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On-line calorimetry as a technique for process monitoring and control in biotechnology

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Abstract

This contribution reviews laboratory-scale investigations carried out on the usefulness of biological heat release measurements, as a means for monitoring and controlling the metabolic state of microbial cultures. Such studies are carried out in high-quality bench-scale calorimeters, but measuring heat generation rates by establishing energy balances ought to be applicable to large-scale bioreactors without resorting to sophisticated instrumentation. The signal received can either be interpreted by more qualitative correlation with the evolution of the culture, or it may be quantitatively exploited – together with other on-line measurements – in order to assess the rates at which various types of metabolisms proceed in the culture. The work described shows how this can be used to keep a culture in a desired metabolic state during fed-batch and transient continuous cultures of the yeast, *S. cerevisae*, and how a bacterial fed-batch culture can be controlled in order to optimize biosynthesis of an antibiotic. © 1997 Elsevier Science B.V.

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1. Introduction

Heat generation and absorption is a truly universal feature of chemical, biochemical, and also biological processes. Even though its extremely general occurrence renders it a very unspecific phenomenon, it is nevertheless clear that quantitative measurements of heat exchange rates might serve as a powerful indicator of the nature of the on-going chemical or biological process [1]. Such an indicator not only constitutes a potentially valuable diagnostic tool for laboratory work, but also holds a promising potential for the control of such processes on an industrial scale.

This is especially important in biotechnology, where computer-based control schemes are often limited by the number of relevant process variables that can be measured on-line and used as continuous input for computer models [2].

Indeed, probes which can be used for such on-line data acquisition have only been developed for a limited number of process variables. In order to improve this situation, the rate of heat generation by the biological process could conceivably be measured quantitatively in industry-size fermenters, without resorting to sophisticated on-line probes [3]. This information could then be used along with other on-line data in process control schemes, provided suitable models are available which relate the heat evolution rate of the cell culture to other relevant process vari-

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ables, such as substrate consumption, growth rate, or oxygen uptake rate.

The quantitative on-line measurement of heat dissipation rates would be facilitated by the fact that industry-sized bioreactors operate nearly adiabatically due to their small surface-to-volume ratio [3] as well as to the poor heat transfer characteristics of the surrounding air. Therefore, if the temperature increases in the cooling water, its flow rate, and the other pertinent energy exchange terms can be measured systematically, and the heat dissipation rate of cellular cultures could be quite easily and quantitatively monitored on-line [3].

Despite the obvious potential of on-line calorimetry for bioprocess monitoring and control, it has rarely been exploited. Published calorimetric data obtained in industrial fermenters are scarce [4–7] and none of these reports pertain to the use of these measurements for control. The reason for this state of affairs probably lies in the difficulty in investigating and developing this concept in laboratory experiments. While commercial micro-calorimeters are clearly sensitive enough to measure the relatively weak heat generation rates accompanying biological processes, they clearly cannot offer the possibility to simulate and maintain realistic process conditions found in technical bioreactors such as pH control, vigorous agitation and aeration, or monitoring the process by either on-line or off-line analyses.

The situation has changed somewhat over the last 15 years with the advent of the so-called 'bench-scale' calorimetric techniques that partially filled this gap. These methods have already been reviewed several times [3,2]. This paper will review the laboratory studies using on-line heat measurements for monitoring and control that have been reported in the literature to-date.

2. Heat as a quantitative indicator of cell metabolism

There are at least three ways to use quantitative heat generation measurements, in order to assess the state of the culture and to decide on corrective actions ensuring proper control of the process. First, it is possible to infer a wealth of information by just qualitatively looking for abrupt changes in thermal power release. All kinds of metabolic events, such as shifts from one substrate to another, shifts from one type of catabolism to another, occurrence of limitations, inhibitions, and overflow metabolism and the like will cause characteristic changes in the heat curve [1]. Given some experience with the respective type of culture, these events can be identified and used for control. This technique has been applied in several of the examples discussed hereafter.

A more quantitative technique consists of monitoring on-line a 'heat yield' or ratio of heat release rate to the rate of production or consumption of a given metabolite.

$$Y_{Q/i} = \left| \frac{q_R}{r_i} \right| \tag{1}$$

This ratio may be interpreted by considering the stoichiometry of the consumption of substrates and the synthesis of products by the microbial culture in terms of a 'macro-chemical' equation. A typical simplified stoichiometry could take the form

$$\mathrm{CH_2O} + Y_{\mathrm{O/S}}\mathrm{O_2} + Y_{\mathrm{N/S}}\mathrm{NH_3} \rightarrow Y_{\mathrm{X/S}}\mathrm{CH_{x_1}O_{x_2}N_{x_3}} + Y_{\mathrm{P/S}}\mathrm{CH_{p_1}O_{p_2}N_{p_3}} + Y_{\mathrm{C/S}}\mathrm{CO_2} + Y_{\mathrm{W/S}}\mathrm{H_2O} \tag{2}$$

where CH_2O represents one C-mole of hexose, the main carbon and energy substrate, $Y_{i/s}$ the stoichiometric coefficients in mole or C-mole per C-mole of substrate, and the two first formulae on the right-hand side represent one C-mole of dried biomass and one C-mole of the major product. The heat yield defined in Eq. (1) represents the molar enthalpy change $\Delta_r H_i$ caused by reaction in Eq. (2) per mole or C-mole of i, where i, can be S (substrate CH_2O), O_2, X (biomass), P (product) etc.

