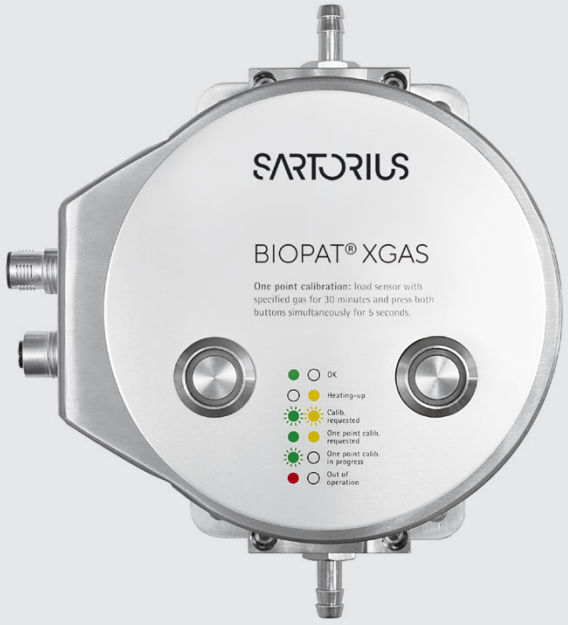
BioPAT® Xgas Metabolic Calculations

Online Off-Gas Analysis



Technical Note

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- Gas measurement cases
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The continued growth of the biotech and biopharmaceutical industry has been driven by the increasing use of therapeutics of large molecules produced by modified organisms.

In biological manufacturing, validation and quality assurance documents submitted to regulatory agencies need to be compliant to a number of stringent criteria. Further, the costs related to any batch failure can become overwhelming. Therefore, accurate data on quality and performance aspects from process development and production is paramount.

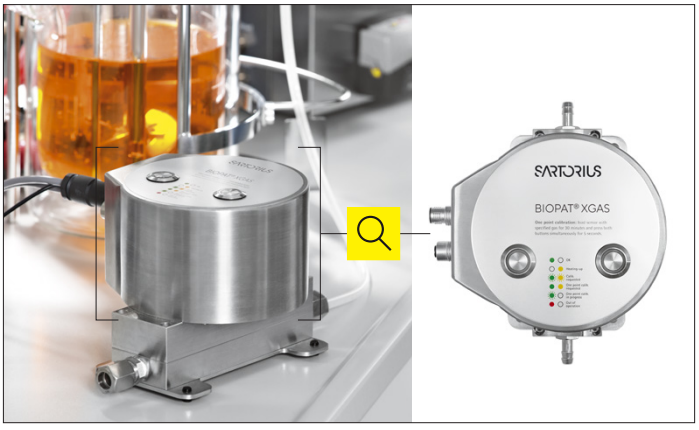
This has led to Lean Six Sigma and automation initiatives to improve process compliance | transparency and rationalize filing with the aid of electronic batch records and electronic signatures.

Software technologies like BioPAT® SIMCA-online can use multi variant data analysis to produce these electronic batch trajectories. However, analytics must still be collected and fed into the software model to provide the assurance everything is on track.

A non-invasive method for collecting critical online information comes from examining the changing gas composition as it passes through an aerobic cell process. The fermentation feed and oxygen is consumed at a measurable rate together with carbon dioxide, biomass and products being produced. These rates can be determined by using a Biostat® in combination with a BioPAT® Xgas. The gassing strategy control on the fermentor allows for accurate determination of gas composition input. The off-gas analyzer when placed outside the sterile barrier of the bioreactor exhaust vent determines the out gas composition. Herein, a detailed description is given on how this information can be used for metabolic calculations and practical advice for Biostat® users.

