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On-line heat flow measurement in laboratory fermenters ☆

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Abstract

A method is described for the on-line measurement of heat production in a laboratory fermenter. The method can be used with standard laboratory fermenters, with minor modifications. The method was used to study the reaction of different yeasts upon addition of glucose pulses.

Keywords: Candida; Glucose pulse; Heat of growth; Microbial calorimetry; *Saccharomyces*

1. Introduction

Measuring the heat produced during microbial processes is of interest from several points of view:

1. Purely scientific.
2. Control of biological processes.
3. Redundancy of measurements.
4. Speed of measurement in the study of dynamic behaviour.

Specialised equipment for the on-line measurement of heat production is costly and not readily available in most microbiological laboratories. Therefore, a method has been developed of using laboratory fermenters with only minor modifications for calorimetric measurements. In this method all variable heat flows to and from the

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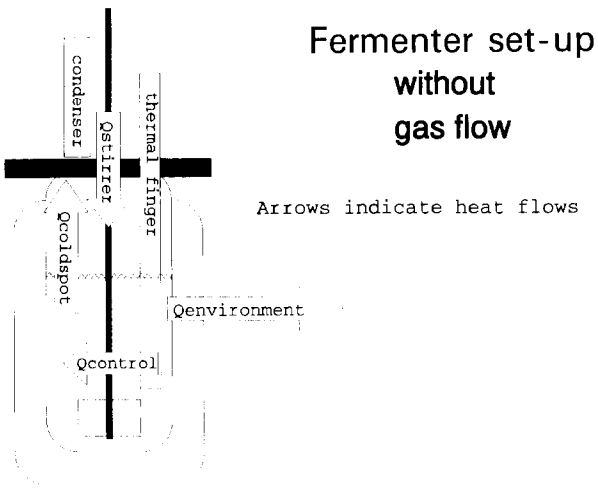

Fig. 1. A. laboratory fermenter, with arrows showing the most important heat flows.

fermenter are measured. Using these measurements, together with knowledge of constant heat flows and instrument characteristics, the heat-flow balance of the fermenter can be solved on-line [1].

2. Methods

2.1. Equipment

The fermenter was equipped with a tubular heat exchanger (thermal finger) to control the temperature of the broth. The inlet and outlet temperatures, the magnitude of the liquid flow through the thermal finger, and the temperature of the fermenter were measured. Fig. 1 shows the fermenter set-up with the main heat flows. The heat exchange with the environment ($Q_{\text{environment}}$) is proportional to the temperature difference between the fermenter and the environment. This was minimised by insulation and estimated from measurements of the temperatures of the fermenter and the environment. Fig. 2 shows the complete set-up and the location of the other temperature measuring points.

The temperature of the water bath was controlled through a simple proportional and integral algorithm, with the temperature of the fermenter as the input variable. As the temperature of the water bath could be varied from 15 K above to 15 K below the desired incubation temperature, a controlled heat flow could be added to or extracted from the fermenter to keep the temperature of the broth within biologically acceptable limits (± 0.2 K), even when there were sudden changes in the biologically produced heat. The tubing between the components of the set-up was constructed with full flow bypasses to minimise transport delays.

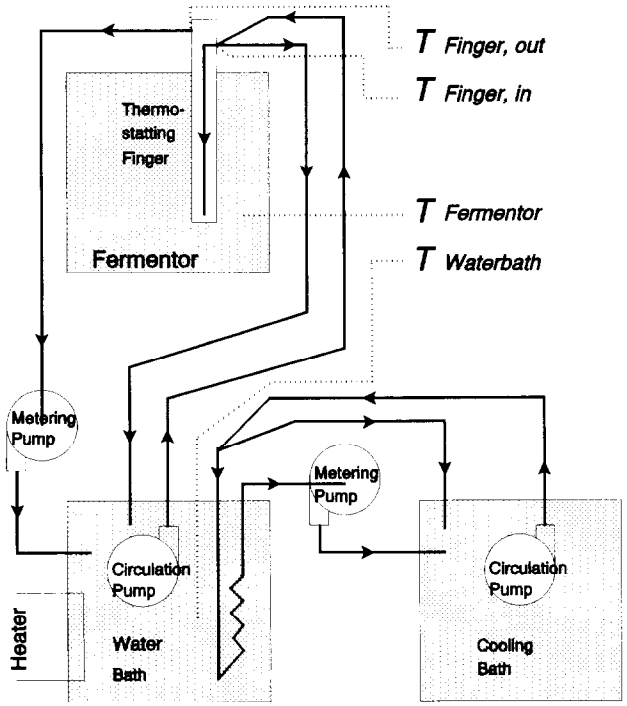

Fig. 2. The complete set-up for making calorimetric measurements in a laboratory fermenter.

Measurements were stored every 4 s. The medium used has been described previously [2]. Glucose (5 g l^{-1}) was used as the source of carbon and energy.

