# Optimized fedbatch fermentation by use of online glucose measurement
# Advanced process control with multivariate feeding strategies

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## INTRODUCTION

In development of intelligent, adaptive substrate feeding strategies it is useful to integrate online-process-data for monitoring the physiological cell status which enables a suitable nutrient supply to increase the total product yield. Therefore we are using an **integrated multivariate glucose measuring tool** (*Trace C2 Control System by Trace Analytics GmbH*) combined with **software solutions** (*BlueVis software by BlueSens gas sensor GmbH*) which allow different types of feeding-strategies. The implementation of a regulator in a controlled fermentation can reduce overflow metabolism by regulating the glucose supply [2; 5]. In this context the use of the integrated-online software opens possibilities for the optimization of fedbatch fermentations by online measurements and process control. The increase of substrate supply can be automated to vary the feeding intervals and prolong the production phase by using “soft-sensors”. Feeding-Profiles which are depending on the current bacterial growth phase can cause a cellular adaption to the supplied mass of substrate. In consequence this work should lead to a variable and **feedback-controlled process regulation (FCPR)** which is dependent on nutrient claims of the cell. By using this FCPR-model a dynamic process control is achieved. The aim of this project is a better understanding of the influence of in-process-glucose-variations on microbial metabolic activities and resulting process optimizations.

## FEEDBACK-CONTROLLED PROCESS REGULATION – (FCPR) STRATEGY

Variations in glucose concentration have a measurable effect towards metabolism which leads to an adaption-process resulting in a differing substrate demand. The regulation strategy reacts to the current substrate consumption of the bacterial culture and enables an adapted substrate feeding. A specific increase of the cell’s own glucose transport systems can be achieved by short timed limitation of glucose. Under glucose limitation, the restricted amount of energy is used as efficient as possible due to the optimization of the affinity of glucose transporters [1; 3; 4].

**Calculation of current substrate consumption**

$$\Delta S = V(t0) * (Cs(t0) - Cs(t1))$$

**Calculation of time to reach adjusted minimal glucose threshold value**

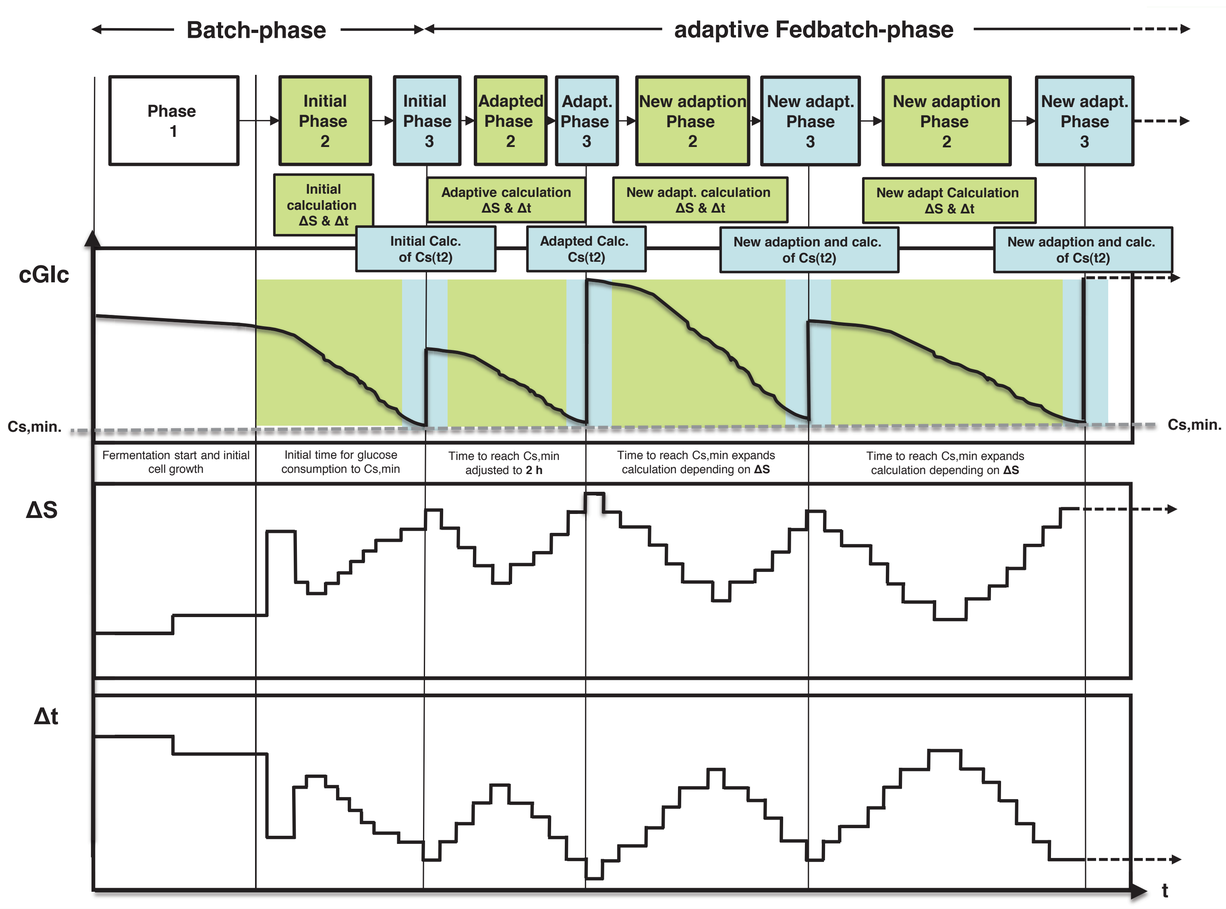
$$\Delta t = \frac{V(t0) * (Cs(t1) - Cs,min)}{\Delta S}$$

