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Calorimetric optimisation of growth and sporulation of *Bacillus thuringiensis* var *galleriae*[1]

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Abstract

Results from the investigation of the cultivation of *Bacillus thuringiensis* var *galleriae* in a bench-scale calorimeter (Bio-RC1) are presented in this work. On-line torque measurements were used to correct the baseline shift resulting from the variation of rheological properties of the medium. Heat measurements, combining with the balances of mass, degree of reduction and energy, are used to estimate the consumption of yeast extract, thus more accurate stoichiometry of the growth reaction in semi-defined medium was determined. The correlations between the rate of heat production and other relevant process variables, during the growth of *B.t.g.*, were explored, and such correlations and stoichiometry of growth form the basis for using on-line data of heat production, together with other continuously measured information, for the optimization of media and the monitoring and control of the growth and sporulation processes of *B.t.g.*, particularly in fed-batch and continuous cultures. © 1998 Elsevier Science B.V.

Keywords: *Bacillus thuringiensis* var *galleriae*; Calorimetry; Energy balance; Mass balance; Stoichiometry

1. Introduction

Continuous on-line measurements of heat evaluated during microbial growth enable the assessment of the main metabolic pathways and events in a cellular culture, therefore, calorimetry has been used as a valuable tool for the monitoring and control of biological processes [1]. For better understanding the metabolism processes and application of the heat signal as a modeling parameter for the control of bioprocesses, the heat generation is often correlated to other process variables which are determined by on/off line methods, such as the concentration of biomass formed, CO_2 production, O_2 consumption, etc.

Media used in industry are often complex, the components of which are difficult to quantify and, hence, cause difficulties in the interpretation of the dynamic processes of cultivation. Heat measurement, combining with mass and energy balances, could provide quantitative information about such microbial growth processes.

During microbial growth in a complex medium, using a biocalorimeter, considerable changes of rheological properties cause a variation of the agitation power which contributes to shifts in the baseline heat

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signal. In order to obtain precise and quantitative information about the metabolic process from heat measurements, this extra heat variation due to the changes of rheological properties in the complex medium has to be corrected for.

In this work, a batch culture of *Bacillus thuringiensis* var *galleriae* on a semi-defined medium was investigated using an isothermal reaction calorimeter (Bio-RC1). A method based on on-line torque measurement was used to correct the baseline shift due to the changes of rheological properties of the medium; the heat measurement, combined with balances of mass, degree of reduction and enthalpy during the batch culture was used to predict the consumption of the yeast extract contained in the medium and, thereby, the yields of biomass could be estimated for the cases with and without considering yeast extract as carbon/energy sources. Quantitative correlations of the heat generation with other relevant process variables were experimentally explored, which enable the heat measurement to be used as a valuable tool for further medium optimization of the growth and sporulation of *Bacillus thuringiensis* var *galleriae* and for bioreactor control.

2. Materials and methods

2.1. Culture conditions

The strain used in this work was *Bacillus thuringiensis* var *galleriae* (*B.t.g.*) which was re-isolated from the dead larvae of *Heliothes armegira*. A semi-defined medium was used, which contained (g/l): glucose, 17.0; yeast extract, 5.0; $(NH_4)_2SO_4$, 8.5; KH_2PO_4, 1.0; $MgCl_2 \cdot 6H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.0005; $ZnSO_4 \cdot 7H_2O$, 0.0005, NaCl, 0.1 and trace elements [2].

2.2. Analytical methods

B. thuringiensis var *galleriae* was grown aerobically in batch cultures in an isothermal reaction calorimeter (RC1, Mettler-Toledo AG, Switzerland), the measurement principle of which has been previously presented and discussed in detail [1,3]. The reactor temperature was set at 30°C, the stirring rate was 800 rpm, and the culture was aerated at a rate of 0.8 vvm. The pH of the culture was controlled by automatic addition of 2 N H_2SO_4 and 2 N NaOH for the growth and sporulation phases, respectively.

