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Using heat-flow measurements for the feed control of a fed batch fermentation of *Saccharomyces cerevisiae*[1]

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Abstract

The glucose feed flow for a fed batch culture of *Saccharomyces cerevisiae* CBS 8066 was controlled on the basis of fast (20 s) heat-flow measurements and/or slow (100 s) outlet gas analysis (RQ). Several criteria were used to judge the performance of different control regimes. All criteria indicated that the combination of heat and RQ-based control loops performed better than the individual control loops. © 1998 Elsevier Science B.V.

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1. Introduction

During bakers' yeast production, high glucose concentrations may cause *Saccharomyces cerevisiae* to switch from purely oxidative to fermentative (oxido–reductive) metabolism [1], with formation of ethanol. This ethanol formation is undesirable, since the ethanol evaporates, which causes loss of energy and carbon source. High glucose concentrations should therefore be avoided. Bakers' yeast is usually produced in a fed-batch process. High glucose concentrations can be avoided by stopping or slowing down the glucose feed pump if the normal process is disturbed. However, production economics demands that the glucose flow is as high as possible (heeding the limitations in turnover capacity of organism and/or fermenter).

In practice, the feed-flow rate is usually controlled on the basis of a pre-calculated 'ideal' profile (Fig. 1) with an initial exponential rise in feed flow as the growth rate of the yeast limits the glucose turnover rate and a constant feed flow when the fermenter characteristics (oxygen transfer and cooling capacity) limit the glucose turnover rate. Corrections to the precalculated feed-flow rate have to be made if the glucose concentration increases to values above the 'fermentation threshold'. Unfortunately, glucose concentrations cannot be measured on-line during an industrial process, therefore the presence of glucose excess is usually detected indirectly via symptoms such as

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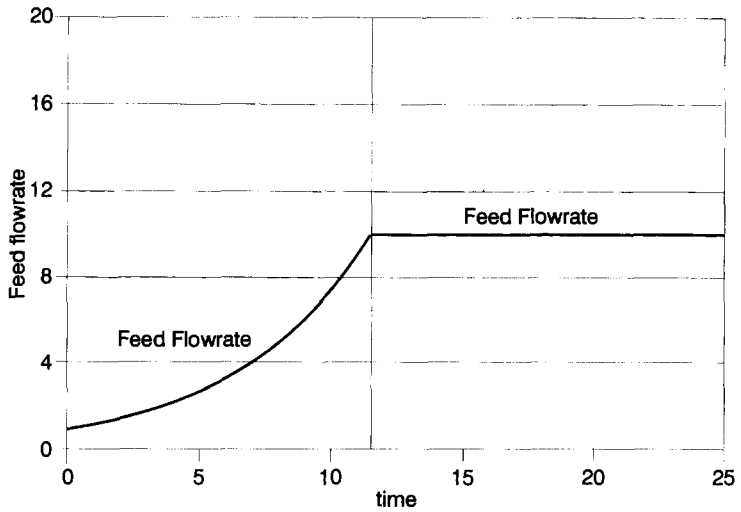

Fig. 1. Precalculated glucose feed-flow rate that is used as the basis on which corrections are made by the control loop(s) that were used. Feed rate is shown in arbitrary units. The actual glucose flow was dependant on the glucose concentration.

ethanol formation or a high Respiratory Quotient. Both these symptoms are easily detected in the fermenter outlet gas, but due to transport phenomena the detection is delayed with respect to the situation in the broth due to transport phenomena. Because of the delay in time (characteristic time 100 s in industrial environment), results from outlet gas analysis can only be used in slow control loops.

To counteract fast disturbances in the glucose flow (due to changes in feed composition or pump malfunction) a fast control loop is necessary. In the present work, a fast control loop was used that controlled the glucose flow rate on the basis of heat-flow measurements. This control loop only uses temperature measurements; it can therefore be made fast, and is completely independent of gas analysis.

