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Monitoring and control of batch and fed-batch cultures of *Saccharomyces cerevisiae* by calorimetry

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Abstract

Heat production rate, measured by a flow-through microcalorimeter, was the only continuous on-line measurement in order to indirectly determine substrate and product concentrations during aerobic batch growth of the yeast *Saccharomyces cerevisiae* with glucose as carbon and energy source. The results showed a very high agreement between indirectly calculated and actually measured concentrations of glucose, ethanol and biomass. Calorimetry was also used for control of the glucose feeding in fed-batch cultures of *S. cerevisiae.* A very simple control strategy was used but still the maximum ethanol concentration was less than 0.2 g/l compared to about 2 g/l during batch growth with the same amount of glucose (5 g/l)

INTRODUCTION

Calorimetry is an excellent tool for monitoring and controlling microbial fermentations. It is very general since cellular growth is always accompanied by production of heat and the measurements are performed continuously on-line without introducing any disturbances to the culture. The amount of heat that is dissipated from a culture is equal to the sum of the enthalpy changes of all metabolic reactions. In other words, the rate of heat production is stoichiometrically related to the rate of substrate consumption and product, including biomass, formation.

The aim of this study was to use cellular heat production rate, measured continuously on-line, to indirectly determine substrate and product concentrations continuously during aerobic batch growth of the yeast *S. cerevisiae* with glucose as the carbon and energy source. Under these conditions *S. cerevisiae* shows a diauxic growth curve due to catabolite repression (refs. 1-2). Initially a mainly fermentative metabolism is used with production of ethanol and a growth rate close to or at maximum. In the subsequent growth period, when glucose is exhausted, the previously formed ethanol is respired and used as the carbon and energy source, resulting in a much slower growth rate. The second aim was to use the calorimetric signal as a control parameter for glucose feeding in fed-batch cultures of *S. cerevisiae* in such a way that ethanol formation is avoided.

MATERIALS AND METHODS

Yeast strain, medium and growth conditions

The yeast strain used was *S. cerevisiae* Y41 (ATCC 38531) and the medium YNB (Difco) supplemented with glucose at a final concentration of 0.5 or 1 % (wt/vol.). The growth conditions are described in refs. 3-4.

Microcalorimetry

The heat production rate was measured with a microcalorimeter (Bioactivity monitor, LKB 2277, Thermometric AB, Järfälla, Sweden) operating in the flow-through mode. The culture was pumped at a rate of 58 or 80 ml/h and before entering the calorimeter this flow was mixed with a second flow of temperature controlled and water saturated air at a rate of 32 or 25 ml/h. These conditions gave an effective volume of the measuring cell of 0.33 or 0.39 ml, respectively.

For a more detailed description of materials and methods see refs. 3-4.

RESULTS AND DISCUSSION

Monitoring of batch growth of *S. cerevisiae*

During the first phase of growth, glucose was consumed by a respiro-fermentative metabolism and ethanol, together with small amounts of glycerol and acetate was produced (Fig. 1). When glucose was depleted there was a second phase of growth where the ethanol was respired. The concentration of acetate fluctuated during the ethanol consuming phase but the maximal concentration was obtained at the point where ethanol was totally consumed. Finally, the acetate was consumed (Fig. 1). These changes in metabolism could be followed with a high resolution by calorimetry.

At the first maximum in the heat production rate curve (Fig. 1) the extracellular glucose concentration was about 2 mM (ref. 5). The following drop of heat production was almost certainly due to an inadequate supply of glucose since the high affinity system for glucose transport has a K_m of 1-2 mM (refs. 6-7). Probably, the decreased rate of glucose transport also induced synthesis of the repressed respiratory enzymes since the RQ was reported to be 1 in the first minimum of the heat production rate (ref. 5) indicating respiration of glucose. Glucose was completely exhausted between the first minimum and the second maximum of the heat production rate curve and the cells started to use ethanol as carbon and energy source (Fig. 1). When ethanol was depleted there was again a sudden drop followed by a plateau in the heat production rate curve (Fig.1). The plateau represented consumption of acetate. If the pH of the culture was kept constant at 4.5 there was no production of acetate during the ethanol consumption period, which was seen by the absence of a plateau in the heat production rate curve when ethanol was depleted.

