

A Guide to the Eppendorf Small Scale Gas Analyzer GA4

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Abstract

This short protocol describes the function and application of Eppendorf's 4-fold gas analyzer module GA4. The GA4 can be used in different processes with the Eppendorf small scale and modular systems together with the process control software DASware® control or as a stand-alone module.

The Eppendorf Off-Gas Analyzer GA4 as a process analytical tool provides information on important metabolic data in real time. It can be used in both microbial

fermentation and cell culture processes. The GA4 provides separate discrete measurement channels for up to four bioreactors measuring oxygen and carbon dioxide content in the exhaust gas flow of the bioreactor.

Critical process values, such as the oxygen transfer rate, carbon dioxide transfer rate, and the respiratory quotient are calculated using the integrated logic. These values give information about the respiratory activity of the organisms.

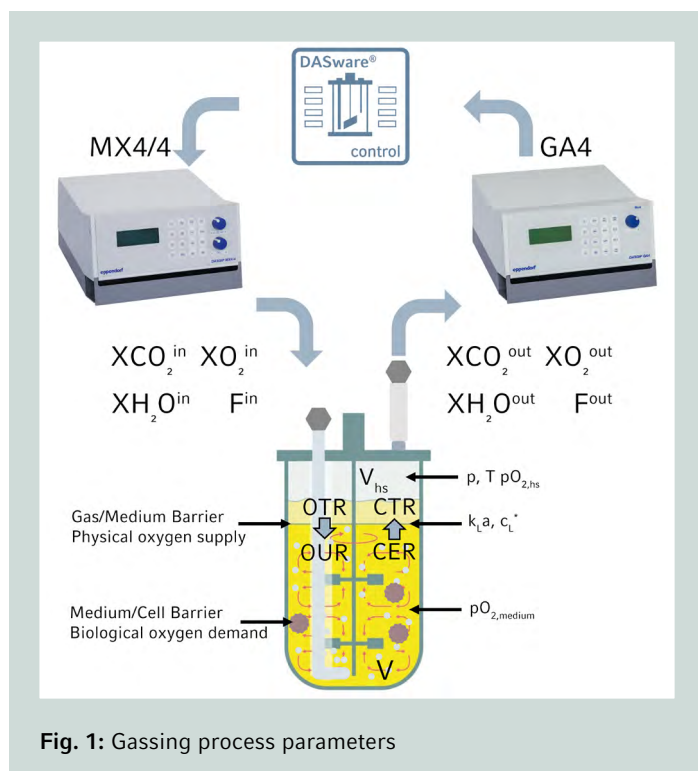
1. Introduction

Real time off-gas data is an important tool for gaining insights into a specific fermentation process by allowing the user to examine carbon evolution, oxygen utilization, and transfer during the fermentation. This information can be used to find changes in metabolism, spot potential problems, or trigger changes in the fermentation process.

The GA4 Off-gas analyzer makes metabolic change identification and the subsequent controlled substrate or nutrition addition possible. Through off-gas analysis, insufficient oxygen or inadequate substrate supply can be identified. Inhibitory properties or conditions such as product, or substrate inhibition or even temperature or pH optima can be investigated.

The GA4 as Process Analytical Technology (PAT) tool provides several key metabolic indices, including oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR), or respiratory quotient (RQ). Processes can be monitored using non-invasive methods to observe the oxygen and carbon dioxide concentrations in the inlet gas and off-gas (exhaust gas) stream outside the sterile barrier [1,2].

Oxygen is a key substrate in aerobic bioprocesses and a continuous supply is needed due to its low solubility in aqueous solutions (Fig. 1).



Respiratory quotient (RQ)

The respiratory quotient (RQ or respiratory coefficient), is a dimensionless number used in calculations of basal metabolic rate (BMR). It is calculated from the ratio of CO₂ produced by the microorganism to O₂ consumed by the microorganism. The Respiratory Quotient can indicate which macronutrients are being metabolized, as different energy pathways consume different substrates including fats, carbohydrates, and proteins. In the human model, if metabolism consists solely of lipids the RQ = 0.7, for proteins RQ = 0.8, and for carbohydrates RQ = 1.0. [4]

$$RQ = \frac{CTR}{OTR}$$

By using the GA4 exhaust gas analyzer it is possible to identify the primary C-source utilized in the process which can be used as trigger and control for nutrient feeds. Furthermore, it can also provide information about product yield, viable cell density and biomass. Furthermore, information can be derived relevant to scale up and mass transfer.

