Application Note

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Real-Time and Online OD600 Measurement for Cell Growth Monitoring

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Abstract

Real time monitoring of cell concentration enables an operator or automated feedback loop to detect and intervene at the point when growth ceases and the process yield is maximized. Real-time analysis for turbidity may also be used to enable alarms, to notify operators of abnormal shifts in the cell growth cycle and therefore preventing any production loss. It is a common request from the industry, to correlate the raw Absorption Unit (AU) values from BioPAT® Fundalux to an industry standard offline OD600 spectrophotometer. This can be accomplished with MFCS SCADA software, where the raw AU values are fed into MFCS, linearized to an OD600 spectrophotometer.

The objective of this guide is to provide direction on how to use data from the Fundalux measurement and how to convert this data into an OD600 value.

Introduction

Continuous total biomass measurement, coupled with other parameters such as DO, pH and temperature, enables the user to understand and optimize bioprocesses. Turbidity sensors can be used to measure the density of total biomass in bacteria, yeast, or mammalian cell cultures directly in the bioreactor.

Collected data from the bioreactor can be used to
- determine best nutrient feed rate
- when to reduce feeding
- when to initiate an induction
- when to stop fermentation and initiate harvest
- inoculate the next bioreactor automatically as soon as a certain cell density is reached in a seed bioreactor

All this leads to an optimal cell growth, consistency of the final product, reduction of production times and elimination of unnecessary process delays.

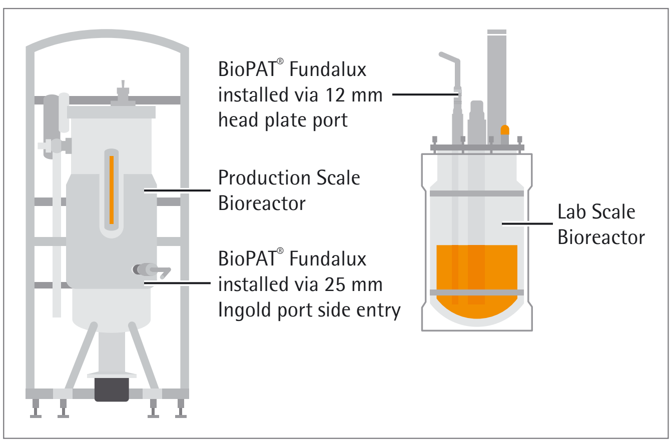


Figure 1: Typical fermentation setup with online cell density measurements using a BioPAT® Fundalux 25 mm probe in a production – and a BioPAT® Fundalux 12 mm probe in a lab scale bioreactor

Turbidity Sensors

To address these requirements, Sartorius offers BioPAT® Fundalux series of photometric probes. These near-infrared (840 nm – 910 nm) absorption probes are specifically designed for use in sanitary fermentation and cell culture applications from R&D to production scale. The seal-less, sapphire window design assures the highest level of sterility. BioPAT® Fundalux probes precisely measure cell growth | total biomass as a function of absorption.

BioPAT® Fundalux series sensors are available in a selection of optical path lengths of 1, 5 and 10 mm. Shorter optical paths are typically used for dense cell cultures such as bacterial and yeast cultures. Longer path lengths are used for mammalian cell cultures. This flexibility allows optimal resolution and superior signal-to-noise ratio for virtually any cell line.

BioPAT® Fundalux 12 mm probes probes can mount conveniently through an industry-standard PG13.5 thread in the bioreactor head plate and the 25 mm probe connections use a standard 25 mm Ingold port side entry. For the 25 mm sensor a horizontal installation is recommended, for easy insertion | removal and to prevent entrapped gas at the top of the port (see figure 1).

BioPAT® Fundalux sensors are designed for CIP | SIP and the 12 mm models can be autoclaved. These probes are suitable for cGMP facilities and conform to FDA requirements. The measured data can be transferred via the serial connection established with the local DCU controller. The AU (absorption unit) output is conveniently displayed on the DCU visual display in real time.

Inline and Real-Time OD600 Measurement

BioPAT® Fundalux probes provide direct, real-time measurement of cell growth without the need for sampling, thereby eliminating volume loss and dilution errors (common with OD600 spectrophotometer), all while reducing the risk of contamination. The in-process measurement can be correlated directly to laboratory analysis methods for cell count and OD600 optical density (see figure 2 and 3). This linearized value can then be displayed in the measurement unit of choice (e.g. WCW, DCW, OD600).

