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BIOTHERMODYNAMIC STUDIES FOR OPTIMIZATION OF CELL-MASS PRODUCTION*

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SUMMARY

Results of thermodynamic studies have been used for optimization of cell-mass production. The yield and the enthalpic efficiency of the cell-mass production are increased due to periodic carbon-substrate supply adapted to the repetitive stages of the synchronized cell population. This effect can be explained by the reduction of the external energy dissipation which is connected to a decreased specific heat production.

INTRODUCTION

Since 1970 many researchers work on instationary cell-mass production (refs. 1–3). They observed a decreased yield except of experiments with a certain periodic carbon-substrate supply adapted to the repetitive states of synchronized cell population (dynamic process control) (refs. 4–6). The latter is connected to an increase of the yield compared to steady-state process. To interprete this effect calorimetric studies of cell growth have been performed.

METHODS

Calculations

The base of the determination of material and energetic quantities is the mass-balance eqn. (1):

$$\left\{ \nu_0\, C_1 H_{1.8} O_{0.5} + \nu_1\, C_k H_l O_m + \nu_2\, O_2 \right\} \longrightarrow \left\{ (\nu_3 - \nu_0)\, C_1 H_{1.8} O_{0.5} + \nu_4\, CO_2 + \nu_5\, H_2O \right\} \quad (1)$$

with $\nu_1 = 1$

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The carbon substrate $C_k H_l O_m$ is converted aerobically into cell mass (average elemental composition $C_1 H_{1.8} O_{0.5}$), carbon dioxide and water. The reaction is irreversible and exothermal. The stoichiometric coefficients ν_i are calculated from the concentrations of the reactands and reaction products.

The yield has been defined as the quotient of experimental and theoretic yield coefficient (refs. 7 and 8) according to eqn. (2):

$$\rho = \frac{Y_{X/S}}{Y_{X/S\ theor}} \times 100 \qquad (2)$$

where ρ yield, $Y_{X/S}$ experimental yield coefficient ($Y_{X/S} = (\nu_3 - \nu_0) / \nu_1$) and $Y_{X/S\ theor}$ theoretic yield coefficient. The theoretic yield coefficient is estimated on the base of the ratio of combustion enthalpies of the carbon substrate and the cell mass, respectively (refs. 9 and 10). The aerobic cell-mass production from sucrose is characterized by a theoretic yield coefficient of 0.78 gram of cell mass produced per gram of sucrose consumed (ratio of combustion enthalpies of sucrose and cell mass 0.89, carbon content of cell mass 0.48, combustion enthalpy of cell mass 21.4×10^3 joule per gram of cell mass).

The enthalpic efficiency is determined according to eqn. (3) described in refs. 11 and 12:

$$\eta_{enth} = \frac{\Delta^{298}_{C}H_X \, Y_{X/S}}{\Delta^{298}_{C}H_S} \times 100 \qquad (3)$$

where η^{enth} enthalpic efficiency, $\Delta_C H_X^{298}$ combustion enthalpy of cell mass, $\Delta_C H_S^{298}$ combustion enthalpy of carbon substrate, and $Y_{X/S}$ yield coefficient.

Furthermore, the reaction affinity and the Gibbs energy change of reaction have to be calculated for the determination of the dissipation function (ref. 13) according to eqns. (4), (4a) and (4b):

$$\Delta_R^T G = -A_R^T = \Delta_R^{298} G - \Delta_R^{298} C_p \int_{298}^{T} \int d \ln T \, d T \qquad (4)$$

with $$\Delta_R^{298} G = \sum_i \nu_i \left(\Delta_f^{298} G^o + R T \ln a_i \right) \qquad (4a)$$

$\nu_i < 0$ for reactands

$\nu_i > 0$ for reaction products

$a_i \simeq c_i$ at $c_i \longrightarrow 0$

and $$\Delta_R^{298} C_p = \sum_i \nu_i C_{pi} \qquad (4b)$$

where A_R^T affinity at temperature T, $\Delta_R^T G$ Gibbs energy change at temperature T, $\Delta_R^{298} G$ Gibbs energy change at 298 K, $\Delta_p^{298} C$ change of specific heat capacity at 298 K, ν_i stoichiometric coefficient, $\Delta_f^{298} G^o$ Gibbs standard formation energy, a activity, c concentration, C_p specific heat capacity, R gas constant and T temperature.

