Application of a self constructed off gas analyser in the education of bioengineers

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Our University of Applied Sciences, FH Campus Wien, offers a degree program in 'Bioengineering'. In the course of this study a fermentation laboratory has to be attended. The aim of this course is the design, operation and analysis of a bioprocess experiment. The students have to use their biological, mathematical and technical skills to solve this exercise.

One of the experiments involved cultivation of the methylotrophic yeast Pichia pastoris (X33); a well known host for recombinant protein expression (Cregg et al. 2000), as well as for applications in white biotechnology (e.g. riboflavin (Marx et al. 2008)). An overnight shake culture was used to inoculate a defined 2 l batch medium (as described in Maurer et al. 2006) with 40 g glucose L-1 as sole carbon source, to a starting optical density (OD600) of 1.0.

The cultivation was carried out in a 5.0 l bioreactor (Minifors, Infors, Bottmingen-Basel, Switzerland; figure 1 B) with a tailored off gas analyser. This off gas analyser consists of a BCP-CO_2, a BCP-O_2 probe (BlueSens, Herten, Germany) and a mass flow controller (Vögtlin, Aesch, Switzerland) with a power supply in a separate control box (figure 1 A). The analogue signals were directly led to an I/O input of the bioreactor and measured as control parameters in the monitoring software (IRIS, Infors).

Figure 1: A) self assembled off gas analyser

Figure 1: B) bioreactor with off gas analyser

The fermentation temperature was controlled at 25°C, pH was controlled at 5.0 with addition of 25% ammonium hydroxide and the dissolved oxygen concentration was maintained above 20% saturation by controlling the

stirrer speed between 250 and 1200 rpm and the air flow between 2.0 and 5.0 l min-1.

Samples were taken frequently over the whole process and analysed as described below. Three aliquots of 10 ml of culture broth were centrifuged and the supernatant saved for HPLC analysis. The pellets were washed in distilled water and recentrifuged, transferred into weighed beakers and dried at 105°C until a constant weight was attained. The biomass concentration was also monitored with an on-line probe (Fogale nanotech, Nimes, France), which had previously been calibrated with dry cell mass data (CDW).

Glucose and ethanol were analysed by HPLC (Shimadzu,

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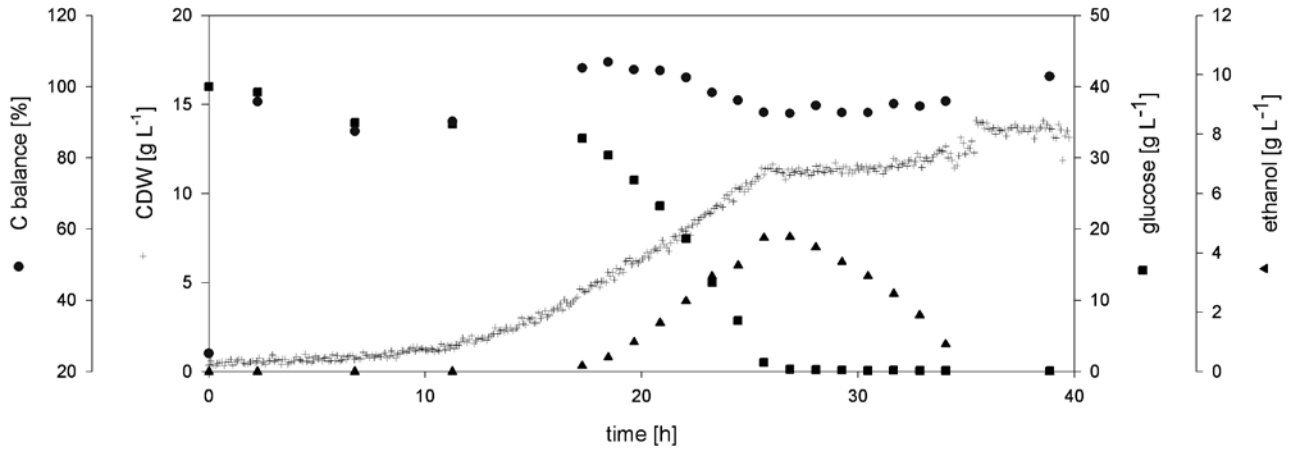

Figure 2: A) Trends of measured cultivation parameters glucose - (squares), ethanol – (triangles) and bio mass concentration (crosses), as well as the carbon balance (circles).