Glucose feed control based on heat-flow measurements has been used before, but was either combined in one control loop with the slow-responding gas analysis [2] or based on measurements in a micro calorimeter outside the fermenter and therefore delayed [3]. The approach taken in our work made it possible to use a fast, heat flow based, control loop either alone or in combination with a slow control loop based on gas analysis.

In the previous work, the response of an yeast culture on glucose excess was studied [4]. The response characteristics regarding gas analysis and heat-flow measurements were used to determine time constants and gain factors for the control loop [5].

A known difficulty with heat-flow measurements is the presence of heat flows that are not dependent on biological processes (eg. stirring heat). This difficulty (zero offset) can be solved by basing the control loop not on the heat-flow measurement itself, but on its time derivative.

Slow changes in the heat flow (hour scale), as could be caused by changes in the viscosity of the broth, are not relevant for the fast control loop. These slow changes can be eliminated from the control if the time derivative is taken over a short period (20 s).

2. Experimental

The fermenter/calorimeter (Fig. 2) has been described before in detail [6,7]. *Saccharomyces cerevisiae* CBS 8066 was fed-batch grown with medium as described by van Urk et al. [8] but with a tenfold higher concentration of all components except the N-source, since the pH was controlled with 4 M ammonia which also served as N-source. Antifoam was added in parallel with the ammonia. The inoculum was taken from a continuous culture at D=0.05 h^{-1} as described by van Urk et al. [8].

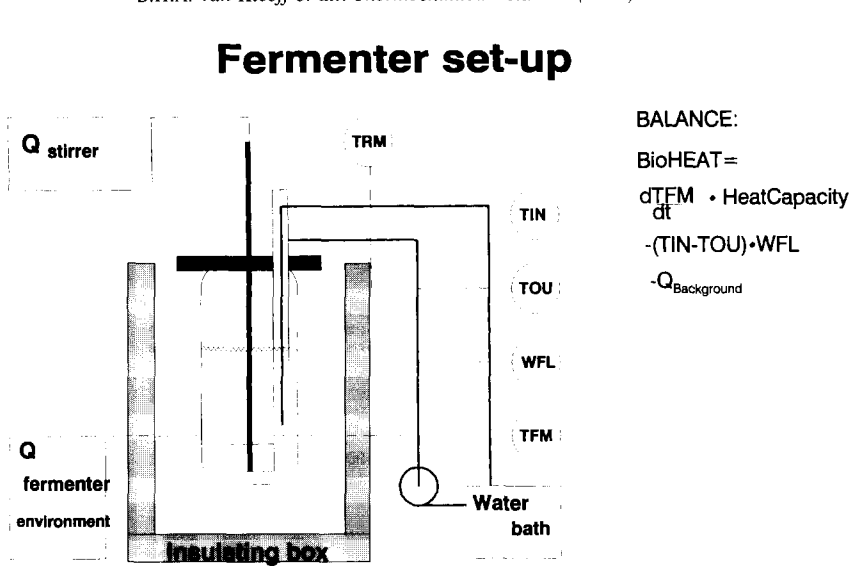

Fig. 2. The fermenter/calorimeter set up that was used during the experiments.

The glucose feed flow was controlled on the basis of a precalculated 'ideal' flow profile. During the experiments, this precalculated feed flow was corrected by control loops using heat-flow measurements and/or gas analysis. Only corrections that decreased the feed flow were allowed by the control algorithms. Moreover, a gradual return to the 'ideal' profile was incorporated in the control algorithms and the pump was not allowed by the algorithms to stop completely, so that a 'maintenance' feed was always provided.

Three different control regimes were tested:

(a) Based on heat-flow measurements alone (heat only).
(b) Based on heat-flow measurements and gas analyses (heat+RQ).
(c) Based on gas analyses alone (RQ only).

The test proceeded as follows:

The fed batch was grown at an initial growth rate of $0.25\ h^{-1}$, under the control regime chosen, until a biomass concentration of approximately $30\ g\ l^{-1}$ and a broth volume of 1 l were reached. Glucose (2 g) was then injected in the fermenter and the response of the control loops and the measured parameters was studied (DOT, pH, heat flow, consumption of alkali, acid and O_2 and production of CO_2).

