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Bench-scale calorimetry in biotechnology

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Abstract

Two calorimetric techniques are reviewed which allow us to monitor and control microbial processes at relatively high cell densities: dynamic and continuous calorimetry. These techniques have been applied to study microbial systems, e.g. the dependence of thermodynamic efficiency and reaction heat on specific growth rate, the reaction heat of mesophilic and thermophilic microorganisms, the oxy-caloric coefficient during aerobic microbial growth, as well as the energetics in the yeast-cell cycle. Such studies are of importance for optimizing biotechnological processes.

INTRODUCTION

The literature contains several monographs and a number of review articles on the description and/or application of calorimeters in several research directions: biochemistry, biophysics and biotechnology. Only a few will be cited here [1–7].

Bench-scale calorimetry [7–13] is a suitable technique for monitoring and control of microbial processes at relatively high cell densities, e.g. production of cell mass, alcohols, organic acids and enzymes. It includes the so-called "dynamic" and "continuous" calorimetry working directly in a bioreactor of 1–10 litre volume.

CALORIMETRIC TECHNIQUES

Dynamic calorimetry

Cooney et al. [10], Heinritz [11] and Minkevich et al. [13] report on a simple technique for measuring the reaction heat of a microbial process by monitoring the culture broth temperature in a bioreactor when the temperature controller is turned off. The total heat production rate in the

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bioreactor measured in this manner can be calculated according to eqns. (1) and (2)

$$\phi_{tot} = \sum_i m^i C_p^i \frac{\Delta\theta}{\Delta t} \tag{1}$$

$$\sum_i m^i C_p^i = (mC_p)_{\text{culture medium}} + (mC_p)_{\text{reactor jar}} + (mC_p)_{\text{stainless steel}} \tag{2}$$

where ϕ_{tot} is total heat production rate in the bioreactor without temperature control, m^i is mass, C_p^i is specific heat capacity, $\Delta\theta$ is temperature difference and Δt is time interval. The value is then corrected for heat loss and gain in the reactor

$$\phi_{tot} = \phi_{mp} + \phi_{agi} - \phi_{surr} - \phi_{sens} - \phi_{evp} \tag{3}$$

where ϕ_{tot} is total heat production rate as before, ϕ_{mp} is heat production rate due to the microbial process, ϕ_{agi} is heat production rate due to agitation, ϕ_{surr} is heat loss rate to the surroundings, ϕ_{sens} is heat loss rate due to the gas stream leaving the reactor with respect to the heat content of the gas stream entering, and ϕ_{evp} is heat loss rate due to the evaporation of water from the culture broth.

If the incoming stream of gas is saturated with water at the temperature of the culture medium, ϕ_{sens} and ϕ_{evp} are negligible. Equation (3) therefore becomes

$$\phi_{tot} = \phi_{mp} + \phi_{agi} - \phi_{surr} \tag{4}$$

The calibration of the calorimeter is carried out on the nutrient medium without cells at specified agitation rates as well as specified rates of the gas stream entering the bioreactor. Thus the heat production rate due to the microbial process can be set equal to zero

$$\phi_{mp} = \phi_{tot} - \phi_{agi} + \phi_{surr} = 0 \tag{5}$$

Thus the heat production rate due to the microbial process at time t is determined by eqns. (6) and (7)

$$(\phi_{mp})_t = (\phi_{tot})_t - (\phi_{tot})_{t<0} \tag{6}$$

with

$$(\phi_{tot})_{t<0} = \phi_{agi} - \phi_{surr} \tag{7}$$

where $t < 0$ is the time at which calibration of the calorimeter is performed. At the time $t = 0$ the measurement of microbial heat production rate is started.

