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Microcalorimetric studies of aerobic growth of Candida maltosa I. Chemostat 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 nutrient limitation, glucose supply, pH value, dilution rate and changes of gas inflow on steady states in chemostat cultures of Candida maltosa grown on glucose. Changes of air and nitrogen inflow caused an increase of specific heat flux and glucose-consumption coefficient in dependence on changing nutrient limitation and the duration of nitrogen inflow. The results are of importance for applying microorganisms in high-performance fermenters with changing gradients of dissolved-oxygen tension.

1. INTRODUCTION

Isothermal flow-microcalorimetry is suitable to get detailled knowledge about the influence of external conditions on aerobic chemostat cultures of yeast strains.
Lamprecht /1/ and Brettel et al. /2,3/ described the dependence of heat flux on dilution rate in chemostat cultures of Saccharomyces cerevisiae grown on glucose. The heat flux rised with dilution rate. The experimental data allow to calculate energy balance equations.

Further calorimetric studies should be aimed at the influence of nutrient limitation, glucose supply, pH value and changes of gas inflow on chemostat cultures of a yeast strain because these conditions are of practical relevance, too.

2. MATERIAL AND METHODS

The mesophilic yeast strain Candida maltosa described in detail elsewhere /4,5/ and the carbon substrate glucose were chosen for the experiments.

The yeast strain was cultivated in a fermenter vessel with gas inflow (air or nitrogen). The nutrient medium flew continuously into the fermenter vessel replacing an equivalent amount of culture broth (chemostat principle /6/). During all experiments the temperature of 32 °C and the volume of the cul-

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ture broth of 0.5 litre were kept constant. The pH value of the culture broth was in the range of 3.5 up to 6.0. The dilution rate was varied from 0.07 up to 0.24 h^{-1}.

The cell-mass concentration was measured photometrically using 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 /7/ 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 l^{-1}) and a high flow rate (0.04 l h^{-1}) were chosen.

3. RESULTS

The specific heat flux and the glucose-consumption coefficient changed in dependence on the nutrient limitation of the chemostat cultures of Candida maltosa grown on glucose (Fig. 1). The lowest values were observed during the glucose-limited process. The values rised from glucose limitation via phosphate limitation to ammonium limitation.

The specific heat flux and the glucose-consumption coefficient increased also with the glucose excess in the case of ammonium-limited chemostat cultures of Candida maltosa (Fig. 2).

Another influence factor on the quantities mentioned above is the pH value of the culture broth as shown in Fig. 3. A pH value of 5.7 induced an increasing efficiency of aerobic cell growth compared to the pH value of 3.5.

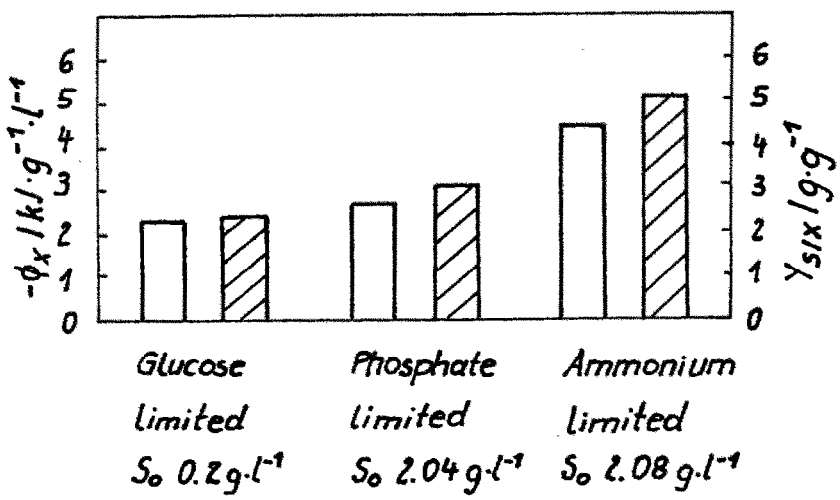

Figure 1. Influence of nutrient limitation on chemostat cultures of Candida maltosa grown on glucose (steady states, ϑ 32 °C, pH value 5.7-5.8, D 0.13-0.14 h^{-1}, □ ϕ_x, ▨ $Y_{s/x}$).

