Application report: Glucose Control

Method: Dialysis
Measurement range: Glucose 0.5 – 20 g/L

TRACE Analytics

INTRODUCTION

Glucose

Glucose is by far the most important substrate for microorganisms and animal celllines in bioprocesses. In over 90 % of all microbial cultivations and in many animal cell cultivations it is used as carbon source. Monitoring and control of glucose concentrations is required for several processes.

The online analyser TRACE C2 Control allows a rapid and precise determination of Glucose and Lactate concentrations inside the bioreactor.

MEASUREMENT PRINCIPLE

The enzyme glucose oxidase (GOD) is used for the detection of glucose. In presence of oxygen, glucose oxidase catalyzes the transformation of β-D-Glucose to D-Glucono-δ-lactone and hydrogen peroxide. The Glucose content is measured indirectly via the formed peroxide, which is re-oxidized to oxygen during the amperometric measurement (Figure 1). The resulting electrical current at the electrode is directly proportional to the amount of oxidized Glucose.

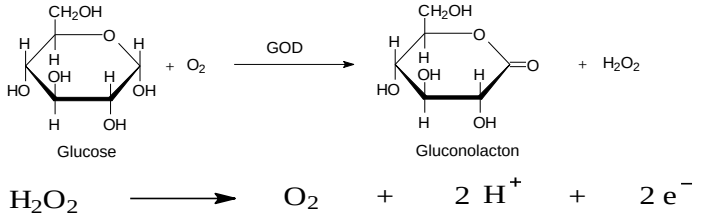

Figure 1. Enzymatical reaction of Glucose

GLUCOSE CONTROL

It is beneficial to control the glucose concentration on a certain level to reduce the formation of by-products. In most of the processes low glucose concentration in the medium will be preferred (substrate limitation).

Controller

TRACE C2 Control is a device that simultaneously measures and controls the substrate concentration. It has two different controller types integrated. These are a PID controller and a Min-Max controller.

PID Controller

Feeding in cell culture cultivation and in microbial fermentation can be reliably controlled with the integrated PID controller. The PID controller consists of three interacting single controllers. This are the proportional controller (**P**), the integral controller (**I**), and the derivative controller (**D**) (Minorsky[1]). The control action of the controller is described by a differential equation (Figure 2).

$$u(t) = K_P \times e(t) + \frac{K_P}{T_N} \times \int_0^t e(\tau)d\tau + K_P \times T_V \frac{d}{dt} e(t)$$

Figure 2. Differential equation of the PID controller

The persistent control deviation of the proportional part of the controller can be balanced with the integral part. With the derivative part of the controller it is possible to react to fast changes of concentrations inside the reactor.

The principle of the PID controller is known since 1922 (Minorsky[1]), but useful rules for adjustment were not available since 1942 (Ziegler and Nicols[2]). Today most of the controllers are adjusted by empirical design. This design can also be used in the field of biotechnology.

Min-Max Controller

This is a simple on-off controller (Figure 3), where the feeding of Glucose is set to a minimal or maximal feed rate. The advantage is that adjusting this controller is very easy.

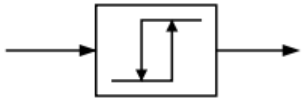

Figure 3. Symbol of an on-off controller

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SYSTEM PERFORMANCE

Disturbance reaction (Step response)
Disturbance reactions were performed in a glass fermenter with a filling volume of 1.5 L. The set point was adjusted to 5 g/L glucose and a glucose consumption rate of 1 g/(L*h) was applied. A feed solution containing 200 g/L glucose was automatically fed with the internal pump. The system was disturbed by addition of water and the glucose concentration of 5 g/L was dropped to 3 g/L. With the step response the parameters of the PID controller were optimized using empirical design (Figure 4). The control range (±10%) can be reached in 15 minutes (green line) instead of 50 minutes (red line).

