Y'_{Q/S}}{\gamma_S/Y'_{X/S} + 3e_{x3}} \tag{10}$$

The first right-hand term of eqn. (10) represents the fraction of the chemical energy available in the substrate that is retained in the biomass. This fraction has been called the "enthalpy efficiency of growth" η_H [45]

$$\eta_H \equiv \frac{\Delta_c H'_X}{\Delta_c H'_S/Y'_{X/S} + 3e_{x3}\Delta_c H'_N} \approx \frac{\gamma_X}{\gamma_S/Y'_{X/S} + 3e_{x3}} \tag{11}$$

The second right-hand term of eqn. (10) shows the fraction of the energy dissipated as heat and can be measured by calorimetry. It is possible to deduce η_H directly from $Y'_{Q/X}$ by substituting eqns. (8) and (11) into eqn. (10)

$$\eta_H = \frac{Q_0\gamma_X}{Q_0\gamma_X + Y'_{Q/X}} \tag{12}$$

Values for enthalpic growth efficiency that were determined from calorimetry are plotted as a function of γ_S in Fig. 8. The enthalpic growth efficiency may also be predicted approximately as a function of γ_S by estimating $Y'_{Q/X}$ in eqn. (12) on the basis of correlation (10). The resulting correlation (13) is shown as solid lines in Fig. 8.

$$\eta_H \approx 0.58 \qquad \text{for } \gamma_S \leqslant 4.67 \tag{13a}$$

$$\eta_H \approx \frac{543}{192\gamma_S + 46} \qquad \text{for } \gamma_S > 4.67 \tag{13b}$$

Figures 7 and 8 show the carbon and the energy assimilation efficiencies, respectively attained in aerobic microbial growth. $Y'_{X/S}$ is analogous to η_H

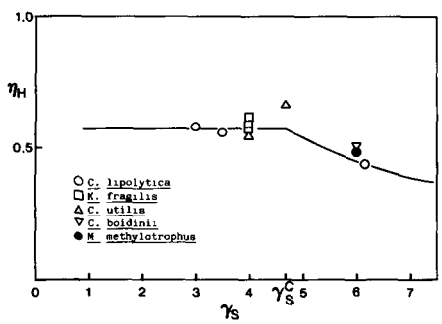

Fig. 8. Relationship between the enthalpic growth efficiency η_H determined calorimetrically and the reductance degree of the substrate γ_S. Redrawn from ref. 51.

in that it indicates the fraction of carbon initially available which is retained in the biomass.

At $\gamma_S < 4.67$, growth occurs on rather oxidized and hence low energy substrates. Energy assimilation efficiencies appear to be optimal and constant in this region, but both the experimental values and the theoretical prediction indicate that microorganisms are not able to retain in the biomass more than about 55–65% of the available chemical energy.

An analogy to a very simple model proposed to predict optimum energy efficiencies in muscles [52] offers some possible underlying reasons for the observed efficiency level. Assuming that in aerobic growth the $T\Delta S$ term is small compared to the enthalpy change during growth, the released heat not only measures $\Delta_G H$ but also the change of free energy, which in turn is a measure of the average driving force for the process. In order to drive growth at a non-zero rate, this force cannot be zero and part of the energy contained in the substrates must be dissipated. The rate of oxidative phosphorylation in muscles has thus been assumed proportional to the dissipated energy divided by the maximum energy dissipation possible for the metabolized substrates [52,53]. In our case, this fraction approximately corresponds to $1 - \eta_H$. During burst activities, muscles are expected to maximize the output of metabolic power, which is the product of the phosphorylation rate times the free energy change in ATP production. In our case the latter would correspond to the energy produced in the form of biomass, which is proportional to η_H. The metabolic output power for aerobic growth would thus be proportional to $\eta_H(1 - \eta_H)$ and be maximal at $\eta_H = 0.5$, as proposed for muscles under stress. In the absence of stress, the organism would be expected to maximize the "economical power output" given by the product of power and efficiency [54]. The resulting function is proportional to $\eta_H^2(1 - \eta_H)$ and has its optimum at $\eta_H = 0.677$.

