Process optimization by the use of substrate feeding strategies with an integrated online glucose measuring tool

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Introduction

In the biotechnological production of market-relevant industrial chemicals, fed-batch fermentation is a common method to obtain high cell densities and thus higher space-time yields. On this occasion, substrate supply is an important parameter for process regulation. Glucose is the most important substrate for microorganisms and animal cell lines in bioprocesses because of its high amount of carbon. A controlled and defined substrate feeding is necessary for an optimal supply of nutrients, which results in a better cell growth and thus better production rates. Excess glucose causes overflow metabolism which results in accumulation of different degradation products during glycolysis. The accumulation of acetate results in decrease cell growth and an inhibition of the metabolic activities [1]. Therefore it is possible to control cell growth and the productivity by using a regulated feeding strategy. Within this subject two methods are compared to decrease unpredictable by-products within a substrate regulation. Previous work showed that a defined level of glucose-concentration (50 mM) provides enough substrate to afford adequate cell growth and productivity and avoids acetate and glucose accumulations caused by overflow metabolism [2].

Regulation principles

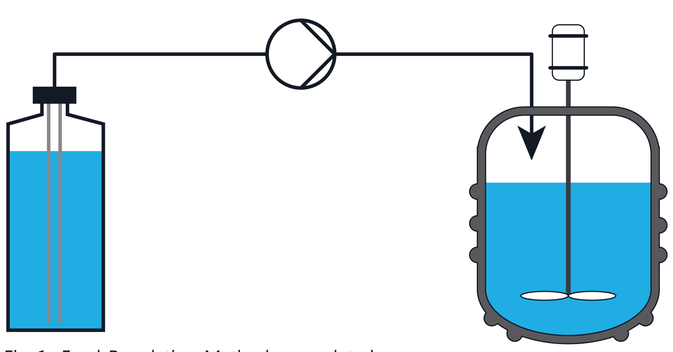

Fig. 1: Feed-Regulation-Method unregulated
A fixed inflow profile is use to keep the substrate concentration on a defined level

- Method based on calculations from experimental data
- Fixed Inflow profile is use to keep the substrate-concentration on a defined level

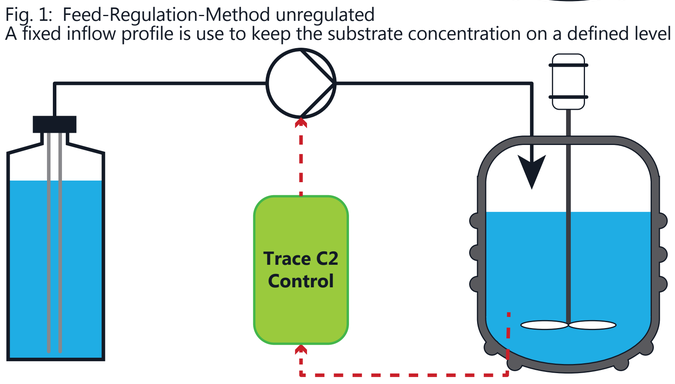

Fig. 2: Feed-on-demand-Regulation-Method online glucose monitoring
The Regulation of the feeding itself can be controlled via PID logic

- Feed on demand regulation
- Integrated glucose monitoring tool Trace C2 Control analysis-system is use to keep the substrate-concentration on a defined level
- The regulation of the feeding itself can be controlled via PID logic.