The GA4 module as PAT can generate more information about critical quality attributes required for comprehensive analysis of the bioprocess.

Various in-process compensations

Pressure Compensation

Most O₂ sensors give a partial pressure reading that may be converted into a concentration. Since the ambient pressure changes with metrological conditions (high-pressure systems or low-pressure systems) and the pressure conditions at the point of measurement (i.e. altitude), the use of (fixed) standard values instead of the actual ambient pressure value can result in an error in the actual XO₂ output concentration during conversion and even greater errors in the OTR and CTR calculations.

The Eppendorf GA4 exhaust gas analyzer utilizes an integrated ambient pressure sensor for online compensation which accounts for a deviating pressure at the point of measurement. Therefore, it minimizes the pressure error concerning the changes in environment, weather or locations.

Humidity Compensation

While gas is passing through a bioreactor the amount of nitrogen will not change. However, gas will absorb water vapor while passing through a bioreactor, so the fractions of the various gas components will change, and therefore the output flow of the gas will change.

Eppendorf's GA4 exhaust gas analyzer supports a humidity sensor for compensation according to the change in gas flow caused by increased water vapor concentration using additional humidity sensors.

Volume Compensation

Changing gas concentrations at the input side of the bioreactor are only measured at the output side of the reactors by the off-gas sensor after a delay.

The GA4 exhaust gas analyzer from Eppendorf supports a volume-based strategy for compensation of these effects.

2. Materials and Methods

Do not operate the GA4 in atmospheres with less than 1 % O₂ if it is equipped with ZrO₂ sensors.

Please ensure that the maximum supply pressure is not above the recommended pressures of the MX and GA4.

Please read the user manuals of the devices for further information. The maximum input pressure for the GA4 is 1.2 bar

Table 1: Technical specifications



Model	DASGIP GA	DASGIP GA4E
Dimension (W x D x H)	300 x 320 x 190 mm / 11.8 x 15.6 x 7.5 in	300 x 320 x 190 mm / 11.8 x 15.6 x 7.5 in
Weight	12.1 kg	12.1 kg
Typical Power consumption	47 W (230 V) / 36 W (115 V)	47 W (230 V) / 36 W (115 V)
Exhaust oxygen measurement		
Channels (O ₂ , CO ₂ , mass flow each)	4	4
Measuring principle	Zirconium Dioxide (ZrO ₂)	Galvanic Cell
Measurement range	1 - 50 %	0 - 100 %
Pressure range	0.8 - 1.2 bar	0.8 - 1.2 bar
Channels	4	4
Exhaust carbon dioxide measurement		
Measurement range	0 - 25 %	0 - 25 %
Pressure range	0.8 - 1.2 bar	0.8 - 1.2 bar
Mass flow measurement		
Measurement range	0 - 300 sL/h	0 - 300 sL/h
	0 - 5 sL/min	0 - 5 sL/min

2.1 Set-up

Details of the setup protocol and safety measures for the GA4 are provided in the manual [1].

2.1.1 Foam Trap



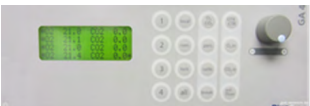
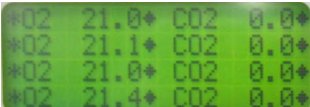
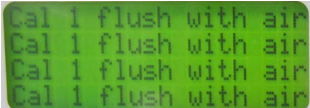
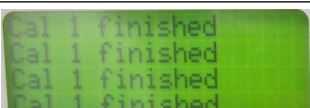