Because of the need to dissipate 40% of the energy through oxidation of the substrate, and because of the low energy content of substrates with $\gamma_S < 4.67$ not much biomass can be formed, and $Y'_{X/S}$ stays below the maximum value of 0.6. More reduced, i.e. more energy-rich, substrates afford a higher yield, but as the degree of reduction of substrates exceeds 4.67 in the right part of the diagram, an upper limit of $Y'_{X/S}$ is reached. The organisms do not seem to be able to retain in the biomass more than about 60% of the carbon atoms available in the substrate. The reason for this seemingly wasteful behaviour must lie in the fact that biological evolution failed to develop ATP yielding oxidation pathways for highly reduced substrates. In order for them to be useful for living cells, such substrates must first be oxidized to the level of the biomass itself, whose degree of reduction ranges from about 4.1 to 4.3. This oxidation releases vast amounts of heat depending on γ_S, but produces no ATP. This explains the increase of $Y'_{Q/X}$ and the reduction in η_H as a function of γ_S. Once the metabolites are at the correct oxidation level, 40% of the carbon atoms

must still be oxidized to CO_2 in order to provide the 40% "useful" energy dissipation necessary for growth. Hence $Y'_{X/S}$ cannot much exceed 60%.

3.2. Heat release and oxygen uptake

It is obvious from Table 2 that the heat yield with respect to oxygen, $Y_{Q/O}$, is more or less constant irrespective of γ_S. The average value found was 440 ± 33 kJ mol^{-1}. This is a confirmation for the well-known rule that the enthalpy change per mole of oxygen consumed, the so-called oxycaloric equivalent $\Delta_r H_O$, adopts nearly the same value for practically all types of aerobic growth processes, regardless of the type of organism or the nature of substrates or products involved [3,9,45,47].

The reason for this is easily seen by rewriting the energy balance (7) for the consumption of one C-mol of substrate and by extending it for the possible formation of a product P. Neither the substrate nor the product are assumed to contain nitrogen, so that $Y'_{N/X} = e_{x3}$.

$$\Delta_c H'_S + Y'_{X/S} e_{x3} \Delta_c H'_N + \Delta_G H'_S = Y'_{X/S} \Delta_c H_X + Y'_{P/S} \Delta_c H'_P \tag{14}$$

where $Y'_{P/S}$ indicates the amount of product formed per C-mole of substrate consumed.

The oxycaloric equivalent can be computed by solving this for $\Delta_G H'_S$ and by dividing by the amount of oxygen taken up per unit of substrate $Y'_{O/S}$. This quantity follows from an oxygen balance as [12]

$$Y'_{O/S} = \tfrac{1}{4}\gamma_S - \tfrac{1}{4}\gamma_X Y'_{X/S} - \tfrac{1}{4}\gamma_P Y'_{P/S} \tag{15}$$

The result is

$$-\Delta_G H_O = 4 \frac{\Delta_c H'_S - Y'_{X/S}(\Delta_c H'_X - e_{x3}\Delta_c H'_X) - Y'_{P/S}\Delta_c H'_P}{\gamma_S - Y'_{X/S}\gamma_X - Y'_{P/S}\gamma_P} \tag{16}$$

For strongly aerobic processes, this equation always yields values around 460 kJ mol^{-1}, no matter what the biomass and the product yields of the particular growth process are. As a matter of fact these yields cancel if eqn. (16) is simplified by substituting eqns. (4) and (5) and with $\gamma_N^0 = 3$. The result is

$$-\Delta_G H_O = 4Q_0 \tag{17}$$

with $Q_0 = 115$ kJ mol^{-1}. This general value has been confirmed in several calorimetric studies, as shown in Table 3. Somewhat higher values were found when oxidizing glucose using an enzyme rather than an organism to oxidize glucose and for chemical oxidation of sulphite [56].

It must be borne in mind that eqn. (17) is only valid for strongly aerobic processes as it would predict a zero heat effect for anaerobiosis. For weakly aerobic or anaerobic processes the denominator of eqn. (16) tends to zero,

TABLE 3

Experimental values for the calorimetric-respirometric ratio $Y_{Q/O}$ during microbial growth

Authors	Ref.	No. of independent measurements reported	γ_S	$Y'_{Q/O}$ (kJ mol^{-1})	Remarks
Cooney et al., 1968	25	8	4	518 ± 12	
Volesky et al., 1982	55	6	4–6.17	447 ± 58	
Volesky et al., 1982	55	1	4	1710	Respiration partially repressed
Birou and von Stockar, 1987	3	12	3–6.13	440 ± 33	
Mossier, 1988	56	3	4	513 ± 15	Cell free enzymatic oxidation of glucose to gluconic acid, included for comparison

but due to the various deviations from the correlation the numerator will always adopt a positive value. For such cases, the oxycaloric equivalent tends to infinity. Thus the high $Y_{Q/O}$ value in Table 3 measured under conditions of partial repression of respiratory metabolism attests to the high fraction of anaerobic, fermentative growth [55].