General Advice for Monitoring Off-gas

The Biostat® hold up time and mixing efficiency determines the lag time and a change is detected from the inlet to the outlet. During this time the gases mixes with the culture and transfer back and forth into the various liquid, cell and gas phases. However, if the inlet oxygen or carbon dioxide gas flow rate is fluctuating in a narrow band of time (2 – 5 minutes) the hold-up time | mixing efficiency will result in a averaging of detected BioPAT® Xgas signal. This results in a higher degree of uncertainty and error. Therefore, it is advised to maintain a wide PID deadband for pO_2 control and have an on | off flow of carbon dioxide to minimize the changes in gas flow rate.



It is highly recommended to have a Biostat® fitted with mass flow controllers (MFC) in order to ensure accurate and reliable metabolic calculations. For example, the continuous and automatic gas flow control of the Biostat® A has a 5% full scale accuracy giving +/-375 mL/min gas flow rate for the maximum air flow of 7.5 L/min. Using a 1L vessel yields an error of 37.5% at 1vvm, 19% on a 2L vessel and 7.5% on a 5L vessel.

Compounding to that, at the beginning of the cultivation where flow rates are lower the error is more significant. Therefore, with such high error bars it is unreasonable to calculate OUR or CER in an acceptable range.

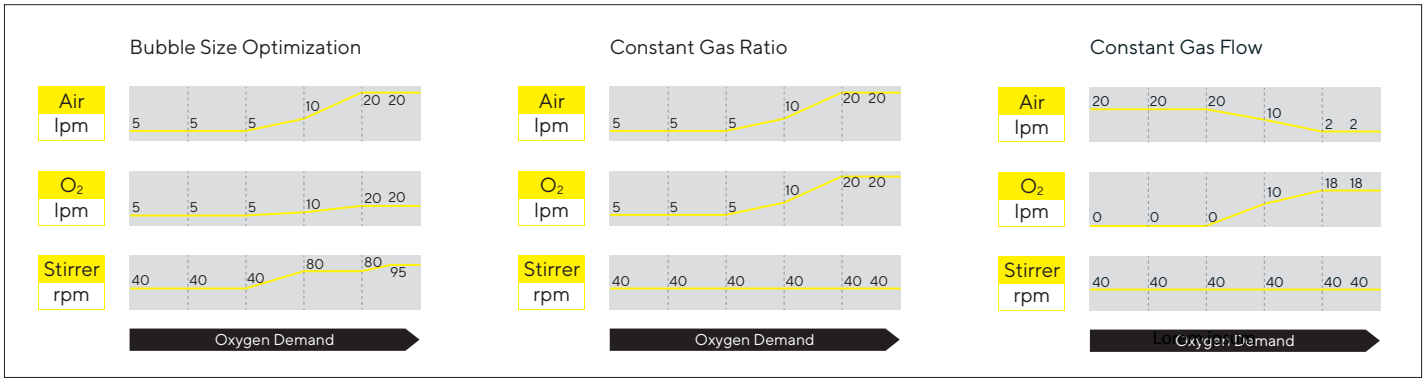


Figure 1: three different modes of gas strategy possible with a Biostat® advance control.

Biostat® Gassing Strategy

The Biostat® advanced pO_2 control allows parallel modification of all bioreactor parameters such as stirrer speed, flow rate for air and oxygen (and other parameters if configured). This simultaneous activation or change allows mimicking of all the common gassing strategies (figure 1) and allows the user to be resource efficient and optimize the gassing process control. Constant gas flow works by decreasing the flow of air and simultaneously increasing oxygen gas at the same level maintaining the same total gas flow rate. Constant gassing ratio fixes both air and oxygen percentage and increases the total flow rate.

Finally, bubble size optimization can fine tune the oxygen percentage and gas-liquid interface by adjusting the impeller speed and total gas flow rate. This flexibility gives the Biostat® user the capability to meet any tradeoff between gas | mass transfer rates, shear forces, foaming and off gas analysis needs.

