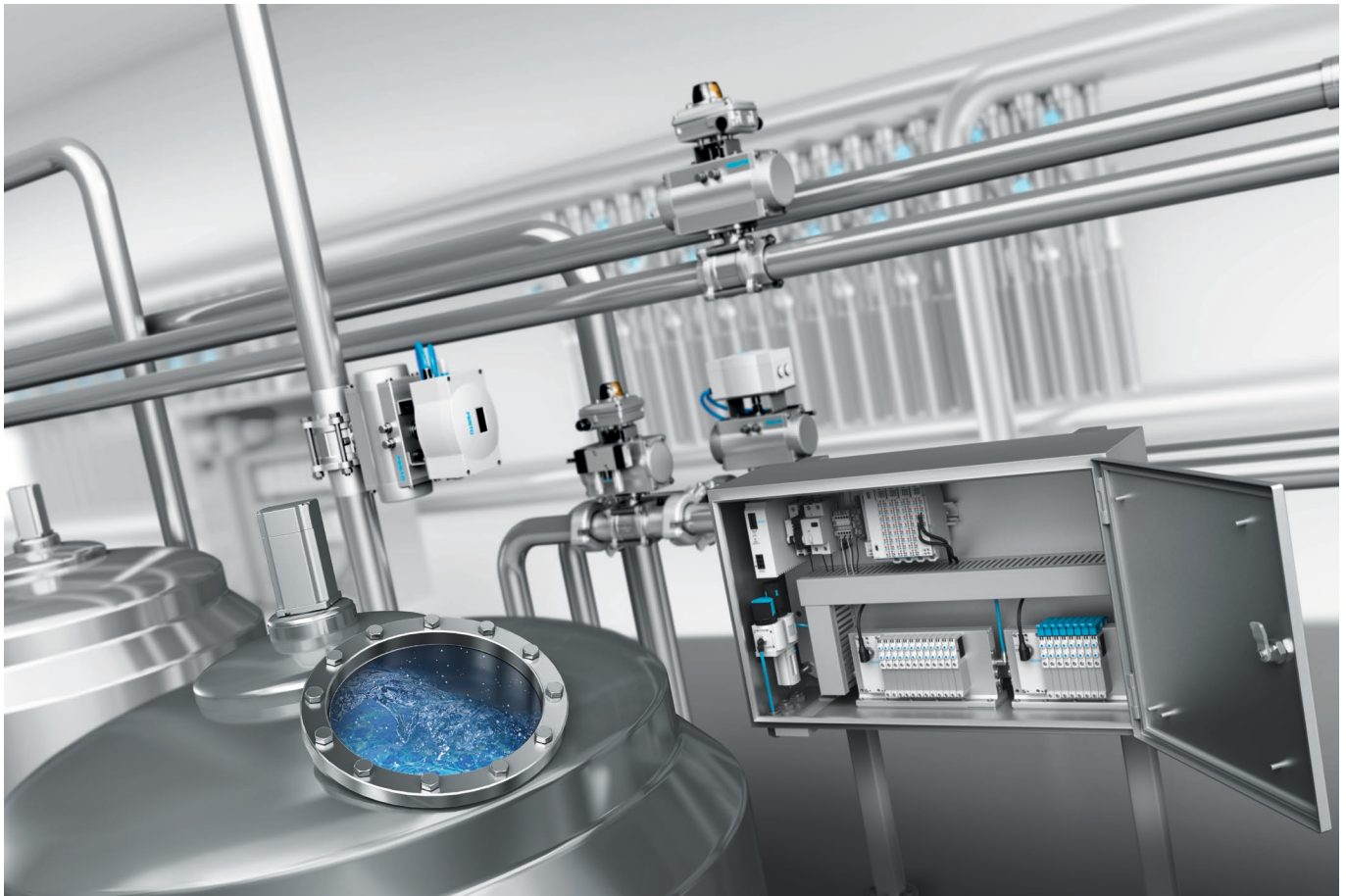


BioMaRe: Biomass calculator

For transparent and efficient cultivation of micro-organisms

FESTO



This white paper addresses the following topics:

- Why is determining the biomass so important but also so difficult?
- How can biomass growth be determined based on existing information?
- Estimating biomass using the example of a soft sensor from Festo

Executive summary

In biotechnology, the content and growth of biomass is an important process variable. There are different ways of quantifying this directly in the reactor. The methods currently used have advantages and disadvantages with regard to optimal process control and costs. This white paper demonstrates how biomass concentration and growth in biotech processes can be calculated using soft sensors and existing measurement signals.