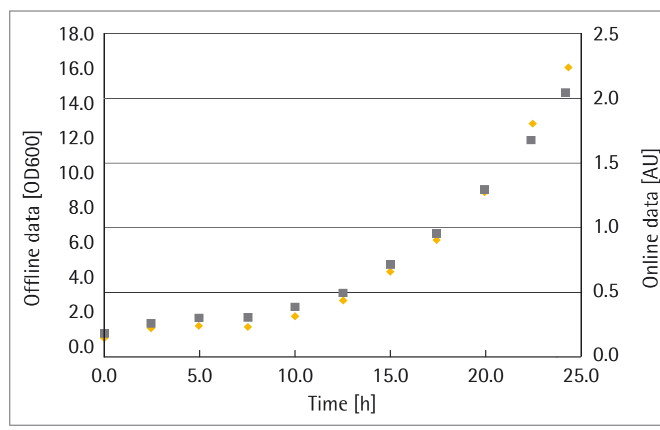


Figure 2: Online and offline data of a 24 h fermentation

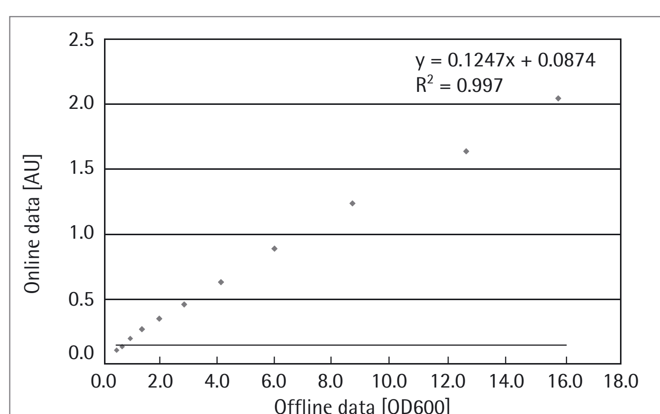


Figure 3: Correlation of online and offline data

How to establish relationship between inline turbidity measurement in AU and OD600 offline measurement

Inline measurement with Fundalux sensors is performed at a wavelength of 840 – 910 nm in order to exclude influences on the measurement signal by the color of the medium.

For OD600 offline measurements the samples need to be diluted to stay within the measurement range of commonly used offline photometers or spectrometers. As a result, offline OD600 signal stays linear over a wider turbidity range in the bioreactor.

Because of these two effects, a correlation between inline and offline measurement needs to be performed for every individual process.

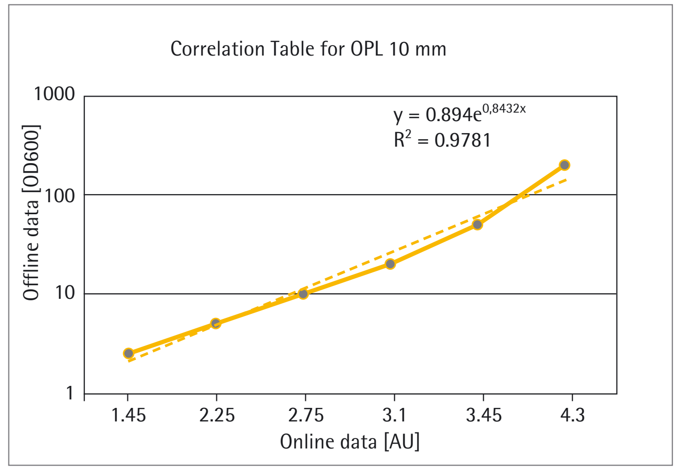
The objective of this guide is to provide direction on how to use data from the Fundalux turbidity measurement and how to correlate this data to the offline OD600 measurements in the lab. Data provided in this guide are only examples and must not be used for direct implementation into your software!

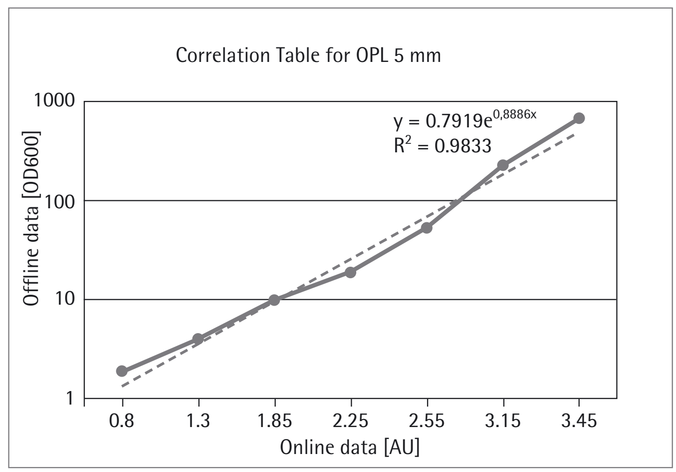
For an exact conversion, it is necessary to capture raw measurement data from the cultivated microorganism and to build a correlation table comparing Fundalux' AU results to OD600 spectrophotometer measurements. In general, it is necessary to gather a minimum of 6 data points, which are evenly spread across the range of total biomass density. All data, which are shown here, are exemplary data and are based on measurements with plastic beads. They are not representative for measurements in a real cultivation. The measured AU range is not the same for every optical path length (OPL), so there is a need to select the right OPL based on the expected OD. The following table shows the relations.