Dialysis measuring method

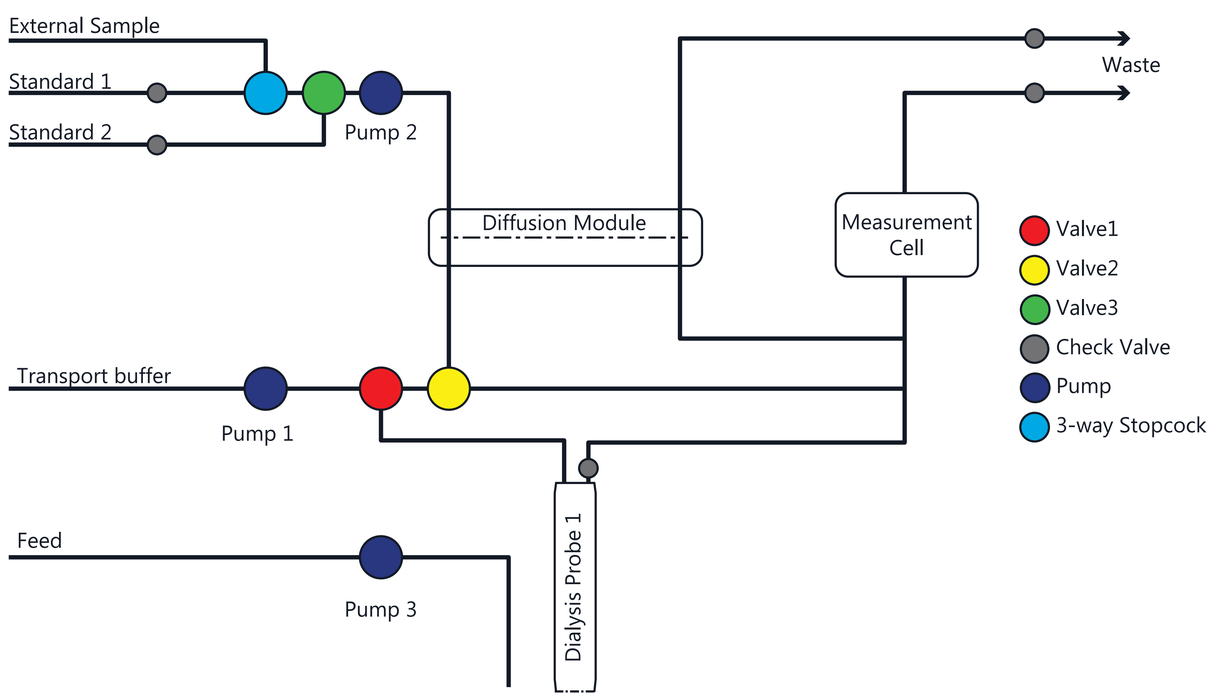

Fig. 3: Measuring principle of the dialysis method (TRACE C2 Control System)
Continuous analysis of the glucose concentration is carried out in combination of enzymatic reaction and electrochemical detection [3].

Results

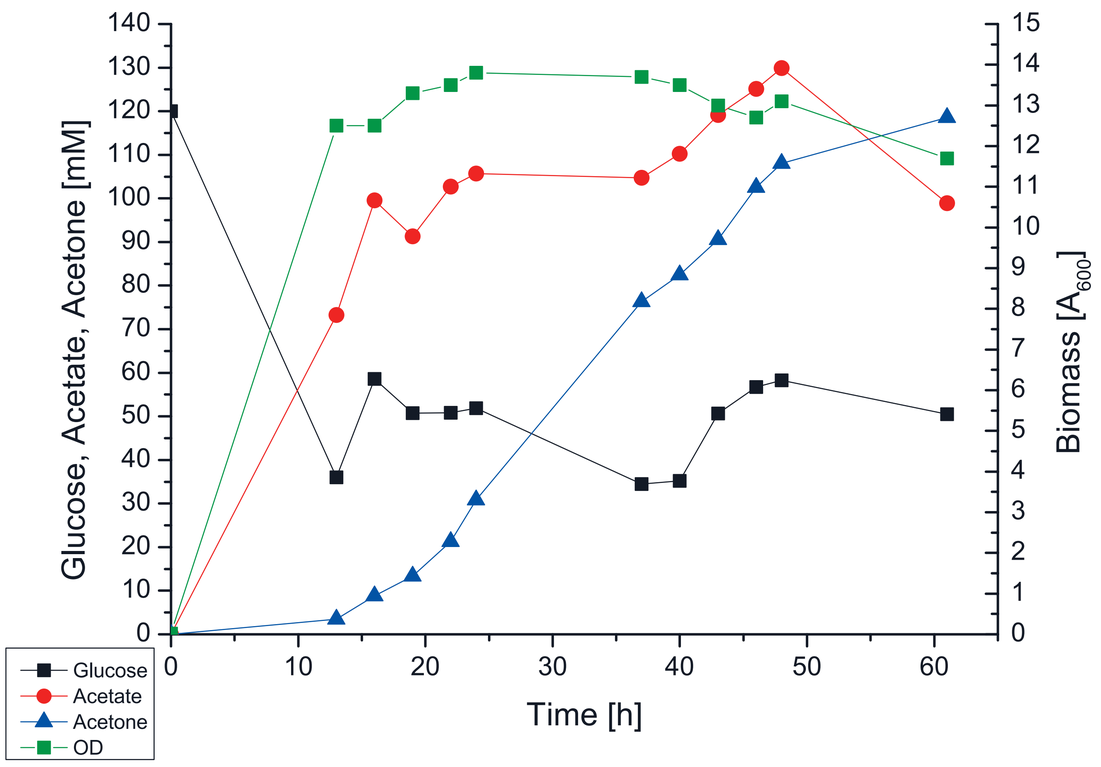

Fig. 4: Fermentation of recombinant *E.coli:* Feed unregulated
Determination of glucose, acetone, acetate, ethanol in mM against time h; fixed inflow profile (Feed: 2, 5 ml / h); filling volume: 750 ml SD8-complex media; organism: *E. coli;* oxygen supply via PTFE membrane; 30°C process temperature; stirring at 800 rpm