Tomorrow's processes will be biochemical

Biochemical processes are increasingly establishing themselves as an alternative to purely chemical production. Advantages such as using renewable raw materials and avoiding by-products that are harmful to the environment are offset by one major disadvantage of biochemical production: its complexity and the associated higher production costs. Many reactions take place simultaneously and influence each other, and numerous factors play a role, although not all of them can be determined easily and directly. To become economically competitive in the long term, the production time needs to be reduced and the yield both per time unit and in absolute terms has to be increased.

This is where automation can make an important contribution. In order to optimise the processes and make them fit for the future, sophisticated sensors as well as advanced process control monitoring systems in real time are needed.

The central role in biochemical processes is taken on by living micro-organisms. In order to guarantee the optimal growth and catalysis, it is crucial that the right conditions are created in the reactor. Apart from the physicochemical variables such as temperature, pH value, oxygen and nutrient concentrations, information about the available biomass and the growth rate are also important variables. They are important for obtaining information about the current process sequence and the development status of the culture. As a result, the addition of nutrients and oxygen and other process events can be predicted better and faster and the process as a whole can be more precisely regulated.

1 — Direct measurement of the biomass

There are several approaches for determining the biomass concentration. Besides the proven methods, which rely on taking physical samples and bear the disadvantage of long time delays, considerable efforts are made towards developing real time sensors. These can provide the required information without interruption and in real time without any laborious manual analysis. It is thus possible to react promptly to process events and intervene with controlled measures.

Optical methods are based on the scattering and absorption of the cell particles that increase as the concentration grows. However, the big disadvantage of these relatively cost-effective sensors is that the high biomass concentrations which are the aim of industrial processes, usually exceed the sensitive measuring range of the opto-electrical sensors, which means that the measurement signal quickly becomes oversaturated. In addition they also record cell particles that have died, thus falsifying the result.

More modern dielectric probes measure the capacitive potential of living cells. The signal obtained can be used to determine the total number of living cells. Since the capacitive potential of the cells is very small, the signal is very susceptible to interference and noise. In addition, the signal quality depends greatly on the installation location and position, which complicates interpretation and transferability. Moreover, direct measuring probes are associated with high investment costs, and require a lot of maintenance and calibration.

2 — Calculating biomass from secondary measurements using soft sensors

Soft sensors are an alternative to direct biomass measurement. These are based on secondary measurement signals that are usually already available. These include measurements that are directly or indirectly influenced by the biomass; they can be used to deduce information about the likely biomass content and its growth behaviour. As the comparison in Table 1 shows, this method can produce good results with low procurement and maintenance costs.

Measuring principle	Time delay	Accuracy*	Procurement costs	Operating costs over lifespan	Calibration costs over lifespan
Drying	~ 2 days	+++	Low	High	Low
Moisture analysis scale	~ 30 min	++	Medium	High	Low
OD/scattering	0	+	Medium	Low	High
Permittivity	0	++	High	Low	High
Soft sensors	0	++	Low	Low	Medium

* + > 10 %, ++ < 10 %, +++ < 5 % relative error

Table 1: Comparison of various biomass measuring principles

3 — The biomass calculator (BioMaRe) from Festo

With components, software and digitalisation solutions from Festo, both the specified direct sensors as well as soft sensors can be used in the process industry. The principle of a biomass soft sensor for biotech processes and the method of integrating one of these sensors are described below:

a) The measuring principle

The BioMaRe understands biomass growth as a rigorous chemical reaction, like the reaction we are familiar with from breathing. Here a carbon source (e.g. sugar) is converted into biomass and carbon dioxide through oxygen consumption, as shown in the diagram in Figure 1. Measuring three of the four components is enough to draw conclusions about the unknown component, since the principle of the conservation of mass also applies to bioreactions. Analysing the components in their elementary composition will result in the system being overdetermined (carbon balance and balance of the reduction equivalents). This redundancy means that data can be cross-checked and basic assumptions can be continuously validated.