3. Results

The tests were repeated at least thrice with very similar results. Typical results are shown in the figures. Fig. 3(a)–(c) shows the action of the control loop(s) on the glucose feed for the three control regimes and the results of the on-line gas analyses after the glucose injection at time 0 (zero). Fig. 4 shows the alkali

Table 1
Yield values (Cmol biomass/Cmol glucose) with different control regimes during 3300 s after adding 2 g (66.6 m Cmol) glucose. Yield value in glucose limited continuous culture=0.6

Control regime →	Heat only	Heat+RQ	RQ only
Yield calculated from CO_2 production and carbon balance	0.30	0.43	0.35
Yield calculated from O_2 consumption and balance of degree of reduction	0.35	0.50	0.43
Yield calculated from alkali consumption and nitrogen balance	0.43	0.47	0.44
Averaged yield value	0.36	0.47	0.41

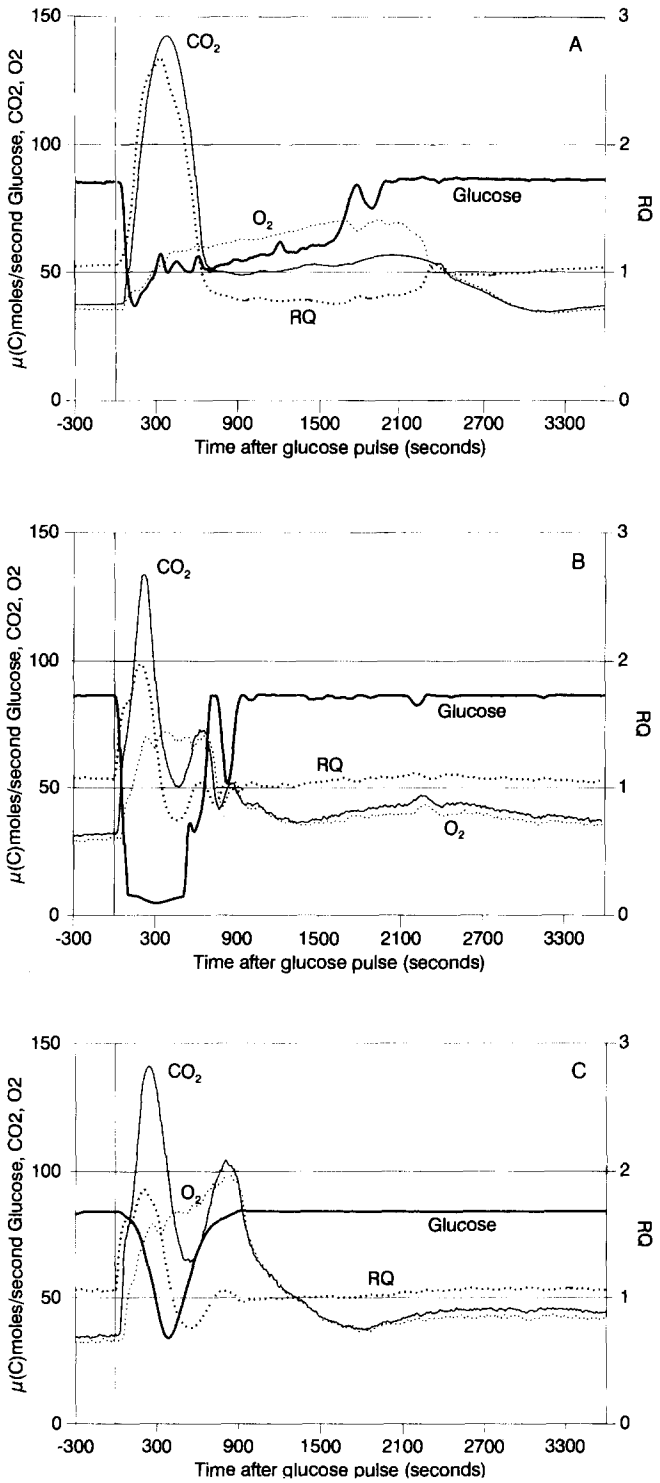

Fig. 3. (a) The glucose feed flow and the results of the outlet gas analysis after a 2 g. glucose pulse at time zero. The control regime was based on heat-flow measurements only (heat only), (b) As Fig. 3(a) but with a control regime based on heat-flow measurements and outlet gas analysis (heat+RQ), (c) As Fig. 3(a) but with a control regime based on outlet gas analysis only (RQ only).