Step	Process Setup	Description
1		The gas analyzer is to be operated with small reactors that are gassed at a flow rate of << 300 sL/h, the reactor can be connected directly to the gas analyzer. The input gas (A) is adjusted using a MFC or rotameter and is sparged through a sterile filter into the bioreactor. The exhaust gas (B) is led through another sterile filter and for safety reasons it is recommended to use a foam trap (C). The exhaust gas can then be connected to the gas analyzer.
2		With large reactors that are gassed at a rate of >300 sL/h, a bypass has to be included in the exhaust setup. So, the exhaust gas line is split into two parts. (B) indicates the exhaust gas line through the sterile filter into the surrounding area. Part of the exhaust gas stream is channeled through another filter, passing a mass flow controller (C) into the trap that leads further to the gas analyzer.

2.1.2 Warming-Up

Step	Process Setup	Description
1		Before initiating experiments with the GA4, the user should flush the GA4 with dried air to reduce humidity that might be accumulated during transport or residual condensate of a previous experiment. This step prolongs its shelf life and prevents damage to the sensors. Therefore, the GA4 tubing for the inlet gas needs to be directly connected to the MX4/4 gas tubing.
2		After approx. 15-30 minutes of flushing, it is safe to activate the GA4. The module will inform the user that it is warming up. This warm-up takes up to 120 minutes.
3		After the warming-up phase the GA4 is ready to use.

2.2 Procedure to execute a One-Point calibration of your Eppendorf GA4 exhaust gas analyzer




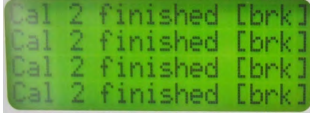

The one-point calibration is required for all GA4 operations. Before starting the calibration, please ensure a warming up period of at least two hours. To execute the one-point calibration the user must sign in as “Operator” or “Manager” in the module.

Step	Process Setup	Description
1		To execute the one-point calibration, the GA4 must be directly connected to a gassing module MX or rotameter/TMFC source. Adjust the gassing rate to 5-50 sL/h. The use of 10 sL/h is recommended.
2		Let the air flush the GA4 for approximately 15 min after the warm-up phase. The measured O ₂ and CO ₂ concentration are shown on the device screen. The gassing rate can be checked using the <O ₂ /CO ₂ > button. Increasing or decreasing concentrations are indicated by ▲ or ▼ arrows.
3		Continue to feed the air and select the channels of the device that need to be calibrated by pressing the <1> ... <4> or <all> button. In this example, all channels are selected as it is indicated by the sun symbol.
4		Then press the button <calib>. The diamond symbol indicates that the calibration for O ₂ and CO ₂ has started and is still ongoing.
5		During calibration, the device display changes automatically between the display shown above in step 4 and the information sheet <Cal 1> flush with air” indicating that the one-point calibration is being performed using compressed air as reference gas. The calibration procedure takes approx. 10 min.
6		The completion of the one-time calibration is indicated by acoustic signal of a “beep” of the device. Further, the screen display shows “Cal 1 finished”, indicating that the one-point calibration is done.
7		The device switches automatically between the window shown under step 6 and the window where the operator can choose to end with the one-point calibration or proceed to the two-point calibration. Pressing the button <calib> the device starts the two-point calibration awaiting the calibration gas standard. The one-point calibration is ended with pressing the button <break>.
8		After pressing the button <break> the one-point calibration is finished and the diamond symbol will disappear. The default information will be shown on the screen.

2.2.1 Procedure to execute a Two-Point calibration of your Eppendorf GA4 exhaust gas analyzer


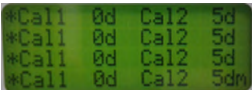

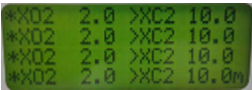

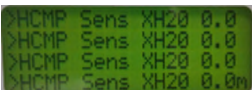
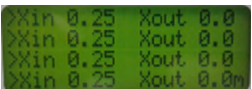
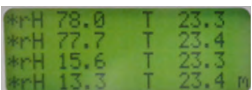
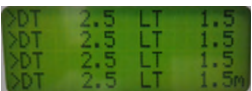

The two-point calibration can be started directly after the one-point calibration is finished. It is recommended for enhanced accuracy.