By measuring the heat generation rate q_R and the consumption/production rate of i on-line. $\Delta_r H_i$ can be continuously monitored. As long as the culture uses a well-defined and constant set of metabolic pathways, $\Delta_r H_i$ will remain constant, whereas a shift to another metabolism will immediately manifest itself in a modified $\Delta_r H_i$.

Yeast cultures growing on glucose are a good example. In 'normal' completely aerobic culture, the energy metabolism is completely oxidative and the culture grows according to the following

stoichiometry:

$$CH_2O + x_3 Y^{OX}_{X/S} NH_3 + Y^{OX}_{O/S} O_2 \rightarrow Y^{OX}_{X/S} CH_{x_1} O_{x_2} N_{x_3} + Y^{OX}_{C/S} CO_2 + Y^{OX}_{W/S} H_2O \quad (3)$$

When grown in the absence of oxygen, the metabolism [8] is reductive and follows the stoichiometry shown in Eq. (4) (assuming that ethanol is the sole fermentation product):

$$CH_2O + x_3 Y^{red}_{X/S} NH_3 \rightarrow Y^{red}_{X/S} CH_{x_1} O_{x_2} N_{x_3} + Y^{red}_{P/S} CH_3O_{0.5} + Y^{red}_{C/S} CO_2 + Y^{red}_{W/S} H_2O \quad (4)$$

where $CH_3O_{0.5}$ stands for 1 C-mole of ethanol.

Eq. (4) is characterized by a much lower biomass yield as compared with Eq. (3) (0.11 vs. 0.6 C-mol/C-mol) but also by a much lower heat evolution. (According to von [1], $\Delta_r H_i$ for Eq. (3) and Eq. (4) is −190 kJ/C-mol and −11.6 kJ/C-mol, respectively).

As explained later, there are several situations in which yeast cultures show a mixed oxido-reductive metabolism with a stoichiometry which is a combination of Eq. (3) and Eq. (4). By monitoring on-line the overall heat effect, $\Delta_r H_i$, it is obviously possible to determine the oxidative and the reductive fractions in the overall metabolism and to detect changes towards one or the other.

Another technique consists in using heat measurement as a substitute for the oxygen uptake rate measurement. This is based on the 'oxycaloric equivalent', $\Delta_r H_{o_2}$, which lies within ±10% of 470 kJ/mol for all oxygen consuming reactions [5], and on the fact that reactions consuming neither oxygen nor other strong oxidants produce negligible amounts of heat as compared to the former. The heat generation can therefore be used as an approximate estimate of the oxygen consumption rate and thus as a rough but quantitative estimation of the rate at which the oxidative metabolism proceeds. Monitoring both heat and oxygen and comparing $\Delta_r H_{o_2}$ with the oxycaloric equivalent of 470 kJ/mol will reveal the absence or presence of other strong oxidants such as nitrates.

3. Control of yeast cultures based on calorimetry

In biotechnological processes, where yeast cells are the production target, as for instance in Baker's yeast production, one will try to maintain an exclusively oxidative metabolism since any shift to a oxido-reductive metabolism will entail an important loss of biomass due to the low value of $Y^{red}_{X/S}$. However, even when grown in aerobic batch cultures, many yeast strains, including the important *Saccharomyces cerevisiae*, exhibit oxido-reductive or even completely reductive metabolism and produce large amounts of ethanol.

The phenomenon occurs because these strains are subject to a catabolite repression effect [9] or because their maximal respiratory capacity is insufficient for complete oxidation – according to Eq. (3) – of all of the glucose they can assimilate. This condition is often referred to as a 'respiratory bottleneck' [10,11]. The excess glucose that cannot be processed by the oxidative stoichiometry then overflows into the reductive pathways, resulting in a growth stoichiometry in accordance with a combination of Eq. (3) and (4).

In order to avoid reductive metabolism, industry produces yeasts in fed-batch cultures designed to avoid the accumulation of a large glucose concentration at any time. Concentrated substrate solution is continuously fed to the culture as fast as possible to ensure fast growth, but just not rapid enough to allow glucose accumulation in the broth and thus to overload the respiratory capacity of the yeast cells.

Optimal control is difficult to achieve in these fed-batch cultures. Because of the exponential growth, the maximal allowable glucose feed rate for completely oxidative metabolism increases exponentially. On the other hand, neither the glucose feed rate, nor the glucose uptake rate, is known exactly; this is due to the lack of on-line sensors for glucose and the variability of glucose concentration in the technical feedstocks.