The uncertainty of the dynamic calorimetric technique is 6.6% at a heat production rate of 20 MJ m^{-3} h^{-1} (5.6 kW m^{-3}) [10]

An advantage of dynamic calorimetry is its relative simplicity. However, the technique requires continuous attention, and automatic control of

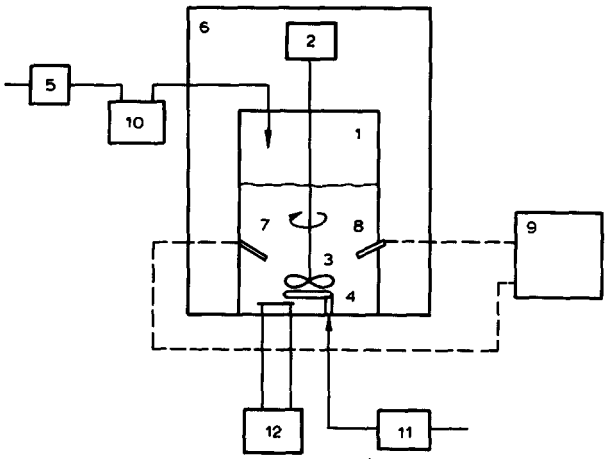

Fig. 1. Reaction calorimeter. 1, Bioreactor with pH control; 2, motor for stirring; 3, stirrer; 4, aeration device; 5, pumps for liquid nutrient medium and lye; 6, thermostatted chamber; 7, temperature sensor; 8, heater; 9, controller; 10, thermostat for adjusting the temperature of liquid nutrient medium; 11, thermostat for adjusting temperature and humidity of air; 12, thermostatted water reservoir in contact with the culture medium.

microbial processes may be not applicable. Additionally, the temperature controller may disturb the microbial process.

Continuous calorimetry

Luong [8] and Heinritz et al. [12] describe a continuously working calorimetric technique. The heat production rate due to the microbial process is determined using a reaction calorimeter which consists of a bioreactor placed in a thermostatted chamber (Fig. 1). The temperature of the chamber is controlled and kept equal to the reaction temperature. The culture medium in the bioreactor is in contact with a water reservoir having a constant temperature below the reaction temperature. The temperature of the culture medium is adjusted to the reaction temperature using an immersion heater. For calculating the heat production due to the microbial process, the voltage and the current of the electric circuit, including the immersion heater, as well as the temperature of the culture medium are measured and controlled. The culture medium is stirred. Additionally, the gas stream entering the bioreactor is thermostatted and saturated with water at the reaction temperature. The heat balance can be written as in eqn. (4).

The reaction calorimeter is calibrated by measuring the above mentioned data in a stirred bioreactor containing nutrient medium without cells. Additionally, a thermostatted and water-saturated gas stream passes through the nutrient medium during calibration. Therefore the total heat production rate at $t < 0$ can be calculated according to eqn. (7).

The heat production rate due to the microbial process follows from eqn. (6).

The overall uncertainty of the continuous calorimetric technique is reported to be $2 \pm 1.3\%$ when the heat production rate varies from 12.2–50 MJ m^{-3} h^{-1} (3.4–13.9 kW m^{-3}) [8]. An advantage of continuous calorimetry compared with dynamic calorimetry is its automatic operation as well as the possibility of automatic control of microbial processes based on heat production rate data.

APPLICATION TO MICROBIAL SYSTEMS

Thermodynamic efficiency and heat yield in dependence on specific growth rate

Thermodynamic considerations assuming the microbial system as a converter of Gibbs energy show a maximum value of thermodynamic efficiency at a specific rate of anabolism of $0.5 \leq J_a/J_{a(max)} \leq 0.88$, where J_a is the rate of anabolism and $J_{a(max)}$ is the maximum rate of anabolism [14]. The thermodynamic efficiency η of the microbial process can be calculated according to ref. 21

$$\eta = -\frac{J_a}{J_c}\frac{\Delta G_a}{\Delta G_c} \tag{8}$$

where J_a is the rate of anabolism, J_c is the rate of catabolism, ΔG_a is the Gibbs energy change in anabolism, and ΔG_c the Gibbs energy change of catabolism. Experimental results of the chemostat culture of *Bacillus stearothermophilus* TP 26 growing on glucose are summarized in Table 1. The thermodynamic efficiency and with it the biomass yield $Y_{X/S}$ and the heat yield $Y_{Q/X}$ possess their extrema at 60% of the maximum specific growth rate under the described conditions.