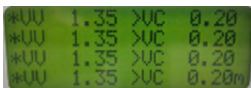
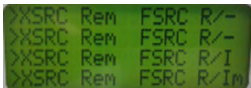
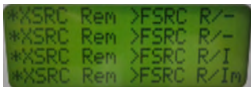

The calibration standard gas can be used together with the MX to provide a steady gas flow, if no other mass flow controller is available. The easiest way is to use the air channel of an available gassing module, for example MX4/4, MX4/1, or MF4.

Step	Process Setup	Description
		On top of the 1-point calibration which is done with dry compressed air, a second gas is needed for the two-point calibration procedure. The recommended calibration gas contents are 2 % O ₂ and 10 % CO ₂ with 88 % N ₂ . Please order from your local supplier. Depending on the supplier, this might be a custom-made gas composition that needs extra inquiry.
8		After setting up the gas flow, the operator should wait at least 15 minutes while the measurement of the calibration standard gas is ongoing. The arrows indicate that the concentrations are changing.
9		After pressing the button <calib> again, the second calibration starts.
10		When the two-point calibration is finished, the GA4 also gives a short acoustic signal with a beep. On the device screen, "Cal 2 finished" is shown and the operator can end the calibration procedure using the button <break>.
11		After pressing the button <break> the two-point calibration is finished and the diamond symbol will disappear. The default information will be shown on the screen.

2.3 Procedure to check your Eppendorf GA4 exhaust gas analyzer settings

After activating the start, and warm up program, and prior to or after calibration of the GA4 module, the user should control and check the setup of the parameters e.g. for compensation strategies, if the parameters meet the specifics of the examination. Wrong settings can lead to up to 20 % errors in measurement and even more in OTR and CTR calculations. The following information is also given with more details in your Eppendorf GA4 manual.

Step	Process Setup	Description
1		When the GA4 is ready to use after the warming phase and calibration, the user should check the most common parameters that the GA4 uses for measurement, calculations and compensation strategies. To check the parameters, press the button <pars>. If the <pars> button is not pressed again after several seconds, then the screen is back to the default view and the next cycle has to be restarted. The values can be changed using the rotary knob if they are free to be changed, indicated by the arrow.
2		If one presses the button <pars> the information given on the display changes. After the first time pressing the <pars> button you find the information of how many days have passed after your last 1-point calibration and your last 2-point calibration of the GA4.
3		After the second time pressing the <pars> button the concentration of the reference gas for the second calibration point – according to the certificate of analysis – should be entered. The oxygen concentration “XO ₂ ” is the first parameter to enter indicated by the arrow directly after the menu window is opened. Typically, an oxygen concentration of 2 % is recommended by the manual.
4		After pressing the button <pars> again the carbon dioxide concentration of the reference gas composition can be entered. Usually a CO ₂ concentration of 10 % is recommended.
5		After pressing the <pars> button again, the setup mode for pressure compensation is shown. There are 3 modes available: automatic “Aut”, manual “Man”, or sensor “Sens”. The right side of the table shows the current measured environmental pressure. The pressure compensation is activated by default and should only be deactivated in special cases.
6		One more press on the button <pars> leads you to the possible choice for humidity compensation “HCMP”. Three modes are available: automatic “Aut”, manual “Man”, or sensor “Sens”. The right side of the table indicates the current measured concentration of humidity in the gas stream. The humidity compensation is activated by default and should only be deactivated in special cases.
7		By pressing the button <pars> again, it gives you the opportunity to enter the humidity of the dry air. You can obtain the value for humidity of the dry air by direct measurement of the input gas connecting the GA4 directly to the mass flow controller. The right column of the table shows the currently measured humidity value of the measured gas.
8		Pressing the button <pars> again will lead to the overview that shows the relative humidity “rH” on the left column and the temperature “T” from the humidity sensor in the measurement bottle on the right column.
9		Next time pressing <pars> will lead you to the setup for the inner diameter of the gas tubing “DT” and the length of the tubing “LT”. Using the rotary knob, you can enter the length of the tubing in meter. The diameter is 2.5 mm by default.
10		Again pressing <pars> the operator can enter the length of the tubing that is 1.5 m by default. For the length of the tubing, it should at least cover the distance between the gassing module and the GA4.