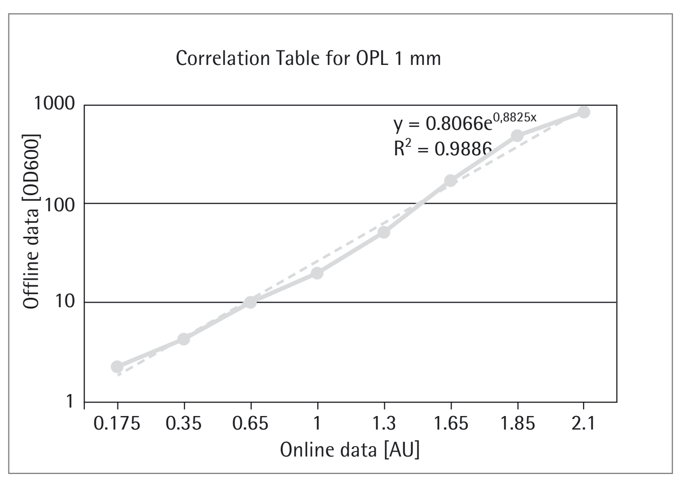
AU (10 mm)	AU (5 mm)	AU (1 mm)	OD600
1.45	0.80	0.18	2.50
2.25	1.30	0.35	5.00
2.75	1.85	0.65	10.00
3.10	2.25	1.00	20.00
3.45	2.55	1.30	50.00
4.30	3.15	1.65	200.00
	3.45	1.85	500.00
		2.10	900.00

Table 1: Relation AU and OD at different OPL

Based on these relations, graphs can be generated. The trend lines are shown in the following graphs. The equations are representing the trend lines accordingly.







In the following example the conversion is described for the sensor with OPL 10 mm. Based on the equation of the trend line the following result is achieved:

$$OD = 0.894e^{0.8432 * AU}$$

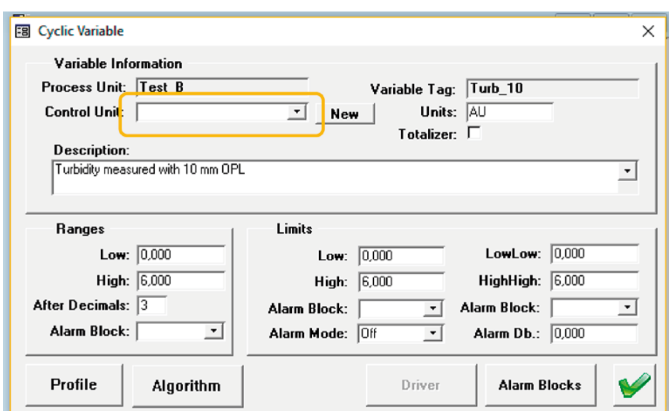
$$AU = \frac{1}{0.8432} * \ln\left(\frac{OD}{0.894}\right)$$

How to use MFCS to automatically convert AU into OD600

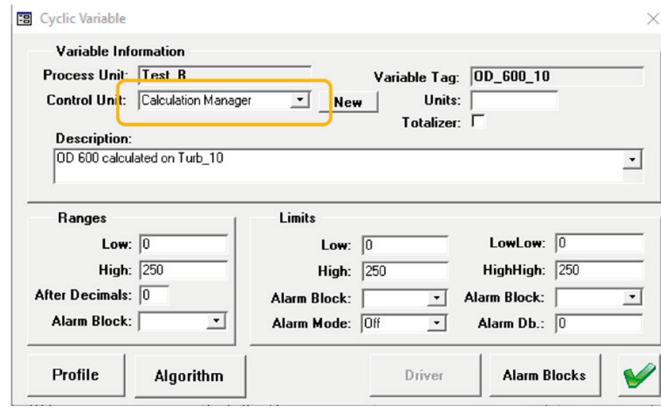
The whole process can also be automated, when the raw AU values are available in the local control unit (DCU) of the BIOSTAT® bioreactor and forwarded to an MFCS SCADA system.