The heat generation under mixed anaerobic/aerobic conditions with decreasing degrees of oxygen supply has been studied in quite some detail for various yeast cultures [57,58]. Mathematical models were also developed which predict the increase of the oxycaloric equivalent as a function of the ratio of oxygen to substrate supply and biological parameters.

4. APPLICATIONS

4.1 Control of fed-batch fermentations by calorimetry

The only attempts to control fermentations by calorimetry appear to be the ones by Randolph et al. [59] and by Larsson et al. [22,60]. Both groups used the production of bakers' yeast as a model process and worked with strains of *Saccharomyces cerevisiae*. Belonging to the so-called aerobic-fermenting yeasts [61], *S. cerevisiae* will grow according to a fermentative metabolism and produce ethanol even in highly aerated conditions if the

substrate concentrations are too high. This behaviour, observed in both batch and continuous cultures at high dilution rates, was originally interpreted as catabolite repression [62–64], but mounting evidence suggests that it may at least partially be due to the existence of a bottleneck in the respiratory pathway that results in an overflow of metabolites into reductive metabolism [65–69].

Whatever the reason for this behaviour, the aerobic production of ethanol in batch cultures drastically lowers the obtainable biomass yield and inhibits growth. In practice, bakers' yeast is therefore produced in fed-batch cultures designed to avoid accumulation of large substrate concentrations at any time. The optimal feeding rate of concentrated substrate solution ought to be as high as possible to ensure fast growth, but not so high as to permit unused glucose to accumulate in the broth and thus to induce ethanol formation. The design of on-line monitoring systems as a basis for feed rate control represents a real challenge to biochemical engineers because the optimal feeding rate increases continuously in an exponential fashion. Furthermore the initial substrate concentration is often unknown and even subject to substantial variations during the culture, due to the ill-defined technical substrate mixtures. On-line calorimetric monitoring of the culture could clearly be used to solve this problem. Gustafsson and her co-workers [22,60] used an LKB flow microcalorimeter to control a fed-batch culture in a 2.5 l aerobic fermenter (see section 2.2 for experimental set-up). The feed control was based on adding individual pulses of concentrated glucose solution corresponding to 0.6 g of glucose each time, an amount sufficiently low to prevent reductive metabolism. Each pulse set off rapid aerobic growth that could clearly be seen on the signal obtained from the calorimeter, as shown in Fig. 9. As soon as the heat generation began to drop a computer triggered another pulse of glucose. As the culture grew, the frequency of glucose additions increased as a result of accelerating substrate consumption. This control scheme successfully suppressed ethanol formation to a residual level of about 0.1–0.2 g l^{-1}. Thus biomass yields of the order of 0.32–0.35 g per gram substrate were attained, which is considerably higher than the values obtained in batch cultures.

The advantage of this control system is its ease of implementation and its simplicity. However it cannot maintain the feed rate at its highest possible value since the glucose concentration is increased only to a preset constant value after each pulse. The feed rate can only be controlled at its optimal value by a control scheme that increases it until reductive metabolism starts to set in.

Such a control scheme may also be based on on-line calorimetric measurements but considerably more information must be acquired on-line in order to detect the onset of fermentative metabolism. The number of variables that must be measured on-line may be determined considering

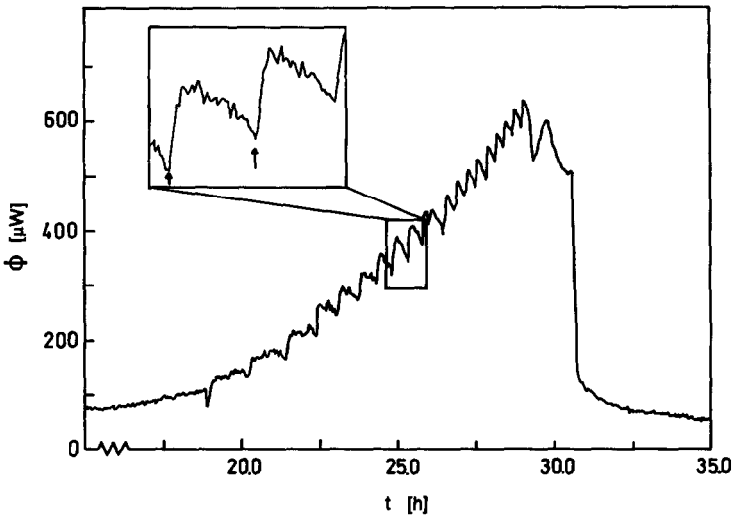

Fig. 9. Heat dissipation rate as a function of time during a fed-batch culture of *Saccharomyces cerevisiae* controlled by calorimetry [60].