11		Pressing <pars> again, opens the menu for the setup of the volume compensation "VCMP". By default, the volume compensation is switched-off. In the right column the actual volume of the vessel is shown.
12		If the volume compensation is enabled as shown in the figure in step 11, by pressing <pars> again, it will open the menu to enter directly the total drained vessel volume "VV". In this case the SR0700DLS is entered with a total drained volume of 1.35 L. The GA4 does not automatically update this volume based on the DASware control setup.
13		With the next time pressing <pars> you can enter the condenser volume "VC" and additional equipment for example foam traps. Here, 0.2 L is entered.
14		The next menu window that is opened by pressing <pars> again will allow the optional setup of the source of the gas composition "XSRC" that is used internally by the GA4 for calculations. For more information please refer to the GA4 manual.
15		The next step through pressing <pars> again gives the opportunity to set the source of the gas flow "FSRC" that is used internally by the GA4 for calculations. For details refer to the GA4 manual.
16		After pressing <pars> several more times or waiting for a short period of time, the screen will be back to the default view.

2.4 *E. coli* Batch fermentation

For the following protocol we used the Application Note No. 408 [5] as the reference for preparation of the chemically defined medium for our *E. coli* batch process. Here we provide summary details and explanations to clarify the process preparation.

2.4.1. *E. coli* strain preparation

The strain *E. coli* ATCC® 25922GFP™ carrying a plasmid encoding the ampicillin resistance gene *bla* was chosen for these studies [6]. For further details please refer to our Application Note 408 [5].

2.4.2. Batch Media preparation

In this protocol we employed the chemically defined medium described in our Application Note 408. To prepare the medium 10x citrate-phosphate buffer, 100 x trace element solution and thiamine (vitamin B) stock solution, and magnesium sulfate stock solution were pre-prepared. DI-Water citrate-buffer and antifoam were filled in the bioreactor and autoclaved. After autoclaving the sterile MgSO₄ stock solution, 100 x trace element solution, thiamine stock solution, glucose solution to reach 90 g/L, and ampicillin to reach 0.1 g/L were aseptically added to reach the final volume (Table 2).

Table 2: General media composition. For the specifics of the media components please refer to Application Note 408 [5].

Component	1 L standard	0.7 L volume
DI-water	474.7 mL·L ⁻¹	385.00 mL
10 x Citrate-phosphate buffer	75 mL·L ⁻¹	52.50 mL
Antifoam	0.3 mL·L ⁻¹	0.21 mL
Glucose + Ampicillin stock solution	334 mL·L ⁻¹	234 mL
MgSO ₄ stock solution	8 mL·L ⁻¹	5.70 mL
100 x trace element solution	8 mL·L ⁻¹	5.70 mL
Thiamine	0.167 mL·L ⁻¹	0.117 mL
Inoculum 10 % (v/v)	100 mL	70 mL

2.4.3. Process Preparation

We used a DASGIP® Bioblock system and DASware control 5 bioprocess control software. The general process parameters are given in Table 3 and the cascade for the dissolved oxygen (DO) is given in Table 4.

As a further reference for the setup of the DASGIP bioprocess system the Short Protocol No. 32 can be used [7]. This protocol explains briefly how to prepare and conduct *E. coli* fermentation processes in the DASbox Mini Bioreactor System and the DASGIP Parallel Bioreactor Systems.

Table 3: Process parameters and setpoints.