Optimal control of feed rate could be achieved by measuring the heat released by the culture in an isothermal reaction calorimeter (RC-1, Mettler-Toledo) as demonstrated by Randolph et al. [12]. In addition to the heat dissipation rate, they continuously measured the CO_2 evolution as well as the ammonia consumption rates by keeping track of the amount of NH_3 that had to be added to maintain a constant pH. By solving the elemental and the energy balances, they were able to determine the glucose consumption rate as well as the rates of production of biomass and CO_2. The oxygen consumption rate was estimated directly

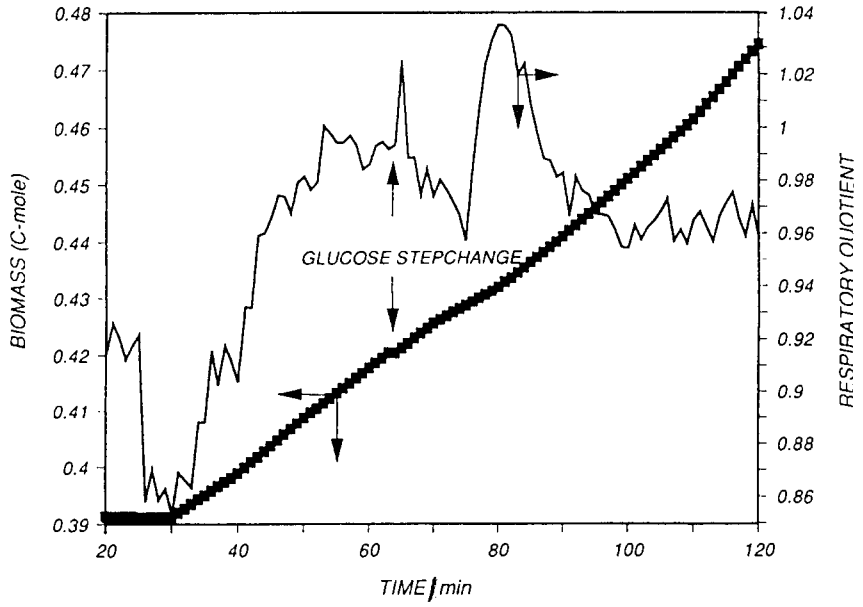

Fig. 1. Heat production rate during a controlled fed-batch experiment of *Saccharomyces cerevisiae* following a feed concentration change from 40 to 50 g l^{-1} glucose (Randolph et al., [12]).

from q_R based on the oxycaloric equivalent of 471 kJ/mol. The ratio r_{O_2}/r_{CO_2}, the respiratory quotient, was continuously calculated and used as a control variable. A controller increased the feed rate of glucose in parallel to the calculated growth rate. If the respiratory quotient (RQ) started to increase above unity, indicating an onset of reductive metabolism, the feed rate was reduced.

Fig. 1 shows the evolution of the calculated respiratory quotient on a very expanded scale for a typical experiment. After stabilization of the RQ to its set-point of 0.97, the glucose-feed concentration was suddenly increased by 25%. After ~20 min, the RQ started to rise above unity, indicating some reductive overflow metabolism. However, the controller reduced the feed rate and the RQ rapidly returned to its set-point, whereas the increase of biomass continued without interruption. This experiment shows that the metabolism could be maintained fully oxidative even under the influence of feed concentration disturbances. In this way, the ethanol formation could be kept below 1 g/l, while high typical biomass yields of 0.4 C-mol were attained.

An even simpler technique was used by Larson et al. [13] in order to control a fed-batch culture of *S. cerevisiae*. They monitored the heat production by continuously pumping a sample stream from the culture through a LKB flow-through calorimeter. 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. 2. 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 ~0.1–0.2 g/l. Thus, biomass yields of the order of 0.32–0.35 g per gram substrate were attained, which is similar to those reported by Randolph et al. [12]. Productivities were, however, lower due to much lower substrate concentrations in the feed and due to the non-optimal control scheme.

In continuous cultures, the respiratory bottleneck of yeasts such as *S. cerevisiae* also leads to a oxido-reductive metabolism at dilution rates higher than a certain threshold, called the critical dilution rate D_{crit}.

