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Calorimetric studies for optimization of high-performance reactors

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Abstract. The isothermal flow-microcalorimetry as well as the bench-scale calorimetry have been used for optimization of microbial reactions in recirculation reactors characterized by periodically changing dissolved oxygen gradients. Thus, the reaction of aerobic growth of Candida maltosa EH15 on periodic changes of air and nitrogen supply has been studied in a chemostat. When the residence time of aerobically growing yeast cells at reduced dissolved oxygen-concentration was smaller or equal to the retardation time of the investigated system no increase of specific heat flux and reciprocal yield has been obtained in comparison to the steady state without perturbations. The experimental results are in agreement with computer-aided calculations on the base of a simple S-E-X model.

INTRODUCTION

Studies of aerobic cell growth are the base of microbial production of many compounds, e.g. cell-mass constituents and extracellular metabolites. Such substances are produced as a rule in high-performance reactors. Cell mass ($C_1H_{1.8}O_{0.5}N_{0.17}P_{0.01}...$), carbon dioxide, water, and other extracellular metabolites ($C_kH_oO_p...$) are formed in an irreversible, autocatalytic, and exothermal reaction from several nutrients, e.g. a carbon substrate ($C_nH_mO_1$), molecular oxygen, ammonium and phosphate ions:

$$a_o C_1H_{1.8}O_{0.5}N_{0.17}P_{0.01}... + bC_nH_1O_m + cO_2 + dNH_4^+ + ePO_4^{3-} + ...$$
$$\longrightarrow (a-a_o)C_1H_{1.8}O_{0.5}N_{0.17}P_{0.01}... + fCO_2 + gH_2O + hC_kH_oO_p...$$
$$\text{with } Q < 0$$

Recirculation reactors which produce space and time dependent concentration gradients improve the material and heat transfer and with it the rate of the microbial reaction in comparison to the stirred vessel (refs. 1 and 2). It seems ,however, that such concentration gradients influence also the yield and energy dissipation of the microbial reaction (ref. 3). Therefore, detailled studies of the dependences of reciprocal yield and specific heat flux on periodically changing oxygen-concentration gradients in recirculation reactors are essential for optimization of microbial reactions. The

isothermal flow-microcalorimetry as well as the bench-scale calorimetry are suitable tools for investigation (refs. 4 - 7).

MATERIALS AND METHODS

For experimental simulation of the conditions in recirculation reactors, the yeast strain Candida maltosa EH15 was cultivated aerobically on a glucose containing nutrient medium (ref. 3) in a chemostat. The dilution rate was 0.14 h^{-1}. The temperature and the pH-value of the culture medium were kept constant as well. The culture medium was exposed to changing air (oxygen) and nitrogen inflow (time ratio 4:1 and duration of nitrogen inflow 120 to 600 sec). The chemostat experiments were carried out in a 0.5 litre stirred reactor coupled to an isothermal flow-microcalorimeter (Fa. LKB) in by-pass. For avoiding exhaustion of dissolved oxygen concentration during the transport of culture medium to the measuring chamber, low cell densities (0.01 to 0.1 g/l) and high flow rates in by-pass (40 ml/h) were chosen.

In order to compare the obtained results with those at higher cell densities (15 g/l) further chemostat experiments were carried out using bench-scale calorimetry (dynamic calorimetry directly in a 5 litre stirred reactor without exhaustion of dissolved oxygen, ref. 7). The yeast strain Candida maltosa EH15 was cultivated on a glucose containing nutrient medium (ref. 8) in a chemostat at a dilution rate of 0.25 h^{-1}, a temperature of 32 °C, and a pH value of 4.2. The culture medium was exposed to changing inflow of air and nitrogen. The time ratio of air and nitrogen inflow varied between 10:1.3 and 4:1. The duration of nitrogen inflow was changed from 1 to 120 sec.

The cell density was determined photometrically using a Specol 11 (Fa. Jenoptik) and/or gravimetrically. The glucose concentration was analyzed enzymatically using the Bioanalyzer YSI 2700 Select (Fa. Kipp & Zonen).

COMPUTER SIMULATION

For prediction of the experimental results, a simple S-E-X model has been realized on a computer system:

$$S \xrightarrow{k_1} E \xrightarrow{k_2} X$$

$$r_E = k_1 \cdot Y_1 \cdot [S] - k_2 \cdot [E]$$

These equations describe the formation of cell mass X from a carbon substrate S via an energy-rich intermediate E in an irreversible, consecutive reaction occurring in an open system.
In order to simulate the influence of repetitive perturbations on an open microbial system the time constant k_1 was changed periodically. It was assumed that (1) the time derivative of the concentration of the energy-rich intermediate is in close connection to the specific heat flux and the yield, (2) the microbial system reacts retardedly to perturbations, and (3) the carbon substrate is not converted oxydatively if the dissolved oxygen-concentration approximates zero (refs. 9 and 10).

