J Ind Microbiol Biotechnol
DOI 10.1007/s10295-006-0176-2

ORIGINAL PAPER

An experimental comparison of respiration measuring techniques in fermenters and shake flasks: exhaust gas analyzer vs. RAMOS device vs. respirometer

Juri M. Seletzky · Ute Noack · Sebastian Hahn · Arnd Knoll · Ghassem Amoabediny · Jochen Büchs

Received: 13 March 2006 / Accepted: 29 August 2006

Abstract Respiration measurement is applied as a universal tool to determine the activity of biological systems. The measurement techniques are difficult to compare, due to the vast variety of devices and analytical procedures commonly in use. They are used in fields as different as microbiology, gene engineering, toxicology, and industrial process monitoring to observe the physiological activity of living systems in environments as diverse as fermenters, shake flasks, lakes and sewage plants. A method is introduced to determine accuracy, quantitation limit, range and precision of different respiration measurement devices. *Corynebacterium glutamicum* cultures were used to compare an exhaust gas analyzer (EGA), a RAMOS device (respiration measurement in shake flasks) and a respirometer. With all measuring devices it was possible to determine the general culture characteristics. The EGA and the RAMOS device produced almost identical results. The scatter of the respirometer was noticeably higher. The EGA is the technique of choice, if the reaction volume is high or a short reaction time is required. The possibility to monitor cultures simultaneously makes the RAMOS device an indispensable tool for media and strain development. If online monitoring is not compulsive, the respiration of the investigated microbial system extremely low, or the sample size small, a respirometer is recommended.

J. M. Seletzky · U. Noack · S. Hahn · A. Knoll · G. Amoabediny · J. Büchs (✉)
Biochemical Engineering, RWTH Aachen University, Sammelbau Biologie, Worringerweg 1, 52056 Aachen, Germany
e-mail: buechs@biovt.rwth-aachen.de

Keywords *Corynebacterium glutamicum* · Exhaust gas analyzer · Oxygen electrode · Oxygen transfer · Respirometry

Introduction

In aerobic cultures, almost every physiological activity is coupled to the respiratory uptake of oxygen, making the oxygen transfer rate (OTR) a valuable parameter to monitor the metabolic activity of biological cultures [1, 2]. The OTR reflects the physiological responses of the microorganisms to different culture conditions such as temperature, pH, osmotic stress, nutrient limitation and inhibition, product or by-product formation and inhibition. Furthermore, it can be used to justify sampling times or the induction time for gene expression.

The comparison and evaluation of different respiration measuring techniques is rendered difficult by the vast variety of devices and analytical procedures commonly in use. The respiration can be measured in reaction vessels as different as fermenters or shake flasks with devices as different as exhaust gas analyzers or respirometers and with sensors as different as magneto-mechanical, electro-chemical, or optical. This study introduces a method to compare and evaluate different respiration measurement devices and analytical procedures considering accuracy, precision, quantitation limit and range. It focuses on the evaluation of the measuring techniques, their advantages and limitations and their possible areas of application. The respiration was measured with three commercially available devices: an exhaust gas analyzer (EGA) coupled to a fermenter, a RAMOS (Respiration Activity Monitoring System) device, which is a novel

Journal : 10295 | Dispatch : 12-9-2006 | Pages : 8
Article No. : 176 | ☐ LE | ☐ TYPESET
MS Code : 176 | ☑ CP | ☑ DISK

technique to monitor the respiration in shake flasks online, and a respirometer.

Measuring the respiration of bioreactors with exhaust gas analyzers (EGA) has been state of the art for years. They are an important tool for process optimization in research and industrial process control. Shake flasks are inexpensive and can be operated simultaneously in large numbers. In large industrial companies, up to several hundred thousand individual experiments may be performed in shake flasks every year [3]. Nevertheless, most experiments are still conducted without any online monitoring hampering focused screening and complicating scale-up by neglecting the effect of oxygen supply and fermentation time on growth and product formation. Only in recent years the monitoring of the OTR in shake flasks [1, 2, 4–8] has become more common. A commercially available technique is the RAMOS device, which enables simultaneous online monitoring of the OTR in several biological cultures under sterile conditions. Respirometry is a wide spread technique to measure the oxygen uptake of various cell suspensions such as bacteria [9–11], microcrustacean [12], or macrophages [13]. It is mainly used to determine the effect of different environmental conditions or the addition of toxic substances or growth factors on the viability. The respiration is usually measured with electro-chemical oxygen electrodes [14], or more recently with optical sensors [8, 15].

Corynebacterium glutamicum [16] a Gram-positive soil bacterium widely used for the industrial production of amino acids was applied as model organism. The organism has the advantage that its growth parameters and cell physiology are generally not influenced by fluid mechanical stress (high-aeration rates, high stirrer speeds) or variations of the dissolved oxygen [17, 18]. Thus, variations of the aeration rate or the stirrer speed should not influence the culture characteristics as long as the dissolved oxygen is above zero.

Materials and methods

Microorganism and cultivation

All experiments were carried out with the wild type of *C. glutamicum* ATCC 13032 [16]. The organism was cultivated with two different media. A complex medium with glucose as carbon source and a defined minimal medium with lactic acid as carbon source to avoid the forming of anaerobic or overflow metabolites.

The complex medium contained per liter (letters in brackets refer to the supplier): 20 g glucose (R), 10 g yeast extract (R), 10 g peptone (M), 2.5 g NaCl (M), 0.25 g $MgSO_4$ (R). The pH was adjusted to 7.2, the glucose was sterilized separately.