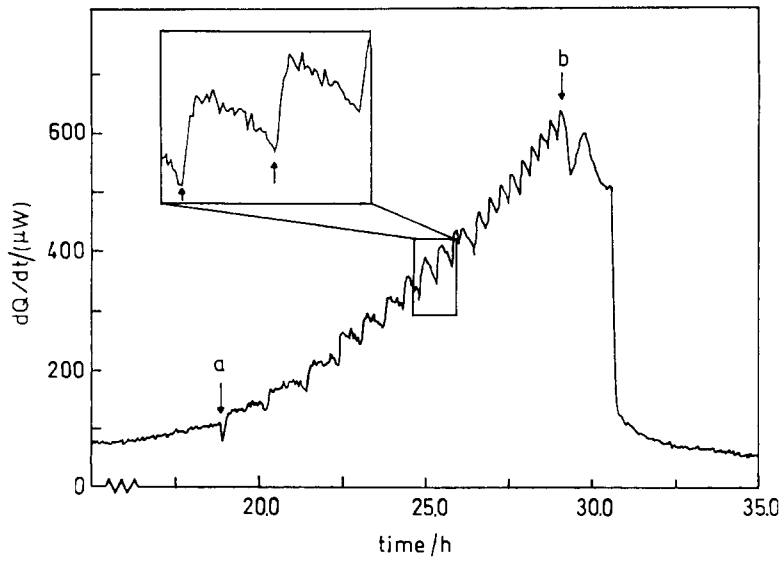

Fig. 2. Heat production rate during a controlled fed-batch experiment of *Saccharomyces cerevisiae* (Larsson et al., [13]).

The high glucose feed rate then overloads the oxidative capacity of the culture and an overflow of excess glucose into the reductive pathways occurs [14,11]. The result is an overall stoichiometry resembling Eq. (2) but which, in fact, is a linear combination of Eqs. (3) and (4) [15].

When grown at dilution rates below D_{crit}, the culture exhibits a fully oxidative metabolism. When such a culture is, however, subjected to a pulse of glucose or to a sudden increase of dilution rate, transient redox metabolism arises and ethanol is formed [16,17]. The overflow of some glucose into the reductive metabolism is due to the fact that an increased amount of glucose fed to the cells can be assimilated immediately [18–21], whereas the respiratory capacity only increases slowly in time. As long as the new dilution rate does not exceed D_{crit}, the metabolism will eventually return to a fully oxidative state.

In very recent experiments, Duboc et al. [22] have studied the question how the increase of the dilution rate in a continuous culture has to be controlled in order to avoid any deviation into reductive metabolism. By measuring the oxygen uptake rate after a step increase of the dilution rate, they determined the kinetics of the adaptation of the respiratory bottleneck. Using the known oxidative stoichiometry shown in Eq. (3), they designed a profile for the increase of dilution rate with time which was adapted to the respiratory dynamics, so that all of the incoming glucose could always be handled by the respiratory capacity (F_{sp} in Fig. 3b). In addition, a feed- back control based on on-line calorimetry was implemented. Using the known value of $\Delta_r Hs$ for Eq. (3), the heat dissipation rate as a function of time was predicted assuming fully oxidative metabolism $q_{R,SP}$ in Fig. 3a. Any deviation of the measured q_R towards lower heat production indicated a shift into the oxido-reductive regime and was automatically corrected by lowering the feed rate.

Fig. 3 shows that partially reductive metabolism set in after about 2.2 h and that the feed rate was decreased. After ~7 h, the measured q_R was again very close to the predicted one, indicating that the metabolism had again become completely oxidative.

The merits and inconveniences of using heat generation as compared to other on-line measurements as a control variable [22] is also addressed. They show that calorimetric measurements react faster and therefore show more details than the other alternatives, which are delayed by mass transfer effects, accumulation terms, mixing phenomenon, and transport through sample lines.

This conclusion is confirmed by van Kleef et al. [23], who studied optimal control of fed-batch cultures

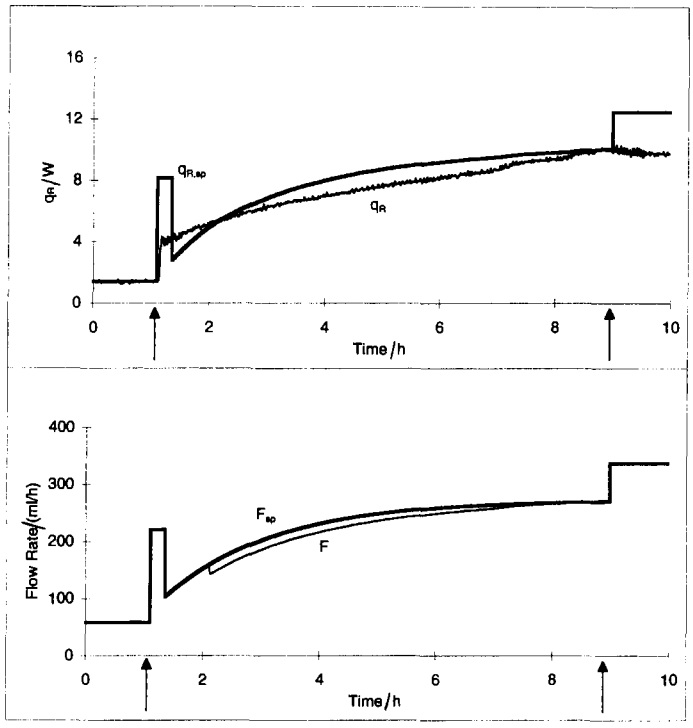

Fig. 3. Feedback control experiment of continuous culture of *Saccharomyces cerevisiae* aimed at controlling the dilution rate increase. The feed flow rate is corrected when the heat production rate is lower than the set-point value (at $t = 2.1h$). Correction stops towards the end of the experiment. A further dilution rate shift was imposed at the end of the experiment to check that the oxidative capacity was at its maximal value (second arrow). Duboc et al., [22].

of the yeast *S. cerevisiae* based on heat, RQ, or both parameters combined. Due to the faster response of the heat measurement, and owing to the relative insensitivity of the off-gas analysis (on which RQ is based), to dilution effects caused by additions, the combination of the measurements worked better than either control parameter by itself. The experiment described by van Kleef [24] is also remarkable in that it has been performed in a conventional laboratory bioreactor without restoring to highly sophisticated calorimetric equipment.