the following stoichiometry:

$$CH_2O + Y'_{O/S}O_2 + Y'_{N/S}NH_3 \rightarrow Y'_{X/S}CH_{e_{x1}}O_{e_{x2}}N_{ex3} + Y'_{P/S}CH_3O_{1/2} + Y'_{C/S}CO_2 + Y'_{W/S}H_2O \quad (18)$$

The yield $Y'_{i/j}$ coefficients appearing in this equation are related to the C-molar rates r'_i of production or consumption of the various species as follows:

$$Y'_{i/j} = \left| \frac{r'_i}{r'_j} \right| \quad (19)$$

A complete description of instantaneous behaviour of the system would involve measuring all seven rates r'_i on-line that correspond to the seven major compounds appearing in eqn. (18) and in addition the heat generation rate ϕ. Fortunately, these rates are not independent of each other because they have to satisfy four elemental balances as well as the energy balance

$$\phi = -\sum_i r'_i \Delta_c H'_i \quad (20)$$

Hence, of the eight rates only three are truly independent of each other, and if three of them are measured continuously, the remaining five can be computed by solving the five balances. Exactly which rates are measured in practice depends on which rates may be easily monitored on-line (generally r'_c, r'_O, ϕ, and r'_N). Heat and oxygen uptake rate are, to a first approxima-

tion, related by a simple proportionality (eqn. 17); hence one may use either r'_O or ϕ, but not both. Heat may be more advantageous than oxygen uptake because of its faster response time and because of greater sensitivity at the laboratory scale.

Using this approach, Randolph et al. [59] achieved optimal control of a fed-batch culture of *S. cerevisiae* in an RCl isothermal reaction calorimeter providing 1.8 l working volume. In addition to the heat evolution rate ϕ, they measured r'_C and r'_N, i.e. the rate of NH_3 addition needed to keep the pH constant. From solving the five balances, other important rates were computed on-line as follows:

$$r'_X = \mu X = r'_N / e_{x3} \tag{21a}$$

$$r'_P = \frac{(r'_N/e_{x3})(\Delta_c H'_X - \Delta_c H'_S) - r'_C \Delta H'_S + \phi}{\Delta_c H'_s - \Delta_c H'_P} \tag{21b}$$

$$r'_S = \frac{(r'_N/e_{x3})(\Delta_c H'_X - \Delta_c H'_P) - r'_C \Delta_c H'_P + \phi}{\Delta_c H'_S - \Delta_c H'_P} \tag{21c}$$

The oxygen uptake rate was calculated directly from ϕ using the oxycaloric equivalent $\Delta_G H'_O$, which was estimated for this culture on the basis of eqn. (16) as 470 kJ mol^{-1}. Subsequent verification by measuring r'_O experimentally during the actual fed-batch cultures yielded a value of 461 kJ mol^{-1}, thereby confirming the consistency of the data.

Based on an initial estimate of biomass and ethanol contained in the system, updated estimates were made at each interval

$$X'(t + \Delta t) = X'(t) + r'_X(t)\Delta t \tag{22a}$$

$$P'(t + \Delta t) = P'(P) + r'_P(t)\Delta t \tag{22b}$$

In agreement with common practice, the respiratory quotient RQ was used as a criterion for the onset of fermentative metabolism

$$RQ = \frac{r'_C}{r'_O} \tag{23}$$

Values of RQ near unity are found during respiratory growth on glucose; fermentative growth is associated with higher values. A set point of $RQ_{sp} = 0.97$ was chosen.