2.2. Heat balance

For any process, the heat balance is

$$\text{Heat accumulation} = \text{Heat flow} + \text{Heat production}$$

For the laboratory fermenter the heat balance is

$$\mathrm{d}T_{\text{fermenter}}/\mathrm{d}t \cdot \text{Heat capacity}_{\text{fermenter}} + \mathrm{d}T_{\text{finger}}/\mathrm{d}t \cdot \text{Heat capacity}_{\text{finger}} = Q_{\text{background}} + Q_{\text{disturbance}} + Q_{\text{control}} + Q_{\text{process}}$$

In this balance, $Q_{\text{background}}$ is the total of heat flows that can be considered to be constant, e.g. the stirring heat if the viscosity of the broth remains constant. Their value must be known from previous calibration experiments. $Q_{\text{disturbance}}$ is the total of heat flows that are not constant but can be measured or calculated, e.g. the heat from addition of acid or alkali for pH control during the experiment. Q_{control} is the

heat flow exchanged between the broth and the thermal finger. This heat flow is equal to the heat gain or heat loss of the liquid flow through the thermal finger. It can be calculated from the measurements of $T_{\text{finger, IN}}$, $T_{\text{finger, OUT}}$, the liquid flow through the finger, and the specific heat of the liquid in the finger. The last term in the equation, Q_{process}, is the heat flow produced by the microbial process.

It should be emphasized that the heat balance can be solved even if the heat accumulation in the fermenter is not zero. Temperature control of the fermenter is of minor importance as long as temperature changes in the broth do not influence the microbial metabolism. Attempts to control the fermenter temperature very precisely are essentially attempts to keep one accumulation term in the heat balance very close to zero. If the temperature of the fermenter can be measured precisely, the accumulation term can be calculated and included in the heat balance. The measurement of the fermenter temperature, or rather of $dT_{\text{fermenter}}/dt$, must be very precise. As the heat capacity of the fermenter when containing broth is large (8 kJ K^{-1} for a typical 1.5 l laboratory fermenter), a standard deviation of 0.01 mK s^{-1} will give a standard deviation of 100 mW in the calculated heat accumulation and, consequently, in the calculated Q_{process}.

3. Results

The response to glucose pulses of two yeast strains grown in continuous culture ($D = 0.066\ h^{-1}$) was studied in the fermenter–calorimeter.

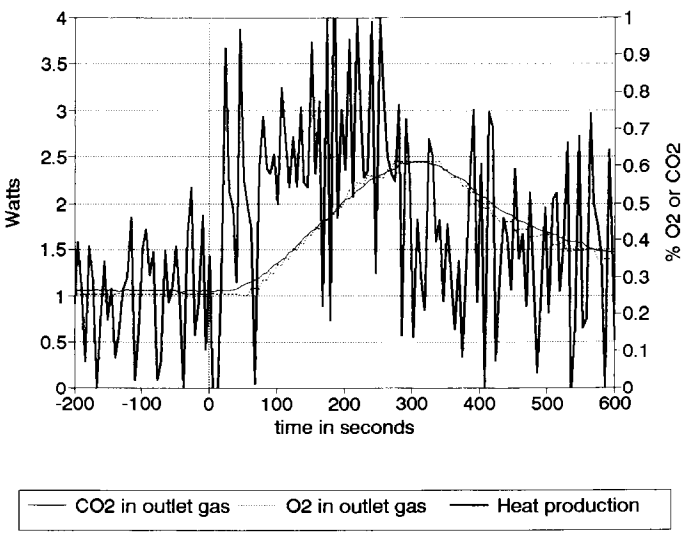

Fig. 3. The response of *Candida utilis* to a 0.2 g glucose pulse given at time 0. No averaging of the heat flow calculations was done.

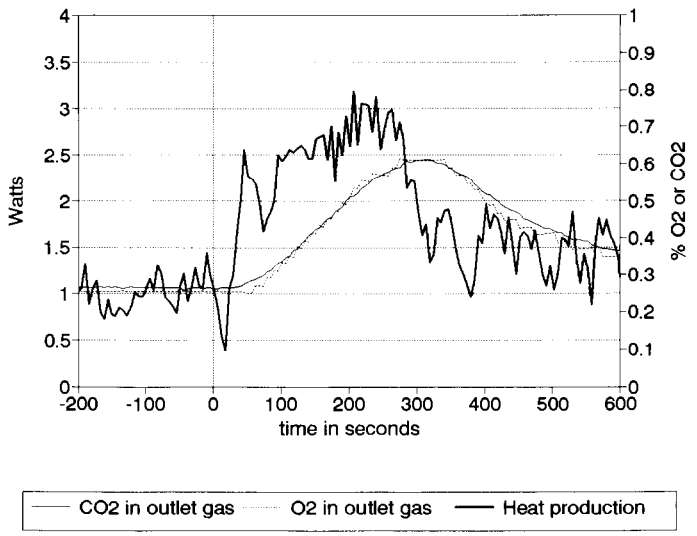

Fig. 4. As Fig. 3, but with an averaging time window of 16 s for the heat-flow calculations.