4. Monitoring and control of a bacterial culture-producing erythromycin

It has been recently demonstrated how on-line calorimetry can be used to monitor and control fed-batch cultures of the filamentous bacteria *Saccharopolyspora erythraea* [25]. A main prerequisite for monitoring filamentous cultures is to correct the power release signal for non-biological effects due to modification of the broth rheology. In isothermal reaction calorimeters, the rheology of the broth affects (i) the heat transmission coefficient UA through the glass wall, which must be known for evaluating the measurement [3], and (ii) the thermal power release by the stirrer, which will be picked up by the calorimeter together with the biological signal.

Although UA can, in principle, be measured during the culture by calibrations, this proved unnecessary because it remained virtually constant. However, the power uptake by the agitator underwent important variations and thus was measured by a torque meter [26]. As can be seen from the example given in Fig. 4, the torque drastically decreased within 25 h into the culture due to the appearance of foam, causing alongside a decrease in the q_R measurement (disturbance No. 1). At 65 h, an antifoam agent was added, causing

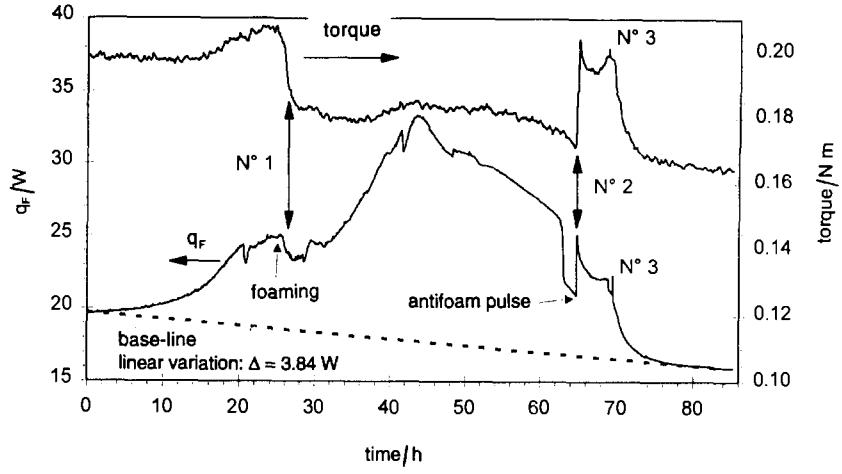

Fig. 4. Heat dissipation rate (q_F) and torque profile during batch culture of *Saccharopolyspora erythraea*. The heat flux measured with the calorimeter is affected by torque and linear baseline variation due to decrease in reactor volume (sampling). (Menoud, [25]).

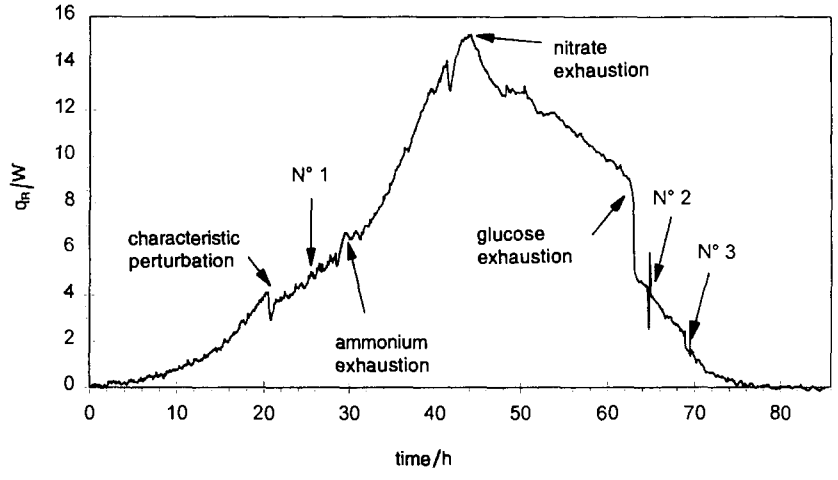

Fig. 5. Heat production rate (q_R) of a batch culture of *Saccharopolyspora erythraea* after correction for baseline and torque variation. In cordrastro with Fig. 4, q_R represents the actual heat dissipated by the microorganisms. Metabolic changes due to ammonium, nitrate or glucose exhaustion are clearly detectable. (Menoud, [25]).

disturbances Nos. 2 and 3 in the thermogram. When the calorimetric baseline was corrected for the power introduced by the stirrer, disturbances Nos. 1–3 disappeared (see Fig. 5).