Parameter	Configuration/Setpoint
Vessel type	SR0700DLS with baffles equipped
Dissolved oxygen (DO)	30 %, controlled by DO cascade
Agitation	Direct drive RE40, max 1600 rpm, controlled by DO cascade
Gassing	4 gas mix by MX4/4 controlled by DO cascade
Temperature	37 °C
pH	7.0 ± 0.1 controlled by 25 % (v/v) ammonium solution
Impeller	2 x Rushton type impeller
Sparger	L-sparger

Table 4: DO Cascade

DO controller output	Agitation [rpm]	Oxygen [%O ₂]	Gas flow [sL·h ⁻¹]
0 % _{DOout}	600	21	42
60 % _{DOout}	1600	21	42
60 % _{DOout}	1600	21	42
100 % _{DOout}	1600	100	42

3. Results & discussion

The goal of most batch, fed-batch, and continuous processes is to achieve the highest possible biomass concentrations in the bioreactor. Under these conditions OTR is one of the key parameters monitored in the design, operation, and scale-up of bioreactors. The OTR value is a typically given specifying parameter for our Eppendorf bioreactors for microbiology determined at the most prominent process condition derived by the sulfite oxidation method.

Stirred tank reactors must be designed so that they have at least the OTR required to maintain the OUR at the required-maximum cell mass. Biologically, the OUR corresponds to the product of biomass-specific oxygen uptake (q_{O_2}) and the concentration of biomass. Since the rates of biological OUR and technical defined OTR must correspond in steady state the product of the equation is technically limited by the OTR.

$$OUR = q_{O_2} \cdot c_x = OTR$$

If the oxygen demand exceeds the maximum OTR the vessel can provide, growth, and product formation become oxygen limiting, and important growth parameters become unrecognizable. Furthermore, shifts in the metabolic pathways can occur.

The GA4 operating parameter values OTR and CTR are shown under the gas analyzer dialog box of the DASware control (Figure 5). Those parameters correspond to the current OUR and CER under the current process condition.

The data can be used directly for process control, and to obtain online information of cell viability. In addition, these data can be used for online calculation of the maximum growth rate.



Fig. 5: Process view of a single unit during an *E. coli* batch fermentation process. The light green data shows the decrease of the dissolved oxygen starting from 100%DO until it reaches the setpoint of 30%DO. The darker red line indicates the increase of the stirring speed which is needed to keep the DO at the setpoint until the oxygen concentration of the bioreactor's inlet air increases. Following this process, pink data lines indicate the trend of the transfer rates. The nearly overlapping OTR (OUR) and CTR (CER) during the process run. Those values are steadily increasing until the carbohydrate to metabolize is no longer available in the growth medium. The sharp decrease indicates here the end of the batch process. The respiratory quotient (RQ) can also be added to the online graph.

For a comprehensive summary, the general process values are shown. Figure 6 displays the OUR (OTR), CER (CTR), and RQ. This indicates the growth of the *E. coli* culture and its metabolic activity. The signal drop towards the end of the process indicates the end of the process itself when the main carbohydrate source was depleted. This change in signal could be used to start a feed for a fed-batch culture. Figure 7 displays the measurement of the off-gas components.

Figure 7 displays the off-gas analyses for the oxygen and carbon dioxide components. Despite the increased oxygen concentration in the input gas stream it is not possible to transfer all the oxygen into the liquid phase as indicated by the increased oxygen concentration in the exhaust gas stream. However, a higher oxygen concentration in the input gas stream is needed so that the OTR can meet the OUR demand of the *E. coli* culture.

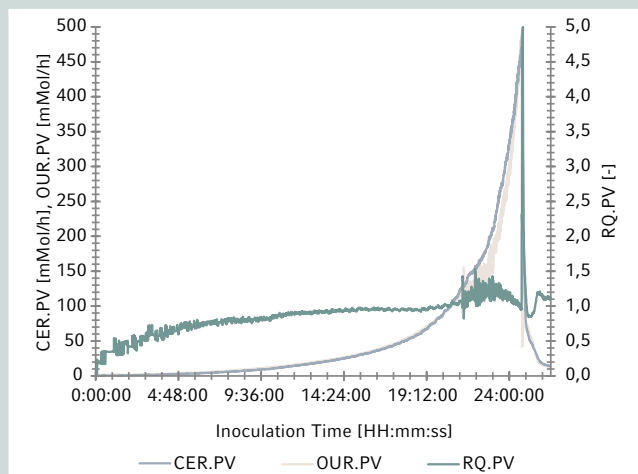


Fig. 6: GA4's calculated transfer rates OUR, CER, and RQ.