In order to maximize biomass production without the formation of ethanol, the control algorithm increased the feed rate F exponentially with time, taking the specific growth rate estimated from eqns. (21a) and (22a) into account, while a gain term provided proportional control of the respiratory quotient RQ.

$$F(t + \Delta t) = F(t)\left[e^{\mu \Delta t}\left(\frac{r'_S - r'_P}{r'_S}\right) + k_C(RQ_{sp} - RQ)\Delta t\right] \tag{24}$$

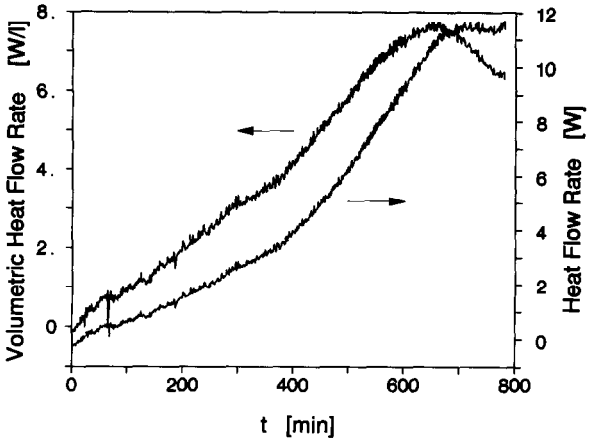

Fig. 10. Total and volumetric heat generation rate during a calorimetrically controlled fed-batch fermentation [59].

Figure 10 shows typical differential heat flow rate curves measured during a fed-batch culture. Towards the end of the culture, an oxygen limitation increased the tendency for reductive metabolism. The resulting increase of RQ was counteracted by the controller by lowering the feed flow rate. This explains the decrease of the volumetric heat generation rate (W l^{-1}). During the whole culture, stable control of RQ between 0.97 and 1 was achieved. When in a separate experiment the glucose concentration in the feed was suddenly increased from 40 to 50 g l^{-1} in order to simulate feed variations occurring in technical operation, RQ rose only to 1.04 and returned to its set point of 0.97 after about 35 min.

Fed-batch cultures were also conducted in repeated drain-and-fill cycles, a common practice in industry in order to minimize reactor downtime. After each fed-batch culture, the calorimeter contents were harvested to a remaining volume of 800 ml and controlled feeding was resumed without adding additional inoculum. Figure 11 compares the actual biomass content as determined from the dry weight with the model calculations based in part on calorimetry and on eqn. (22a). Despite the fact that the current biomass value used by the computer was never readjusted after inputting an initial value, the controller model kept track of the biomass correctly over many cycles. By calorimetric control of the RQ the formation of ethanol was avoided and its concentration never exceeded 1 g l^{-1}. The observed biomass yield of 0.401 C-mol per C-mol substrate is similar to the one reported by Larsson et al. [60].

However, Randolph et al. [59] obtained much higher biomass productivities by about an order of magnitude. This was in part due to the use of higher total substrate concentrations (up to 30 g l^{-1}) than in ref. 60 (5–10 g l^{-1}) and by continuously pushing the feed rate up to its optimal value by means of a more sophisticated measuring and control algorithm.

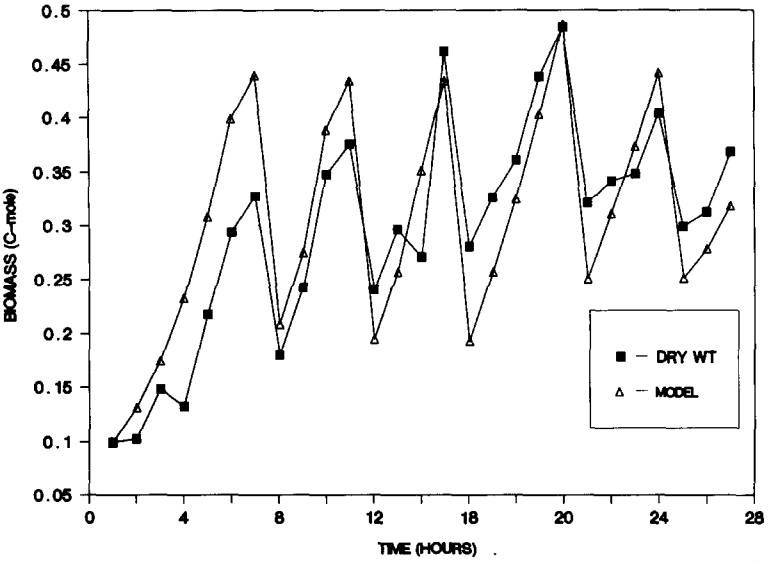

Fig. 11. Comparison of biomass estimations from on-line control model and off-line dry weight determinations during a repeated fed-batch experiment [59].