Figs. 6 and 7 show the evolution of a typical culture of *S. erythraea* on a complex, industrial medium containing glucose (50 g/l), ammonium sulfate (4.5 g/l), and a complex extract of cottonseed flower (30 g/l), Menoud, [25]). Fig. 6 shows the profiles for the concentrations of glucose, dry biomass, ammonium ions and erythromycin as determined by off-line analyses. The curve connecting the keys for ammonium represents an on-line measurement based on the consumption of NaOH necessary to neutralize the protons liberated by the assimilation of ammonia. The thermogram of the culture is shown in Fig. 7. It contains the following set of characteristic features, which were found in a qualitatively similar way in all

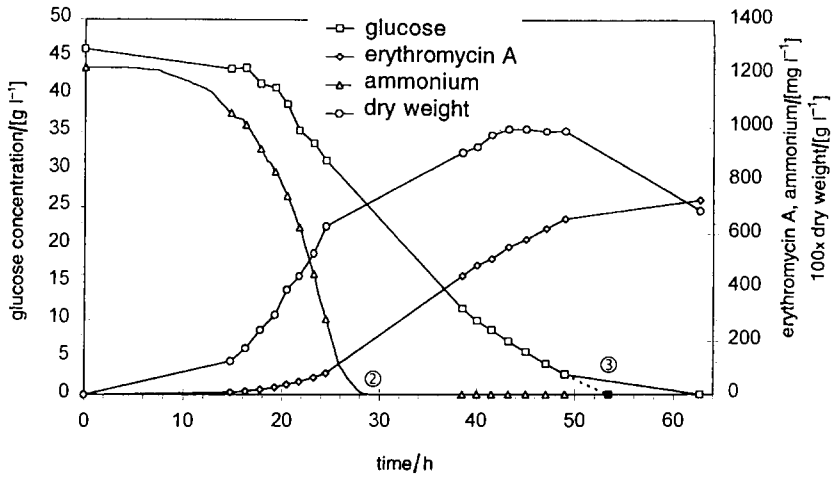

Fig. 6. Batch culture of *Saccharopolyspora erythraea* on glucose and ammonium (initial concentration of 4.47 g l^{-1}). Profile of glucose, erythromycin A, ammonium and dry weight increase. 2 – indicates ammonium exhaustion, and 3 – glucose exhaustion. (Menoud, [25]).

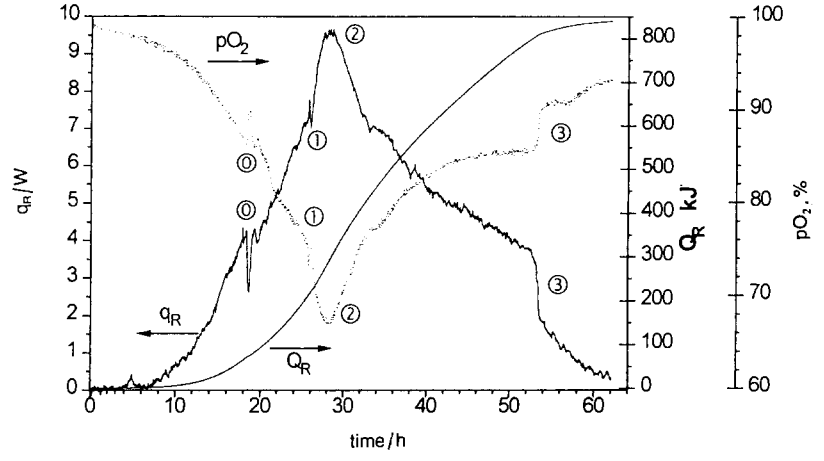

Fig. 7. Heat production rate pO_2 and total heat released during a batch culture of *Saccharopolyspora erythraea* on glucose and ammonium (same experiment as in Fig. 6.). 0 and 1 indicate two characteristic perturbations that were observed for all the experiments, and 2 indicates ammonium exhaustion and Æ glucose exhaustion as shown in Fig. 6. (Menoud, [25]).

thermograms, and which could be used to identify particular events.

At the time labeled '0', a characteristic decrease of 1.4 ± 0.14W occurred during 45 min; this was probably due to the exhaustion of an unidentified substrate contained in the cottonseed flower extract. When the residual ammonium concentration became limiting, a characteristic peak of ~1 W, followed by a period of high heat generation, was systematically observed (see label '1'). This is most probably due to the tendency of microbial energy metabolism to uncouple under energy sufficient conditions [27]. This intensive heat production continued for ~2 h until the ammonium ions were completely exhausted (see Fig. 6), after which the heat generation started gradually to decrease ('2'). After these events, growth continued though slowly, drawing on an unidentified nitrogen source in the cottonseed flour. Although some erythromycin was produced before the events '1' and '2', the main production usually occurred after these and

continued until glucose became exhausted and biosynthesis came to a halt. Glucose exhaustion was also revealed precisely by the thermogram in the form of an almost instantaneous drop in heat production, marked '3', after which the heat production slowly decreased to the baseline.