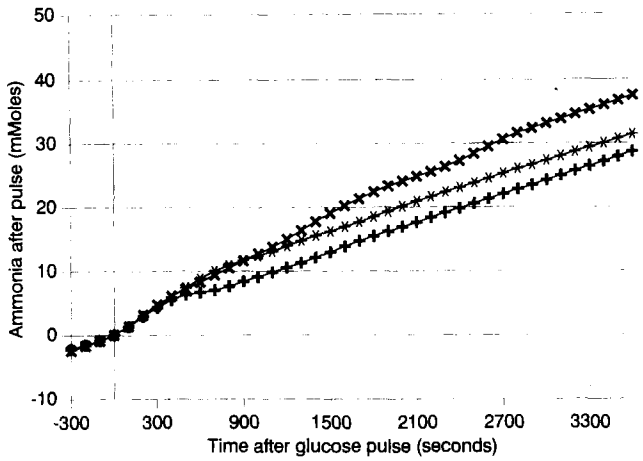

Fig. 4. Alkali consumption during glucose pulse experiments. X: control regime based on heat-flow measurements only (heat only), +: control regime based on heat-flow measurements and outlet gas analysis (heat+RQ), *: control regime based on outlet gas analysis only (RQ only).

consumption during the glucose pulse experiments for the three control regimes.

Table 1 summarizes the yield values that were calculated for the yeast from different measurements and balances. Direct measurement of the yield (from biomass determination) was not reliable because the increase in biomass during the glucose pulse experiments is very small as compared to the biomass already present.

4. Discussion

On the information that glucose excess is present in the broth, an experienced human operator would take the following control action:

- Decrease the glucose flow as quickly as possible.
- Restore the glucose flow to the original value when the glucose excess has been processed by the yeast (as indicated by the RQ return to the value before the glucose excess).

With the control regime based on heat-flow measurements only (Fig. 3(a)), the glucose flow decreases swiftly but not completely (the gain factor in this fast control loop could not be increased further without losing stability).

With the control regime based on heat+RQ (Fig. 3(b)), the combined action of the control loops drives the glucose flow swiftly (due to the fast – heat flow based – control loop) to an almost complete stop (due to the additional contribution of the slow – RQ based – control loop). This type of action closely resembles the action that would have been taken by an experienced human operator!

With the control regime based on RQ only (Fig. 3(c)), the glucose flow is decreased slowly and not completely (the gain factor in this slow control loop, also, could not be increased further without losing stability).

Judged from Fig. 3(a)–(c) the control regime based on heat+RQ performs better (more like an experienced human operator) than the individual control loops.

If the control regime performs well, the alkali consumption for pH control (and as N-source) should be disturbed as little as possible by the glucose injection. Extra alkali consumption indicates the formation of acetic acid which is difficult to metabolise. Its formation should therefore be avoided. From Fig. 4 it is clear that with the heat+RQ control regime the disturbance in the alkali consumption is less than with the other control regimes. Again the control regime based on heat+RQ can be judged to perform better than the individual control loops.

During yeast production, the yield of yeast on glucose should be kept high even in the presence of disturbances. As stated before, the yield could not be determined directly from mass determinations. However the values given in Table 1, calculated from different measurements and balances, indicate that the control regime based on heat+RQ performs better than the individual control loops.

5. Conclusion

In fed batch yeast production, it is possible to use heat-flow measurements to control the glucose feed flow in the presence of disturbances. Combination of the (fast) heat flow based control with (slow) control based on outlet gas analyses (RQ) can give results that are better than the results obtained with the individual control loops.

Acknowledgements

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