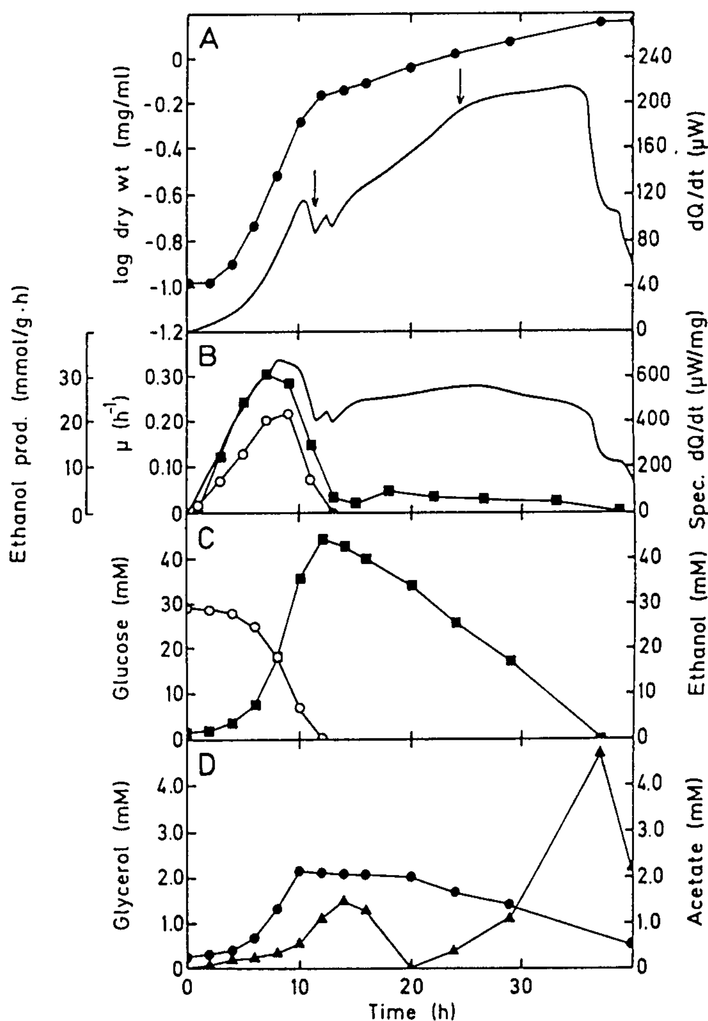

Figure 1. Changes in measured parameters during growth without pH adjustment of *S. cerevisiae* on glucose. (A) Dry weight (●) and recorded heat production rate, dQ/dt (-), with an effective volume of the measuring chamber of 0.33 ml. (B) Specific growth rate, μ (■), specific ethanol production rate (O) and specific heat production rate, (spec. dQ/dt), (-). (C) Extracellular glucose (O) and ethanol (■) concentrations. (D) Extracellular glycerol (●) and acetate (▲) concentrations. Results from a typical experiment are shown (from Ref. 3). Reprinted by permission from John Wiley and Sons, Inc. Biotechnol. Bioeng., authors Larsson, Blomberg, Gustafsson. Copyright © John Wiley and Sons, Inc. 1991.

Indirect determination of substrate and product concentrations during batch growth of *S. cerevisiae*

By using pre-determined heat and growth yields with the rate of heat production as the only continuous on-line measurement it was possible to calculate indirectly substrate and product concentrations continuously during aerobic batch growth of *S. cerevisiae* with glucose as carbon and energy source.

The heat yields (ΔQ_X, kJ/C-mol) were determined as the amount of heat produced (ΔQ, kJ/l) per amount of biomass produced (ΔX, C-mol/l). The growth yields ($Y_{X/S}$, C-mol/C-mol) were determined as the amount of biomass produced per amount of substrate consumed (ΔS, C-mol/l). The yields were assumed to be constant within each phase of growth (respiro-fermentative, respiratory). This is of course an over-simplification but for this purpose it proved sufficient. However, the yields changed when the cells switched from respiro-fermentative to respiratory metabolism. The heat production rate was used to monitor the growth process and hence to determine when to change yield values in the calculations. This was done at the first minimum in the heat production rate curve shown by the first arrow in Fig.1 where the previously reported RQ of 1 (ref. 5) indicated a change from respiro-fermentative to fully respiratory metabolism. In addition, since the cultures were not pH adjusted, the pH decreased to about 2.6 in the late respiratory phase and at this stage there was a further increase in heat yield and decrease in growth yield. This was shown as a change of slope of the heat production rate curve and the change of yield values are indicated by the second arrow in Fig.1. If the pH was kept constant (4.5) the yields remained the same throughout the respiratory growth period and there was no change in the slope of the heat production rate curve. The heat and growth yields were determined from a number of experiments (w/o pH adjustments). The average values for the heat yield were 251 kJ/C-mol (respiro-fermentative), 1243 kJ/C-mol (early respiratory) and 1900 kJ/C-mol (late respiratory), respectively. The average values for the corresponding growth yields were 0.130, 0.380 and 0.284 C-mol/C-mol, respectively.