It is obvious from Fig. 7, that all these events can also been found on the response curve of the dissolved oxygen probe. If the volumetric mass transfer coefficient $k_L a$ and the oxygen concentration in the bubbles were known, this could be translated into a precise measurement of oxygen uptake rate:

$$r_{O_2} = k_L a(C^*_{O_2} - C_{O_2}) \quad (5)$$

Both factors, however, vary due to several reasons, including the complex variable rheology of the culture. Dissolved oxygen measurements are thus at best a qualitative indicator, whereas the thermal power release can be exploited via enthalpy balances [25].

Repeating the experiment reported in Figs. 6 and 7 at several initial ammonium ion concentrations showed that increasing the concentration of $(NH_4)_2SO_4$ from 1.75 to 8 g/l leads to a higher build-up of biomass and, thus, to an overall increase of erythromycin production rate by a factor of 2, but also resulted in a reduction of the overall product yield and final concentration from 900 to 423 mg/l. The reason was a clear decrease of the specific erythromycin productivity during the growth phase (before event '2'), which can be explained by an inhibition of the biosynthesis by the nitrogen source [28,29].

There could, therefore, be room for improvement by adding $(NH_4)_2\ SO_4$ in a fed-batch manner, with the aim of building up high cell densities, and yet keeping the ammonium ion concentration low at all times. The result of such a fed-batch culture is shown on Figs. 8 and Fig. 9. It was started with an initial ammonium sulfate concentration of only 0.5 g/l but with a high glucose concentration of 100 g/l. The thermal power release (Fig. 9) indicated the onset of growth and went through the characteristic disturbance ('0') observed in the previous experiment. When it indicated ammonium ion limitation by going through the pronounced maximum labeled ('1'), the ammonium sulfate concentration was restored to 0.5 g/l by automatic addition (Fig. 8). A period of readjustment with low activity followed ('3'). Then, growth vigorously resumed and the heat generation went through the characteristic rapid peak ('4') and the period of intense heat production ('1'), again indicating imminent ammonia exhaustion. After five pulses leading to five similar cycles, the exhaustion of glucose caused a rapid breakdown of the heat production by about 6 W, but the erythromycin can be seen to have reached a level of 1200 mg/l. This concentration is not surprising in view of the high amount of glucose processed

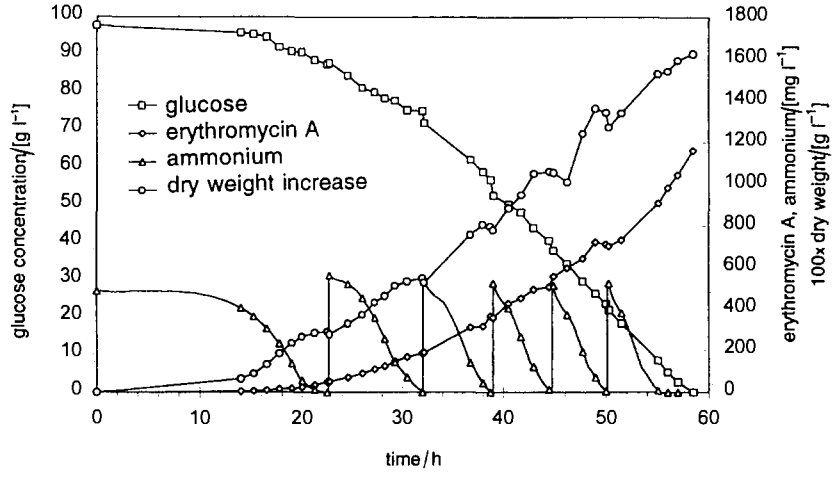

Fig. 8. Controlled fed-batch culture of *Saccharopolyspora erythraea* by pulsing ammonium. Ammonium depletion was detected by the heat production signal (as illustrated in Fig. 9) and was compensated for by an ammonium pulse. (Menoud, [25]).