Volumetric Gas Transfer

The rate at which oxygen and other gasses transfer from the gas phase into the liquid phase, on to the cells and out again is described by the volumetric transfer rate. A typical measurement for a bioreactor is the rate at which oxygen transfers from the gas phase to the liquid phase; the volumetric oxygen transfer rate. This depends on two things; the concentration gradient ($C_{O2,\alpha}$- C_{O2} driving force) and k_La. Measuring and calculating the concentration difference between the liquid and gas phase is relatively simple whereas measuring and calculating "k_L" and "a" independently is rather more challenging. Basically, "k_L" can be described as the resistance oxygen observes transferring from the gas to liquid phase and the term "a" can be described as the interfacial gas-liquid surface area per unit volume. Many things influence k_La and throughout a bioprocess batch it dynamically changes its value, meaning the Biostat® has to maintain dissolved oxygen set point by changing gassing strategy parameters to meet the cells growing oxygen uptake rate (OUR). In a steady state the oxygen transfer rate (OTR) will equal the OUR.

$$k_La = OTR / (C_{O2,\alpha} - C_{O2})$$

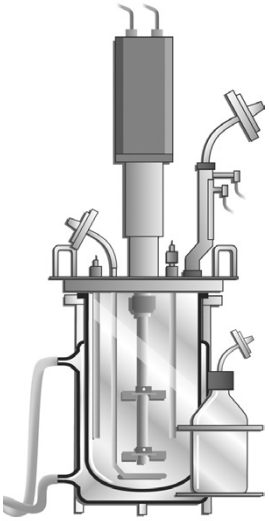
Gas measurement cases

Gassing with air

Typical microbial applications will simply use process air through a ring sparger to aerate an aerobic cultivation in order to provide sufficient dissolved oxygen. The Biostat® vessel agitation rate and system pressure (only on steel vessel with pressure rating) can be used to increase the volumetric oxygen transfer rate when dissolved oxygen is limited or falls below the pO_2 set point.

Fortunately, these factors do not influence (or are accounted for by either the MFCs or BioPAT® Xgas) the measurement or control of the Biostat® gas flow rate and thus metabolic calculation remains accurate (case 1).

Gas in



Gas out

$F_{Air,\alpha}$
Gas % volume
$N_{2,\alpha}$ = 79.07% (v/v)
$O_{2,\alpha}$ = 20.90% (v/v)
$C_{O2,\alpha}$ = 0.03% (v/v)

V = Bioreactor liquid in volume (L)*

* either fixed, manually imputed or measured online by gravametic analysis

Measured by BioPAT® Xgas
$O_{2,\varphi}$ and $CO_{2,\varphi}$

$N_{2,\varphi}$ = 100 - $O_{2,\varphi}$ - $C_{O2,\varphi}$
$F_{G,\alpha}$ = $F_{G,\alpha}$ * $N_{2,\alpha}$ / $N_{2,\varphi}$

K_{CO2} = 26.59 min.mmol/l/h
K_{O2} = 26.44 min.mmol/l/h

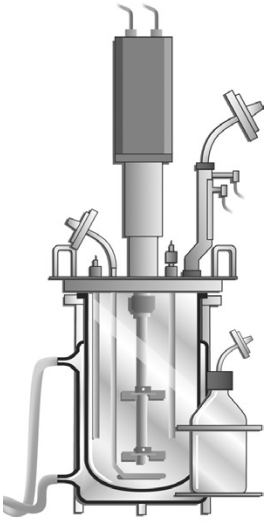
Case 1: Gassing with air

O_2-Enrichment

The O_2-Enrichment either uses a 3/2-way solenoid valve to select either air or O_2 flow or individual MFCs to control the ratio of the two gases to the sparger. O_2 is pulsed via a solenoid valve or MFC, enriching the oxygen percentage of the air to maintain the pO_2 set point.

The MFCs can be integrated to measure and control the total gas flow rate via manual adjustment or automatically in conjunction with the controller. Therefore, the additional oxygen in the inlet gas composition must be accounted for in the gas composition calculation (Case 2).