TABLE 1

Studies of chemostat culture of *Bacillus stearothermophilus* TP 26 on glucose by means of continuous calorimetry. Temperature 68 °C, pH value 6.8, maximum specific growth rate 0.5 h^{-1}

D (h^{-1})	η (%)	$Y_{X/S}$ (g g^{-1})	$-Y_{Q/X}$ (kJ g^{-1})	μ/μ_{max}
0.4	2.3	0.14	34.6	0.8
0.3	3.7	0.32	22.6	0.6
0.14	1.7	0.22	47.6	0.28

D dilution rate; η, thermodynamic efficiency; $Y_{X/S}$, biomass yield; $Y_{Q/X}$, heat yield; μ, specific growth rate; μ_{max} maximum specific growth rate.

TABLE 2

Comparison of the heat yield $Y_{Q/X}$ of aerobic microbial growth in a chemostat at two different dilution rates

Microorganism	Carbon substrate	θ (°C)	pH value	D (h^{-1})	$Y_{X/S}$ ($g\,g^{-1}$)	$-Y_{Q/X}$ ($kJ\,g^{-1}$)
Candida maltosa EH15	Glucose	32	4.2	0.14	0.43	17.8
Candida maltosa EH15	Glucose	32	4.2	0.25	0.45	15.2
Bacillus stearothermophilus TP26	Glucose	68	6.8	0.14	0.22	47.6
Bacillus stearothermophilus TP26	Glucose	68	6.8	0.22	0.32	22.6

Abbreviations as in Table 1.

Heat yield of the growth of mesophilic and thermophilic microorganisms

The strains *Candida maltosa* EH15 (mesophile) and *Bacillus stearothermophilus* TP26 (thermophile) were cultivated on glucose according to the chemostat principle. Heat measurements were carried out using dynamic and continuous calorimetry. Table 2 shows the experimental results. As a rule, thermophilic microorganisms show a higher heat yield and a lower biomass yield during aerobic growth than mesophiles.

Oxy-caloric coefficient of aerobic microbial growth

The oxy-caloric coefficient (ratio of heat production rate to oxygen consumption rate) is closely connected with the biomass yield and energetic efficiencies of aerobic microbial growth. Its theoretical value of -14.38 kJ g^{-1} O_2 (-3.44 kcal g^{-1} O_2) has been deduced from the theory of mass energy balances [13,15,16]. If carbon dioxide, water and cell mass are the only reaction products of aerobic microbial growth, and energy is produced exclusively in the respiration chain of microorganisms, the experimental value of the oxy-caloric coefficient approximate the theoretical value. This fact was shown by Minkevich et al. [13] using dynamic calorimetry during semicontinuous culture of *Candida maltosa* EH15 on ethanol (Fig. 2). The oxy-caloric coefficient was near its theoretical value during three yeast cell cycles.

Cooney et al. [10] determined an oxy-caloric coefficient of -16.2 ± 0.39 kJ g^{-1} O_2 in experiments with bacteria and yeasts. They treated the experimental data from dynamic calorimetry by least-squares linear regression of experimental data. Heinritz et al. [12] measured an oxy-caloric coefficient of -12.7 ± 1.4 kJ g^{-1} O_2 in a chemostat culture of thermophilic bacteria on glucose at 68 °C and pH value of 6.8 by means of continuous calorimetry.