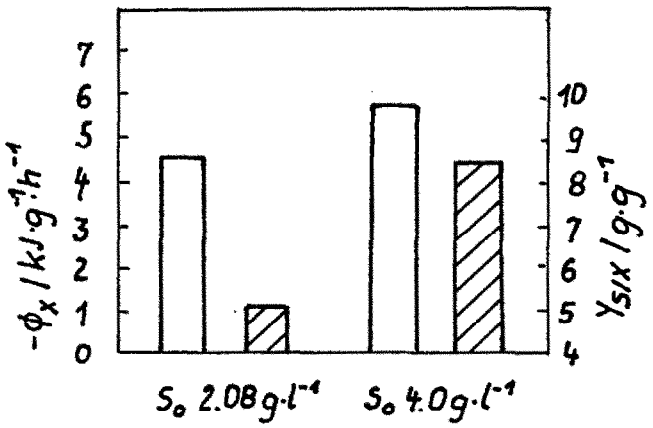

Figure 2. Influence of glucose excess on ammonium-limited chemostat cultures of Candida maltosa grown on glucose (steady states, ϑ 32 °C, pH value 5.7-6.0, D 0.13-0.15 h^{-1}, □ ϕ_x, ▨ $Y_{s/x}$).

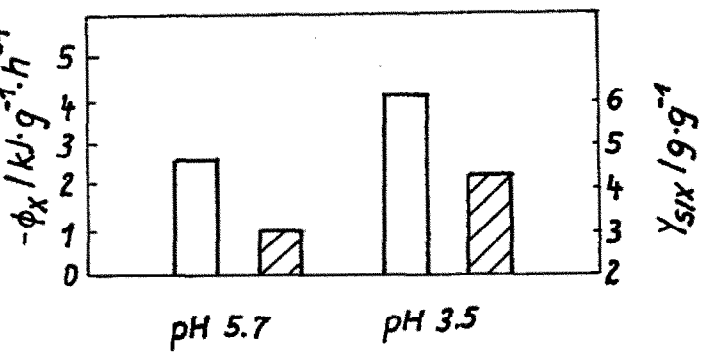

Figure 3. Influence of pH value on phosphate-limited chemostat cultures of Candida maltosa grown on glucose (steady states, ϑ 32 °C, S_o 1.75-2.04 g·l^{-1}, D 0.14 h^{-1}, □ ϕ_x, ▨ $Y_{s/x}$).

The dependence of chemostat cultures of Candida maltosa on dilution rate ranging from 0.07 to 0.24 h^{-1} is demonstrated in Fig. 4. Whereas the specific heat flux rised with dilution rate the glucose-consumption coefficient decreased. This result is in agreement with the experiments of Brettel et al. /2,3/.

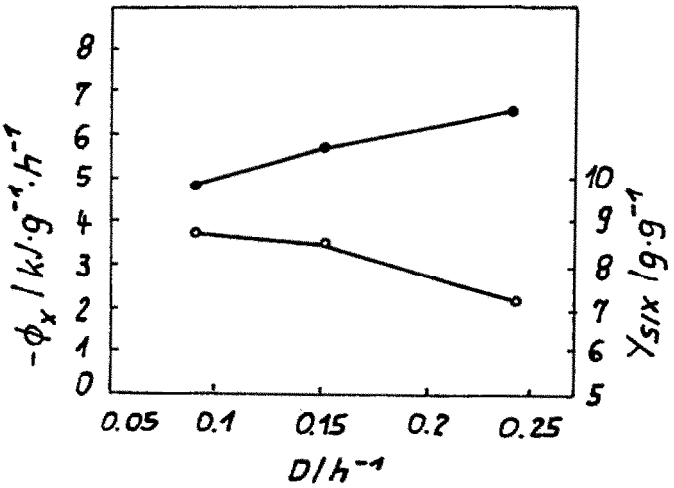

Figure 4. Influence of dilution rate on ammonium-limited chemostat cultures of Candida maltosa grown on glucose (steady states, Θ 32 °C, pH value 5.9-6.0, S_o 4.0 g·l^{-1}, -●- ϕ_x, -o- $Y_{S/X}$).