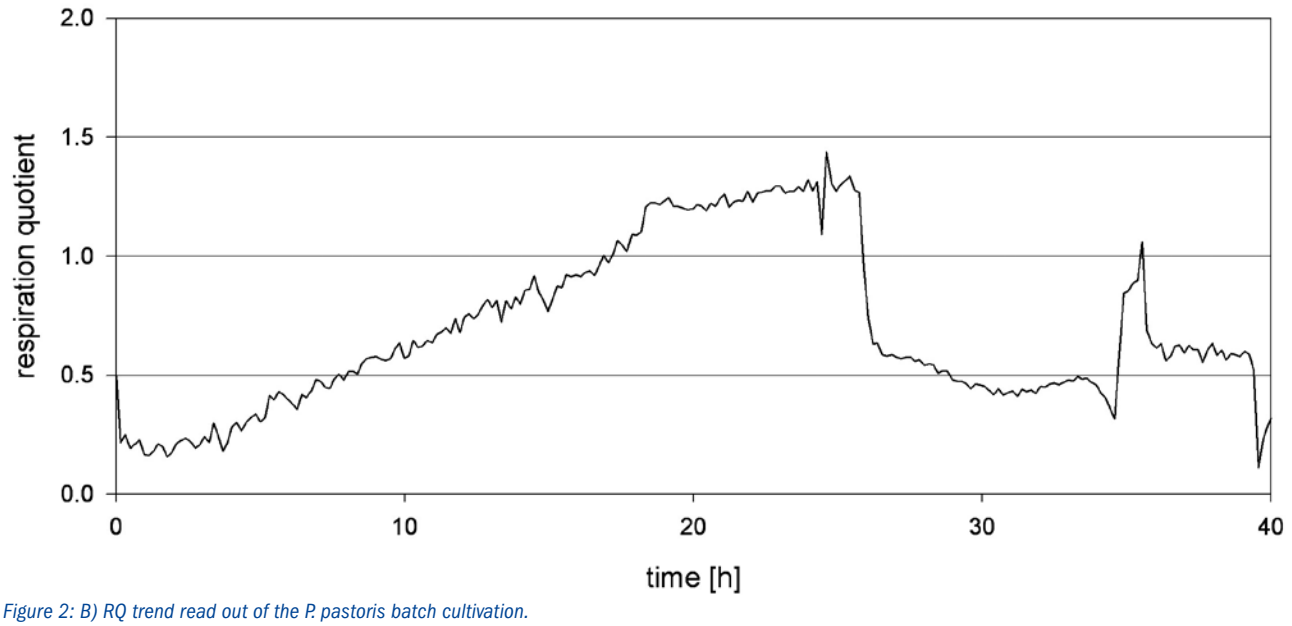

Figure 2: B) RQ trend read out of the P. pastoris batch cultivation.

Japan) using an ion exchange column Aminex HPX-87H (Bio Rad). The mobile phase was 15 mM sulphuric acid.

The aim of the exercise was the calculation of typical fermentation parameters such as biomass concentration, substrate uptake rate, specific growth rate, and so on, as well as the respiratory quotient (RQ) and the over all carbon balance (OCB). Using the universal gas equation and the recorded oxygen and carbon dioxide concentration [%] and the air flow data. The students were able to calculate the oxygen uptake rate (OUR), the carbon dioxide evolution rate (CER) and hence the required RQ and OCB.

Figure 2 A shows the diauxic behaviour of this yeast strain, first using up glucose as preferred substrate (specific glucose uptake rate qGlucose= 0.44 g g-1 h-1) and forming ethanol with a rate of qP ethanol= 0.08 g g-1 h-1 as by product. After a first stationary phase the ethanol was utilised with a rate of qethanol = 0.04 g g-1 h-1. The online measurement of the oxygen and carbon dioxide concentrations enabled the simultaneous determination of the shift based on the calculated RQ, which changed from 1.2 during the aerobic glucose consumption to 0.5 during the ethanol utilization. The carbon utilisation was therefore balanced with a tolerance of 93-105%. These online measurements therefore serve as teaching vehicles enabling the students to grasp application and value of off-gas analysis.

Literature

Cregg, J., J. Cereghino, J. Shi & D. Higgins (2000) Recombinant protein expression in Pichia pastoris. Mol Biotechnol, 16, 23-52. | Marx, H., D. Mattanovich & M. Sauer (2008) Overexpression of the riboflavin biosynthetic pathway in Pichia pastoris. Microb Cell Fact, 7, 23. | Maurer, M., M. Kuehleitner, B. Gasser & D. Mattanovich (2006) Versatile modeling and optimization of fed batch processes for the production of secreted heterologous proteins with Pichia pastoris. MICROBIAL CELL FACTORIES, 5, -.