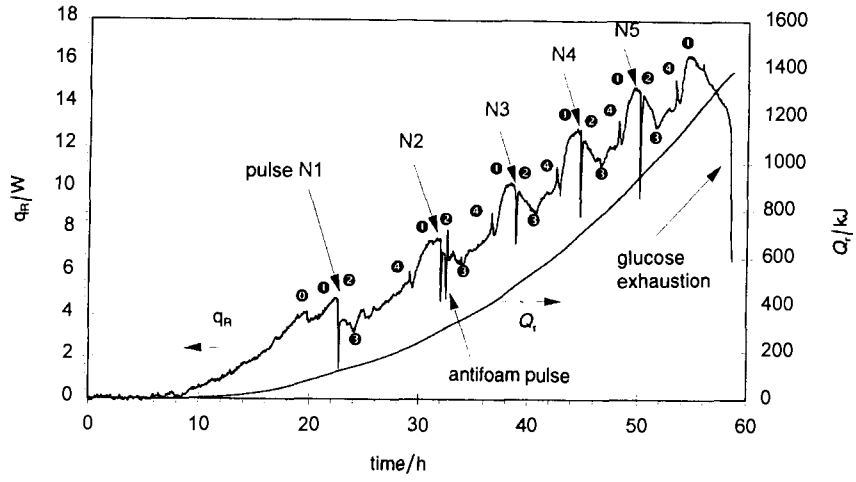

Fig. 9. Heat production rate and total heat released during controlled fed-batch culture of *Saccharopolyspora erythraea* by pulsing ammonium as shown in Fig. 8. The labels '2' to '3' correspond to the same events as in Figs. 6 and 7. (Menoud, [25]).

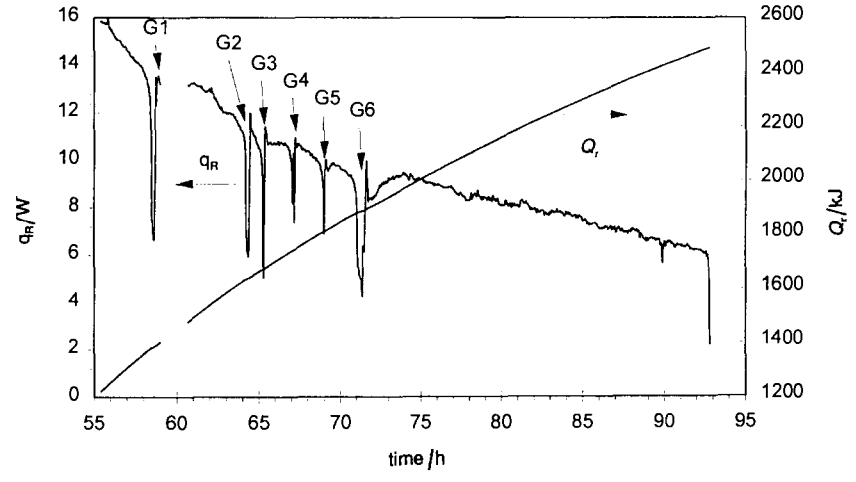

Fig. 10. Heat production rate and total heat released during controlled fed-batch culture of *Saccharopolyspora erythraea* by pulsing glucose after ammonium depletion of the culture shown in Figs. 8 and 9. Depletion of glucose is easily detected by the sharp heat production rate decrease and was followed by the injection of a glucose pulse. (Menoud, [25]).

but the specific productivity was maintained at the high values typical for cultures with low ammonia content.

The same culture was used to investigate whether the biomass formed could be simulated to continue producing antibiotics by feeding it with additional glucose. A total of 46.3 g/l of additional glucose was delivered to the culture in six pulses, which were added each time the characteristic drop in the thermogram indicated exhaustion of glucose. As can be seen in Fig. 10, the glucose pulses restored the heat production rate to the state immediately before the drop, but the slow overall decline of the thermal activity typical for the production phase after nitrogen exhaustion could not be halted. Biomass concentration stayed constant at ~15 g/l during these pulses, but the erythromycin concentration doubled to 2300 mg/l. This type of extended fed-batch culture on glucose is expected to be especially attractive because of catabolite repression and adverse rheology effects that

would occur if all the glucose would be present in the medium from the beginning.

5. Conclusions

Laboratory work shows that heat dissipation may be used as a reliable and quantitative indicator of the state of the metabolism of microbial cultures. A prerequisite is the on-line correction of the signal for the heat liberated by the stirrer, which can be highly variable during batch cultures because of rheological modifications in the broth. One possible use of the signal is based on simply correlating the heat dissipation qualitatively with the evolution of the culture. This enables the identification of pertinent metabolic events by analyzing the heat production rate as a function of time. It has been shown that it is possible to control fed-batch cultures based on this technique. A similar qualitative analysis could be performed in some cases based on the response of a dissolved oxygen probe. Other signals, such as CO_2 evolution or O_2 uptake rates calculated from off-gas analysis, are too slow for this purpose. But in contrast to DO signals, heat release measurements can also be exploited quantitatively, thus permitting mathematical assessment of the rates at which various types of metabolic activities occur. This is a basis for constructing robust controllers and rational process control.

Most of such experiments have been performed at the laboratory scale, using high quality, sensitive bench-scale calorimetric equipment such as isothermal reaction calorimeters. It was shown, however, that conventional bioreactors, converted to bench-scale calorimeters by relatively simple means can also yield valuable calorimetric data if the process is correctly modelled. Establishing energy balances around technical scale bioreactors may be comparatively easier due to the fact that many side effects such as heat loss will be attenuated at larger scale. The potential, merits and limitations of this concept has however, yet to be demonstrated at industrial scale.

Acknowledgements

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