4.2 Calorimetric measurement of oxygen transfer rates and mass transfer coefficients in bioreactors

One of the major engineering challenges in highly aerobic biotechnical operations is to ensure adequate oxygen transfer from the gaseous to the liquid phase. The rate of oxygen transfer (OTR), which has to match the oxygen consumption rate of the culture r_O (mol s^{-1} m^{-3}) depends on the difference between the actual O_2 concentration C_L in the liquid broth and the saturation concentration C_L^* as follows:

$$\text{OTR} = k_L a(C_L^* - C_L) \tag{25}$$

where k_L stands for the actual mass transfer coefficient (m s^{-1}) and a for the interfacial area per unit volume of liquid (m^2 m^{-3}), respectively. The product $k_L a$ is called the volumetric mass transfer coefficients (s^{-1}) and determines the aeration capacity of a particular bioreactor under given operating conditions.

For technical purposes, it is important to characterize bioreactors by measuring $k_L a$. One well known method consists of replacing the biological culture by a sulphite solution, which in the presence of Co^{2+} ions as catalyst can simulate the oxygen uptake of microorganisms by being oxidized to sulphate

$$2SO_3^{2-} + O_2 \rightarrow 2SO_4^{2-} \tag{26}$$

Under appropriate experimental conditions [70] the reaction is sufficiently fast to maintain C_L at zero. The oxygen transfer rate is then constant

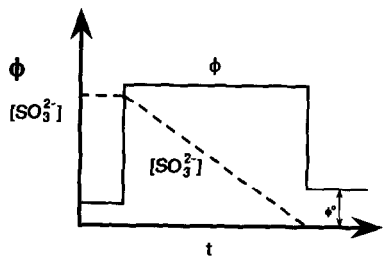

Fig. 12. Reactant concentration and heat dissipation rate as a function of time as expected for a zero order reaction such as the oxidation of sulphite.

according to eqn. (25) and becomes zero order in sulphite. The sulphite concentration is expected to decrease linearly as shown schematically in Fig. 12. If the remaining sulphite concentration is titrated at regular intervals by iodometry, the reaction and OTR may be determined from the slope of such a plot and $k_L a$ can be evaluated using eqn. (25) and assuming $C_L = O$. This method has been widely used for determining the $k_L a$ that can be attained in small and medium sized bioreactors [70–72] and even in large scale aeration tanks for biological waste treatment [73].

It has been demonstrated that calorimetry can be used to replace the classical iodometric titration [74]. The oxycaloric equivalent for this reaction has been determined on the basis of eqn. (16) [56] $\Delta_r H_O = -545$ kJ mol^{-1} which is considerably higher than the 460 kJ mol^{-1} expected from eqn. (17). The experimental mean value measured in an Isothermal Reaction Calorimeter was 562 ± 1 kJ mol [56].

During sulphite oxidation Mossier et al. [74] obtained perfectly flat heat flow rate curves as expected (see Fig. 12) for a zero order reaction. After deducting the base line ϕ^0, instantaneous values for $k_L a$ may be computed from the dissipated power as

$$k_L a = \frac{\phi - \phi^0}{C_L^* \Delta_r H_O} \tag{27}$$

TABLE 4

Comparison of $k_L a$ measured by different methods according to Mossier [56]

N [a] (min^{-1})	$k_L a^{cal}$ [b] (10^3 s)$^{-1}$	$k_L a^{stat}$ [c] (10^3 s)$^{-1}$	$k_L a^{gas}$ [d] (10^3 s)$^{-1}$
500	59.3	68	59
700	128	159	124
900	259	–	267

[a] N, agitation speed, [b] $k_L a^{cal}$, measured by calorimetry. [c] $k_L a^{stat}$ measured by static gassing in experiments, [d] $k_L a^{gas}$, measured by O_2 balancing.