Gas in



Gas out

$F_{G,\alpha}$
$N_{2,\alpha}$ = $(F_{Air,\alpha} \times 79.07 + F_{O2,\alpha} \times 0^{a}) / F_{G,\alpha}$
$O_{2,\alpha}$ = $(F_{Air,\alpha} \times 20.9 + F_{O2,\alpha} \times 100^{b}) / F_{G,\alpha}$
$CO_{2,\alpha}$ = $(F_{Air,\alpha} \times 0.03 + F_{O2,\alpha} \times 0^{c}) / F_{G,\alpha}$

[a] Percentage of nitrogen in oxygen cylinder gas
[b] Percentage of oxygen in oxygen cylinder gas
[c] Percentage of carbon dioxide in oxygen cylinder gas

$F_{G,\varphi}$, Y_{φ}
$N_{2,\varphi}$, $O_{2,\varphi}$, $CO_{2,\varphi}$

$N_{2,\varphi}$ = $100 - O_{2,\varphi} - CO_{2,\varphi}$

$F_{G,\varphi}$ = $F_{G,\alpha} * N_{2,\alpha} / N_{2,\varphi}$

K_{CO2} = 26.59 min.mmol/l/h
K_{O2} = 26.44 min.mmol/l/h

Case 2: O_2-Enrichement

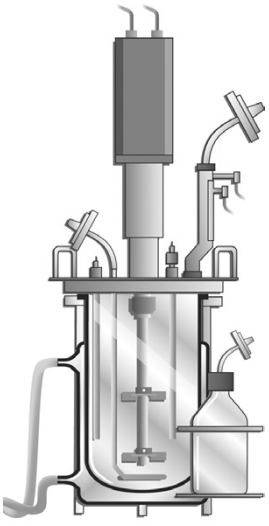
Advanced additive flow

Cell culture operations typically use 4 main gases; air, oxygen, carbon dioxide and nitrogen. Each gas servers a function and can be used at various stages throughout the process for controlling critical parameters. Advanced additive flow gassing strategy is furthermore system-dependent allowing gasses (Air, O_2, N_2 and CO_2) to be directed to the sparger or routed to overlay. All gasses in use must be included in the inlet flow gas composition calculation. Please take note of the component percentages of a mixed composition gas cylinders as this can have a significant impact on the calculated metabolic value if oxygen or carbon dioxide is not accounted for (case 3).

Please note the accuracy of a mixed composition gas cylinder and use this value for the metabolic calculation. Occasionally, cylinders have specification accuracy of +/-2% component gas which can result in significant affects to the calculated metabolic values and in turn change the desired control loop.

An advantage of having the combination of a combined ring | microsparged and gas overlay Biostat® is that outlet gas concentrations can be diluted by increasing air flow to the overlay. This will have minimal impact on gas liquid transfer rates but the gas dilution factor can be included into the metabolic calculations ensuring there is no saturation of the BioPAT® Xgas oxygen or carbon dioxide sensors.

Gas in



Gas out

$F_{G,\alpha}$

$N_{2,\alpha} = (F_{Air,\alpha}{}^{*} \times 79.07 + F_{O2,\alpha} \times 0^{b} + F_{CO2,\alpha} \times 0^{c} + F_{N2,\alpha} \times 100^{a}) / F_{G,\alpha}$

[a] Percentage of nitrogen in cylinder gas

* Combined overlay and sparged air flow rate

$O_{2,\alpha} = (F_{Air,\alpha} \times 20.9 + F_{O2,\alpha} \times 100^{b} + F_{CO2,\alpha} \times 0^{c} + F_{N2,\alpha} \times 0^{a}) / F_{G,\alpha}$

[b] Percentage of oxygen in cylinder gas

$CO_{2,\alpha} = (F_{Air,\alpha} \times 0.03 + F_{O2,\alpha} \times 0^{b} + F_{CO2,\alpha} \times 100^{c} + F_{N2,\alpha} + 0^{a}) / F_{G,\alpha}$

