A Generic Biomass Soft Sensor and Its Application in Bioprocess Development

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Abstract

Biomass concentration is a key process variable, which is used to identify trends and initiate process events in microbial bioprocesses. Real-time data on the biomass is essential to implement advanced control strategies, including the control of biomass-specific nutrient uptake

Introduction

In developing a bioprocess, researchers incur significant costs to determine the optimal culture conditions and install the process control technology to maintain them [1, 2, 3]. They routinely monitor and control the temperature, pH, and availability of oxygen in the culture, but these are not the only relevant process parameters. A time-resolved determination of the biomass concentration is also of great importance, enabling them to not only monitor cell growth, but also to establish an optimal supply of substances, like nutrients, co-factors, and inducers. The demand for them changes over time as the culture grows. To manage the availability of nutrients we must know how much of them is consumed. The nutrient supply can be described by the biomass-specific nutrient uptake rate at a given point in time (qs(t)) (Box 1).

There are several methods to determine the biomass concentration in a microbial bioprocess. The choice of the best method for high-cell-density fermentations depends strongly on the demands of the application. At the production stage, robustness and accuracy are the main demands, while cost and transferability are of limited concern [4]. Methods based on light scattering

Christoph Herwig’s group at Technische Universität Wien (TU Wien) has developed a biomass soft sensor based on readily available measurements and process parameters. They used the soft sensor to control various substrate uptake rates in parallel small-scale bioreactors.

*Fig. 1: In many bioprocesses carbon is metabolized to biomass and \( \text{CO}_2 \) by oxidative transformation. Out of known or measured amounts of carbon (substrate), \( \text{O}_2 \) and \( \text{CO}_2 \) that are introduced to and leave the bioreactor, a soft sensor can calculate the biomass.*
and transmittance, like optical density measurement, are widely used. They are inexpensive and easy to implement, but their application range is limited to certain biomass concentrations.

Researchers at TU Wien developed a biomass soft sensor as an alternative method for biomass determination. The term soft sensor combines the words software and sensor. The idea behind it is to use easy-to-determine parameters to calculate variables that are more difficult to obtain by direct measurement. The soft sensor delivers readings like a conventional hardware sensor, but uses a mathematical model to determine the sensor signal based on other measurements [5].

The soft sensor for biomass determination described here uses mass balancing. It is based on the hypothesis that in a bioprocess carbon is metabolized to biomass and CO₂ by oxidative transformation. This applies to the most industrially used bacterial, mammalian, and yeast cell lines. From the amount of carbon that goes into the bioreactor (quantified, for example, by measuring the amount of substrate that is fed in) and leaves the bioreactor (quantified, for example, by measuring the CO₂ concentration in the exhaust), we can estimate the biomass concentration (Fig. 1). Feeds and outflows of the bioreactor are quantified using standard online measurements, and the software calculates the biomass based on a data model.

This application note describes the successful implementation of such a biomass soft sensor in an Eppendorf DASbox® Mini Bioreactor System using the DASware® control software. The biomass estimate was used to evaluate and control the biomass-specific substrate uptake rates of different nutrients in high-density E. coli fermentations. The combination of the biomass soft sensor with an optical density sensor enables full automation of the multi-bioreactor system for bacterial processes.

### Material and Methods

The project comprised two steps. First, the researchers implemented the soft sensor. Then they used it to set up control loops to control the biomass-specific uptake rates of different substrates in E. coli high-density fermentation processes (Fig. 3). For example, they show results on the simultaneous control of the biomass-specific uptake rates of glucose and lactose.

#### Soft sensor implementation

**Setup of data model**

As the first step of the soft sensor implementation the
researchers set up a data model that describes the oxidative transformation of carbon to biomass and CO₂. The mass balance system is depicted in Figure 2. One can solve the material balances of carbon and free electrons (C balance and DoR balance). The rates of substrate uptake, carbon evolution, oxygen uptake, and biomass formation have to be estimated [6]. Table 1 summarizes the measurements and constants which are used to do so. The substrate uptake rate is determined from the pump flow rate. The rates of carbon evolution and oxygen uptake are calculated from exhaust composition and mass flow.