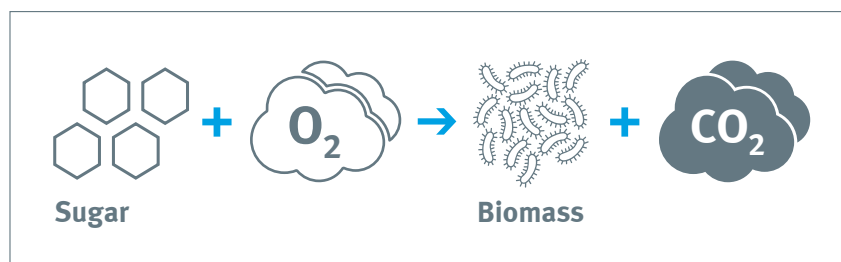


Figure 1: Diagram of indirect biomass determination

b) The general conditions

Information about the addition of sugar as well as the oxygen consumption and the formation of CO_2 is needed to operate the biomass calculator. In biotech plants, these variables are generally collected by measuring the mass flow as well as analysing the exhaust air flow. In addition to these time-based measurement signals and the substrate concentration in the feed flow, the other main requirement for solving the elementary balances is the molecular composition of the biomass and of the substrate. A list of all parameters and their origin as well as default values can be found in Table 2.

Name	Unit	Origin	Default value	Type
Nutrient feed	l/h	Flow measurement/ scale	-	Time-based
Gas feed	l/h	Flow measurement	-	Time-based
Exhaust gas flow	l/h	Flow measurement	-	Time-based
O_2 in exhaust gas	%	Exhaust gas analyser	-	Time-based
CO_2 in exhaust gas	%	Exhaust gas analyser	-	Time-based
Filling volume	L	Volume balance	-	Time-based
Substrate concentration in feed	g/l	Media recipe	10 - 600	Constant
Oxygen content of moist reactor air	%	O_2 content in exhaust gas under operating conditions without cells	20.7	Constant (reactor-dependent)
O_2 content of air	%	Air composition	21.0	Constant
CO_2 content of air	%	Air composition	0.0039	Constant
C-mol weight of substrate	g/C-mol	Chemical formula	30 (glucose)	Constant
C-mol weight of biomass	g/C-mol	Determination of C proportion	~ 26	Constant
Y_s	-	Reduction equivalent of substrate	4 (glucose)	Constant
Y_{O_2}	-	Reduction equivalent of oxygen	-4	Constant
Y_x	-	Reduction equivalent of biomass	~ 4.13	Constant

Table 2: Time-based and constant input variables required to calculate the biomass using a soft sensor

In a fed-batch, where the substrate solution is continuously fed in, the biomass formation can be calculated using the available measurements, assuming that there are no accumulations of residual substrate (carbon source) in the reactor.

c) The soft sensor implementation

In order to put a biomass soft sensor into operation, the process variables required for this must be picked up in real time so they can be processed in the algorithm. Ideally the soft sensor should be integrated directly in the programmable logic controller (PLC) that regulates the entire bioreactor. In other words, this is where all the sensor signals of the bioprocess are received and processed so that the field devices can be optimally actuated. As a result, the sensor information required for the soft sensor is directly accessible and the interaction with the soft sensor can take place directly via the existing operating screen. However, the soft sensor can also be operated on a separate processing unit like a PC or a separate PLC, as in our concrete example. The acquisition of the sensor information must then be configured separately, e.g. via an OPC UA network.

Figure 2 shows the integration of the BioMaRe at a bioreactor with the mass and information flows as well as the required real-time sensors.