[c] Percentage of carbon dioxide in cylinder gas

$F_{G,\varphi}$, Y_{φ}
$N_{2,\varphi}$, $O_{2,\varphi}$, $CO_{2,\varphi}$

$N_{2,\varphi} = 100 - O_{2,\varphi} - CO_{2,\varphi}$

$F_{G,\varphi} = F_{G,\alpha} * N_{2,\alpha} / N_{2,\varphi}$

$K_{CO2} = 26.59$ min.mmol/l/h
$K_{O2} = 26.44$ min.mmol/l/h

Case 3: Advanced additive flow

Metabolic Calculations

In cases 1 to 3 the data from the inlet gas composition and the outlet is placed into the following equations to calculate;

$$OUR = (F_{G,\alpha} * O2_{\alpha'} - F_{G,\varphi} * O2_{\varphi'}) / V * K_{O2} \quad mmol/L/hr$$
$$CER = (F_{G,\varphi} * CO2_{\varphi'} - F_{G,\alpha} * CO2_{\alpha'}) / V * K_{CO2} \quad mmol/L/hr$$

$$RQ = CER / OUR$$

Oxygen demand
OUR is dependent on the specific oxygen uptake rate coefficient (q_{O2}[$mmol_{O2}$/gDCW*h])of the organism and varies depending on the conditions, cell line and cell type (table 1). Microbial fermentations generally have higher q_{O2} values when compared to mammalian cell culture. The resulting higher O_2 demand lowers the relative error when calculating metabolic constants, thus providing a better signal to noise ratio. Despite this, off-gas monitoring of high cell density cultivations yields the same valuable information if the inherent gas measurement error at the inlet and outlet is kept to a minimum.

Both the Biostat®'s MFCs and the BioPAT® Xgas are integrated and work together to be dedicated analysis system for one cultivation that feeds real-time online data into BioPAT® MFCS for control loop opportunities.

Table 1: common cell types and their corresponding specific oxygen uptake coefficient values

Cell type	q_{O2} values
E. coli – Bacterial	20 $mmol_{O2}$/(gDCW*h)
P. Pastoris – Yeast	13 $mmol_{O2}$/(gDCW*h)
CHO – Mammalian cells	1×10^{-4} $mmol_{O2}$/(million.cell*h)

Data Interpretation

For a complete glucose conversion RQ will be 1 (six molecules of oxygen are used and six molecules of carbon dioxide produced). In other cases, the nutrient feed sources may have a different stoichiometric ratio of O_2 consumption and CO_2 production. Therefore, confirming the chemical decomposition of the nutrient source is needed to understand the theoretical output value of RQ.

This calculation may also be dynamic as fermentations often switch feed sources to induce a particular metabolic production pathway.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Table 2: Sartorius Stedim model fed-batch process conditions

Temperature-Set point	37 °C
pH-Set point	6.8
pO_2-Setpoint	20%
Organism	*Escherichia coli* BL21(DE3)
Initial glucose conc.	10 g/L
Initial OD_{600}	1.0
Initial DCW	0.03
Initial working volume	3.2 L
Volume feed	1.3 L
μ_{set}	0.15 h^{-1}

In order to demonstrate the application and performance of the BioPAT® Xgas, Sartorius Stedim have model biological processes for testing new Biostat® and BioPAT® equipment. One such process is *Escherichia coli* fed-batch cultivation (table 2) in chemically defined media. The process was performed in a gen 1 – Biostat® B 5L Univessel®. Figure 2, 3 and 4 show the parameter tracking by BioPAT® MFCS of the process from 0 to 27 hours. The first 7 hours of the process runs in batch mode, switching to fed-batch when a defined OD_{600} is measured. Figure 2, shows the PID controller (agitation 1st cascade) activating after 5 hours when the pO_2 falls to 20%. After 24 hours the gassing strategy switches from gassing with air to oxygen enrichment. This maintains the oxygen set-point when the defined maximum stirrer rate has been reached. Figure 3 plots the exponential growth rate measured by off-line sampling of the OD_{600} reaching a maximum of 220 (dry cell weight 66 g/L). A calculation of the specific growth rate is also included. Overlaid on Figure 3 is the raw pO_2 percentage data measured by the dissolved oxygen probe.