The additional system redundancy allows for system reconciliation. The residuals ε are assigned to the rates according to their measurement error. A statistical test (h-value) evaluates the system integrity, which applies if the residuals can be explained solely by measurement errors [7].

In this application, the researchers designed the soft sensor to work in strictly C-source-limited fed-batches or

Table 1: Needed measurements and constants for rate estimation.

<table>
<thead>
<tr>
<th>Rate</th>
<th>Description</th>
<th>Measurement</th>
<th>Constant parameter</th>
<th>Assumed error</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_s ) [C-mol/h]</td>
<td>Substrate uptake rate</td>
<td>Pump flow rate [L/h]</td>
<td>C-concentration (C-mol/L)</td>
<td>2 %</td>
</tr>
<tr>
<td>( r_{co2} ) [mol/h]</td>
<td>Carbon evolution rate (CER)</td>
<td>CO₂ and O₂ concentrations (off gas analyzer [%] and mass flow [sL/h])</td>
<td>In-gas composition, evaporation</td>
<td>3 %</td>
</tr>
<tr>
<td>( r_o2 ) [mol/h]</td>
<td>Oxygen uptake rate (OUR)</td>
<td></td>
<td></td>
<td>3 %</td>
</tr>
<tr>
<td>( r_x ) [C-mol/h]</td>
<td>Biomass formation rate</td>
<td>Calculated by soft sensor</td>
<td>Elemental composition of biomass (g/C-mol)</td>
<td>( \approx10 % )*</td>
</tr>
</tbody>
</table>
continuous cultures where the product and possible by-products can be neglected.

Creation and integration of soft sensor algorithm
Once they had established the data model, the researchers created the soft sensor algorithm in MATLAB®. They incorporated it into the DASware control software through a Visual Basic control script that accesses the function. The results are stored, and can be visualized directly, within the DASware control software (Fig. 4) without the need for further customization or the establishment of OPC connections. Additional features like automated phase transition from batch to fed batch and induction as well as control options were included.

Figure 3 summarizes how the soft sensor is integrated into the bioprocessing environment.

Soft sensor calibration
The bioprocesses were run in two phases. In the initial batch phase the cultures grew to a cell dry weight (CDW) of up to 10 g/L. During this phase, the biomass concentration can be measured accurately with optical density sensors. At the end of the batch phase, the actual biomass concentration measured with the OD sensor was used to start the soft sensor.

High-density fermentation
Culture conditions
The researchers conducted the fermentations in a DASbox® Mini Bioreactor System (Eppendorf AG, Germany) equipped with glass vessels or BioBLU® 0.3f Single-Use Vessels. Both vessel types have a maximum working volume of 250 mL.

They used a recombinant *E. coli* strain, which bears a green fluorescent protein (GFP) gene under the control of the LAC promoter. The bacteria were cultivated in a chemically defined medium with a limited amount of C-source (10 g/L glucose).

After C-source depletion, glucose and inductor (lactose) were fed to the culture with a DASGIP® MP8 multi pump module (Eppendorf AG, Germany).

The pH was maintained at 7.2 by NH₃ addition, which served as an additional N-source. Air saturation was kept over 30 % by increasing the stirrer speed and oxygen concentration in the inflow air, which was supplied with 2 VVM through an L-sparger. The five M18 ports were occupied by pH, DO, and OD sensors (Eppendorf DASGIP OD4 Module, 880 nm), gas inlet and gas outlet. Three dip tubes were used for feed, base addition, and offline sampling.

The exhausted gas was analyzed by a DASGIP GA4 gas sensor module (Eppendorf AG, Germany) with a ZrO₂ sensor for O₂ and infrared CO₂ sensor technology.