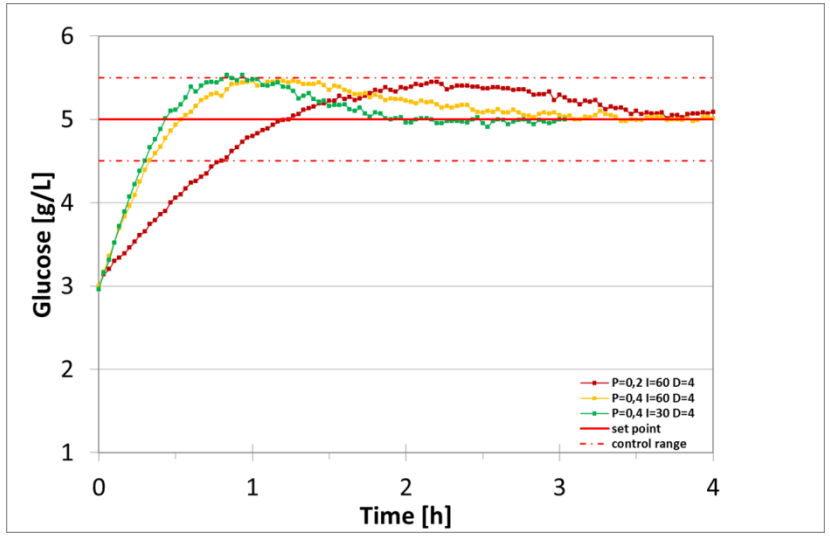

Figure 4. Different adjustments of the PID controller and the influence on step response.

Glucose control in a recombinant *E.Coli* fermentation (Acetone production)
With an optimal setting of the controller a recombinant *E.coli* fed-batch cultivation was performed. The selected PID settings were P=0.15, I=45, and D=2. Starting with a glucose concentration of about 20 g/L. During the batch phase the cells start to consume substrate. The set point for the fed-batch control was adjusted to 7.5 g/L glucose.

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The set-point was reached and maintained shortly after starting the feeding (vertical line in Figure 5). The online glucose concentrations (blue line in Figure 5) were confirmed by offline measurements (HPLC).
With this feeding strategy the acetone production is comparable with other feeding strategies, but a much lower amount of toxic by-products is formed.

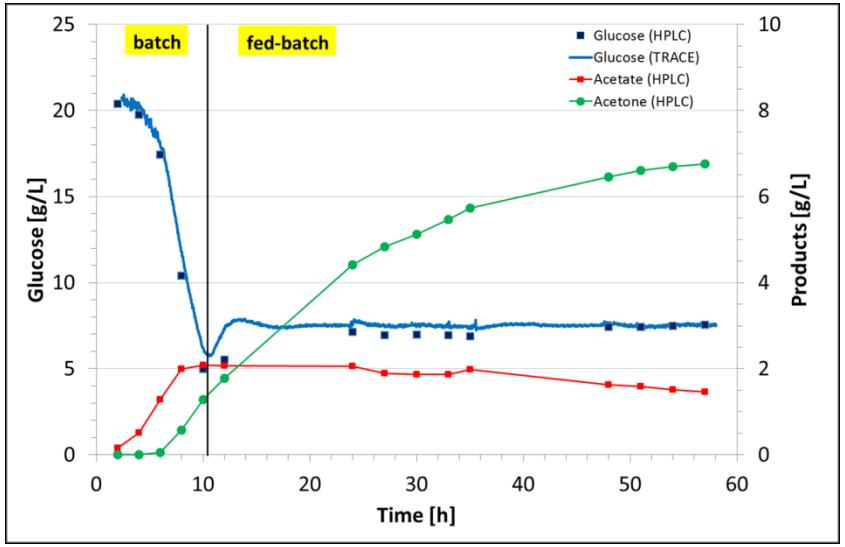

Figure 5. Acetone production with a recombinant *E.coli*

Feeding without online glucose monitoring causes increased acetate formation due to overflow metabolism. The formation of the by-product acetate can be reduced more than three times with feed control by using TRACE C2 Control.

Literature
[1] Minorsky, Nicolas: Directional stability of automatically steered bodies; J. Amer. Soc of Naval Engineers 34 (1922), pp. 280–309.
[2] Ziegler,J.G. and Nicols, N.B.: Optimum settings for automatic controllers; Trans. ASME, 64 (1942), pp.759-768