The heat signal from the calorimeter was measured continuously and on-line. A torquemeter (TG-02, Vibro-Meter SA, Switzerland) was employed to continuously measure the stirring power supplied to the calorimeter contents [4].

The concentrations of CO_2 and O_2 in the exit gas were determined by infra-red and paramagnetic analyzers, respectively, (Servomex, Crowborough, UK); concentrations of glucose and acetic acid were determined using standard enzyme assays (Boehringer Mannaheim, Germany); biomass concentration was determined by dry-weight measurement: 5 ml culture-samples were filtered on membranes (0.2 μm), dried over night at 105°C prior to weighing.

3. Results and discussion

3.1. Heat production of the growth and baseline correction through on-line torque measurements

Because the rheological properties of the medium change during the culturing process, the agitation power, which contributes to the baseline of the heat measurement, varies accordingly in order to keep a constant agitation speed in the reactor. This is particularly true when growth occurs on a complex medium, and/or the aeration rate is high and/or a high agitation speed is used. However, the agitation power is proportionally correlated to the torque which can be measured with a torque meter linked between the agitation motor and the rotor [4]. Therefore, torque measurements enable corrections to the baseline to be made, and thus a more correct and precise profile of heat generation can be obtained.

Heat-flux curves, with and without correction for torque measurements, are shown in Fig. 1. From Fig. 1, it can be seen that the cultivation process contained two distinct patterns: the first reflects the growth phase, the fall of the heat flux after ca. 5 h implied a limitation of some nutrient(s). However, this limitation was due to neither glucose nor ammonia since between 60–80% of the initial medium concentration remained. The second phase corresponds to the sporulation process, however, the reason for the peak

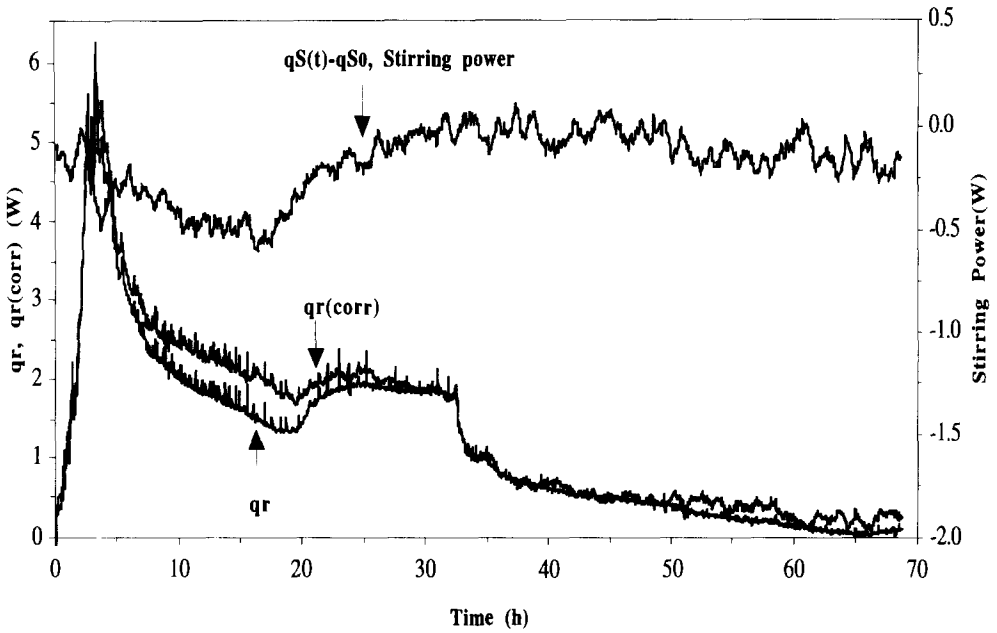

Fig. 1. Curves of heat production rates (with and without corrections) and stirring power during the batch culture of *B.t.g.* in a semi-defined medium. (q_r: rate of heat production of growth; q_r (corr): rate of heat production of growth, corrected for torque measurement; $q_S(t)$: stirring power at time t during growth; q_{S0}: constant stirring power before inoculation (baseline));

in the calorimetric signal during this phase is difficult to explain. The latter may be due to the endogenous oxidization of the storage substances accumulated in the cells or the breakdown of components of the yeast extract by enzymes formed during the sporulation process. Biomass concentration did not increase during this phase. The exact processes occurring in the latter phase remain to be resolved. Nevertheless, the heat measurement was shown as a valuable tool to assess the main pathways and events during the growth process.