In conclusion, the use of the GA4 off-gas analyzer as PAT possibilities offers a number of significant and useful applications. The specific oxygen uptake q_{O_2} of a microorganism can be recorded, allowing an organism with low oxygen demand and high ability for product formation can be identified.

Another function supported by the GA4 gas analyzer is the maintenance of a specific OUR, CER, or RQ during the

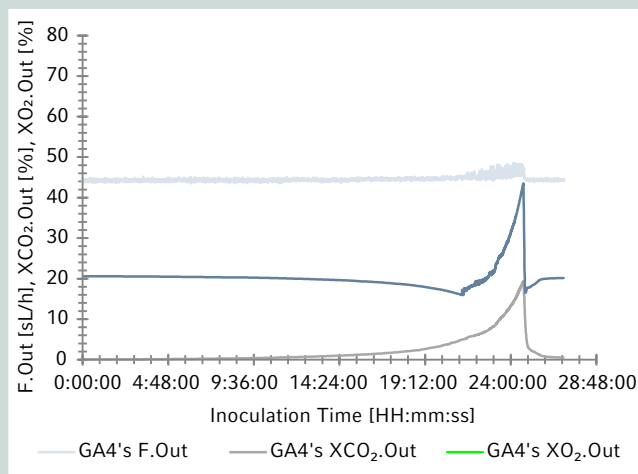


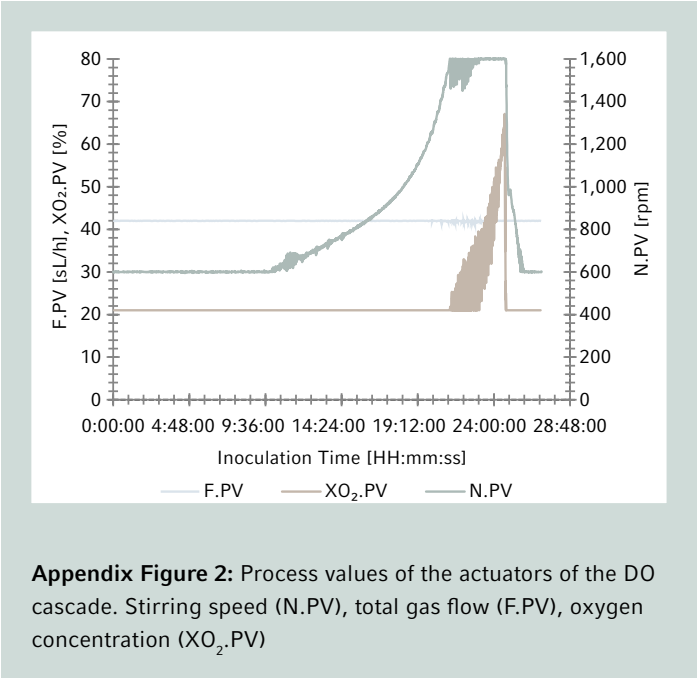
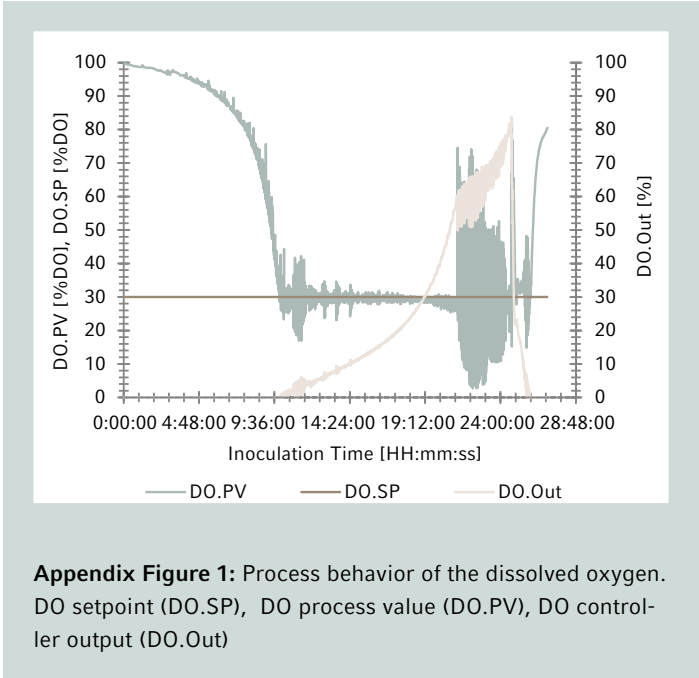
Fig. 7: Displays the off-gas analyses for the oxygen, carbon dioxide and gas flow components.