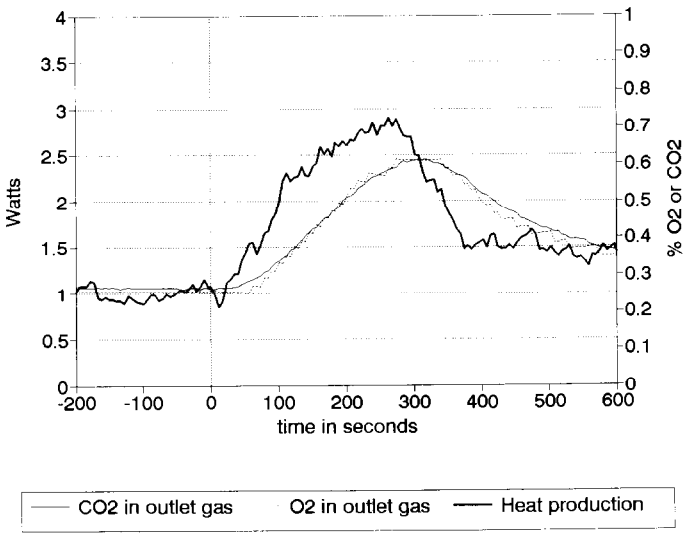

Fig. 5. As Fig. 4, but with an averaging time window of 64 s.

Glucose catabolism in yeast can proceed via different pathways.

Respiration

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2807\ kJ$$

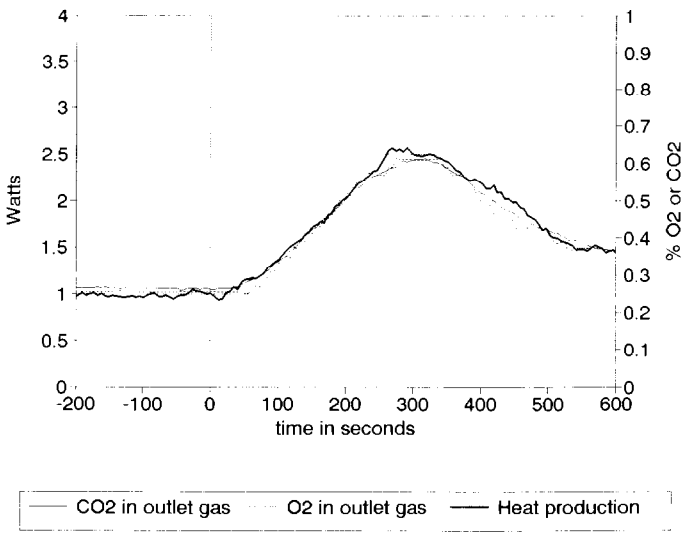

Fig. 6. As Fig. 4, but with an averaging time window of 180 s.

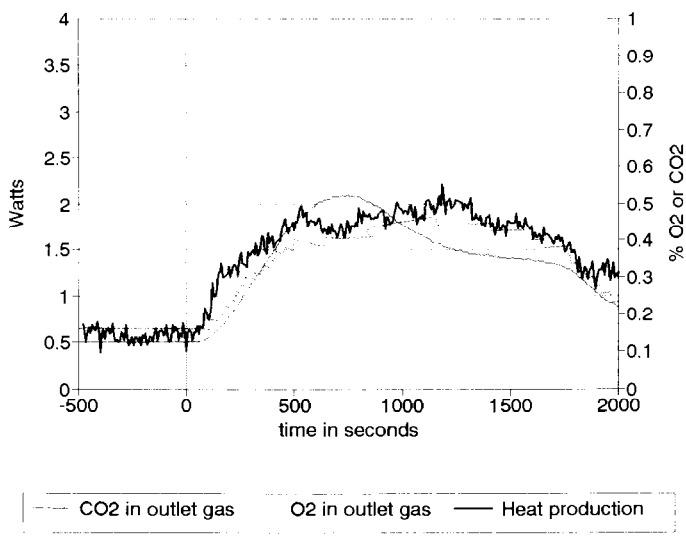

Fig. 7. The response of *Saccharomyces cerevisiae* to a 0.2 g glucose pulse given at time 0. Averaging time window 64 s.

Fermentation

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2CH_3CH_2OH + 69\ kJ$$

The ethanol formed during the fermentation can be oxidised.

