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MICROCALORIMETRIC INVESTIGATIONS OF THE GROWTH OF YEAST. ENERGY BALANCE OF BATCH AND CONTINUOUS CULTURES.

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Abstract

Growth of yeast cells (Saccharomyces cerevisiae) at the expense of glucose or ethanol in batch and chemostat cultures was studied by flow-microcalorimetry. With glucose media and with batch cultures on ethanol the energy balances were completely established, whereas as with chemostat cultures on ethanol a lack of about 25% of the energy-input occured which was not converted into biomass or dissipated as heat.

Introduction

In two previous papers (Brettel et al. 1980, 1981a) we described an experimental device for measuring the heat evolution of aerobic batch and chemostat cultures of yeast cells. The system consisted of a fermentor vessel linked to a flow-calorimeter. That way the measured rates of heat production could directly be correlated to the metabolic events within the culture.

In this paper we present results of the overall energy balances of batch and chemostat cultures growing on glucose or ethanol media.

Methods

The apparatus and the organism used were described in details in Brettel et al. 1980 and 1981a. In all experiments a diploid strain of Saccharomyces cerevisiae was used which grew in a synthetic medium with either 1% glucose or with 1% ethanol. Portions of the culture volume were continuously pumped through a tube system into the calorimeter and back to the fermentor vessel (Fig.1). With the chemostat cultures the fermentor was continuously fed with fresh medium at a constant rate F, whereas the same amount of suspension flew out through an overflow tube (left part of Fig.1). Steady state growth with a constant specific growth rate $\mu = D = F/V$ (D = dilution rate, V = volume of the culture) were obtained in glucose media up to rates of 0.4 h^{-1} and in ethanol media up to D = 0.2 h^{-1}. The calorimeter was a LKB-instrument (type 10700-1) with a sensitivity of 56.6 µV/mW. Samples of the

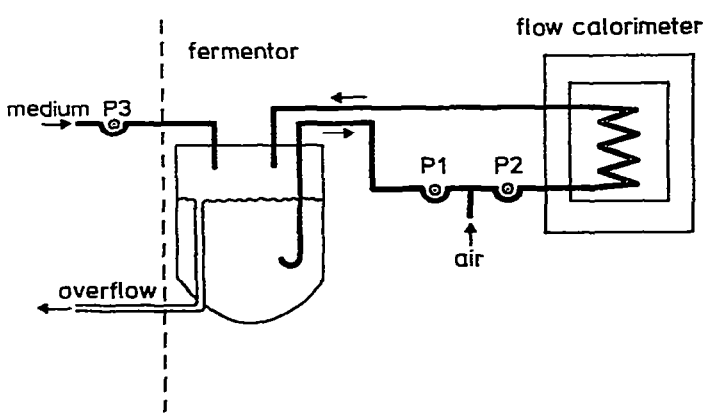

Fig.1: Diagram of the experimental device. P1, P2 and P3 are peristaltic pumps. The left part of the arrangement separated by the dotted line, represents the inflow and the outflow of the culture vessel, when it is run as a chemostat.

suspension were taken at a branch in the tubing for microscopic counting, determination of cell dry weight, glucose and ethanol concentrations and oxygen consumption rates. Samples were drawn from the grown biomass too and burnt in a bomb calorimeter (Philipson type) to obtain the energy content of the crop (enthalpy of formation ΔH_c).

The rate of heat production $\dot{Q} = dQ/dt$ were corrected due to their time lag between fermentor and calorimeter detector, the values of ethanol concentration due to its evaporation during culturing (Brettel et al. 1981a, 1981b).

Results

Batch cultures

Fig.2 shows the calorimetric curves (power-time-curve $\dot{Q}(t)$) and the correspondent courses of the dry mass for both media. With glucose as sole energy and carbon source an at least triphasic growth occurs. After an initial exponential growth at the expense of aerobic fermentation of glucose (first peak in the thermogram) a second exponential phase follows during which the accumulated ethanol is respired (second large peak

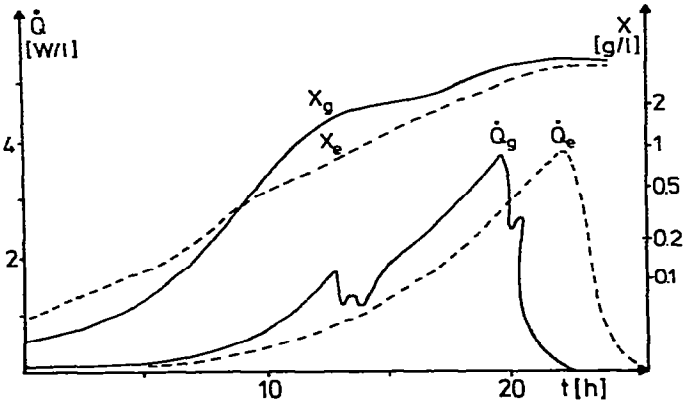

Fig.2:
Course of heat production rate $\dot{Q}$ and dry biomass X during batch experiments. The indices e and g stand for glucose and ethanol media.

in the thermogram). During the first growth phase a further intermediate metabolite, acetate, is built up which is degraded after the consumption of ethanol giving rise to a third sharp peak at the very end of the heat production curve.