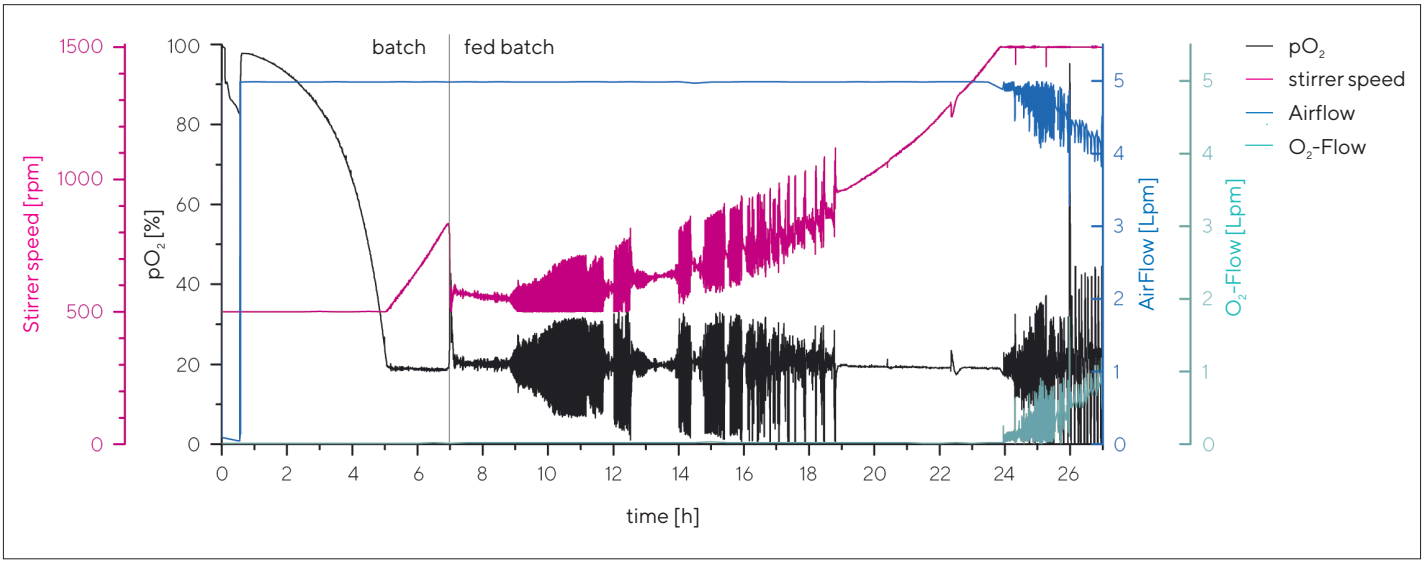


Figure 2: Graphical plot of a 27 hour fed-batch fermentation of *E.coli* tracking the parameters in BioPAT® MFCS

As the BioPAT® Xgas is dedicated to the 5L vessel exhaust gas outlet and the data from the Biostat® B's MFC is logged into BioPAT® MFCS, this allows the dynamic calculation of OUR, CER and RQ shown in Figure 4. This calculation combines; Case 1 and Case 2) factors in changes in bio-reactor liquid volume, air and oxygen gas flow rate (inlet gas composition) as well as pressure and humidity compensation by the BioPAT® Xgas giving an accurate measure of cellular metabolic status as it changes over time.

This allows the capability to implement control loop triggers to start feeds or build more data into your process model for improved understanding and process control.