Calculations

Biomass production. The biomass produced (ΔX, C-mol/l) during a time period was calculated by dividing the amount of heat produced (ΔQ, kJ/l) during the time period by the heat yield (ΔQ_X, kJ/C-mol).

$$\Delta X = \Delta Q / \Delta Q_X$$

Substrate consumption. The glucose consumed (ΔS, C-mol/l) during a time period was calculated by dividing the biomass produced (ΔX, C-mol/l) during the time period by the growth yield ($Y_{X/S}$, C-mol/C-mol).

$$\Delta S = \Delta X / Y_{X/S}$$

The ethanol consumed during respiratory growth was calculated in the same way but using another value for the growth yield.

Product formation. To calculate the ethanol produced during growth on glucose, the fraction of substrate used for catabolism ($\Delta_k S$, C-mol/l) and for anabolism ($\Delta_a S$, C-mol/l) must be calculated. This was done by using the concept of degree of reduction since the degree of reduction of the substrate (γ_S) times the fraction of the substrate used for anabolism is equal to the degree of reduction of the biomass (γ_X) times the biomass produced (ΔX, C-mol/l).

$$\Delta_a S \times \gamma_S = \Delta X \times \gamma_X$$

The degree of reduction is calculated as γ_i = 4C+H-2O-3N, where C, H, O, N denote the atomic coefficients of carbon, hydrogen, oxygen and nitrogen, respectively. The factor of -3N compensates for the electrons donated to the biomass from ammonia. The degree of reduction of the biomass was 4.16.

Since both the total amount of substrate (ΔS, C-mol/l) as well as the fraction of substrate used for anabolism ($\Delta_a S$, C-mol/l) is known the fraction of substrate used for catabolism ($\Delta_k S$, C-mol/l) is also known. The catabolic fraction was further divided in a respiratory ($\Delta_{kr} S$, C-mol/l) and a fermentative ($\Delta_{kf} S$, C-mol/l) part because of the difference in heat production (ΔQ, kJ/l) for the two routes

$$\Delta Q = \Delta_{kr} H \times \Delta_{kr} S + \Delta_{kf} H \times \Delta_{kf} S$$

where $\Delta_{kr} H$ (kJ/C-mol) and $\Delta_{kf} H$ (kJ/C-mol) are the respiratory and fermentative enthalphy changes, respectively.

By measuring the amount of heat produced (ΔQ, kJ/l) over a time period, the amount of glucose used for fermentation ($\Delta_{kf} S$, C-mol/l) and hence the amount of ethanol produced for the time period was calculated.

$$\Delta_{kf} S = (\Delta Q - \Delta_{kr} H \times \Delta_k S) / (\Delta_{kf} H - \Delta_{kr} H)$$

The agreement between the indirectly calculated and the measured values of glucose, ethanol and biomass concentrations is shown in Fig. 2. As a further test of the validity of this procedure, the yield values obtained for non-pH adjusted cultures were applied to calculate the changes in substrate and product concentrations during a pH adjusted culture. The only difference was that there was no change in yield values during the respiratory growth phase. The timescale of this culture was markedly different. Compared to the non-pH adjusted culture shown in Figs. 1 and 2, the respiro-fermentative phase lasted several hours longer whereas the respiratory phase was about twice as fast. In spite of this, there was still a very close resemblance between indirectly calculated and measured values (Fig. 3)

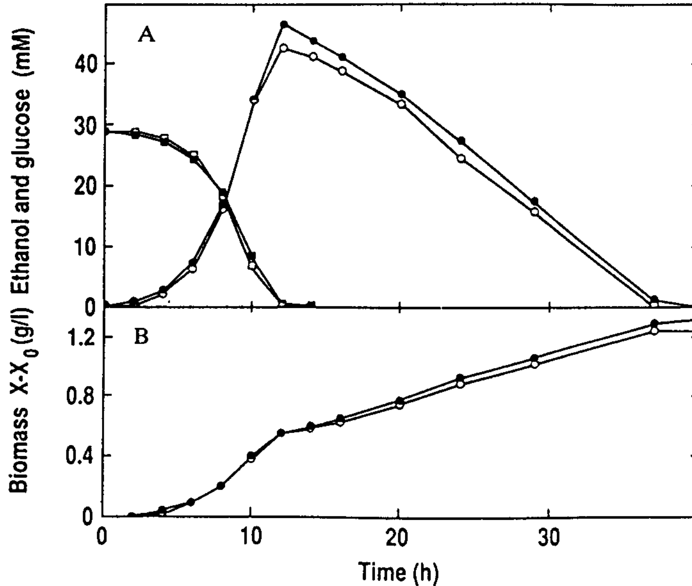

Figure 2. Comparison of measured versus calculated glucose and ethanol (A) and biomass (B) concentrations during growth of *S. cerevisiae* on glucose in a non-pH adjusted culture. (A) Measured ethanol (O) and glucose (❑) concentrations. Ethanol (●) and glucose (■) concentrations calculated from the measured heat production. (B) Measured biomass concentration (O). Biomass concentration calculated from the measured heat production (●) (from Ref. 3). Reprinted by permission from John Wiley and Sons, Inc. Biotechnol. Bioeng., authors Larsson, Blomberg, Gustafsson. Copyright © John Wiley and Sons, Inc. 1991.