With ethanol as sole energy and carbon source only one growth phase occurs in spite of the presence of intermediate acetate. The course of the energy balance throughout the growth of the cultures was set up by using the measured parameter $\dot{Q}(t)$ for the heat production rate, $X(t)$ for cell production, $c_g(t)$ for glucose concentration, $c_e(t)$ for ethanol concentration, and the separately determined values ΔH_c for the energy content of cells, glucose, ethanol and acetate. For a 1% glucose medium the relations for the energy input and output

$$E_{in} = c_g(0) \cdot \Delta H_c(\text{glucose}) = 140.8 \text{ kJ/l}$$

$$E_{out} = X(t) \cdot \Delta H_c(\text{cells}) + \int \dot{Q}(t)dt + c_e(t) \cdot \Delta H_c(\text{ethanol}) + c_g(t) \cdot \Delta H_c(\text{glucose})$$

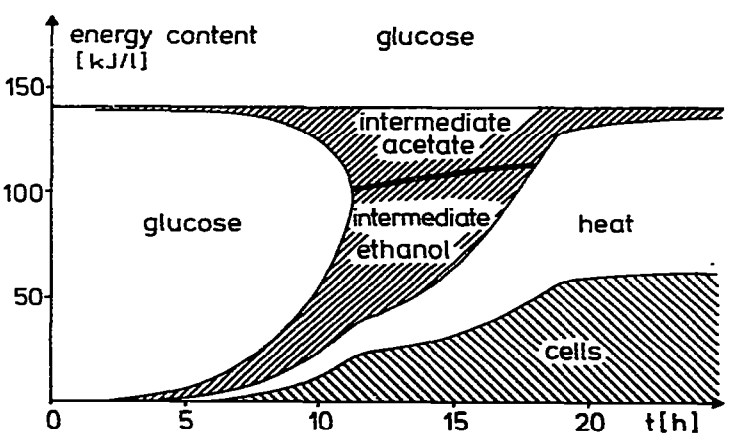

Fig.3: Energy balance of a batch culture in glucose medium

are shown in Fig.3. The concentration of acetate was not measured quantitatively but assumed to be responsible for the whole intermediate deficiency in the energy budget.

For ethanol media the relations

$$E_{in} = c_e(0) \cdot \Delta H_c(\text{ethanol}) = 296 \text{ kJ/l}$$

$$E_{out} = X(t) \cdot \Delta H_c(\text{cells}) + \int \dot{Q}(t)\,dt + c_e(t) \cdot \Delta H_c(\text{ethanol})$$

are shown in Fig.4.

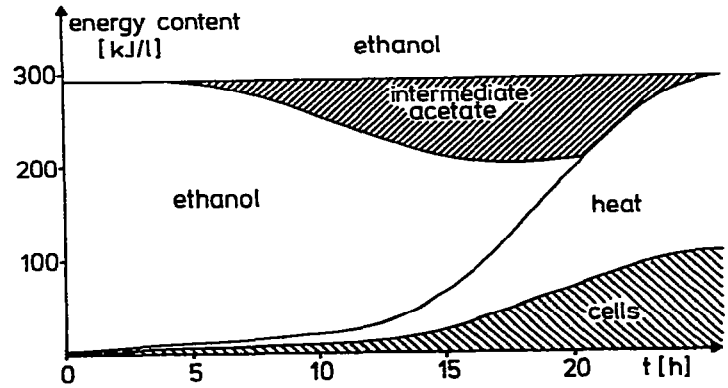

Fig.4:
Energy balance of a batch culture in ethanol medium

Continuous cultures

Fig.5 represents the measured heat production rates and cell concentrations in dependence of the adjusted dilution rate D. The course of the cell mass is nearly constant up to D = 0.35 h^{-1} for glucose media and up to D = 0.2 h^{-1} for ethanol media, indicating steady state conditions over this range. The rates of heat production increase linearily with D due to the linearily increasing concentration of substrate (glucose or ethanol, respectively). An energy flow budget of the system can be set up by the following relations.

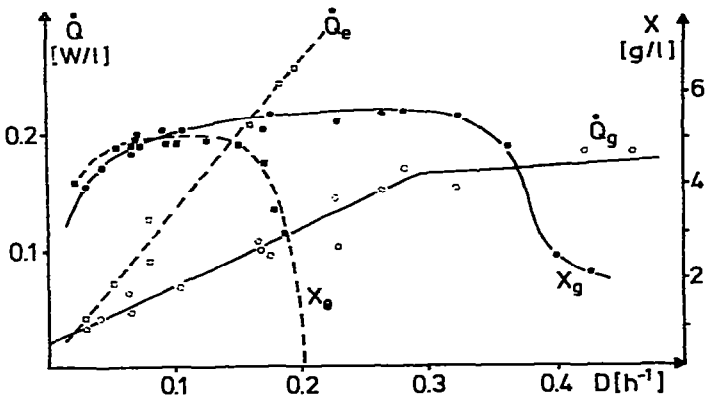

Fig.5:
Heat production rate and dry mass measured in continuous cultures on glucose media (index g) and on ethanol media (index e) with different dilution rates D.

