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Calorimetry of technical microbial reactions

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Abstract - Heat generation is a very conspicuous phenomenon in large scale bioreactors and could in principle quite easily be measured by the temperature increase of the cooling water. Taking yeast cultures growing on a mixed oxido-reductive metabolism for various reasons as an example, this paper demonstrates how heat dissipation measurements may be used in combination with one other on-line measurement in order to obtain a quantitative estimation of the nature of the on-going metabolism and of the current overall stoichiometry. The application of such measurements to controlling fed-batch cultures is also discussed.

INTRODUCTION

Heat is a universal and unavoidable by product of all biological phenomena, including those that are exploited in biotechnology at technical scale. Any change in growth rate, metabolism, biocatalytic activities and in other biological phenomena occurring in technical reactors will invariably affect the rate at which heat is released. Yet heat effects in cellular cultures often go unnoticed when one is working with conventional laboratory equipment because most of the heat released by the culture is lost to the environment too quickly to give rise to a perceivable temperature increase. This, however, is completely different at large scale (ref. 1). 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. 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 in industrial fermentors. Systematic monitoring of heat generation rates by the culture in large scale bioreactors would clearly be of considerable benefit for process optimization and control. The information contained in this signal could be used together with data on other relevant process parameters to obtain quantitative on-line estimates of the activity, the metabolism and the state of the culture.

The aim of this contribution is to explore this philosophy on the basis of laboratory scale experiments. Yeast cultures growing according to different forms of oxido-reductive metabolism in response to various single and dual limitations will serve as an example in case.

CALORIMETRIC EQUIPMENT

In order to make use of heat release measurements in bioprocess control algorithms, suitable models must be available which relate the heat evolution rate of the culture to other relevant process variables, such as substrate consumption, growth rate or oxygen up-take. Numerous workers have studied these relationships in calorimetric experiments at the laboratory scale. The need for maintaining technically relevant, strictly controlled culture conditions made it difficult to obtain meaningful results in microcalorimeters (refs. 2-4).

The work presented in this paper has therefore been carried out in so-called "isothermal reaction calorimeters". Several units were purchased from Ciba-Geigy AG, Basle and from Mettler-Toledo AG, Greifensee, Switzerland, who markets them under the name RC1 for chemical work. The instruments were then modified for biological work as described elsewhere (ref. 5). Isothermal reaction calorimeters are composed of a jacketed glass reactor, with a typical nominal volume of 2 l, which may be operated much in the same way as an ordinary laboratory fermentor. Some of the culture parameters such as T, pH, dissolved oxygen concentration, and off-gas composition are measured continuously as a basis for rigorous control of the culture conditions. Other process and biochemical variables may be monitored as desired by off-line analysis of samples. The instruments were operated as chemostat and in the fed-batch modes during the experiments described in the next two sections, respectively.

In addition, isothermal reaction calorimeters are designed for continuous monitoring of the heat generation rate with a resolution of about 50 mW after modifying them for biological work. The calorimetric measuring technique has already been described several times (ref. 1).

HEAT AS A QUANTITATIVE INDICATOR OF CELL METABOLISM

Yeasts often grow according to a mixed oxido-reductive metabolism (ref. 6). The overall, apparent stoichiometry resulting from this mixed metabolism may be written as a "quasi-chemical" reaction using C-molar notation (ref. 7):

$$CH_2O + Y'_{o/s}\,O_2 + x_3\,Y'_{x/s}\,NH_3 \rightarrow$$

$$Y'_{x/s}\,CH_{x_1}O_{x_2}N_{x_3} + Y'_{P/s}\,CH_3O_{1/2} + Y'_{c/s}\,CO_2 + Y'_{w/s}\,H_2O \qquad (1)$$

All the stoichiometric coefficients, i.e. the Y-values in Eq. (1), will depend on the relative mixture of oxidative (respiratory) and reductive (fermentative) metabolism and will vary in response to a modification of this mixture. The same is true for the various consumption and production rates, such as for glucose (r_s'), oxygen (r_o'), biomass (μX) and CO_2 (r_c'). The heat evolution rate (q) will likewise be affected. Continuous yeast cultures can be forced to grow according to a mixed metabolism with varying extents of fermentation by limiting the oxygen supply to a chemostat culture to various levels. At low enough O_2 supply rates, the culture will become dually limited by glucose and O_2 (ref. 7) and will have to adapt $Y'_{o/s}$ in Eq. (1) to the ratio of O_2/glucose supplied to the culture. Facultative anaerobes do this by respiring an amount of glucose corresponding to the available O_2 and by degrading the rest according to the fermentative pathway.

The supply ratio O_2/S thus determines the relative importance of oxidative to reductive metabolism and hence all the Y-values in Eq. (1). The effect on this ratio on the "heat yield" $Y'_{Q/X}$, i.e. the amount of heat released per C-mole of biomass grown, is shown in Figure 1 for a *K. fragilis* culture (ref. 8). At high values of this ratio, i.e. in the right hand part of the figure, O_2 is not limiting and $Y'_{Q/X}$ has a constant value, indicative of fully oxidative growth. On the left side, $Y'_{Q/X}$ decreases in a hyperbolic fashion as oxygen becomes limiting and the O_2/S supply ratio is gradually reduced to zero.