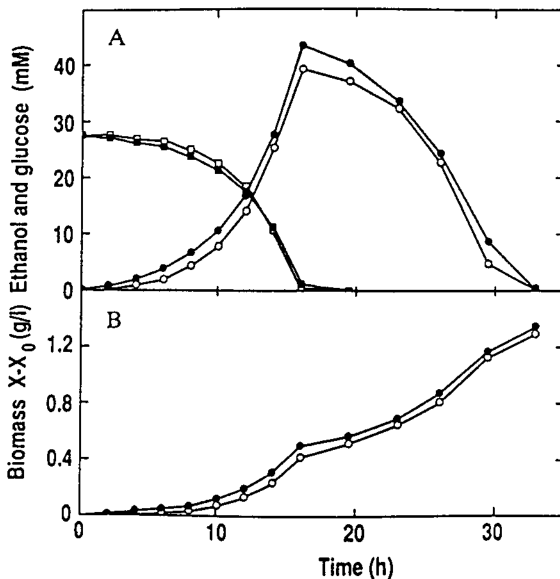

Figure 3. Comparison of measured versus calculated glucose and ethanol (A) and biomass (B) concentrations during growth of *S. cerevisiae* on glucose in a pH-adjusted culture (symbols as in Figure 2) (from Ref. 3). Reprinted by permission from John Wiley and Sons, Inc. Biotechnol. Bioeng., authors Larsson, Blomberg, Gustafsson. Copyright © John Wiley and Sons, Inc. 1991.

Calorimetric control of fed-batch cultures of *S. cerevisiae*

The calorimetric signal was used as a control parameter for glucose feeding in fed-batch cultures of *S. cerevisiae*. When the available glucose was consumed by the cells the heat production rate dropped. The decreased calorimetric signal was sensored by a computer which started a pump for another pulse of glucose (Fig. 4). Each pulse contained a sufficiently small amount of glucose (0.2 g/l) to avoid catabolite repression and hence ethanol formation. This cycle was repeated until all the glucose was added. By this very simple control strategy it was possible to keep the maximal ethanol concentration below 0.2 g/l compared to 2 g/l obtained for batch cultures with the same amount of glucose (5 g/l)

The growth yields for fed-batch cultivations were more than 30 % higher than for batch cultures. However, energy balance calculations showed that a large part of the increase could be explained by the evaporation of ethanol during batch cultivations. If evaporation of ethanol was assumed to account for all the discrepancy of the energy balance and the growth yields were corrected accordingly, the increase in growth yield for fed-batch cultures was about 10 %.

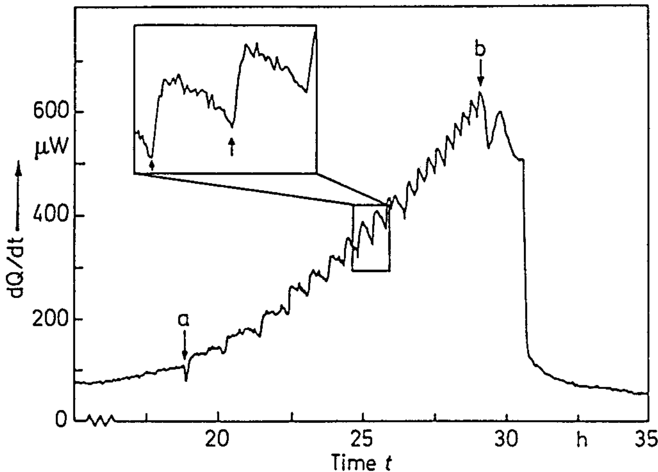

Figure 4. Heat production rate (dQ/dt) during fed-batch growth of *S. cerevisiae* with glucose (total amount 5 g/l) as the carbon and energy source. The calorimetric measuring cell had an effective volume of 0.39 ml. Start of glucose additions (a), glucose depletion (b) (from ref. 4). Reprinted by permission from Springer-Verlag. Bioproc. Eng., authors Larsson, Liden, Niklasson, Gustafsson. Copyright © Springer-Verlag 1991.

CONCLUSIONS

Calorimetry has a great potential for process control. It is clear that in some cases it could be replaced by for instance exhaust gas analysis. However, calorimetry is more general since there are anaerobic processes which proceed without formation of gaseous products. It could also provide additional and complementary information if applied simultaneously with gas analysis.

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