**Report from**

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# On-line biomass estimation of yeast fed batches using off gas analysis of carbon dioxide and oxygen

**Introduction**

Cultivation of cells is the most important unit operation in nearly all biotechnological production processes. Measurement, monitoring and control of cultivations are therefore a permanent topic in the industrial and scientific community.

The biomass, the productive fraction of the process, is one of the most interesting parameters. Many techniques have been developed to measure the biomass concentration, using different physical principles, mathematical models or combinations thereof, reviewed by Olsson L. (1997).

We evaluated the oxygen uptake rate (OUR) and the carbon dioxide evolution rate (CER) as potential values for a biomass software sensor, an approach described by Petkov and Davis (1996). Because the yeast *Pichia pastoris* is in our scientific focus, we determined the necessary model coefficients in chemostat cultivations with glycerol or glucose as carbon source. The resulted models for the biomass evolution were tested in a batch and standard fed batch.

Such application can be very beneficial for all bioreactors equipped with an off gas analyser, avoiding an invasive biomass sensor. This approach further meets the aim of “process analytical technology” (PAT), monitoring the quality of a process by quantification of our “cell factories”.

**Machinery assembly**

A 5.0 L bioreactor system (Minifors, Infors, Bottmingen-Basel, Switzerland; Figure 1) has been used for the cultivations. Compared to the standard setup, the system has been extended by the implementation of the balance signals for feed, harvest and base consumption, a mass flow controller (Vögtlin, Aesch, Switzerland) for the air supply and the off gas analyser BlueInOne (BlueSens, Herten, Germany) measuring carbon dioxide concentration, oxygen concentration, absolute humidity and temperature. The analogue signals were directly led to an I/O input of the bioreactor and locked as parameters in the monitoring software (IRIS, Infors).

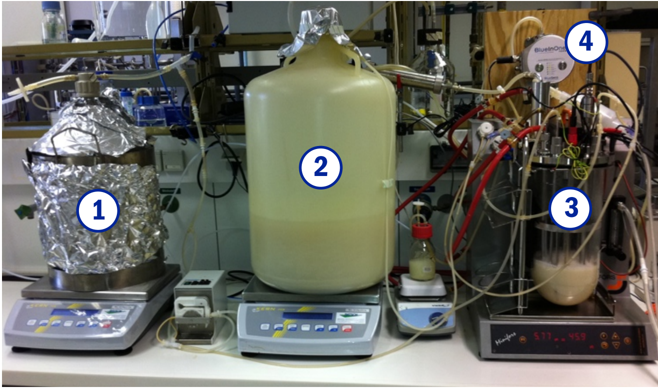

Figure 1: Experimental setup for the chemostat cultivations. 1. Feed balance, 2. Harvest balance, 3. Bioreactor and 4. BlueInOne off gas analyzer

**Material and Methods**

The *P. pastoris* strain SMD1168H (protease deficient) was used in this study. For the pre culture a shake flask containing 100 mL of YPG medium (per liter: 10 g yeast extract, 10 g peptone, 10 g glycerol) was inoculated with the *P. pastoris* strain, and incubated at 28°C for approximately 24 hours and agitated at 180 rpm. This culture was used to inoculate the starting volume in the bioreactor to a starting optical density of 2.0.

The cultivation temperature was controlled at 25°C, pH was controlled at 5.85 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 adjustion of the air flow from 4.2 to 15.0 L min$^{-1}$.

Synthetic batch, chemostat and fed batch medium were used as described in Maurer et al. (2006), just varying the carbon source glycerol or glucose. The chemostat cultures were performed at specific growth rates ranging from rates 0.025 to 0.15 h$^{-1}$. Steady state samples were taken after 5 residence times each. The fed batch was performed according to a standard protocol with a constant feed rate of 9.25 g fed batch medium L$^{-1}$ batch h$^{-1}$ (Gasser et al. 2006). For the biomass determination 5 mL of culture broth was centrifuged, the pellet was washed once with distillated water transferred to a pre weighted beakers. The beakers were dried at 105°C to constant weight. All samples were analyzed in duplicates.