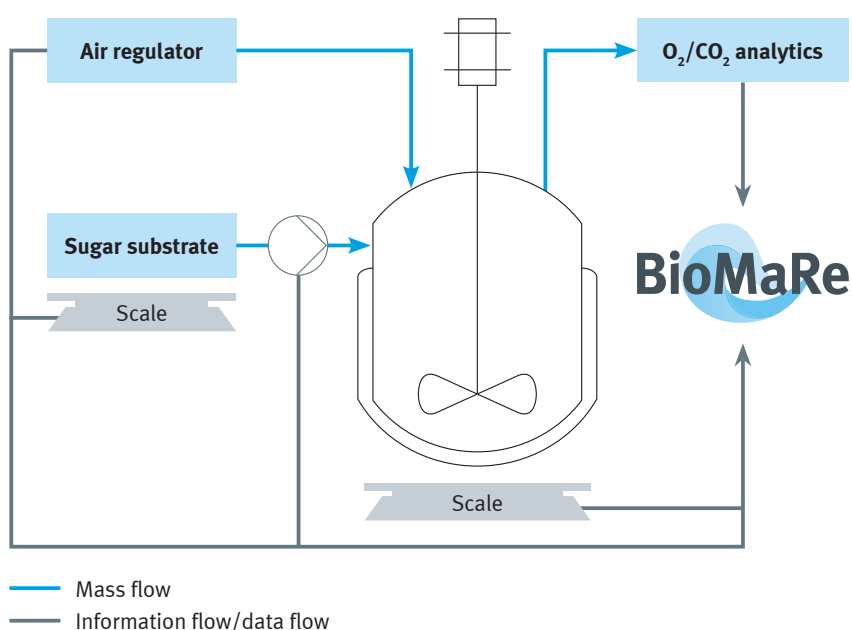


Figure 2: Implementation diagram for a biomass soft sensor

4 — Using the BioMaRe

The BioMaRe was used in a case study to estimate the growth of an industry-relevant, recombinant *E. coli* during a bioprocess. The test setup on a laboratory scale can be seen in Figure 3.

A process control approach typical for the biopharmaceutical industry was chosen for the test. After an initial batch process with a small amount of sugar as a basis, the transition was made to a fed-batch. After a sufficient biomass concentration was reached, production was started by adding an induction solution and the substrate addition rate was reduced.



Figure 3: Test setup and testing of the BioMaRe on a laboratory scale

As can be seen in Figure 4, it was possible to determine the CO₂ emission rate (CER), the oxygen uptake rate (OUR) and the substrate addition using the measured mass flows (scale for the substrate, flow rate measurement for gases) as well as to analyse the exhaust gas composition. Based on this, the specific growth rate (μ) was calculated using the balancing method. The signal calculated in real time (μ BioMaRe) corresponds very well to the measured μ . The growth is only overestimated at the end of the process, because the calculation algorithms used do not take cell death and cell lysis into consideration.