3.2. Stoichiometry of the growth of Bacillus thuringiensis var galleriae

The macroscopic equation for the growth of *B.t.g.* can be described as following:

$$S + Y_{YE/S}YE + Y_{N/S}NH_3 + Y_{O/S}O_2 \rightarrow Y_{X/S}X + Y_{C/S}CO_2 + Y_{P/S}P + Y_{W/S}H_2O \quad (1)$$

where S, YE, X and P represent the substrate (glucose), yeast extract, biomass formed, and product (acetic acid), respectively. All components are expressed in C-mol, which means that each chemical formula of a component was reduced to the basis of one carbon atom. The number of carbon atoms, degree of reduction and enthalpy are conserved during the growth process in a batch culture [5,6]. In the case of dynamic experiments, if n_i represents the amount of the ith component that has been consumed or produced, then for any time t, the balance for carbon atom, degree of reduction and energy for the above growth process can be formulated as the following:

Carbon balance

$$n_S + n_{YE} = n_X + n_{CO_2} + n_P \quad (2)$$

Degree of reduction balance

$$n_S \cdot \gamma_S + n_{YE} \cdot \gamma_{YE} + n_{O_2} \cdot \gamma_{O_2} = n_X \cdot \gamma_X + n_P \cdot \gamma_P \quad (3)$$

where γ_i is the degree of reduction, defined for any substance i of the generalized chemical formula ($CH_{e_{i,H}}O_{e_{i,O}}N_{e_{i,N}}$) as

$$\gamma_i = 4 + e_{i,H} - 2e_{i,O} - 3e_{i,N} \quad (4)$$

Enthalpy balance

$$n_S \cdot \Delta_C H_S^* + n_{YE} \cdot \Delta_C H_{YE}^* = n_X \cdot \Delta_C H_X^* + n_P \cdot \Delta_C H_P^* + Q \quad (5)$$

where ΔH_i^* is the modified enthalpy of combustion, defined for any substance i of the generalized chemical formula ($CH_{e_{i,H}}O_{e_{i,O}}N_{e_{i,N}}$) as

$$\Delta_C H_i^* = \Delta_C H_i^0 - e_{i,N} \cdot \Delta_C H_{NH_3}^0 \quad (6)$$

The molecular weight, degrees of reduction and enthalpies of combustion of substrates and products involved in this growth reaction are readily available from tabulated data [7]; for biomass ($CH_{1.7}O_{0.57}N_{0.17}$) and yeast extract, the molecular weight, degrees of reduction and enthalpies of combustion are 28.00 (g C-mol^{-1}), 4.2 and 483.0 (kJ C-mol^{-1}); 28.93 (g C-mol^{-1}), 4.1 and 471.5 (kJ C-mol^{-1}), respectively [8]. Therefore, from the above balances, i.e. Eqs. (2),(3) and (5), the yeast extract taken up during the growth of *B.t.g.* was independently calculated to be 0.0565, 0.0772 and 0.0653 C-mol, from which an average value of 0.0663 C-mol was obtained. Consequently, the yield coefficient of the biomass formed ($Y_{X/S}$), with and without considering the consumption of yeast extract, can be estimated as 0.645 and 0.771 (C-mol C-mol^{-1}), respectively.