It is well known that perturbations caused by changes of external conditions induce rising values of glucose-consumption coefficient and yield coefficient, respectively /8-12/. Therefore, further experiments were carried out to study the influence of repetitive changes of air and nitrogen inflow on chemostat cultures of Candida maltosa in order to simulate gradients of dissolved-oxygen tension occuring in high-performance fermenters /12,13/. Fig. 5 shows the increase of specific heat flux and glucose-consumption coefficient caused by a change of air and nitrogen inflow.
The influence of repetitive changes of air and nitrogen inflow on ammonium-limited chemostat cultures of Candida maltosa is demonstrated in Fig. 6. Each change was observed to be coupled to an increase of specific heat flux and glucose-consumption coefficient.
Table 1 shows the dependence of specific heat flux and glucose-consumption coefficient on changing nutrient limitations during chemostat cultures of Candida maltosa perturbed by changes of air and nitrogen inflow. The maximum increase of both quantities (37-40 %) was observed in the case of potassium-limited chemostat culture of Candida maltosa with glucose excess.
The increase of both quantities was also dependent on the duration of nitrogen inflow (Table 2). Maximum values were observed at a duration of nitrogen inflow of 300 seconds.

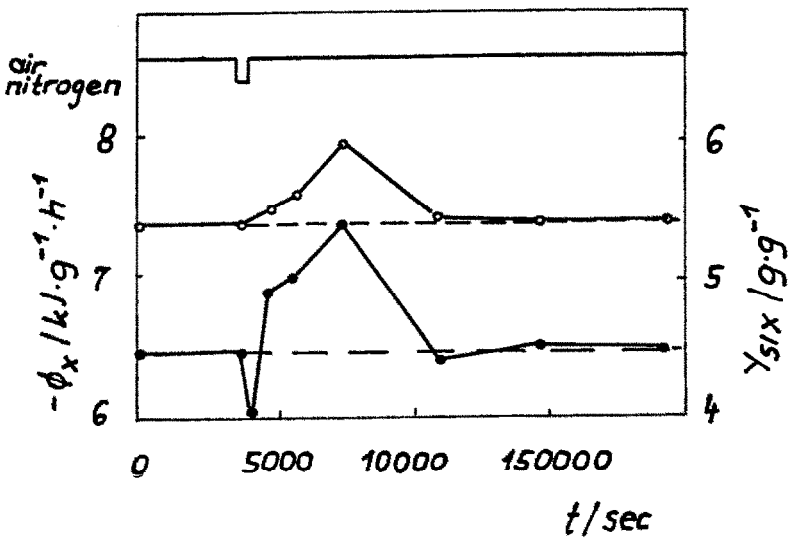

Figure 5. Influence of a change of gas inflow (air/nitrogen/air) on phosphate-limited chemostat cultures of Candida maltosa grown on glucose (Θ 32 °C, pH value 3.5, S_o 1.75 g·l^{-1}, D 0.13 h^{-1}, - - - steady-state values, -●- ϕ_x, -o- $Y_{s/x}$).