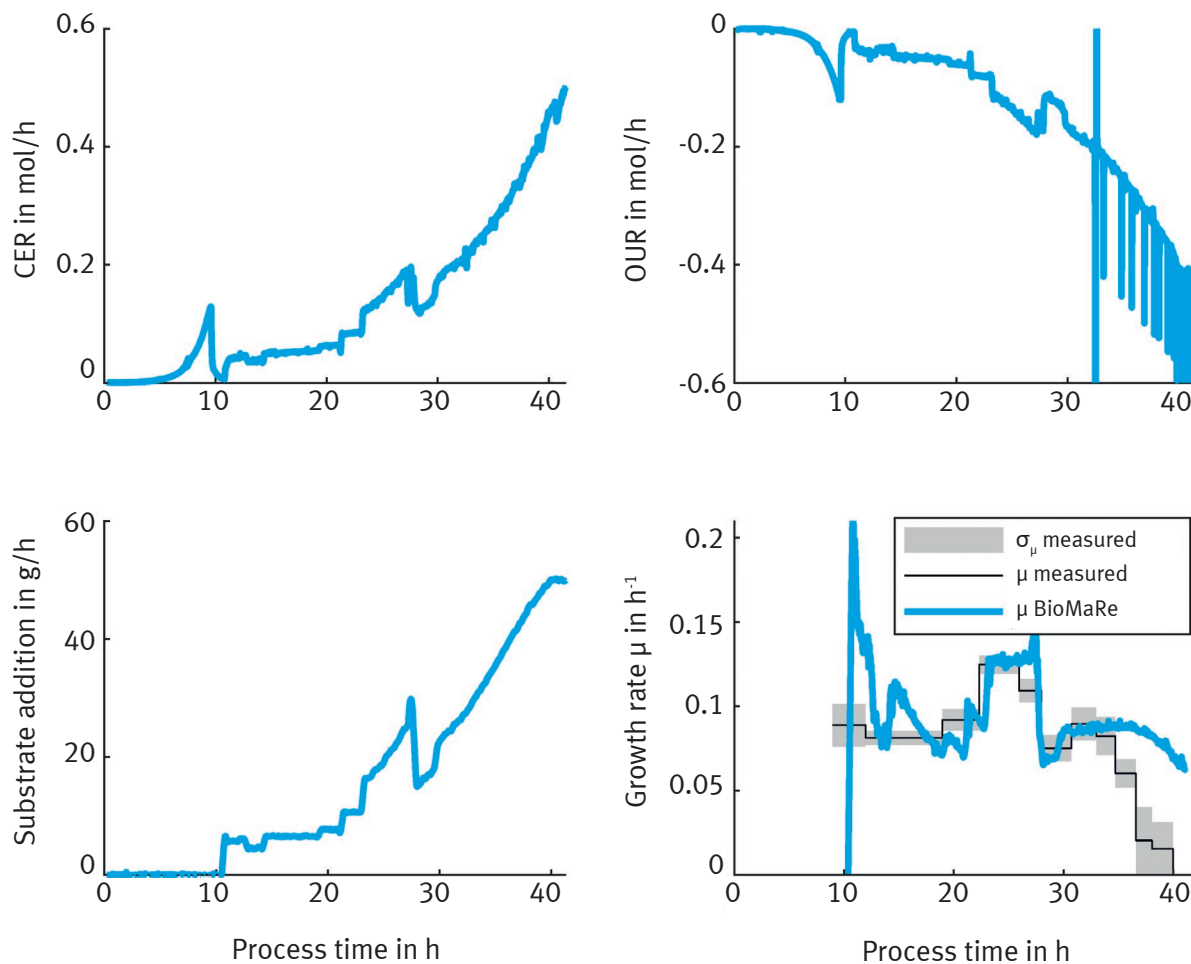


Figure 4: Measured CO_2 emission rate (CER), oxygen uptake rate (OUR) as well as substrate addition and resulting specific growth rate (μ BioMaRe) Using the reference measurement approach (drying of offline samples), the specific biomass growth was represented as a comparison (μ measured) as well as its uncertainty based on a triple measurement (σ_μ measured).

The biomass calculator provides useful information about the current biomass concentration and the specific growth. It does this without any manual analysis or delay. This enables decisions to be made about process regulation for more stable, optimised and faster process control. For example, the biomass concentration could be very easily read off from the display menu as shown in Figure 5 and the production phase could be started without delay once the target biomass concentration (30 g/L) was reached.

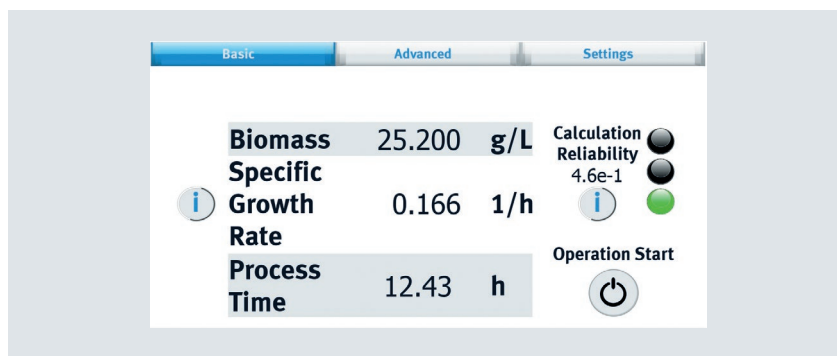


Figure 5: User interface and simple BioMaRe display menu. Information about biomass concentration, specific growth rate and the calculation reliability can be read off as a traffic light system.

