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# On-line detection of baseline variations through torque measurements in isothermal reaction calorimeters ☆

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**Abstract**

In addition to on-line detection of events in biotechnology, calorimetry could be used to control processes. In the latter case, a correct and instantaneous heat signal must be available. This would only be possible if a correct evaluation of baseline evolution was possible. In this paper it is shown that torque measurements are a powerful tool for detecting and correcting baseline shifts and for eliminating external perturbations during cultures of a filamentous bacterium.

*Keywords:* Baseline evaluation; Isothermal reaction calorimeter; *Saccharopolyspora*

## List of symbols

| | |
|---|---|
| $A$ | heat transfer surface in $m^2$ |
| $M_t$ | torque or moment of torsion in (N m) |
| $n$ | stirring speed in $s^{-1}$ |
| $T_R$ | reactor temperature in °C |
| $T_J$ | jacket temperature in °C |
| $U$ | global heat transfer coefficient in (W $K^{-1}$ $m^{-2}$) |

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*Greek symbols*

$\phi_A$ heat dissipation due to agitation in W
$\phi_B$ baseline signal in W
$\phi_L$ external heat effects in W
$\phi_R$ heat signal to microbial metabolism in W
$\phi_{MEAS}$ measured heat production rate in W
$\phi_{R,vol}$ volumetric microbial heat-production rate in (W $l^{-1}$)

## 1. Introduction

Calorimetry is a powerful technique for studying the behaviour of micro-organisms and rapidly detecting events occurring during a fermentation. The bench-scale calorimeter RC1 (Mettler-Toledo AG, Switzerland) modified for biological use offers on-line heat-production rate measurements that could be used for process control [1,2].

In order to obtain the correct heat signal, the baseline behaviour has to be determined. In the case where the initial and final baseline levels are different, the thermogram must be corrected for this variation. This is usually done off-line at the end of the experiment by re-drawing the baseline assuming either a linear variation or an evolution according to a more sophisticated model [3,4]. Baseline variation may however occur at any point in time during the experiment. An ideal calorimetric experiment would, in principle, require continuous monitoring of the baseline and on-line correction of the measured power release. This paper shows how this may be done for those baseline variations that result from variations in the power uptake by the stirrer, using on-line torque measurements.

## 2. Materials and methods

Calorimetric measurements were performed in an isothermal reaction calorimeter (RC1, Mettler-Toledo AG, Switzerland) composed of a 2 l jacketed reactor [1,2].

The measured heat-production rate ($\phi_{MEAS}$ in W) is given by

$$\phi_{MEAS} = U \cdot A \cdot (T_R - T_J) \quad (1)$$

where $U$ is the global heat transfer coefficient in (W $K^{-1}$ $m^{-2}$), $A$ is the heat transfer surface in $m^2$, $T_R$ is the reactor temperature in °C, and $T_J$ is the jacket temperature in °C.

The $U \cdot A$ factor was determined using an internal electrical heater before inoculation and at the end of the experiment, after cells had been killed.

The heat signal due to microbial metabolism ($\phi_R$; W) is defined as

$$\phi_R = \phi_{MEAS} - \phi_B \quad (2)$$

It can also be expressed as the volumetric heat production rate $\phi_{R,vol}$ in (W $l^{-1}$).

The baseline signal ($\phi_B$) is composed of two terms: the first term is heat dissipation due to agitation ($\phi_A$) that could vary with time depending on the physical properties of the broth (viscosity change, foaming, etc.); the second term is due to external heat effects ($\phi_L$) that are maintained constant during an experiment (heat losses to the environment, thermostated cover, evaporation effects).

Torque was measured using a torquemeter (TG-02, Vibro-Meter SA, Fribourg, Switzerland) which had a nominal range of 0–1 N m. To enable correct torque measurements, the motor, the torquemeter, and the agitator had to be aligned very precisely. In order to avoid effects of minor misalignments, aluminium miniature couplings (BSD Thomas, Meier & Co., Niedergösgen, Switzerland) were used.

Torque measurements give heat-production rates due to agitation ($\phi_A$ in W) as

$$\phi_A = M_t \cdot 2 \cdot \pi \cdot n \tag{3}$$

where $M_t$ is the torque or moment of torsion in (N m), and $n$ is the stirring speed in $s^{-1}$.

With an agitation of 1000 rpm, the proportional factor ($2 \cdot \pi \cdot n$) is equal to 104.7 $s^{-1}$, which means that a 0.05 N m variation would induce a 5 W perturbation in the heat signal.

