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Microcalorimetric studies of aerobic growth of Candida maltosa II. Batch cultures

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Abstract

An isothermal flow-microcalorimeter was coupled to a fermenter in a by-pass. It was used to study the influence of initial glucose concentration, nutrient limitation and changes of gas inflow (air and nitrogen) on batch cultures of Candida maltosa grown on glucose.

1. INTRODUCTION

Isothermal flow-microcalorimetry is suitable to get detailled knowledge about the dynamics of cell popuations in aerobic batch cultures of yeast strains /1-3/. The experimental results will explain calorimetric studies of chemostat cultures of Candida maltosa grown on glucose described in detail elsewhere /4/.

2. MATERIAL AND METHODS

The mesophilic yeast strain Candida maltosa /3,5/ and the carbon substrate glucose were chosen for the experiments.

The yeast strain was cultivated discontinuously in a fermenter vessel with an inflow of gas (air or nitrogen). During all experiments the temperature of 32 °C and the volume of the culture broth of 0.5 litre were kept constant. The pH value of the culture broth was in the range of 2.6 up to 6.25.

The cell-mass concentration was measured photometrically with a Spekol 11 (firm Carl Zeiss Jena). The glucose concentration was analyzed enzymatically using a test kit (firm Arzneimittelwerk Dresden).

The isothermal flow-microcalorimeter 10700-1 (firm LKB Jarfälla) described in detail elsewhere /6/ was coupled to the fermenter vessel in a by-pass. For avoiding exhaustion of dissolved-oxygen tension during the transport of the culture broth to the measuring chamber a low cell concentration (0.01 up to 0.1 $g \cdot l^{-1}$) and a high flow rate (0.04 $l \cdot h^{-1}$) were chosen.

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3. RESULTS

Figure 1 shows the aerobic growth of Candida maltosa grown a synthetic glucose medium in a batch culture.

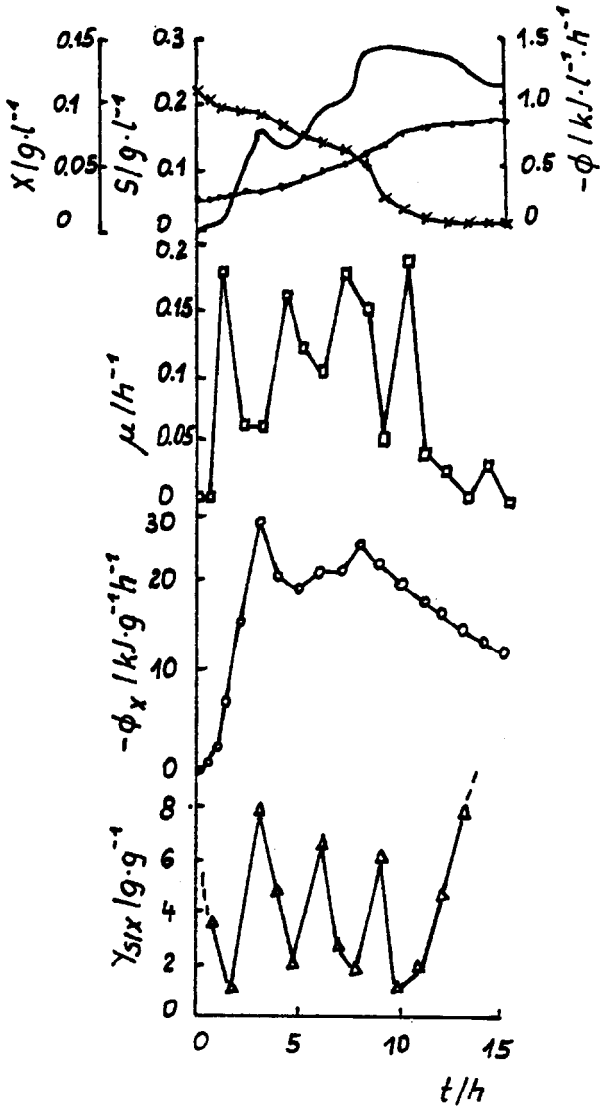

Figure 1. Glucose-limited batch culture of Candida maltosa grown on a synthetic glucose medium (Θ 32°, pH value adjusted to 6.25 using phosphate buffer, S_0 0.22 g·l^{-1}, -●- X, -x- S, —— ϕ, -□- μ, -o- ϕ_x, -Δ- $Y_{S/X}$).