The biomass concentration curve can be seen in Figure 6. Compared with the reference measurement (drying of offline samples), the soft sensor demonstrates very high correspondence in the growth phase and in the first part of the production phase (1.3 % relative, mean square error). In the final hours of the production phase, the discrepancy between the calculated biomass signal and the measurement increases progressively. This can be traced back to a behaviour that is not yet modelled in the BioMaRe algorithm at the moment: as a result of the increased cell stress during the product formation, there is more lysis of cells and a decline in living biomass.

The measurement based on permittivity shown next to it is better at indicating the cell death; however, it exhibits severe fluctuations during the first process phases, whereby the overall error is a comparable 6 %.

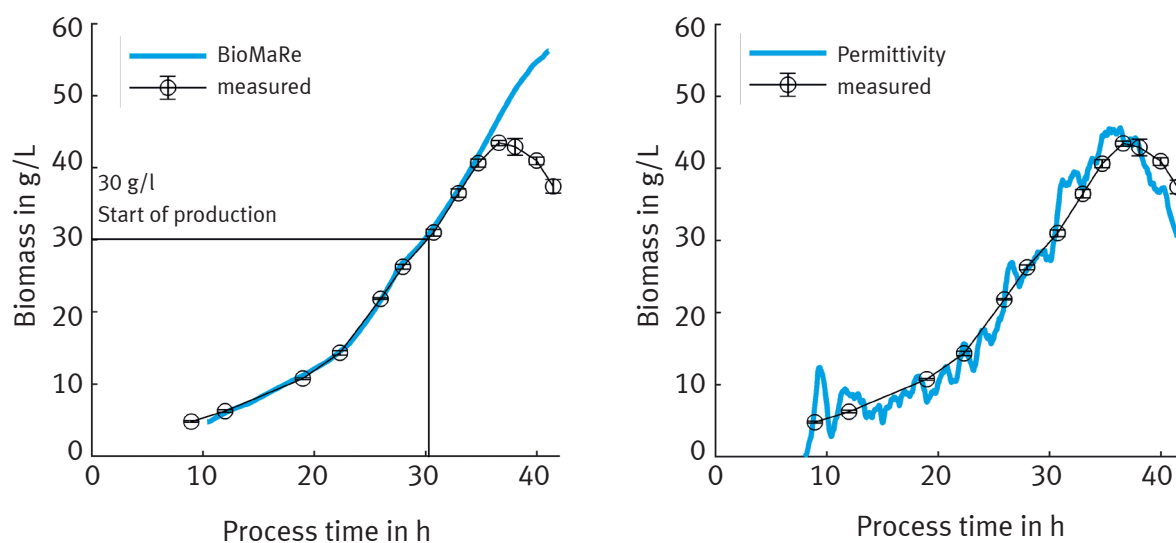


Figure 6: Calculated biomass (BioMaRe) on the left and permittivity on the right compared with the measured biomass achieved by drying offline samples. Up until the time when there is significant cell death, the mean deviation with the BioMaRe is just 1.3 % and the deviation taking into account the overall process is 5.6 %. Cell death can be better recorded with measurements based on permittivity, but the signal is not as stable and overall the deviation is a comparable 6 %.

Conclusion

By integrating soft sensors and using existing measurement signals, conclusions can be drawn about important but difficult to measure states in the process. In biotechnology, the biomass concentration as well as the growth rate can be determined under specific conditions using soft sensors. Unlike other measuring principles, it is not necessary to install measurement probes directly in the bioreactor; this reduces the risk of contamination and the investment costs. The low-maintenance operation and the use of generally valid elementary balances results in a robust and transferable measurement that achieves an accuracy comparable with modern online measuring devices. Complete automation of the process sequences with no manual sampling produces more robust and shorter processes as well as an increase in the specific production output. As a result, plant utilisation goes up and production costs come down.

This research paper was produced in cooperation with the Vienna University of Technology and the Innovation Alliance for Functionally Optimised Biosurfactants, with funding from the Federal Ministry of Education and Research. If you have any questions or would like further information, please get in touch with our contact person Dr. Daniel Wibbing directly (details below).

Publisher/author:

Festo SE & Co. KG

Dr. Daniel Wibbing

E-mail: daniel.wibbing@festo.com

Further information about automation solutions for the biotech and pharmaceutical industries can be found online:

→ www.festo.com/biotech