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Implementation of a new method for online glucose control in a 10 L CHO bioprocess

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Introduction

More than 75% of recombinant therapeutic proteins are produced using mammallien cell culture process. During the last 15 years a great deal of effort has been spend towards monitoring and controling such processes. Due to the complexity of the utilized biological system there are many important process parameters to be monitored and controlled. The glucose concentration - as the main carbon source - is one of the most important ones. In this contribution, a simple method for online glucuse measurement is presented. The glucose concentration is directly measured based on an enzymatic reaction. This method is then used to control the glucose level in a fed-batch fermentation at a constant level.

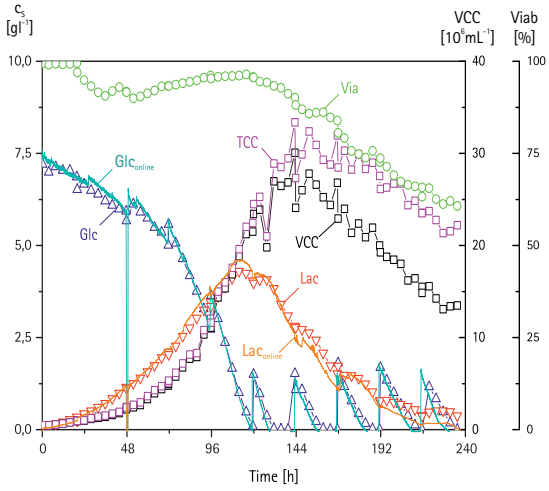

Fig. 1: Progression of most important offline variables of a typical fed batch process

CHO-K1 Cultivation Process

CHO-K1 cells are cultivated in a 7.5 L CHOMACS CD Medium containing 7.5 g/L glucose. The initial cell concentrations is 0.4 Mio cells/mL. pO2 is controlled at a setpoint of 40% via gasmix sparging and agitation control. An open loop feeding strategy is applied where the process is fed every 24 hours with feed medium containing 40 g/L glucose and 5.5 g/L glutamine. The most important offline variables of a typical fed-batch process are plotted in figure 1. Metabolites are measured with a YSI 2900 analyzer. Total and viable cell concentrations is measured using a CEDEX device. For online monitoring of glucose and lactate a dialysis probe with a 19 mm standard port from TRACE analytics is used. The analytes in the transport solution are measured at-line based on an enzymatic reaction (glucoseoxidase). The measurement frequency can be as low as 2 min. Considering the reaction time constants in mammalian cell culture processes this method can therefore be considered an online measurement.

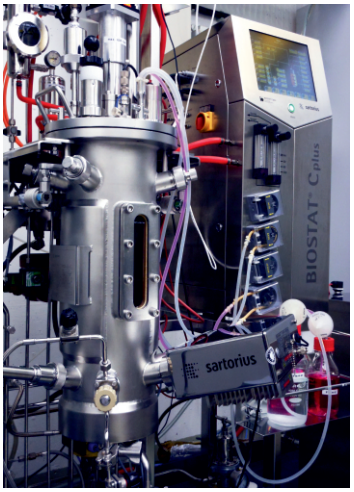

Fig. 2: Stainless steel stirred CSTR BIOSTAT Cplus 10

Setup

The technical set up is shown in figure 3. The measurement signal is forwarded from the TRACE device to the BlueVis software via a MODBUS/TCP interface. The signal is written on the software integrated OPC server which is accessed by the SCADA System MFCS. All process data are collected by the data managementsoftware SIPAT (Siemens).

The control algorithm is programmed in a python enviroment. Every 3 minutes a new measurement value is acquired. In case of two successive values below the set point, the feed pump will pump a defined volume of 5 mL glucose feed into the bioreactor. The glucose concentration in the feed is 75 g/L.

As soon as the concentration of glucose had reached the desired set point during the process, the feed solution for the open loop feed (Feed A) was replaced by another feed without glucose (Feed B) and the glucose feed (Feed Glc) for closed loop control. Hence the media was still supplied with important amino acids.

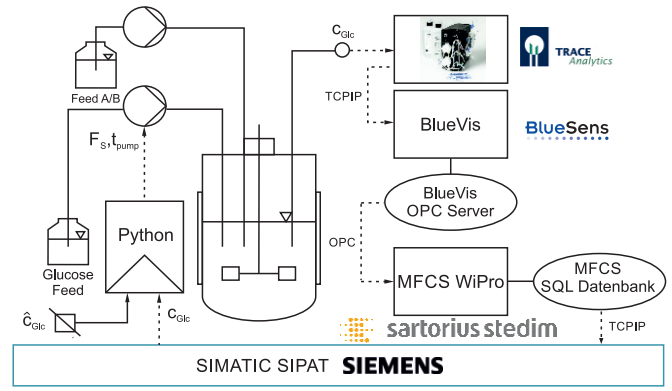

Fig. 3: Technical setup for glucose control in a 10 L CHO cultivation process in a stainless steel vessel

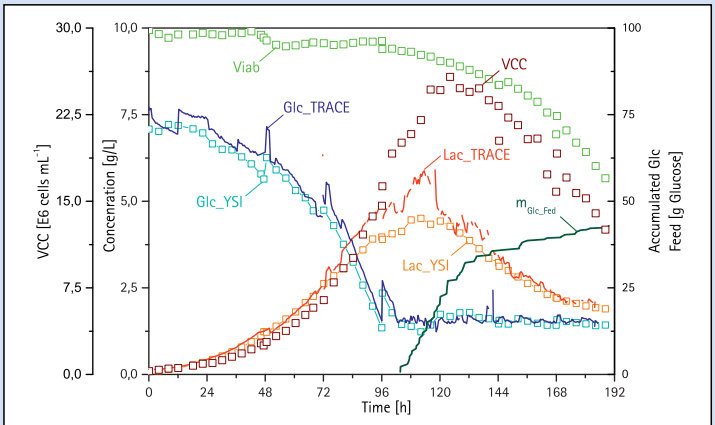

Fig. 4: Fed-Batch process with controlled glucose level at 1.5 g/L glucose

Discussion

As can be seen from figure 4, glucose was succesfully controlled at a setpoint of 1.5 g/L in a range of ± 0.1 g/L. The level of glucose consumption is constant during the whole feeding. However there is a shift in the glucose consumption rate at 132 hours which is caused by a decrease of viable cell concentration. This event can be derived from the slope of the accumulated glucose which was added to the reactor (m_{Glc_Fed}). The viability of cells starts to decrease after 5 days, when the lactate concentration peaks at 4.5 g/L according to offline data. Too high lactate concentrations can have an adverse effect on cell growth. In order to reduce the maximal lactate concentration and the metabolic shift to lactate consumption might be shifted to earlier process times. This might be achieved by replacing Feed A by Feed B one day earlier and by controlling the glucose level at a set-point of 0.5 g/L. By implementing the demonstrated IT- and process design, glucose control was realized in a automated, knowledge based fashion demonstrating the power of the chosen process data management system.