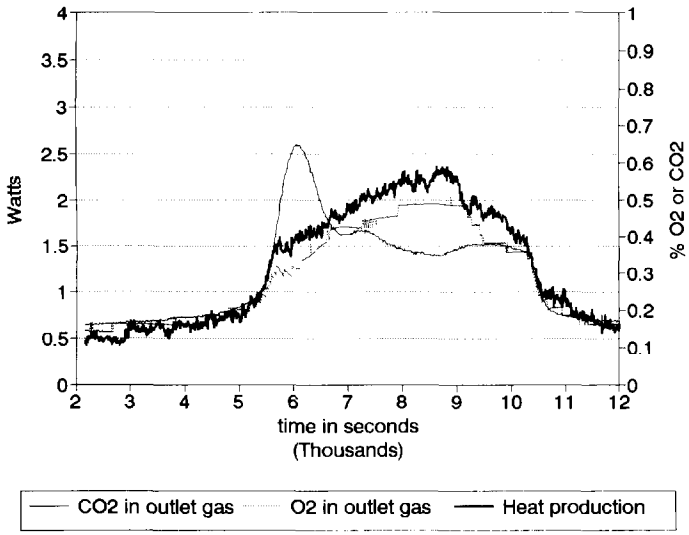

Fig. 8. A spontaneous oscillation in *Saccharomyces cerevisiae*. Averaging time window 64 s.

Ethanol oxidation

$$2CH_3CH_2OH + 6O_2 \rightarrow 4CO_2 + 6H_2O + 2738\ kJ$$

The yeast strain *Candida utilis* CBS 621, while under aerobic conditions, will show only respiration. The response to a 0.2 g glucose pulse injected at time 0 in a continuous culture is shown in Figs. 3–6.

The yeast strain *Saccharomyces cerevisiae* CBS 8066, while under aerobic conditions will show respiration as well as fermentation if the glucose concentration is high enough. The response to 0.2 g glucose pulse injected at time 0 in a continuous culture is shown in Fig. 7.

The yeast strain *Saccharomyces cerevisiae* CBS 8066, in continuous culture, shows spontaneous oscillations. An oscillation is shown in Fig. 8.

4. Discussion

If the heat calculations are not averaged over a longer time window than the 4 s between readings, a very noisy result is obtained (Fig. 3). With continuous cultures this noise is far higher than with blank (not inoculated) experiments. This suggests that at least part of the noise is caused by short-term variations in heat production. It should be rememberd that, with continuous cultures in small-scale laboratory fermenters, the growth medium is added drop-wise with a time interval of several seconds.

The effects of different averaging time windows was studied. In this study only those measurements that were known at the time of evaluation were used. Using

only measurements of this type will shift the heat curve to a later time; however, it is the only averaging method that can be used on-line if the heat measurement has to be used for control purposes. Increasing the averaging time window will decrease the noise (Figs. 4 and 5) at the expense of the response time, until at 180 s averaging time (Fig. 6) the calculated heat shows a curve which is practically identical to those of the O_2 uptake and the CO_2 production measurements that are shown as reference. With this long averaging time the specific shape of the heat curve changes into the common bell-shaped profile which results from any measurement (even of a sharp pulsed phenomenon) if it is averaged over a sufficiently long period of time. However, measuring heat gives a choice between a fast but noisy response and a slow but smooth response, while monitoring O_2 and CO_2 will always give a slow response because of mixing in the headspace of the fermenter (which is equivalent to an ever-present averaging mechanism). Moreover, the O_2 and the CO_2 curves are always preceded by a time delay due to the transport time in the connecting tubing. Although it is possible to correct mathematically for the averaging mechanism it is not possible to correct on-line for the transport delay.

With *Candida utilis* the carbon balance during the glucose pulse fitted to within 5%. The standard rule that one mole of O_2 uptake should equal 460 kJ of heat production was also followed to within 5%.

With *Saccharomyces cerevisiae* (Fig. 7) the carbon balance during the glucose pulse did not fit at all. About six times the expected amount of CO_2 was produced. Comparing this pulse response with the behaviour observed during a spontaneous oscillation (Fig. 8) suggests that the small glucose pulse initiates an oscillation. Following a suggestion by Auberson et al. [3], it may be that the ethanol produced during the first phase of the pulse induces budding and degradation of the reserve material.

5. Conclusions

It has been shown that it is possible to use laboratory fermenters, to which minor modifications have been made, for calorimetric measurements. Noise in the measured heat accumulation should be kept as low as possible. Averaging the measurement over a large time window will lower the noise but increase the response time.

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References

[1] B.H.A. van Kleeff, J.G. Kuenen and J.J. Heijnen, Biotechnol. Bioeng., 41 (1993) 541.
[2] H. van Urk, P.R. Mak, W.A. Scheffers and J.P. van Dijken, Yeast, 4 (1988) 283.
[3] L.C.M. Auberson, T. Kanbier and U. von Stockar, J. Biotechnol., 29 (1993) 205.