In other experiments, the dependence of the oxy-caloric coefficient on environmental conditions and the state of the cell (population) state has been studied [17,18]. Experimental results of the investigations using dy-

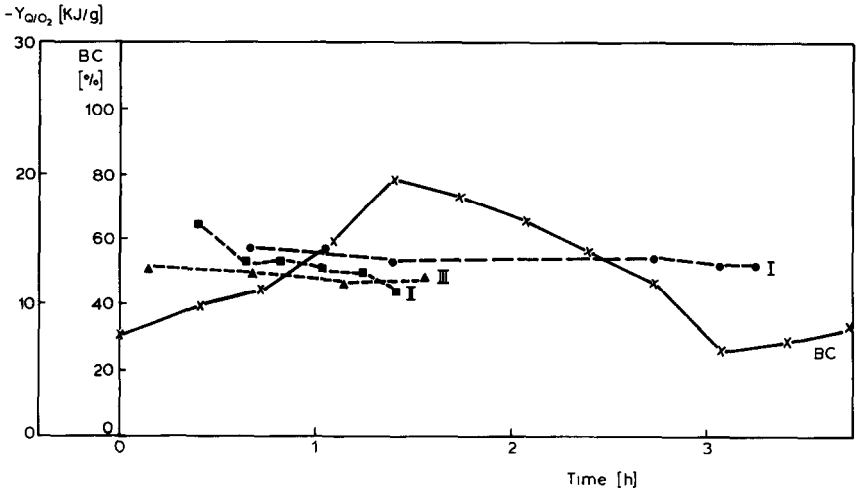

Fig. 2. Semicontinuous culture of a synchronized yeast population on ethanol (temperature, 32 ° C; pH value, 4.2; dynamic calorimetry). Y_{Q/O_2}, oxy-caloric coefficient; BC, percentage of budding cells. (I, II and III are values of three different yeast cell cycles.)

namic calorimetry are shown in Fig. 3. The detected periodic changes of the oxy-caloric coefficient can be explained by its dependence on the cell state in the yeast cell cycle [17]. The increase of the oxy-caloric coefficient in the single cell state of the yeast cell cycle is caused by ethanol production and a second energy-delivering mechanism. The described experimental results are in agreement with the microcalorimetric studies of Brettel et al. [19,20].

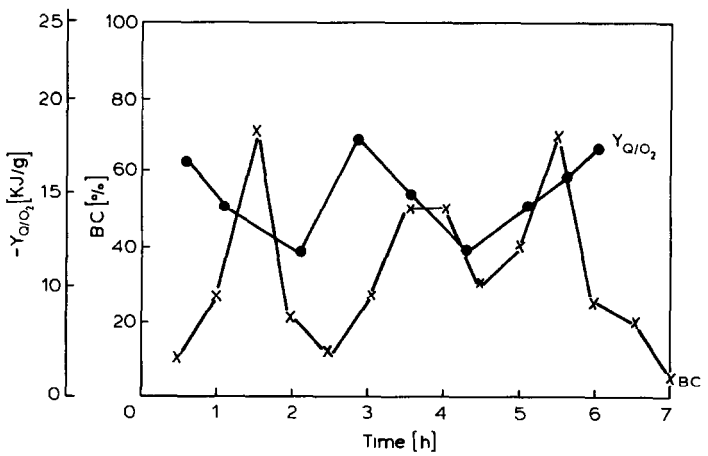

Fig. 3. Batch culture of a synchronized yeast population on glucose (temperature, 32 ° C; pH value, 4.2; dynamic calorimetry). Y_{Q/O_2}, oxy-caloric coefficient; BC, percentage of budding cells.