The results of computer simulation (Figs. 1 and 2) show the dependence of percentage increase of specific heat flux and reciprocal yield on the duration and frequency of repetitive perturbations. When the perturbation time is smaller than or equal to the retardation time of the microbial system the quantities mentioned above correspond to the steady-state data. Consequently, both quantities rise and pass maxima if the perturbation time exceeds the retardation time of the microbial system.

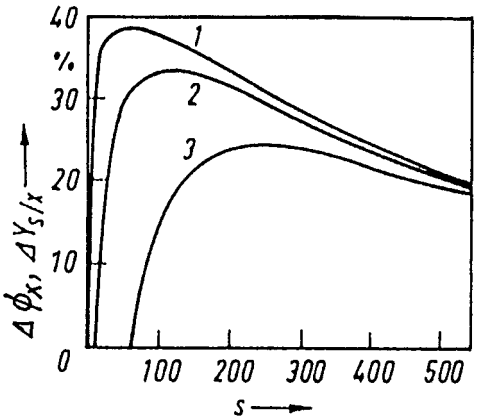

Figure 1. Computer simulation of the influence of perturbation time (s) on the percentage increase of specific heat flux ($\Delta \phi_X$) and reciprocal yield ($\Delta Y_{S/X}$) at varying retardation time 1-1.2 sec, 2- 12 sec, and 3- 60 sec (with $Y_1=2$ and $k_2=0.25\ min^{-1}$)

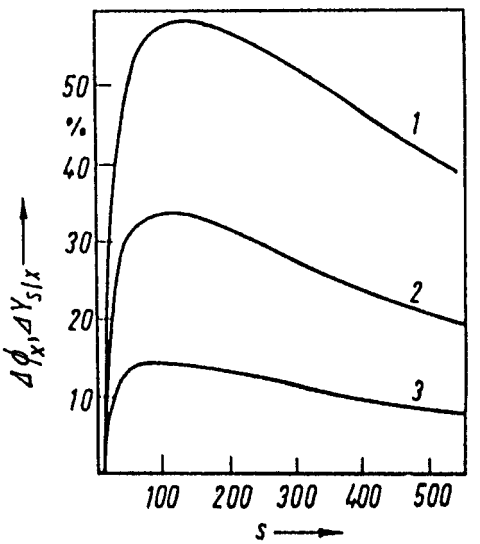

Figure 2. Computer simulation of the influence of perturbation time (s) on the percentage increase of specific heat flux ($\Delta \phi_X$) and reciprocal yield ($\Delta Y_{S/X}$) at varying perturbation frequency 1- 2:1, 2- 4:1, and 3- 10:1 (with $Y_1=2$ and $k_2=0.25\ min^{-1}$)

EXPERIMENTAL RESULTS AND DISCUSSION

Isothermal flow-microcalorimetry

The influence of repetitive changes of air and nitrogen inflow on the steady-state cultivation of Candida maltosa EH15 is demonstrated in Fig. 3. The specific heat flux and the reciprocal yield pass maxima at a duration of nitrogen inflow of 300 sec and a ratio of air to nitrogen inflow of 4:1.
This result is in agreement with the predicted dependencies of computer simulation. It can be interpreted by the reaction of batch cultures of Candida maltosa EH15 to interruption of aeration (ref. 11).

Dynamic calorimetry

Fig. 4 shows the percentage increase of specific heat flux and reciprocal yield caused by repetitive changes of air and nitrogen inflow during a chemostat culture of Candida maltosa DEH15. When