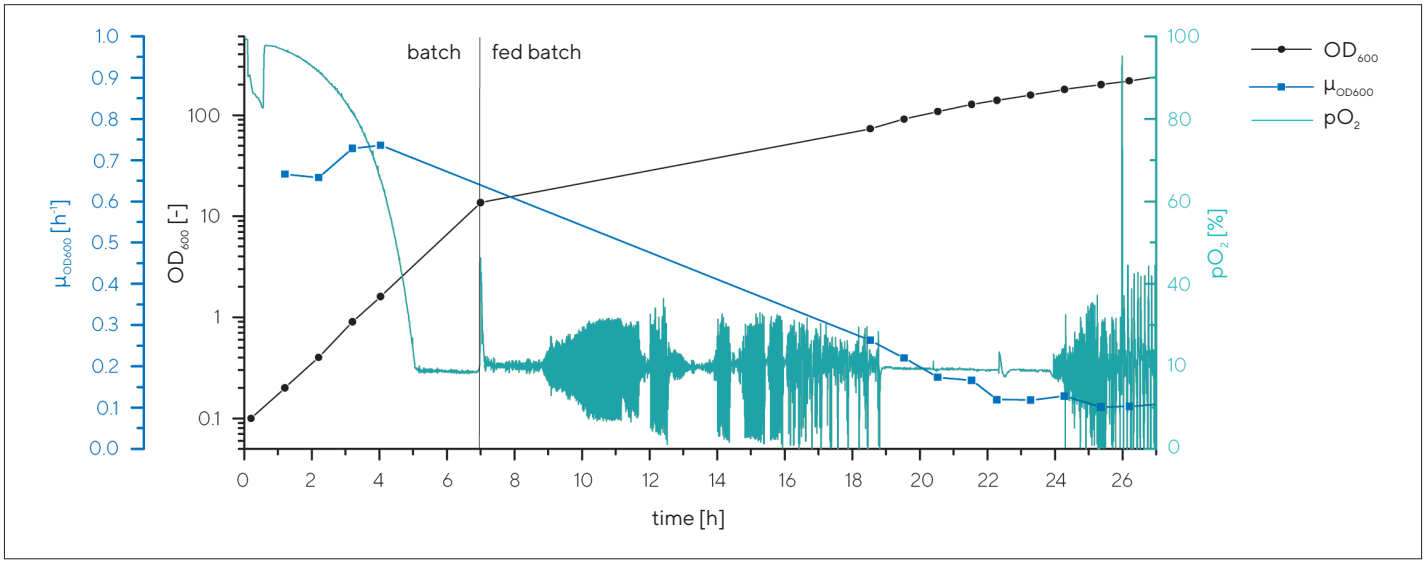


Figure 3: Graphical plot of a 27 hour fed-batch fermentation of *E.coli* tracking the online and off-line parameters in BioPAT® MFCS