Offline measurements
To determine the cell dry weight (CDW) the researchers centrifuged 2 mL of culture broth (4,500 x g, 4°C, 10 min), washed the cell pellet with a 0.1 % NaCl solution, and subsequently dried it at 105°C for 48 h.

Cell-free samples of the cultivation broth were analyzed
for concentrations of substrates and metabolites using HPLC (Agilent Technologies, USA) with a SUPELCOGEL™ C-610 H ion exchange column (Sigma-Aldrich, USA) and a refractive-index detector (Agilent Technologies, USA). The mobile phase was 0.1 % H₃PO₄ with a constant flow rate of 0.5 mL/min, and the system was run isocratically at 30°C.

Results

To demonstrate the functionality of the system, the researchers carried out experiments with a recombinant *E. coli* strain bearing a GFP gene under the control of the LAC promoter. They aimed to simultaneously control the availability of lactose and glucose, to equilibrate the bacterial growth, energy maintenance, and productivity.

Lactose was used instead of IPTG to induce the GFP hosting LAC operon. Lactose is metabolized by the host strain, so for continuous protein production it must be continuously added to the culture.

The researchers tested different glucose-to-lactose ratios in four parallel fermentations. Each fermentation run used the same setpoint for the glucose uptake rate $q_{s_{\text{glu}}}$ but varied the lactose uptake rate $q_{s_{\text{lac}}}$. To control $qs$, the biomass concentration was estimated online using the soft sensor. Based on this data, the pump flow rate was automatically adjusted to control the supply of a mixed feed containing lactose and glucose.

Figure 5 shows optical density and the soft sensor-based biomass concentration estimates of one fermentation. The biomass concentration deriving from offline measurements (black crosses), OD correlation (blue line) and soft sensor (orange line) are depicted.

The data derived from the biomass soft sensor showed good alignment with the reference measurements. The biomass soft sensor application achieved an accuracy of < 10%, which is within its working range [5]. The soft

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**Fig. 5:** Biomass concentration estimates.
Crosses: Biomass concentration (cell dry weight, CDW) deriving from offline measurements.
Blue line: OD correlation.
Orange line: Results from soft sensor.
NRMSE: Normalized root mean square error

**Fig. 6:** Setpoints and time courses of $q_{s_{\text{glu}}}$ (solid lines) and $q_{s_{\text{lac}}}$ (dashed lines) in the four fermentation runs (bioreactor A-D). spt: setpoint
Conclusion

The researchers implemented a relatively simple and straightforward method for biomass estimation in a small-scale parallel bioreactor system. The soft sensor works for strictly C-source-limited fed-batch and continuous cultures. The researchers used it to simultaneously control the uptake rates of a substrate and an inducer. The results obtained are fully scalable and applicable in industry without the need for expensive additional measuring equipment. Besides manual offline sampling and sample volume reduction, the described fermentations could run fully automatically.

In bioprocess development, the control of biomass-specific substrate uptake rates is a valuable tool to determine the optimal supply of nutrients and/or inducers. The researchers performed similar studies with Pichia pastoris strains to tune the pAOX promoter. Repressing glycerol was used as the C-source and methanol as the inducer.

The Eppendorf bioprocess control software DASware control 5 (former version DASGIP control 4.5) offers the interface for the integration of soft sensors. The soft sensor algorithm and process control strategies are developed by the end-user.
Literature


### Ordering information

<table>
<thead>
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<th>Description</th>
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<tr>
<td>DASbox® Mini Bioreactor System for Microbial Applications, max. 25 sL/h gassing</td>
<td>76DX04MB</td>
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<td>DASbox® Mini Bioreactor, 2 Rushton-type impellers, 60 – 250 mL, overhead drive</td>
<td>76SR0250ODLS</td>
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<td>BioBLU® 0.3f Single-Use Vessel, fermentation, 2 Rushton-type impellers, sterile, 4-pack</td>
<td>78903509</td>
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