Similar effects are obtained in *S. cerevisiae* if one increases the dilution rate beyond a certain value known as D_{crit} (refs. 6 and 7). Even if such cultures are supplied with large quantities of O_2, they cannot respire all incoming glucose due to a biologically limited respiration capacity. As D is increased, a growing part of the substrate is therefore reductively degraded, and $Y'_{o/s}$ decreases. Mixed oxido-reductive growth may also be induced by limiting the nitrogen supply (ref. 9) or by a combination of several of these factors. In all cases the heat yield has been shown to be affected in a characteristic way (ref. 7).

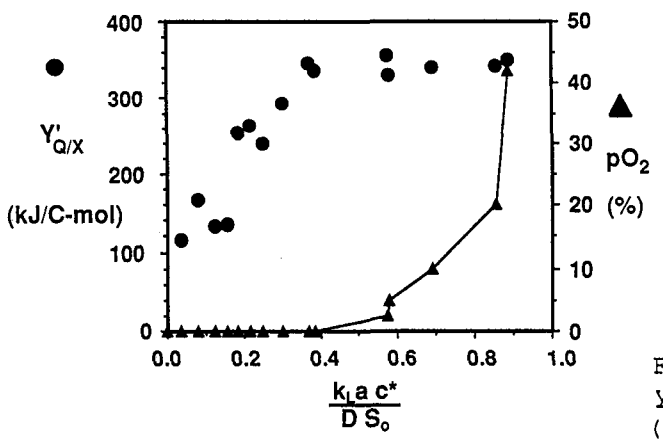

Figure 1. Heat dissipated per C-mol of biomass grown (●) and dissolved oxygen concentration (△) for a continuous culture of *K.fragilis* as a function of the ratio of oxygen to glucose supply. Recalculated from ref.8.

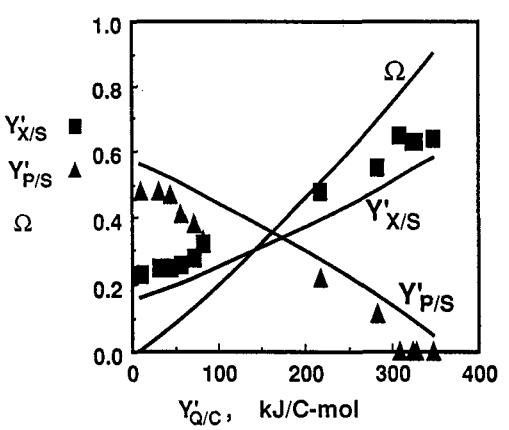

Figure 2. Aerobicity (Ω), biomass yield (Y'X/S) and ethanol yield (Y'P/S) as a function of the heat dissipated per mole of carbon dioxide evolved for a continuous culture of *S.cerevisiae*. Solid lines: computed from unified stoechiometric model (ref. 10), points: measured values. Redrawn from ref.10.

In all these cases, the relative importance of oxidative to fermentative metabolism may be characterized by the ratio of the actual $Y'_{O/S}$ value to the one obtained for fully respiratory growth (refs 7-8):

$$\Omega = Y'_{O/S} / Y'^{ox}_{O/S} \qquad (2)$$

This ratio has been called the aerobicity of the culture and varies from $\Omega = 1$ for fully oxidative to $\Omega = 0$ for purely reductive growth. If its value is known, any Y-value appearing in Eq. (1) or any ratio of two consumption or production rates may be predicted using a unified stoichiometric model (ref. 10). Conversely, Ω may be evaluated continuously on the basis of a Y-value or 2 measured rates.

Among the most convenient rates for on-line monitoring are the heat and the CO_2 evolution rates. The ratio of these rates, $Y'_{Q/C}$, is also an indicator of Ω and thus of the whole stoichiometry. Figure 2 shows Ω, $Y'_{X/S}$ and $Y'_{P/S}$ as calculated from, the $Y'_{Q/C}$ unified stoichiometric model (solid lines). Also plotted are the really observed stoichiometric coefficients $Y'_{X/S}$ and $Y'_{P/S}$ (points) as a function of measured values of $Y'_{Q/C}$ for *S. cerevisiae* cultures. It may be concluded that reasonable estimates of $Y'_{X/S}$ and $Y'_{P/S}$ would have been obtained from the measurement of $Y'_{Q/C}$ alone, although these measurements seem to underestimate the aerobicity consistently by a certain amount.