3.3. Correlations between the heat production rate and other process variables during the growth

The correlations between the rate of heat production and other relevant process variables during the growth of *B.t.g.* were explored. These are useful for further work on the optimization of the medium and the control of bioreactor by means of calorimetry. The profiles of the heat flux (q_r) and the percentages of carbon dioxide (%CO_2) and oxygen (%O_2) in the gas phase, measured continuously during the growth have been shown in Fig. 2, from which the rates of carbon dioxide production (CPU) and oxygen uptake (OUR) were calculated as functions of time, and compared with the rate of heat production (q_r), as shown in Fig. 3. It can be found that these three on-line measured parameters (q_r, CPR and OUR) correlate well with each other, with a respiratory quotient (RQ) of 0.989 (Fig. 3). The ratio between heat generation and oxygen consumption, $Y_{Q/O}$, during the growth was calculated to be 408.5 kJ/mol (Fig. 4), which is close to the values determined in the aerobic growth of other microorganisms [9].

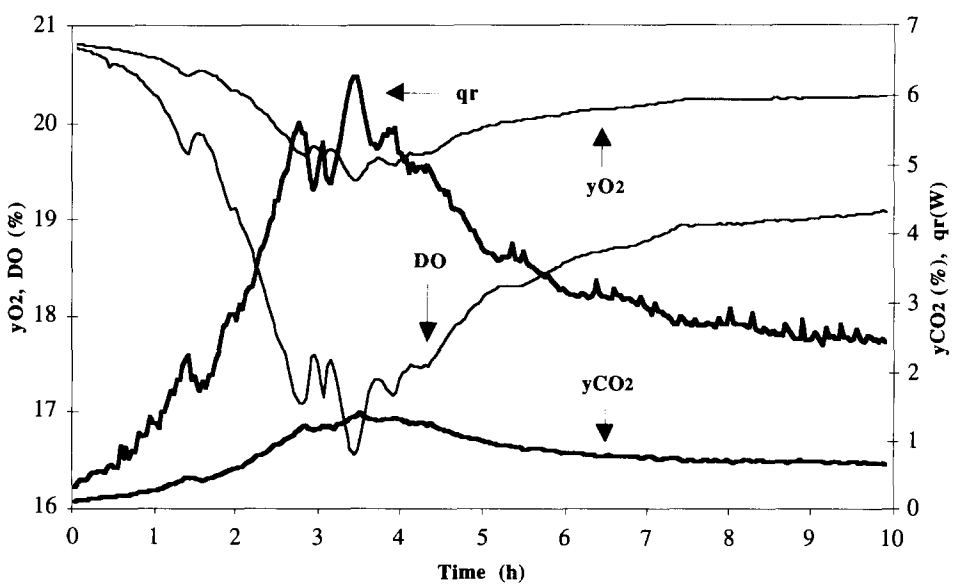

Fig. 2. Heat, DO, %O_2, and CO_2 profiles during the batch culture of *B.t.g.* in a semi-defined medium. (q_r: rate of heat production of growth; yCO_2, yO_2: percentages of CO_2, O_2 in the gas phase; DO: percent of oxygen dissolved in the medium)