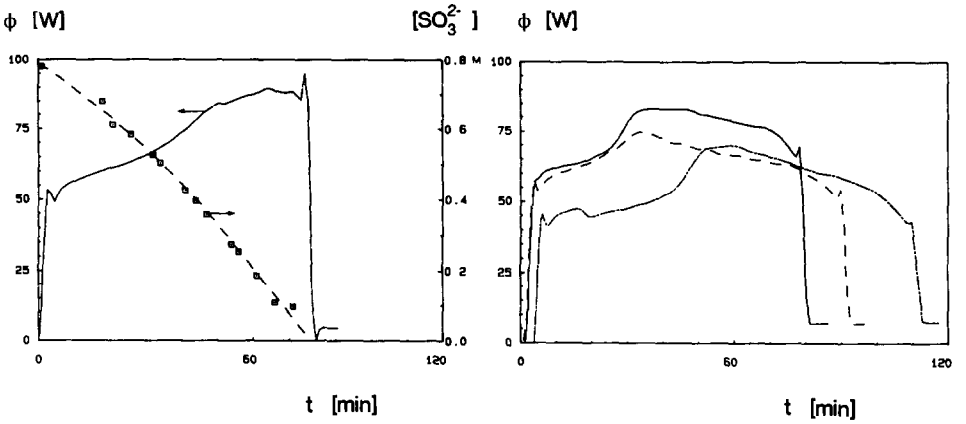

Fig. 13. Heat dissipation rate during sulphite oxidation in the fast reaction regime. Heat generation and sulfite consumption at (left) $[CoSO_4] = 0.5$ mM, (right) $[CoSO_4] = 1$mM [74].

This was experimentally verified. Table 4 compares $k_L a$ values obtained by calorimetry ($k_L a^{cal}$) with other methods.

The same method has also been widely used to measure the interfacial area a separately. To do this, the catalyst concentration is increased by several orders of magnitude to make the Hatta number greater than 3. The oxygen transfer is then given by

$$OTR = a\sqrt{\tfrac{3}{2}}\, k_2 D C_L^{*3} \tag{28}$$

where k_2 and D represent the chemical rate constant and the diffusivity, respectively, rather than by eqn. (25) [75]. k_2 and D can be measured in wetted wall absorbers [74], or values may be taken from the literature.

Figure 13 shows attempts to measure a by calorimetry. As is clearly seen, the reaction order is far from zero, and the method reveals itself as unsuitable for the purpose at hand. The increase of the transfer rate during the oxidation reflects itself as a slight curvature of the titrated sulphite concentration, which could have gone unnoticed if the experiment had been monitored by titration alone, as is common practice. Figure 13 also clearly shows that the results are not reproducible, probably due to catalyst instability.

Partly on the basis of this calorimetric investigation, the authors recommend that the popular sulphite method as a means for measuring interfacial areas in bioreactors should be abandoned. This work demonstrates the potential of isothermal reaction calorimetry for characterizing the aeration capacity of bioreactors. As opposed to conventional techniques, calorimetry yields instantaneous information on transfer rates and thus offers new insight.

5. CONCLUSIONS

In order to collect useful calorimetric data for biotechnological purposes at the laboratory scale, calorimeters must be available in which biological processes can be studied under strictly controlled conditions that resemble those maintained in large scale bioreactors. Typical controlled process variables include pH, dissolved oxygen concentration, redox potential, concentrations of metabolites and the like. In addition, such calorimeters must permit the monitoring of many other variables, either by inserting probes or by sampling the reactor contents. It must also be possible to operate them under open, steady or even non-steady conditions.

Several calorimeter designs have been proposed and tested to meet these criteria, yet only two of them have been and still are used extensively for biotechnology related studies. One method consists of coupling a flow calorimeter to a bench-scale fermenter; the other is based on isothermal reaction calorimetry.

Results obtained under biotechnology-relevant conditions can contribute new results to even quite fundamental research topics, such as bioenergetics in highly aerobic microbial growth. The results obtained in this area have just begun to reveal the enormous number of research opportunities that could be explored with such techniques. Bench-scale calorimetry and similar methods could be used in the future to extend bioenergetic investigations to hydrogen-oxidizing bacteria, anaerobic respiration, aerobic and especially anaerobic chemoautolithotrophes (methanogenesis), and the like, all of which are difficult to study in conventional microcalorimeters due to the presence of several phases. The possibility of performing calorimetric chemostat experiments can be used to study spontaneously synchronizing and oscillating cultures whilst transient responses to various types of step changes could provide a novel tool for investigating inhibition, induction and repression kinetics.

The wealth of quantitative knowledge generated by calorimetric measurements under technically realistic conditions may be applied at production scale for bioprocess control. In large scale bioreactors, the heat generation is a very conspicuous phenomenon that could quite easily be quantitatively monitored. No attempts have been published as yet to control large bioreactors based on measured heat dissipation rates, but the present report shows how the feasibility of this idea has been illustrated by fed-batch experiments on the bench-scale.

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