CONTROL OF FED-BATCH FERMENTATIONS BY CALORIMETRY

The tendency of the yeast *S. cerevisiae* for mixed oxido-reductive growth in the presence of large amounts of glucose, which was discussed in the previous section, has a major implication on the industrial production of baker's yeast. If the yeast is grown in normal batch cultures, aerobic production of ethanol will dramatically lower the biomass yield and inhibit growth. In practice, baker's yeast is therefore produced in fed-batch cultures designed to avoid the accumulation of large glucose concentrations at any time. Concentrated substrate solution is continuously fed to the culture as fast as possible to ensure fast growth, but just not fast enough to allow glucose accumulation in the broth and thus to overload the respiratory capacity of the yeast cells.

Using an approach similar to the one discussed in the previous section, Randolph et al. (ref. 11) showed that optimal control of the feed rate could be achieved based on measuring the heat released by the culture in a RC1. In addition to the heat dissipation rate, they continuously measured the CO_2 evolution rate and the ammonia consumption rate by keeping track of amount of NH_3 that had to be added to keep the pH constant. From solving the 4 elemental and the energy balances, they were able to determine the consumption and production rates of the remainder of the 7 major entities appearing in Eq. (1). A control algorithm increased the glucose feed rate exponentially until an onset of reductive metabolism was detected based on these 3 measurements.

Figure 3 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. This was counteracted by the controller by lowering the feed flow rate. This explains the decrease of the volumetric heat generation rate (W/l). During the whole culture the calculated respiratory quotient ($=r_C'/r_O'$) remained between 0.97 and 1, thereby indicating purely oxidative metabolism, while the growth rate was pushed to a maximum. 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

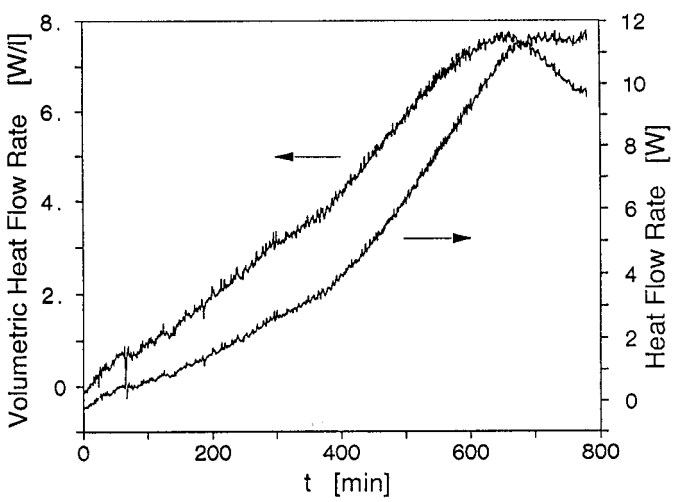

Figure 3. Total and volumetric heat generation rate during a calorimetrically controlled fed-batch fermentation (ref. 11).

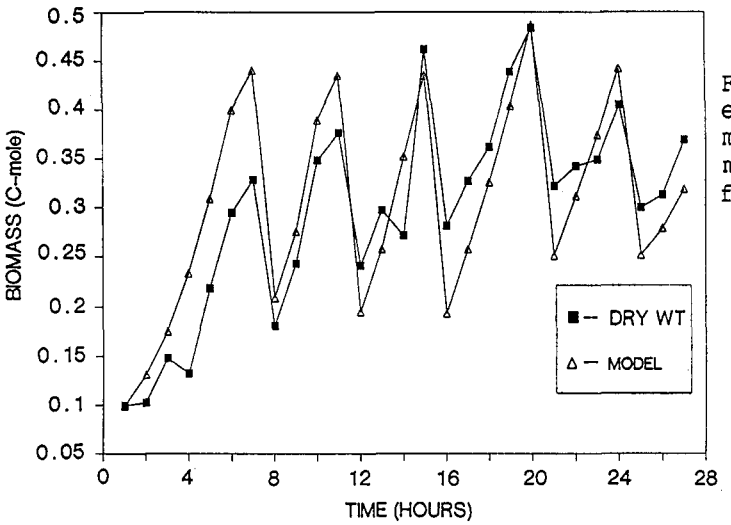

Figure 4. Comparison of biomass estimations from on-line control model and off-line dry weight measurements during a repeated fed-batch experiment (ref. 11).

remaining volume of 800 ml and controlled feeding was resumed without adding additional inoculum. Figure 4 compares the actual biomass content as determined from the dry weight with the model calculations based in part on calorimetry. 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 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 typical for purely oxidative growth.

CONCLUSIONS

The example of oxido-reductive metabolism in yeast developed in this paper shows that heat dissipation measurements can in combination with other convenient on-line measurements and be used as a quantitative indicator of the metabolism, of the whole apparent growth stoichiometry, and of the activity of the culture. Such measurements thus provide a convenient basis for process control.

SYMBOLS

$Y'_{i/j}$	Stoichiometric coefficient or yield for compound i per C-mol of j
r_i'	Conversion rate of i, C-mol s^{-1} m^{-3} or mol s^{-1} m^{-3}
Ω	Aerobicity of the culture, Eq. (2)

Subscripts and superscripts

S	Substrate (glucose)	C	CO_2
P	Product (ethanol)	O	O_2
X	Biomass	ox	Fully oxidative

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