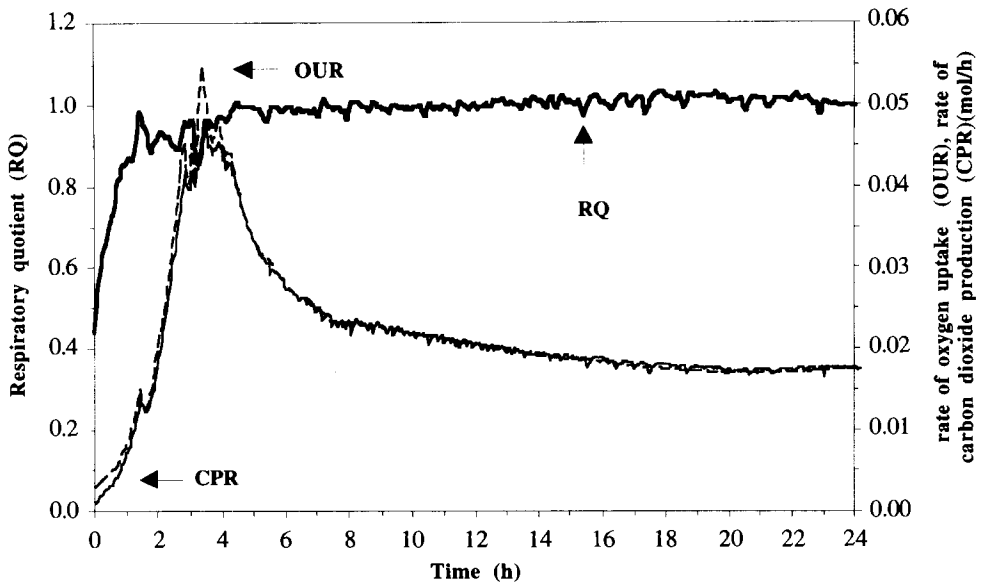

Fig. 3. Rates of oxygen uptake and carbon dioxide production and the respiratory quotient during the batch culture of *B.t.g.* in a semi-defined medium. (CPU: rate of carbon dioxide production; OUR: rate of oxygen uptake; RQ: respiratory quotient)

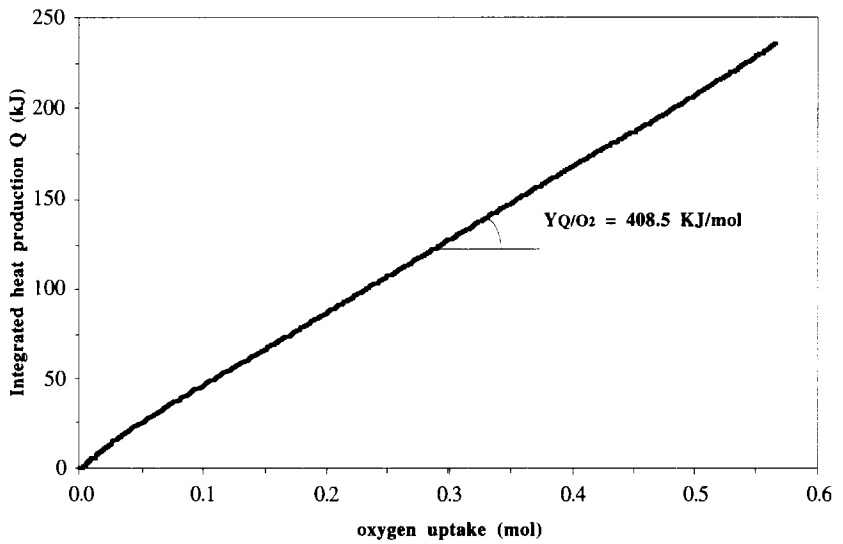

Fig. 4. Correlation of the integrated heat production to the oxygen consumption in a batch culture of *B.t.g.* in a semi-defined medium. (Y_{Q/O_2}: heat yield related to oxygen production.)

4. Conclusions

Results from the calorimetric investigations of the cultivation of *Bacillus thuringiensis* var *galleriae* presented in this work have shown that the bench-scale calorimetry is a powerful tool for the monitoring and control of fermentation processes even in a complex medium. The baseline shift resulting from the variation of rheological properties of the medium can be corrected through on-line torque measurements. Heat measurements, combining with balances for mass, degree of reduction and energy, are very useful

for the estimation of a more accurate stoichiometry of the growth reaction. The rate of heat production during growth is correlated with the other relevant process variables, thus, on-line data of heat released by microbial cultures, which is suggested as a prior parameter for its faster response, together with other continuously measured information can be used for the optimization of media and the monitoring and control of the growth and sporulation processes, such as *B.t.g.*, particularly in fed-batch and continuous cultures.

5. List of symbols

$Y_{i/j}$	yield of compound i related to compound j, C-mol C-mol^{-1}
n_i	number of moles of compound i expressed, C-mol formula in mol
γ_i	degree of reduction of compound i defined with respect to NH_3 (Eq. (4))
$\Delta_C H_i$	standard enthalpy of combustion of compound i, kJ C-mol^{-1}
$\Delta_C H_i^*$	enthalpy of combustion of compound i, defined with respect to NH_3 (Eq. (6)), kJ C-mol^{-1}
Q	integrated heat production of growth, kJ

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