entire feed process by means of a correspondingly controlled substrate feed and composition. In this configuration the microbial growth and activated metabolic pathway remain steady, and the pathway can be manually switched. In DAS-ware control software, this can be achieved by scripting or by building a user-defined loop to control the feed pumps.

Literature

- [1] DASGIP® GA4 Off-Gas Analyzer User Manual
- [2] Winckler S., Krueger R., Schnitzler T., Zang W., Fischer R., Biselli M., 2013. A sensitive monitoring system for mammalian cell cultivation processes: a PAT approach Bioprocess Biosyst Eng. DOI: <https://doi.org/10.1007/s00449-013-1062-8>
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Appendix



Ordering information

Description	Order no.
DASGIP® GA4 Exhaust Analyzing Module , including accessories for 4 vessels, including analog I/O option	
O ₂ 1 - 50 % and CO ₂ 0 - 25 %	76DGGA4X
O ₂ 0 - 100 % and CO ₂ 0 - 25 % (GA4E)	76DGGA4EX
DASGIP® GA4 Stand-Alone Exhaust Analyzing Module , including analog I/O option, including accessories, without relative humidity measurement	
for 1 vessel, O ₂ 1 - 50 % and CO ₂ 0 - 25 %	76DMGA1X
for 1 vessel, O ₂ 0 - 100 % and CO ₂ 0 - 25 % (GA1E)	76DMGA1EX
for 2 vessels, O ₂ 1 - 50 % and CO ₂ 0 - 25 %	76DMGA2X
for 2 vessels, O ₂ 0 - 100 % and CO ₂ 0 - 25 % (GA2E)	76DMGA2EX
for 4 vessels, O ₂ 1 - 50 % and CO ₂ 0 - 25 %	76DMGA4X
for 4 vessels, O ₂ 0 - 100 % and CO ₂ 0 - 25 % (GA4E)	76DMGA4EX
Accessories	
Kit to Compensate Relative Humidity and Temperature , for DASGIP® GA4, including accessories	
for 1 vessel	76DGGA1RHT
for 2 vessel	76DGGA2RHT
for 4 vessel	76DGGA4RHT
DASGIP® MX4/4 Stand-Alone Gas Mixing Module , for 4 vessels, mass flow controller	
0.1 – 50 sL/h, 0.1 – 40 sL/h CO ₂ , incl. 2x 30 m gas tube and EasyAccess Software	76DMMX44
0.5 – 250 sL/h, 0.5 – 150 sL/h CO ₂ (MX4/4H), incl. 2x 30 m gas tube and EasyAccess Software	76DMMX44H
DASGIP® MX4/1 Stand-Alone Gas Mixing Module , for 1 vessel, mass flow controller	
20 – 600 sL/h, incl. 2x 30 m gas tube and EasyAccess Software	76DMMX41F600
10 – 300 sL/h, incl. 2x 30 m gas tube and EasyAccess Software	76DMMX41F300
4 – 120 sL/h, incl. 2x 30 m gas tube and EasyAccess Software	76DMMX41F120
1 – 30 sL/h, incl. 2x 30 m gas tube and EasyAccess Software	76DMMX41F030
40 – 1200 sL/h, incl. 2x 30 m gas tube and EasyAccess Software	76DMMX41F1200

Your local distributor: www.eppendorf.com/contact

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