**Method of Calculation**

The oxygen uptake rate (*OUR*) [g h$^{-1}$] is calculated by the equation of the ideal gas law (1) and the difference of the inlet and outlet concentration of oxygen.

$$OUR = \frac{p \cdot M_{O_2}}{R \cdot T}\left(F_{AIR,in} \cdot \frac{c_{O,cal}}{100} - F_{AIR,out} \cdot \frac{c_{O_2,out}}{100}\right) \quad (1)$$

The carbon dioxide evolution rate (*CER*) [g h$^{-1}$] is calculated applying the difference of the outlet and inlet carbon dioxide concentration.

$$CER = \frac{p \cdot M_{CO_2}}{R \cdot T}\left(F_{AIR,out} \cdot \frac{c_{CO_2,out}}{100} - F_{AIR,in} \cdot \frac{c_{CO,cal}}{100}\right) \quad (2)$$

Because just the incoming air flow ($F_{AIR,in}$) is measured and controlled, the off air flow ($F_{AIR,out}$) has to be calculated with a compensation based on the nitrogen balance:

$$F_{AIR,out} = F_{AIR,in} \cdot \frac{\left(1 - \frac{c_{O_2CAL}}{100} - \frac{c_{CO_2,CAL}}{100}\right)}{\left(1 - \frac{c_{O_2,out}}{100} - \frac{c_{CO_2,out}}{100} - \frac{c_{H_2O,out}}{100}\right)} \quad (3)$$

The further parameters are the pressure* *p* [kPa], the molar mass of oxygen $M_{O_2}$ = 32.00 [g mol$^{-1}$], or the molar mass of carbon dioxide $M_{CO_2}$ = 44.00 [g mol$^{-1}$], the ideal gas constant *R* = 8.314472 [J mol$^{-1}$ K$^{-1}$] and the temperature* *T* [K] (*...measured by BlueInOne).

Results and Discussion

We based our biomass prediction on the oxygen balance, or the carbon balance for aerobic growth (Petkov and Davis 1996), respectively.

$$OUR = m_{O_2/X} \cdot X + \frac{1}{Y_{X/O}}\frac{dX}{dt} \quad (4)$$

$$CER = m_{CO_2/X} \cdot X + \frac{1}{Y_{X/CO}}\frac{dX}{dt} \quad (5)$$

For steady state conditions the equations (4) and (5) could by formed as follows:

$$OUR = \left( m_{O_2/X} + \frac{1}{Y_{X/O}} \cdot D \right) \cdot X \qquad (6)$$

$$CER = \left( m_{CO_2/X} + \frac{1}{Y_{X/CO}} \cdot D \right) \cdot X \qquad (7)$$

The correlation between oxygen uptake rate (OUR) and the carbon dioxide evolution rate (CER)in relation to the specific growth rate $\mu$ was experimentally determined in chemostat cultures (Figure 2). The dependence of OUR on $\mu$ and CER on $\mu$ is described in equation (6) and (7). The values for the maintenance coefficients for oxygen $m_{O/X}$ or carbon dioxide $m_{CO/X}$ , as well as the yield coefficients of oxygen $Y_{X/O}$ or carbon dioxide $Y_{X/CO}$ were derived by the method of least squares, i.e. the parameters $m_{O/X}$ and $Y_{X/O}$ were modified in such a manner that the sum of the deviations from the experimental data squared reached a minimum. An additional condition is that they are greater than zero. The results are summarized in Table 1.

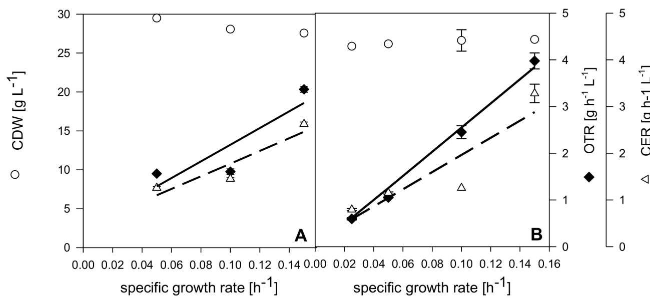


Figure 2: Biomass concentration (open circles), OUR (black diamonds) and CER (open triangles) for A) glycerol and B) glucose limiting chemostats, as well as the respective approximations, OUR model (solid line) and CER model (dashed line).

| | Glycerol | Glucose |
|---|---|---|
| $m_{O/X}$ [g g$^{-1}$] | 0.010 | 0.000 |
| $Y_{X/O}$ [g g$^{-1}$] | 1.471 | 1.039 |
| $m_{CO/X}$ [g g$^{-1}$] | 0.012 | 0.006 |
| $Y_{X/CO}$ [g g$^{-1}$] | 1.926 | 1.466 |

Table 1: Values of maintenance coefficients and yield coefficients

Biomass software sensor calculation

The OUR and CER was measured during the cultivations. Assuming that the coefficients (Table 1) are constant the equation 4 and 5 could be integrated to yield the biomass X (t).