**Calculation of glucose concentration to feed for optimal substrate supply**

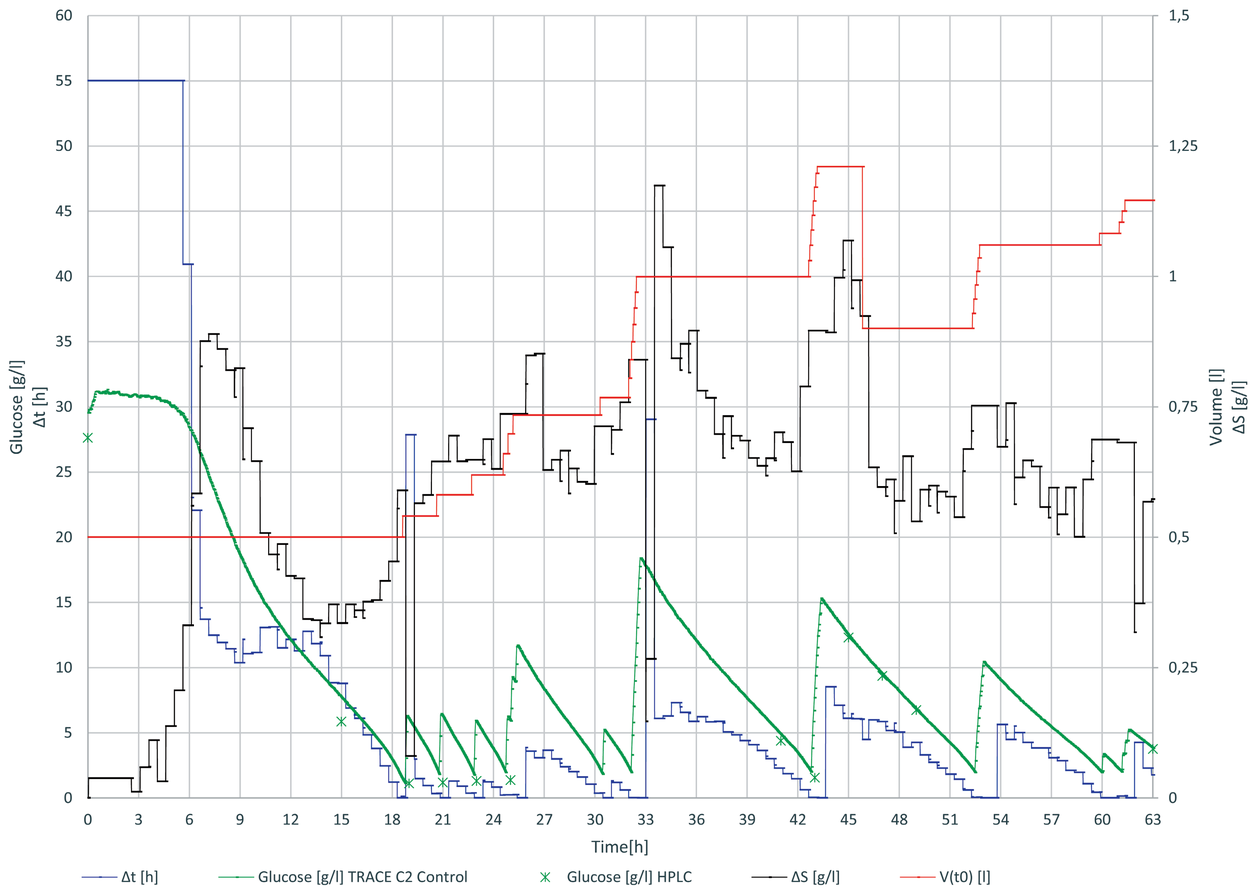
$$Cs(t2) = \frac{(\Delta S * \Delta(t1))}{(V(t0) + V(t1))\,\frac{\Delta S}{Cs,Feed}} + Cs,min$$