1. Implement turbidity value of the DCU in the MFCS configuration

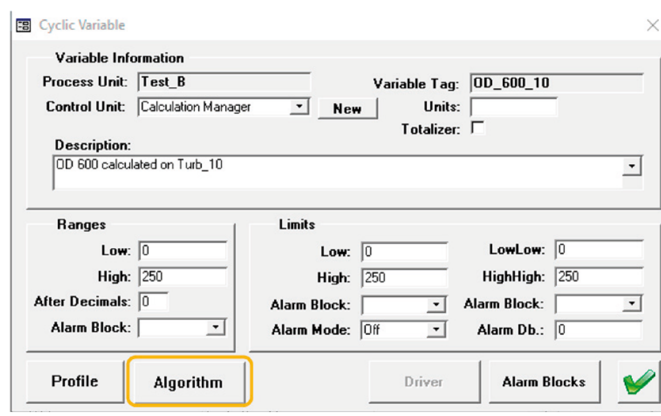
The AU value of the Fundalux sensor represents the raw value. This value has a range from 0 to 6 AU and is submitted by the DCU. In MFCS you have to configure the value as a new "Cyclic Variable" (e.g. "Turb_10"). As "Control Unit" select the respective BIOSTAT® system:



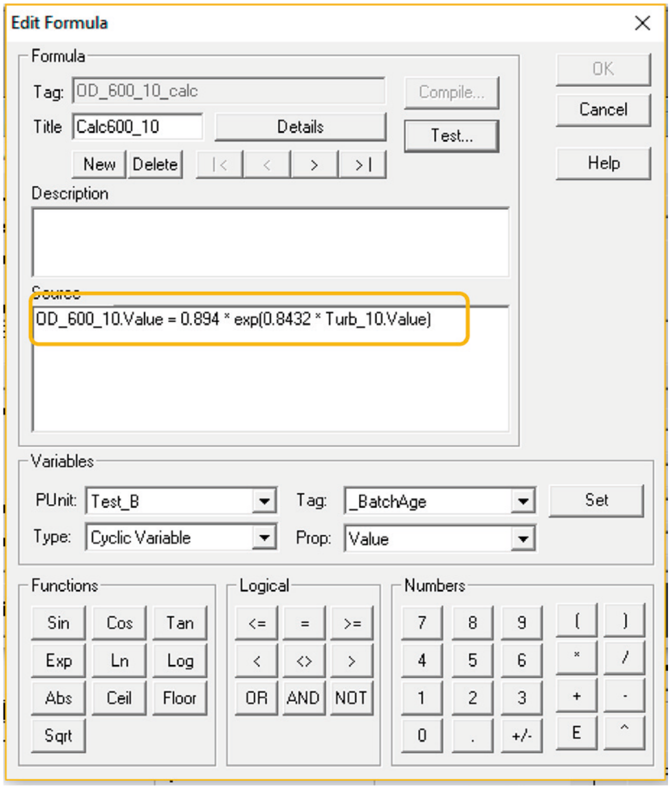
2. Create OD600 soft sensor
Based on the variable created in the first step, an additional "Cyclic Variable" can be configured (e.g. "OD_600_10").
As "Control Unit" you need to select "Calculation manager":



After setting the ranges and limits of the new variable, you need to define the algorithm. Click "Algorithm":



The equation in the "Source"-field represents the relationship between AU and OD600.



Perform a backup of the system to make the values available. During fermentation the calculation manager gives out a new OD value immediately after receiving an AU value from the DCU.

Such an automated calculation can be used as basis for an automated control loop.

Conclusion Fermentation and Cell Growth Monitoring

Autoclavable or CIP | SIP compatible, BioPAT® Fundalux probes are suitable for use in laboratory, pilot, or production scale bioreactors. The approach provides the process operator with real-time knowledge needed to optimize a bioprocess run and at the same time it makes offline sampling with OD600 spectrophotometers redundant. BioPAT® Fundalux sensors provide a stable and reliable measurement, even in systems with a high degree of aeration or agitation. Reliable, continuous, inline measurement eliminates laboratory sampling and is more consistent, sensitive and cost-effective than classical sampling, sample handling and offline analysis methods. In combination with MFCS SCADA an OD600 correlation with the inline turbidity measurement can easily be implemented.

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