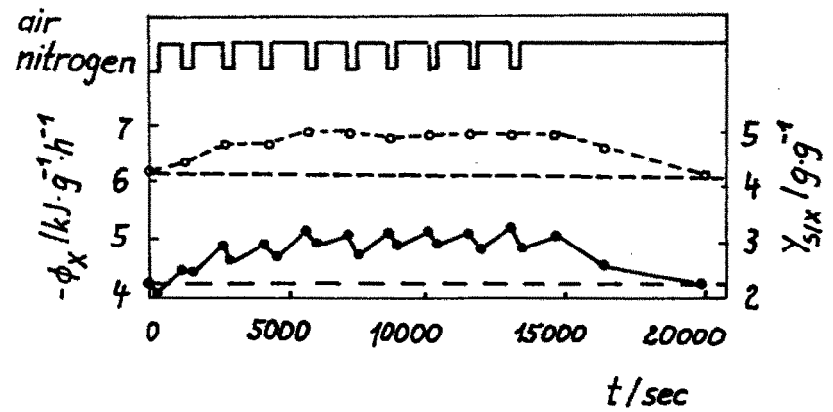

Figure 6. Influence of repetitive changes of gas inflow on phosphate-limited chemostat cultures of Candida maltosa grown on glucose (Θ 32 °C, pH 3.5, S_o 1.75 g·l^{-1}, D 0.14 h^{-1}, - - - steady state, -●- ϕ_x, -o- $Y_{s/x}$).

Table 1
Influence of repetitive changes of gas inflow during chemostat cultures of Candida maltosa in dependence on different nutrient limitations (ϑ 32 °C, duration of nitrogen input 300 sec, ratio of nitrogen and air inflow 1:4, * steady state, ** transient state)

Nutrient limitation	D /h^{-1}	S_o /$g \cdot l^{-1}$	pH	$-\phi_x$ /$kJ \cdot g^{-1} \cdot h^{-1}$	$\Delta\phi_x$ /%	$Y_{s/x}$ /$g \cdot g^{-1}$	$\Delta Y_{s/x}$ /%	
Glucose-limited	0.22	0.2	6.4	4.15	8.1	2.40	7.1	**
	0.22	0.2	6.4	3.84		2.24		*
Phosphate-limited	0.14	2.1	3.5	4.18	17.7	5.25	19.3	**
	0.14	2.1	3.5	3.55		4.40		*
Potassium-limited	0.14	2.08	3.5	7.41	40.1	8.23	37.2	**
	0.14	2.08	3.5	5.29		6.0		*

Table 2
Influence of repetitive changes of gas inflow on phosphate-limited chemostat cultures of Candida maltosa (ϑ 32 °C, pH 3.5, S_o 2.1 $g \cdot l^{-1}$, D 0.14 h^{-1}, ratio of nitrogen and air inflow 1:4, * steady state, ** transient state)

Duration of nitrogen inflow /sec	$-\phi_x$ /$kJ \cdot g^{-1} \cdot h^{-1}$	$\Delta\phi_x$ /%	$Y_{s/x}$ /$g \cdot g^{-1}$	$\Delta Y_{s/x}$ /%	
120	3.80	7.0	4.72	7.3	**
	3.55		4.40		*
300	4.18	17.7	5.25	19.3	**
	3.55		4.40		*
600	4.06	14.4	5.07	15.2	**
	3.55		4.40		*

4. SUMMARIZING CONCLUSIONS

The lowest values of specific heat flux and glucose-consumption coefficient have been obtained in steady states of chemostat cultures of Candida maltosa grown on glucose. They are dependent on the dilution rate, the pH value, the glucose supply and the nutrient limitation. Repetitive changes of gas inflow (air/nitrogen/air) caused a decrease of the efficiency of aerobic growth of the yeast strain in dependence on the duration of nitrogen inflow and the kind of changing nutrient-limitations. This fact is of importance for applying microorganisms in high-performance fermenters with changing gradients of dissolved-oxygen tension.

5. SYMBOLS

Θ	temperature (in °C)
S_0	glucose concentration of nutrient medium (in $g \cdot l^{-1}$)
D	dilution rate (in h^{-1})
ϕ_x	specific heat flux (in $kJ\ g^{-1}h^{-1}$)
$\Delta\ \phi_x$	increase of specific heat flux (in %)
$Y_{s/x}$	glucose consumption coefficient (in $g \cdot g^{-1}$)
$\Delta\ Y_{s/x}$	increase of glucose-consumption coefficient (in %)

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