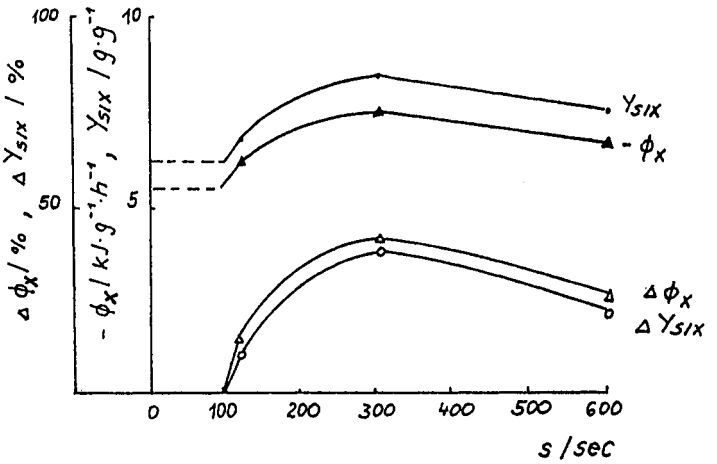

Figure 3. Influence of periodically changing air and nitrogen inflow on a chemostat culture of Candida maltosa EH15 at a varying duration of nitrogen inflow (s) (Θ 32 °C; pH 3.5; S_o 2.1 g/l; D 0.14 h^{-1}; ratio of air and nitrogen inflow 4:1)

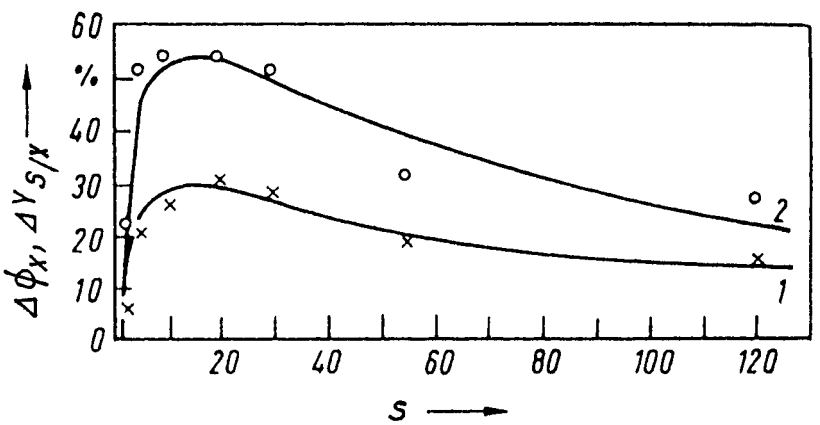

Figure 4. Influence of periodic changing air and nitrogen inflow on the percentage increase of specific heat flux ($\Delta \phi_x$) and reciprocal yield ($\Delta Y_{s/x}$) during chemostat culture of Candida maltosa EH15 at varying duration of nitrogen inflow (s) and ratio of air to nitrogen inflow 1- 10:1.3 and 2- 4:1
o, x experimental values
____ calculated values (Y_1=3; k_2=0.028 min^{-1})

the duration of nitrogen inflow is greater than the retardation time of the microbial system both quantities mentioned above rise and pass maxima at a duration of nitrogen inflow of 5 to 20 sec. Whereas the retardation time at lower cell densities is in the range of 100 sec it amounts to 2 sec at higher cell densities (compare Figs. 3 and 4). This refers to the dependence of retardation time on the exhaustion rate of dissolved oxygen in the culture medium.

The theoretically calculated solid lines in Fig. 4 based on a simple S-E-X model and the assumption that Y_1 is equal to 3 and the time constant k_2 amounts to 0.25 min^{-1} fits in the experimental data determined by dynamic calorimetry directly in the reactor. These dependencies confirm the experimental results measured by isothermal flow-microcalorimetry at low cell densities (Fig. 3). Thus, it is obvious that isothermal flow-microcalorimetry is suitable for optimization of aerobic microbial processes if special measuring conditions, e.g. low cell densities and sufficient dissolved oxygen, are guaranteed.

CONCLUSIONS

The experiments show as a rule an increase of the specific heat flux and the reciprocal yield of the microbial steady-state process if periodically changing dissolved oxygen gradients occur. Such a reaction to repetitive perturbations can be avoided if the perturbation time is smaller than or equal to the retardation time of the microbial system. The experimental data are in agreement with calculated data based on a simple S-E-X model.
These results show us that the development of high-performance reactors has to consider the microbial system and its properties besides the hydrodynamic reactor characteristics.

SYMBOLS

a, a_o, b, c, d e, f, g, h	stoichometric coefficients
$Y_{s/x}$	reciprocal yield
$\Delta Y_{s/x}$	percentage increase of reciprocal yield
$-\phi_x$	specific heat flux
$\Delta \phi_x$	percentage increase of specific heat flux
s	perturbation time, duration of nitrogen inflow
S	carbon substrate
E	energy-rich intermediate
X	cell mass
Y_1	yield
k_1, k_2	time constants
Θ	temperature
pH	pH value
S_o	carbon substrate concentration of nutrient medium
D	dilution rate

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