$$P_{in} = D \cdot c_g(O) \cdot \Delta H_c(\text{glucose}) = 39.5\ D\ W/l$$

$$P_{out} = D \cdot X(t) \cdot \Delta H_c(\text{cells}) + \dot{Q}(t) + D \cdot c_g(\text{out}) \cdot \Delta H_c(\text{gluc}) + D \cdot c_e(\text{out}) \cdot \Delta H_c(\text{ethanol})$$

where $c_g(\text{out})$ and $c_e(\text{out})$ represent the concentrations of glucose and ethanol in the outflowing suspensions which are not used by the cells. Fig.6 shows these relations for the ethanol media.

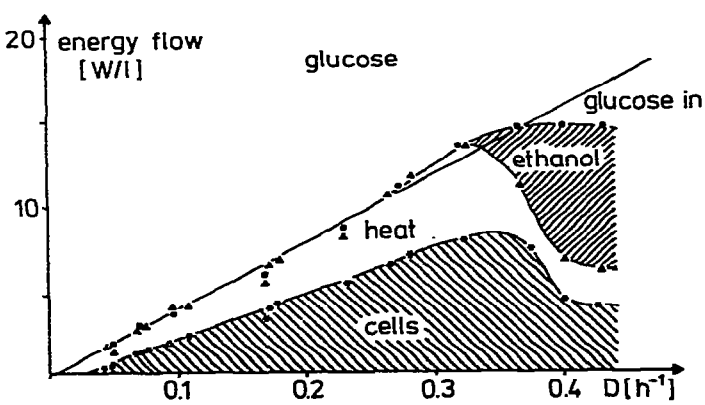

Fig.6:
Energy flow balance of continuous cultures in glucose media.

$$P_{in} = D \cdot c_e(0) \cdot \Delta H_c(\text{ethanol}) = 82.22\ D \quad W/l$$

$$P_{out} = D \cdot X(t) \cdot \Delta H_c(\text{cells}) + \dot{Q}(t) + D \cdot c_e(\text{out}) \cdot \Delta H_c(\text{ethanol}).$$

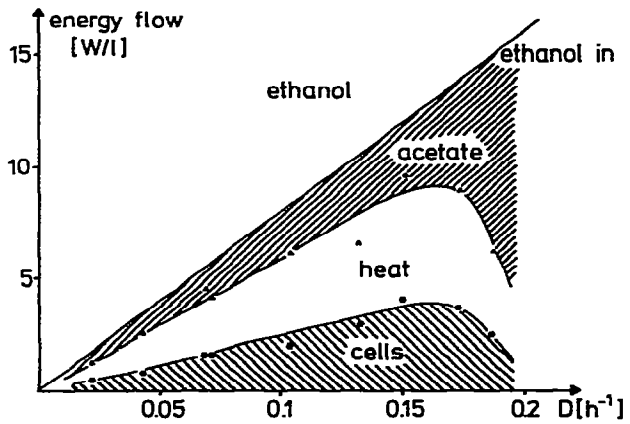

Fig.7:
Energy flow balance of continuous cultures in ethanol media

Discussion

Fig.3,4,6 and the table prove that the energy balances in glucose media and in batch cultures on ethanol are established. Only with the continuous cultures on ethanol a part of about 25% of the energy input occurs in form of acetate which remains unused within the culture. Therefore with these cultures only 23-34% of the energy of the medium can be converted into cell material. This proportion is lower than in the corresponding batch culture and reflects thus the opposite situation as in glucose cultures. It is obvious that in batch cultures a higher proportion of the input energy is transformed into heat than in continuous cultures, and on the other hand in ethanol media a higher proportion than in glucose media. Con-

sidering the dissipation of heat as lost energy the growth of yeast cells in a continuous culture on glucose seems to be the most economic culture type.

Table: Percentage distribution of the energy content in batch cultures and of the energy flow in continuous cultures. The variations with the continuous cultures are due to their dependence on the dilution rate D.

	$\frac{E_{cells}}{E_{in}}$	$\frac{Q}{E_{in}}$	$\frac{E_{cells} + Q}{E_{in}}$	$\frac{E_{cells}}{E_{cells} + Q}$
Batch culture glucose	0.44	0.55	0.99	0.65 0.36
Batch culture ethanol	0.34	0.67	1.02	0.34
Continuous culture glucose	0.57 - 0.64	0.45 - 0.38	1.02	0.56 - 0.64
Continuous culture ethanol	0.23 - 0.34	0.51 - 0.43	0.74 - 0.77	0.31 - 0.43

Furthermore the relative growth efficiency $E_{cells}/(E_{cells}+ Q)$ in glucose media is nearly double that of ethanol media. It is remarkable that these values are of the same magnitude within the same media in spite of the different culture con-

ditions. This holds even too for the batch culture in glucose medium, where two growth phases due to depletion of glucose and ethanol succeed one another.

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