The Gibbs standard formation energy and the specific heat capacity are given in refs. 11, 14 and 15.

The absolute value of Gibbs energy change is equal to the reaction affinity which is in close connection with the dissipation function, equation (5):

$$\Psi = \frac{r}{X} A_R^T \qquad (5)$$

where Ψ dissipation function, A_R^T reaction affinity at temperature T, X cell-mass concentration and r reaction rate.

The dissipation function can be devided into two parts if the system state is far from thermodynamic equilibrium, eqn. (6):

$$\Psi = \Psi_u + \Psi_d \qquad (6)$$

where Ψ dissipation function, Ψ_d external energy dissipation determined calorimetrically and Ψ_u a function which is in close connection to the dissipation function (ref. 16).

EXPERIMENTAL

Conditions of cell growth

Aerobic growth of Candida maltosa on a sucrose containing nutrient medium in a chemostat (continuous process with external control) was chosen as a model. For the formation of 1 gram of cell mass 370 mg NH_4Cl, 36 mg KOH, 57 mg H_3PO_4, 18.2 mg $MgSO_4 \times 7\ H_2O$, 0.8 mg $MnSO_4 \times 7\ H_2O$, 0.95 mg $ZnCl_2$ and 0.63 mg $CuSO_4 \times 5\ H_2O$ were added. The nutrient medium contained also 0.1 mass percent of yeast extract and 1 to 2 mass percent of sucrose. The pH value of 4.2, the temperature of 32 °C, the aeration rate

of 50 l l^{-1} h^{-1}, the stirring velocity of 1800 rpm and the dilution rate of 0.25 h^{-1} were kept constant. The cell growth was limited by the sucrose concentration. Whereas the sucrose concentration of 2 mass percent was kept constant during steady-state cell growth it was changed periodically between 1 and 2 mass percent during dynamic process control. The period of sucrose supply was equal to the cell-doubling time during dynamic process control. For optimization of dynamic process control the time ratio of increased to reduced sucrose supply has been varied (increased value = sucrose supply of steady state; reduced value = one half of sucrose supply of steady state).

Dynamic calorimetry

The heat due to cell growth was determined from the temperature increase of culture medium during repetitive interruptions of cooling with no temperature control according to the principle of dynamic calorimetry (refs.17 and 18). Temperature measurements were carried out using a Beckmann thermometer (10^{-2} degrees) from Glaswerke Ilmenau in an isolated reactor system. For calibration, the temperature increase of the aerated nutrient medium due to stirring in the reactor was determined at 32 °C and a pH value of 4.2. The humidity and the temperature of the air was adjusted in all experiments. Eqns. (7) to (10) show us the way to calculate the heat of cell growth (ref. 17):

$$Q_{prod} = \sum_i m_i C_{pi} \frac{\Delta T}{\Delta t} \qquad (7)$$

$$\sum_i m_i C_{pi} = (m C_p)_{\text{culture medium}} + (m C_p)_{\text{reactor jar}} + (mC_p)_{\text{stainless steel}} \quad (8)$$

where Q_{prod} heat production in the reactor with no temperature control, m_i mass, C_{pi} specific heat capacity, ΔT temperature difference and Δt time interval.