**Legend:**

| | | |
|---|---|---|
| $\Delta S$ | = | substrate-consumption-rate g/l |
| $V(t0)$ | = | volume at time 0 |
| $Cs(t0)$ | = | substrate concentration at t0 time |
| $Cs(t1)$ | = | substrate concentration at t1 time |
| $Cs, min$ | = | minimal substrate threshold value |
| $\Delta t$ | = | time span to reach Cs,min depending on ΔS |
| $\Delta(t1)$ | = | pre-adjusted time interval |
| $V(t1)$ | = | volume at t1 time |
| $Cs, Feed$ | = | substrate concentration of feed-medium |
| $Cs(t2)$ | = | substrate concentration to be added to the culture to reach Cs, min in adjusted time span |




## RESULTS: FED-BATCH FERMENTATION WITH ADAPTIVE FEEDING STRATEGY




## CONCLUSION AND OUTLOOK

- theoretical feed-regulation concept was developed
- successful transmission of “soft-sensor” based regulation strategy to real microbial fermentation process was shown
- adaptive and automated glucose feeding dependent on current substrate consumption of bacterial culture was possible
- automation and real-time calculation of substrate supply showed some deviations in real fermentation process from theoretical model
- further approaches will optimize the automated calculation of needed substrate

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TRACE Analytics
BlueSens
SPONSORED BY THE
Federal Ministry of Education and Research
FORSCHUNG AN FACHHOCHSCHULEN

LITERATURE:
[1] Ferenci, T. 2001. Hungry bacteria-definition and properties of a nutritional state; Environ. Microbiol. 3; (pages 605-611)
[2] Hass, Pörtner 2008. Praxis der Bioprozesstechnik; Spektrum 2 Auflage
[3] Ihssen J.; Egli T. 2005. Global physiological analysis of carbon- and energy-limited growing Escherichia coli confirms a high degree of catabolic flexibility and preparedness for mixed substrate utilization; Environ. Microbiol. 7 (1568-1581)
[4] Lin H.Y., Mathiszik B., Xu B., Enfors S.O., Neubauer P. 2001. Determination of the maximum specific uptake capacities for glucose and oxygen in glucose-limited fed-batch cultivations of Escherichia Coli; Department of Automatic Control, Institute of Technology TOC vol. 73, Issue 5 (pages: 347-357)
[5] Luli, G.W.; Strohl W.R. 1990. Comparison of Growth, Acetate Production, and Acetate Inhibition of Escherichia coli Strains in Batch and Fed-Batch Fermentations; Department of Microbiology, The Ohio State University, 484 West 12th Avenue, Columbus, Ohio