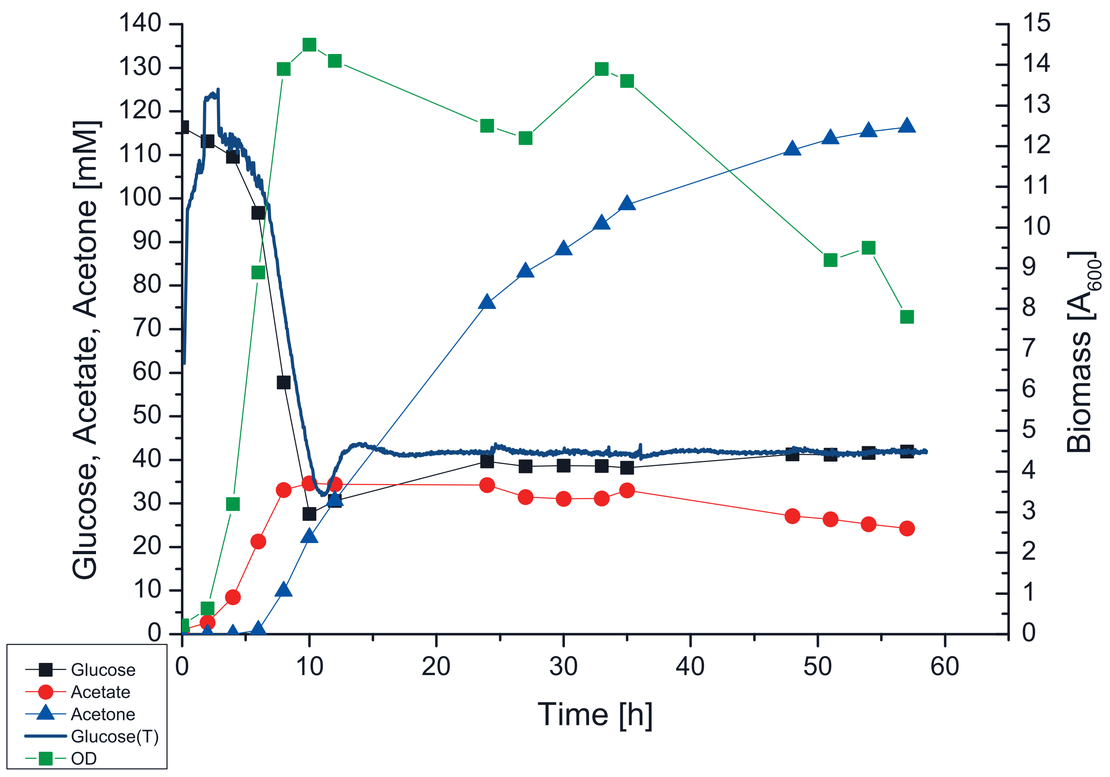

Fig. 5: Fermentation of recombinant *E.coli:* Feed-on-demand
Determination of glucose, acetone, acetate, ethanol in mM against time h; Glucose Feed by Trace C2 Control analysis-system; PID adjustment: P: 0, 15; I: 45 minutes; D: 2 minutes; filling volume: 500 ml SD8-complex media; organism: *E. coli;* oxygen supply via PTFE membrane; 30°C process temperature; stirring at 800 rpm

- By using the fixed inflow profile the glucose concentration fluctuates significantly
- Exact setting of the glucose concentration is not possible
- Acetate concentration is three times higher → toxicity
- Vague feed control → measurement of online glucose concentration not possible
- The time of the feeding must be estimated

- Online glucose measurement of the Trace C2 analysis-system
- Substrate-concentration is triggered by the system on an optimal level
- Less acetate production → lower amount of toxic by-product

Conclusion

- Feed control without online glucose monitoring causes acetate formation due to overflow metabolism
- Online glucose monitoring possible by using Trace C2 Control system
- Interactive modification of the Trace C2 Control software generates a dynamic feeding
- Fed-batch strategy reacts to the needs of the bacterial culture and creates adapted substrate feeding

Outlook

The most used feeding strategies are based on "trial and error" principles. The here introduced regulation model could lead to optimized fedZ-atch processes because it can be transferred on other cultivations resulting in increased productivity. By using an integrated glucose measuring tool combined with software solutions, different types of fed-batch strategies are possible and will be tested in additional approaches.

References

[1] Luli; Strohl (1990): Comparison of growth, acetate production, and acetate inhibition of Escherichia coli strains in batch and fed-batch fermentations; Appl. Environ. Microbiol. (vol. 56 no. 4; 1004-1011)

[2] Bittner (2014): Integrative Methode zur beschleunigten Entwicklung von Bio-Produktionsverfahren am Beispiel der nachhaltigen Herstellung einer Industriechemikalie

[3] TRACE Analytics GmbH (2014): Technische Dokumentation Trace C2 Control (Version 1)

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