Figure 2 shows the biomass trends of a glycerol batch.

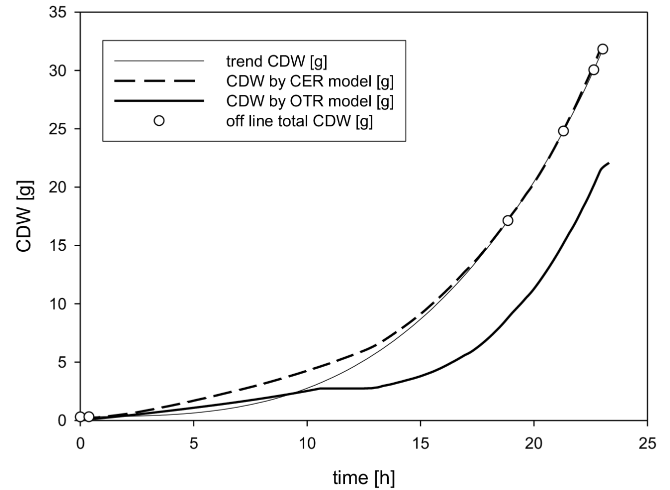


Figure 3: Biomass trend of a glycerol batch: off line total cell dry matter (open circles), smoothed regression of CDW (thin line), biomass trends calculated by OUR model (solid line) and CER model (dashed line).

The prediction by the CER model shows a great correlation with the off line biomass data. During the experiment an error of the OUR locking occurred between hour 10 and 12.5, however the missing data can not explain the weak fitting of the OUR model trends during the first ten hours. At least form hour 15 till the end of the batch the OUR biomass trend follows a nearly identical slope as the off line data.

A glycerol batch followed by a glucose fed batch is illustrated in Figure 4. The applied predictions by the glycerol OUR and glycerol CER model could well predict the biomass at the end of the batch. However, the predictions for the fed batch failed completely. That can have several reasons, the coefficients could not be determined correctly and the additional conditions are set wrong or the model equation holds not for the glucose consumption. To overcome this more effort has to be put into the error determination. The maintenance coefficients and yields should be

determined in chemostats with different biomass concentrations or fed batch cultivations as well as remaining substrate and by products should by measured.
In summary the biomass prediction by the CER model is a promising tool for biomass estimation in glycerol batch cultivations and worth for a permanent implementation in PLS.

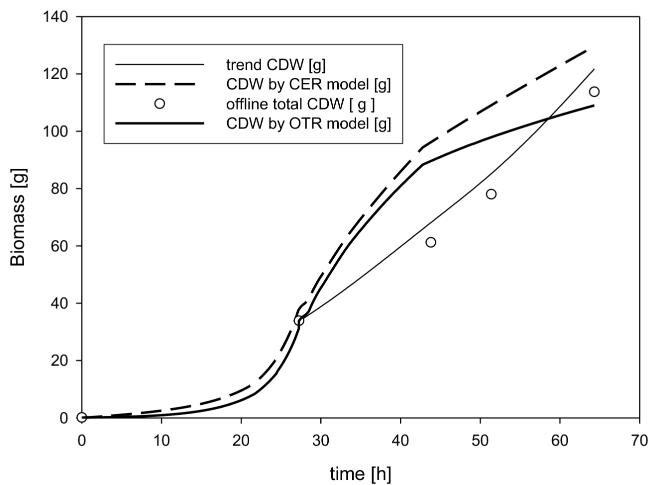


Figure 4: Biomass trend of a glycerol batch followed by a glucose fed batch: Off line total cell dry matter (open circles), smoothed regression of CDW (thin line), biomass trends calculated by OUR model (solid line) and CER model (dashed line).

**Literature**

Gasser, B., M. Maurer, J. Gach, R. Kunert & D. Mattanovich (2006) Engineering of Pichia pastoris for improved production of antibody fragments. *Biotechnol Bioeng,* 94, 353-61.

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The University of Applied Sciences Vienna, Department of Bioengineering, educates students in the field of applied biotechnology. Further we have a strong focus on research and development in the field of microbial strain improvement for recombinant protein production, metabolic engineering bioprocess engineering and bioinformatics. The scope of the projects within bioprocess engineering reaches from monitoring and modelling to optimization and control of biotechnological processes.

**Project team**

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