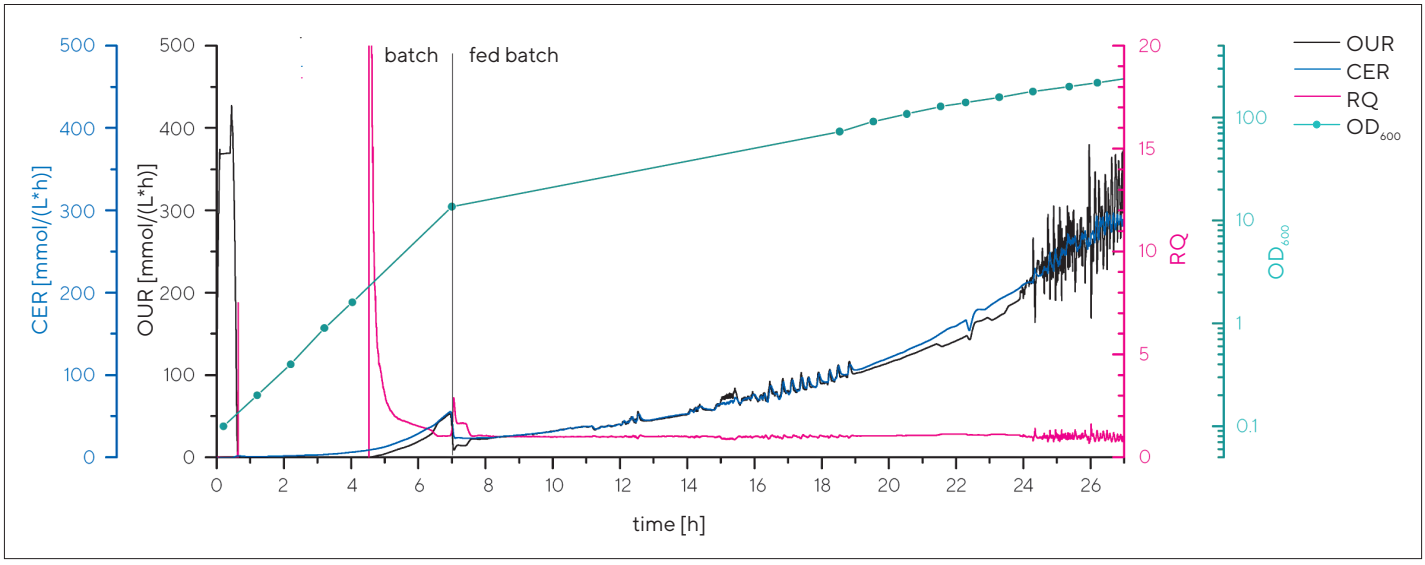


Figure 4: Graphical plot of a 27 hour fed-batch fermentation of *E.coli* tracking the auto-calculated and off-line parameters in BioPAT® MFCS

Calculating error

BioPAT® Xgas humidity sensor corrects for the humidity change that occurs when the dry air feed gas is sparged through the bioreactor liquid. Without this correction, errors are introduced into the headspace data due to dilution by the additional water vapor and gas pressure. This results in the O_2 and CO_2 sensors having an accuracy of 0.2% full scale and 3% Rdg. These error values are used to distinguish a percentage accuracy which varies proportionally to the measured span (reading) from one which is a fixed percentage of the maximum measurement reading (full scale). Additionally, any error in the gas flow measurement and gas composition must also be factored into the calculation.

Example	
A measurement reading from the BioPAT® Xgas of:	18.45% O_2 and 3.5% CO_2
The error for the O_2 reading would range from:	[19.04% to 17.86% // 4 significant figures]
The error for the C_{O2} reading would range from:	[3.612% to 3.388% // 4 significant figures]

Acronym key

OUR – Oxygen uptake rate
CER – Carbon dioxide emission rate
OTR – Oxygen transfer rate
RQ – Respiration coefficient
DCW – Dry cell weight
MFC – Mass flow controller
OD_{600} – Optical density at 600 nm
μ_{OD600} – Specific growth rate at 600 nm
$F_{G,\alpha}$ – Inlet flow of total gas
$N_{2,\alpha}$ – Inlet percentage of nirtogen
$O_{2,\alpha}$ – Inlet percentage of oxygen
$CO_{2,\alpha}$ – Inlet percentage of carbon dioxide
$F_{O2,\alpha}$ – Inlet flow of oxygen
$F_{Air,\alpha}$ – Inlet flow of air
$F_{N2,\alpha}$ – Inlet flow of nitrogen
$F_{CO2,\alpha}$ – Inlet flow of carbon dioxide
$F_{G,\varphi}$ – Outlet flow of total gas
$N_{2,\varphi}$ – Outlet percentage of nitrogen
$O_{2,\varphi}$ – Outlet percentage of oxygen
$CO_{2,\varphi}$ – Outlet percentage of carbon dioxide
K_{CO2} – CO_2 mass transfer coefficient
K_{O2} – O_2 mass transfer coefficient
V – Volume of liquid in bioreactor
q_{O2} – specific oxygen uptake coefficient

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