The relationship between torque measurement and heat production was verified in a series of experiments where the main parameters were varied as follows: working volume 1.1–1.7 l, stirring speed 100–1000 rpm, with or without aeration. This correlation is illustrated in Fig. 1.

Batch cultures of a filamentous bacterium, *Saccharopolyspora erythraea* P1060 (formerly *Streptomyces erythreus*) provided by Ciba-Geigy AG (Basel, Switzerland), were performed. This industrial mutant strain was selected for its hyperproductivity of a broad-spectrum antibiotic, erythromycin A [5]. The operating conditions were: temperature 32.0°C; agitation 1000 rpm; pH 7.0, automatically

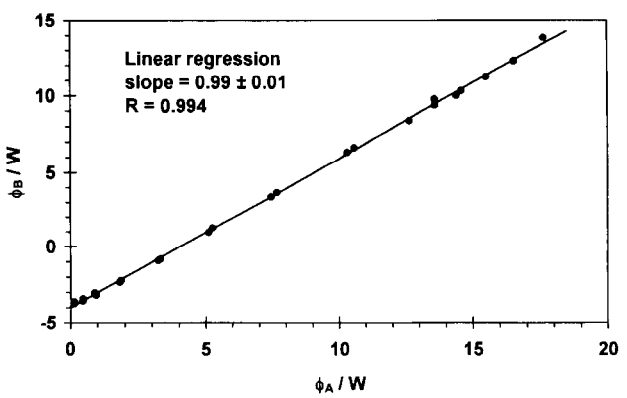


Fig. 1. Correlation between power due to agitation $\phi_A$ and baseline signal $\phi_B$ for a range of volumes, agitation speeds and aeration rates.

controlled by the addition of 2 M NaOH or 2 M $H_2SO_4$; aeration flow rate 1.0 vvm.

The growth medium [5] consisted of: glucose as main energy and carbon source (50–100 g $l^{-1}$); $NH_4NO_3$ 5.76 g $l^{-1}$; NaCl 5 g $l^{-1}$; $KH_2PO_4$ 1.6 g $l^{-1}$; Pharmamedia (Sigma, St-Louis, USA) supernatant 30 g $l^{-1}$; propanol as precursor 1% v/v; antifoam (SAG 471, Ciba-Geigy AG, Basel, Switzerland) 1.0–1.5 g $l^{-1}$; and trace mineral salt solution. The medium was sterilised for 20 min at 121°C. Glucose, propanol and erythromycin A concentrations were determined by HPLC; $NH_4^+$ was assayed enzymatically (Boehringer, Mannheim, Germany); and $NO_3^-$ was determined using a spectrophotometric method (Technicon autoanalyser, Tarrytown, USA).

## 3. Results and discussion

Batch cultures of *Saccharopolyspora erythraea* were performed in the RC1 calorimeter. A typical measured heat signal and torque measurement are shown in Fig. 2. Between inoculation and cell death, the baseline was not constant, with a large drift of 3.5 W, although the $U \cdot A$ coefficient was constant ($\Delta = \pm 1\%$). This baseline drift represented almost 20% of the maximum heat-production rate and could not be neglected. In order to evaluate correctly the heat signal, a realistic baseline correction must be applied [3,4]. Therefore, assumptions such as a constant agitation power [6] or a linear baseline variation are not valid.

During such experiments, three main perturbations were usually observed that could not be explained by additional nutrient analysis. Perturbation 1 always occurred 20–21 h after inoculation, during the exponential phase. Perturbation 2 occurred simultaneously with the beginning of foaming and correlated with a large decrease in the torque signal. Perturbation 3 corresponds to an antifoam pulse which induced a large torque increase and simultaneous heat-signal increase.

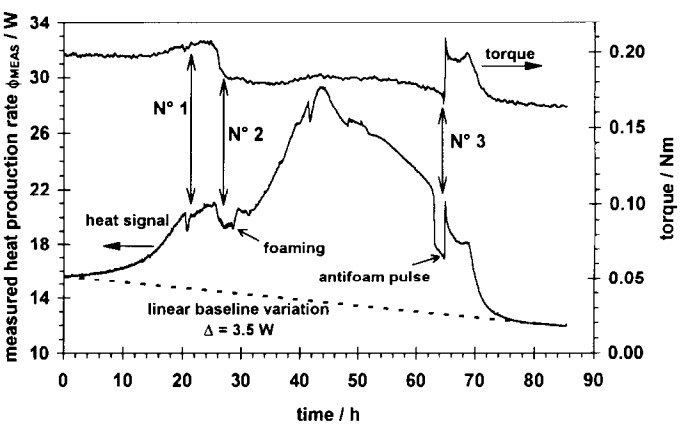


Fig. 2. Measured heat-production rate $\phi_{MEAS}$ and torque measurement during a culture of *Saccharopolyspora erythraea*. (1–3) The main perturbations in the heat signal (see text).