The lag phase of the batch culture is characterized by a rise of heat flow and with it specific heat flow immediately after inoculation. Glucose is consumed simultaneously. The increase of the quantities mentioned above is dependent on the initial glucose concentration (compare Table 1). These results are in agreement with calorimetric studies of Stoward /7/ as well as Forrest and Walker /8/.

Table 1
Influence of initial glucose concentration on lag-phase behavior of Candida maltosa (ϑ 32 °C)

S_o /g·l^{-1}	$\frac{\Delta X}{\Delta t}$ /g·l^{-1}·h^{-1}	$\frac{\Delta S}{\Delta t}$ /g·l^{-1}·h^{-1}	$-\phi_x$ /kJ·g^{-1}·h^{-1}	Remarks
0.22	0	0.1	0 up to 1.24	pH value 6.25 comp. Fig. 1
2.0	0	0.4	0 up to 6.69	pH value 6.25 comp. Fig. 2
7.0	0	0.12	0 up to 16.79	pH value 4.35 → 4.05

The time dependence of the heat flux shows a double peak characterizing the metabolism of Candida maltosa (compare Fig. 1). Brettel et al. /2/ described also a double peak of the time dependence of heat flux during the aerobic growth of Saccharomyces cerevisiae on a synthetic glucose medium in a batch culture.
The time dependencies of cell concentration, glucose concentration, specific growth rate, glucose-consumption coefficient as well as heat flux and specific heat flux deviate from the well known exponential growth functions. The specific growth rate and the glucose-consumption coefficient change periodically. The periods are in the order of magnitude of hours and seem to correlate with the cell-doubling time. Bley et al. /9/ explained the periodic changes of the glucose-consumption coefficient during a batch culture of a yeast strain by a partial synchronization of cell functions.
The influence of nutrient limitation in stationary phase of batch cultures of Candida maltosa is demonstrated in Figs. 1 and 2. Whereas the glucose limitation caused a rise of the glucose-consumption coefficient and a decrease of the specific heat flux (compare Fig.1), the ammonium limitation is connected to a glucose-consumption coefficient of 3.3 g g^{-1} after passing a maximum as well as to a decrease of the specific heat flux (compare Fig. 2).

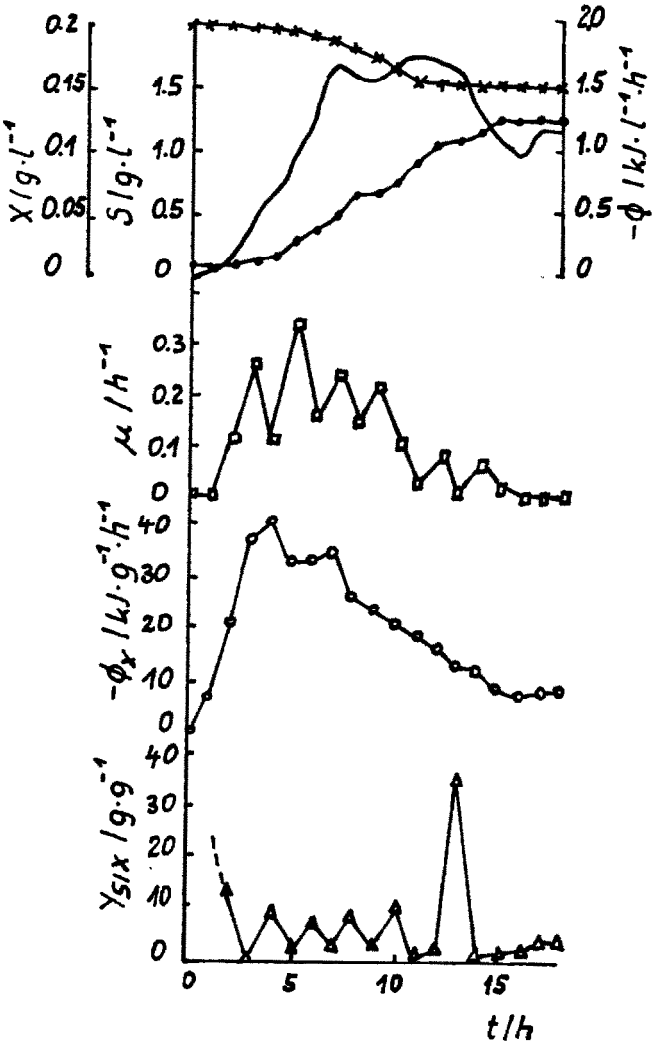

Figure 2. Ammonium-limited batch culture of Candida maltosa grown on a synthetic glucose medium (θ 32 °, pH value adjusted to 6.25 using phosphate buffer, S_o 2 g·l^{-1}, -●- X, -x- S, —— ϕ, -□- μ, -o- ϕ_x, -Δ- $Y_{s/x}$).