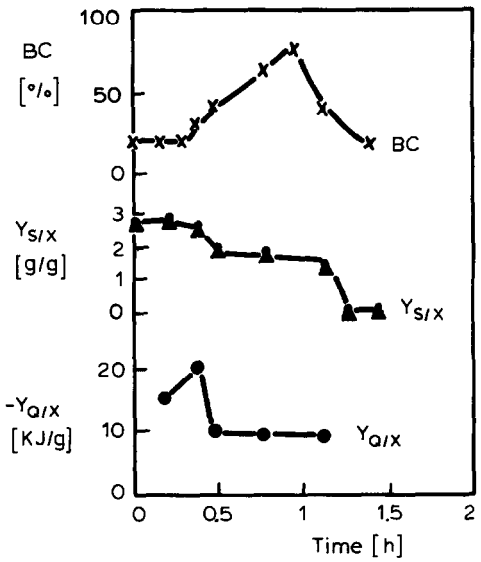

Fig. 4. Semicontinuous culture of a synchronized yeast population on sucrose limited by the carbon substrate concentration (temperature 32 ° C, pH value 4.2, dynamic calorimetry). BC, percentage of budding cells; $Y_{S/X}$, carbon substrate consumption; $Y_{Q/X}$, heat yield.

Mass–energy balances in the yeast cell cycle

The cell states of the yeast cell cycle have been studied using a synchronized yeast cell population. After one doubling of cells, one half of the culture medium was replaced by fresh nutrient medium sufficient for the next cell doubling. The heat production rate during the repetitive cell cycles was measured by means of dynamic calorimetry.

The experimental results of synchronized growth of *Lodderomyces elongisporus* DEH15 on sucrose are shown in Figs. 4 and 5 and also in Table 3.

Two different cell states could be observed during the cell cycle: the single cell state and the budding cell state. The single cell state is characterized by a high heat yield and also a high carbon substrate consumption. These values pass maxima at the transition from the single cell state to the budding cell state and reach minimum values during budding (see Fig. 4).

The described dependences were also detected in the case of synchronized growth of *Lodderomyces elongisporus* DEH15 limited by the phosphate ion concentration (Fig. 5). In the phosphate ion limited experiment with carbon substrate in excess the heat yield and the carbon substrate consumption were higher in the single cell state when compared with the values of the carbon substrate limited experiment (see Table 3).

The carbon substrate consumption and heat yield in budding cell state are minimal and are similar in both experiments (see Figs. 4 and 5 and

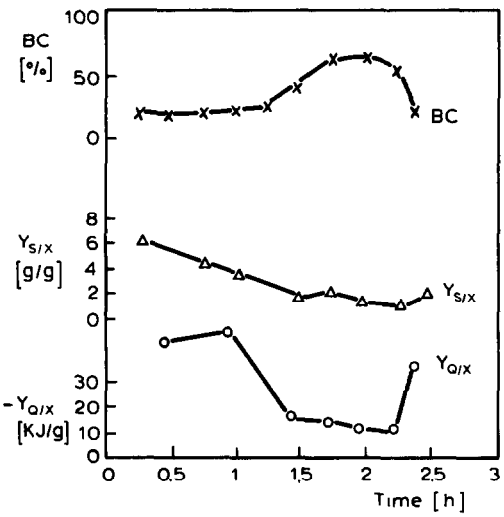

Fig. 5. Semicontinuous culture of a synchronized yeast population on sucrose limited by the phosphate ion concentration (temperature 32 °C, pH value 4.2, dynamic calorimetry). BC, percentage of budding cells; $Y_{S/X}$, carbon substrate consumption; $Y_{Q/X}$, heat yield.

TABLE 3

Characterization of cell states of the yeast cell cycle (see also Figs. 4 and 5)

	Carbon substrate limitation		Phosphate ion limitation	
	SCS	BCS	SCS	BCS
$\bar{Y}_{S/X}$ (g g^{-1})	2.8	1.9	3.8	1.9
$-\bar{Y}_{Q/X}$ (kJ g^{-1})	20.3	8.3	50	8.4

$\bar{Y}_{S/X}$, average carbon substrate consumption; $\bar{Y}_{Q/X}$, average heat yield; SCS, single cell state; BCS, budding cell state.

Table 3). Thus, the quantities mentioned above can be influenced in the single cell state of the yeast cell cycle by changing the rate of the carbon substrate feed. From these results, possibilities for optimization of biotechnological processes aimed at the production of biomass, organic acids and alcohols have been deduced [17,18].

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