The heat production in the reactor measured in this manner was then corrected for heat loss and gains on the reactor:

$$Q_{\text{cell growth}} = Q_{prod} - Q_{agi} + Q_{surr} + Q_{sens} + Q_{evp} \quad (9)$$

where $Q_{\text{cell growth}}$ heat of cell growth, Q_{prod} heat production in the reactor with no temperature control, Q_{agi} heat of agitation, Q_{surr} heat loss to the surroundings, Q_{sens} heat gained by the gas stream leaving the reactor with respect to the heat content of the gas stream entering and Q_{evp} heat loss due to evaporation of water from the culture medium.

If the incoming stream of air is saturated with water at the temperature of the culture broth, Q_{sens} and Q_{evp} are negligible. Eqn. (9), therefore, becomes:

$$Q_{\text{cell growth}} = Q_{prod} - Q_{agi} + Q_{surr} \quad (10)$$

RESULTS

Studies of yeast-cell cycle

Calorimetric studies of yeast-cell growth show two stages of yeast-cell cycle in first approximation: The single-cell stage and the budding-cell stage (Table I).

TABLE I

Characterization of cell stages in the yeast-cell cycle (Candida maltosa grown on sucrose)

	sucrose limitation		sucrose excess	
	SCS	BCS	SCS	BCS
μ / h^{-1} /	0.4	0.6	0.2	0.6
$Y_{X/S}$ / g g^{-1} /	0.36	0.53	0.26	0.53
ϱ / % /	45.8	67.5	32.9	67.5
$Y_{Q/X}$ / kJ g^{-1} /	- 20.3	- 8.3	- 45.0	- 8.5
ψ_d / kJ g^{-1} /	8.1	5.0	9.2	5.1
η_{enth} / % /	45.9	67.6	32.9	67.6

μ specific growth rate, $Y_{X/S}$ Yield coefficient, ϱ yield, $Y_{Q/X}$ specific heat production, ψ_d external energy dissipation, η_{enth} enthalpic efficiency, SCS single-cell stage, BCS budding-cell stage

The single-cell stage is characterized by decreased yield and enthalpic efficiency as well as increased specific heat production and external energy dissipation compared to corresponding values in budding-cell state. The quantities mentioned above are

dependent on the carbon-substrate supply.

These results are in agreement with experiments of v. Meyenburg (ref. 19) and Bley et al. (ref. 20). They indicate that cell-mass production should be optimized by adapting the carbon-substrate supply to the repetitive cell stages of yeast-cell cycle. This could be shown by periodic adaptive carbon-substrate supply to synchronized growth of Candida maltosa in a continuous process.

Dynamic process control

Periodic adaptive carbon-substrate supply to a synchronized cell population (dynamic process control) causes increased yield at constant average specific growth rate compared to the corresponding steady-state process (Table II). The optimum period of adaptive carbon-substrate supply is in the range of the cell-doubling time (about 3 h at a dilution rate of 0.25 h^{-1}). The optimum time ratio of increased to reduced carbon-substrate supply was detected as 1:2. The increase of the yield due to dynamic process control is coupled to increased enthalpic efficiency. This result can be explained by the reduction of the external energy dissipation. It is in agreement with the decrease of specific heat production.

TABLE II

Dynamic process control compared to the corresponding steady-state process

I Dynamic process control (period 3 h , 2 h 100 %, 1 h 50 %)

IIDynamic process control (period 3 h , 1 h 100 %, 2 h 50 %)

	Process control concept		
	Dynamic process		Steady-state process
	I	II	
D / h^{-1} /	0.26	0.26	0.25
t_d / h /	2.7	2.7	2.8
$Y_{X/S}$ / g g^{-1} /	0.53	0.48	0.45
ς / % /	70.1	65.2	62.3
$Y_{Q/X}$ / kJ g^{-1} /	- 7.6	- 9.8	- 11.8
ψ_d / kJ g^{-1} /	2.6	3.5	3.8
η_{enth} / % /	72.0	65.1	62.2

D dilution rate, t_d cell-doubling time, $Y_{X/S}$ yield coefficient, ς yield, $Y_{Q/X}$ specific heat production, ψ_d external energy dissipation, η_{enth} enthalpic efficiency

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