In analogy to the lag phase behavior of Candida maltosa a change of gas inflow air/nitrogen/air during aerobic growth of the yeast strain induces rising of heat flux and with it specific heat flux as well as glucose-consumption coefficient (Fig. 3). The increase of the quantities mentioned above is also dependent on the glucose concentration in the culture broth. These results explain the time dependencies of specific heat flux and glucose-consumption coefficient induced by repetitive changes of gas inflow during chemostat cultures of Candida maltosa /4/.

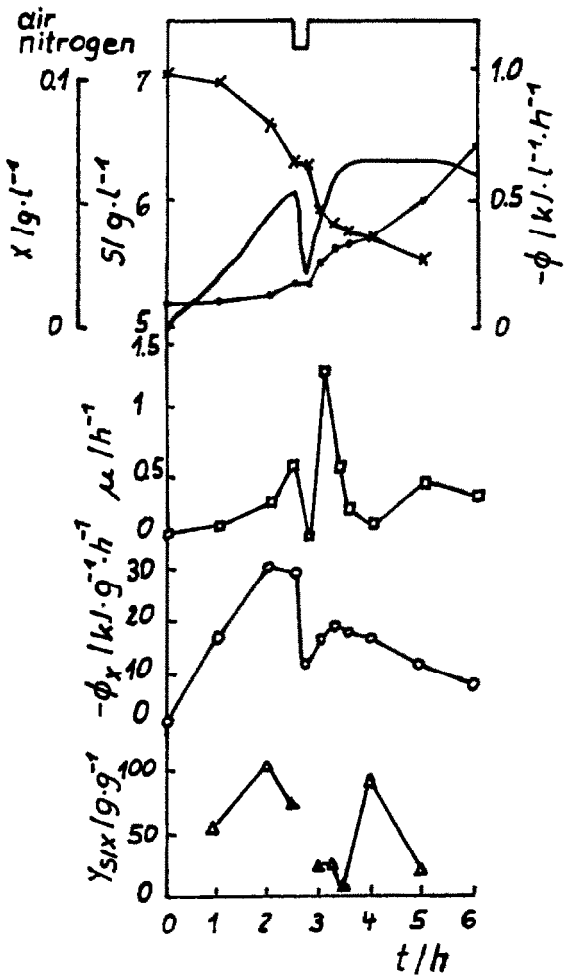

Figure 3. Influence of a change of gas inflow air/nitrogen/air on a batch culture of Candida maltosa grown on a sythetic glucose medium (Θ 32 °, pH-value 4.35 → 2.6, S_0 7 g·l⁻¹, -●- X, -x- S, —— O, -□- μ, -o- ϕ_x, -Δ- $Y_{S/X}$).

4. SUMMARIZING CONCLUSIONS

Calorimetric studies of batch cultures of Candida maltosa are suitable for describing the dynamics of yeast-cell populations. The lag phase of batch culture of Candida maltosa is characterized by an increased specific heat flux coupled to a consumption of glucose depending on initial glucose concentration. In analogy a change of the gas inflow (air/nitrogen/air) causes a rise of the specific heat flux and the glucose-consumption coefficient depending on glucose concentration in the culture broth, too.
The time dependencies of all measured quantities, especially of the specific growth rate and the glucose-consumption coefficient, deviate from the well known exponential growth functions during batch cultures of Candida maltosa.This fact can be explained by a partial synchronization of cell functions.

6. SYMBOLS

t	time (in h)
Θ	temperature (in °C)
X	cell concentration (in $g \cdot l^{-1}$)
S	glucose concentration (in $g \cdot l^{-1}$)
S_o	initial glucose concentration (in $g \cdot l^{-1}$)
μ	specific growth rate (in h^{-1})
ϕ	heat flux (in $kJ \cdot l^{-1} \cdot h^{-1}$)
ϕ_X	specific heat flux (in $kJ \cdot g^{-1} \cdot h^{-1}$)
$Y_{S/X}$	glucose-consumption coefficient (in $g \cdot g^{-1}$)

7. REFERENCES

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