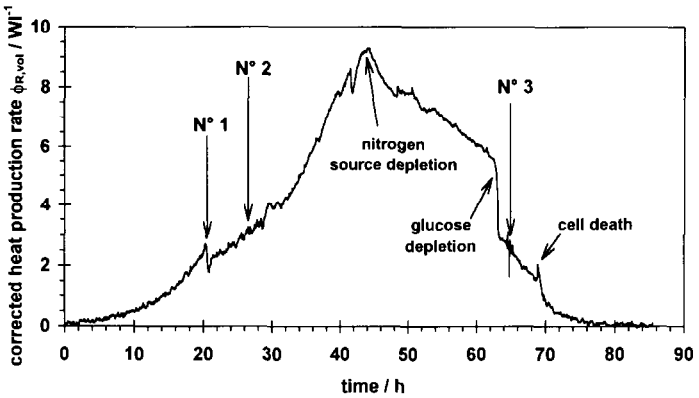


Fig. 3. Corrected volumetric heat-production rate $\phi_{R,vol}$ during a culture of *Saccharopolyspora erythraea*.

In order to correct the measured heat signal with the best baseline evaluation it is necessary to: (a) correlate the torque measurement with the power input due to agitation (Eq. (2)); (b) subtract $\phi_A$ from $\phi_{MEAS}$; (c) other external heat effects (thermostated cover, heat losses) are maintained constant during an experiment and can be easily subtracted; and (d) divide the obtained heat signal by the working volume to get $\phi_{R,vol}$ in (W l$^{-1}$).

The result of this procedure is given in Fig. 3. The efficiency of baseline estimation through torque measurement was excellent. The drift of the baseline between inoculation and cell death is effectively equal to zero. One can note the disappearance of perturbation 2, which was due to antifoam titration followed by foaming, and perturbation 3, which correlated with antifoam pulse and breaking of foam. Perturbation 1 remained and was related to an unidentified biological event. Moreover, other metabolic events, such as nitrogen source depletion and glucose depletion were detected by calorimetry.

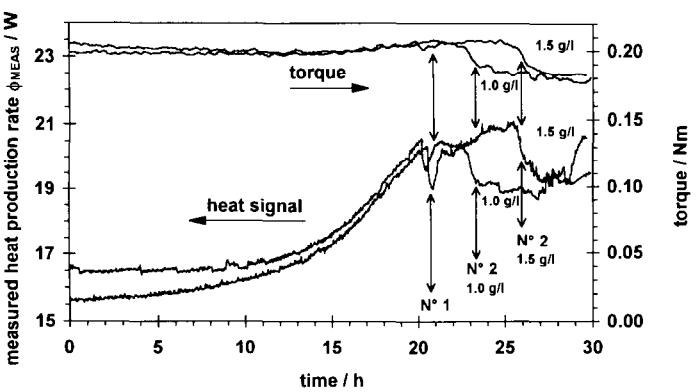


Fig. 4. Comparison of two different initial antifoam concentrations (1.0 and 1.5 g l$^{-1}$): effects on the torque and the measured heat signals $\phi_{MEAS}$.

Fig. 4 compares two measured heat signals for two different initial antifoam concentrations (1.0 and 1.5 g $l^{-1}$). Perturbation 1 occurred simultaneously in both experiments, but perturbation 2 was delayed in the case of high antifoam concentration, thus confirming the important influence of antifoam and foaming on torque and, therefore, on the heat-production rate.

## 4. Conclusions

Torque measurements are a powerful tool for detecting baseline shifts and eliminating external perturbations. It was shown that torque could be used to correct on-line the measured heat-production rate. The corrected signal could be used for on-line control of the process. In particular, torque changes due to antifoam titration will enable optimal control of antifoam addition. Antifoam concentration should be as low as possible, considering downstream processing problems due to antifoam residues.

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