Complex medium plate cultures were used to inoculate complex medium precultures. The precultures were harvested after 10 h. Complex medium main cultures were directly inoculated from the precultures with a biomass concentration of 0.3 g/l. For minimal medium main cultures the precultures were washed twice in 9 g/l NaCl. The pellet was resuspended in minimal medium and the main culture inoculated with a biomass concentration of 0.42 g/l. The temperature for all cultivations was 30°C.

The minimal medium contained per liter (letters in brackets refer to the supplier): 10 g lactic acid (R), 20 g $(NH_4)_2SO_4$ (R), 1 g KH_2PO_4 (R), 2 g K_2HPO_4 (F), 0.25 g $MgSO_4{\cdot}7H_2O$ (R), 30 mg $(HO)_2C_6H_3COOH$ (R), 10 mg $CaCl_2{\cdot}H_2O$ (A), 10 mg $MnSO_4{\cdot}H_2O$ (M), 10 mg $FeSO_4{\cdot}7H_2O$ (S), 1 mg $ZnSO_4{\cdot}7H_2O$ (F), 0.2 mg $CuSO_4$ (M), 0.2 mg biotin (R), 0.02 mg $NiCl_2{\cdot}7H_2O$ (R). The pH was adjusted to 7 with NaOH (R). The trace elements, the biotin, the 3,4-dihydroxybenzoic acid ($(HO)_2C_6H_3COOH$) were sterile filtered, the lactic acid was autoclaved separately.

The chemicals were supplied by the following companies (underlined letters refer to the abbreviations used in the media descriptions): AppliChem, Darmstadt, Germany; Fluka, St. Gallen, Switzerland; Merck, Darmstadt, Germany; Roth, Karlsruhe, Germany; Sigma-Aldrich, St. Louis, MO, USA

Analytical procedures and culture conditions

Exhaust gas analyzer and fermentations

Figure 1a schematically depicts the analytical procedure to determine the OTR with an exhaust gas analyzer (EGA). The OTR is calculated by specifying the oxygen concentration difference between the inlet gas stream (O_{2in}) and the outlet gas stream (O_{2out}). The OTR was measured with a magneto-mechanical EGA (Advance Optima, Magnos 106, ABB Automation, Frankfurt, Germany). The outlet gas stream is dried by a cooler and the volume flow (0.5 l/min) through the EGA is kept constant with a thermal mass flow controller (5850TR, Brooks, Hatfield, PA, USA), thus, variations of the aeration rate cannot influence the EGA. It was calibrated prior to each experiment with nitrogen and a test gas (25% O_2, 5% CO_2 and 70% N_2). The OTR was recorded every 2 min. Fermentations were carried out in a laboratory fermenter (Biostat M, Braun Biotech, Melsungen, Germany) total capacity 1.5 l, working volume 1 l, specific aeration

Journal : 10295 Dispatch : 12-9-2006 Pages : 8
Article No. : 176 LE TYPESET
MS Code : 176 CP DISK

rate 2 vvm, rushton turbine (four blades, diameter 47 mm, blade height 9 mm). The inlet gas stream of the fermenter was controlled with a thermal mass flow controller (5850TR, Brooks, Hatfield, PA, USA). Additional fermentations were carried out in a 50 l fermenter (LP351, Bioengineering AG, Wald, Switzerland) total capacity 50 l, working volume 15 l, specific aeration rate 0.5 vvm, three rushton turbines of that one submersed (six blades, diameter 120 mm, blade height 25 mm), four baffles (height 600 mm, width 30 mm) a more detailed description of the fermenter is given by [19]. The dissolved oxygen (DO_2) was maintained above 30% by adjusting the stirrer speed. In case of excessive foam the antifoam agent (Plurafac LF 1300, BASF, Ludwigshafen, Germany) was added.

RAMOS and shaken cultures

Figure 1b schematically depicts the analytical procedure to determine the OTR in shake flasks employing the RAMOS technology developed by Anderlei [1, 4]. The OTR is measured by periodically repeating an automated measuring cycle. A measuring cycle is composed of a measuring phase (Δt) (flask closed airtight, 10 min) and a rinsing phase (continuous air flow, 20 min). Thus, recreating the average oxygen supply of a standard flask with cotton plug [4]. The OTR is calculated from the decrease of the partial oxygen pressure (ΔpO_2) in the headspace of the shake flask (gas volume V_g).

The OTR was measured with a RAMOS device (Hitec Zang, Herzogenrath, Germany) utilizing an electro-chemical oxygen sensor. To allow the monitoring of weakly respiring cell cultures the accuracy and the precision of the measurement is increased by recalibrating the oxygen sensors before each measuring phase using the steady state gas composition at the end of the rinsing phase [1, 4]. To avoid oxygen limited culture conditions the operating conditions were selected according to the shake flask model of Maier and Büchs [20]. All shaken cultures were cultivated on an orbital shaker (Lab-Shaker LS-W, Adolf Kühner AG, Birsfelden, Switzerland) with a shaking diameter (d_0) of 50 mm, a shaking frequency (n) of 300 rpm and a filling volume (Vl) of 10 ml. RAMOS cultivations were carried out in unbaffled 250 ml measuring flasks [2]. The temperature was kept constant at 30°C by placing the RAMOS device in a thermo-constant room.

Respirometry and solubility

Figure 1c schematically depicts the analytical procedure used to determine the OTR with a respirometer. A sample taken from any culture vessel is aerated and afterwards the decrease of the dissolved oxygen (DO_2)

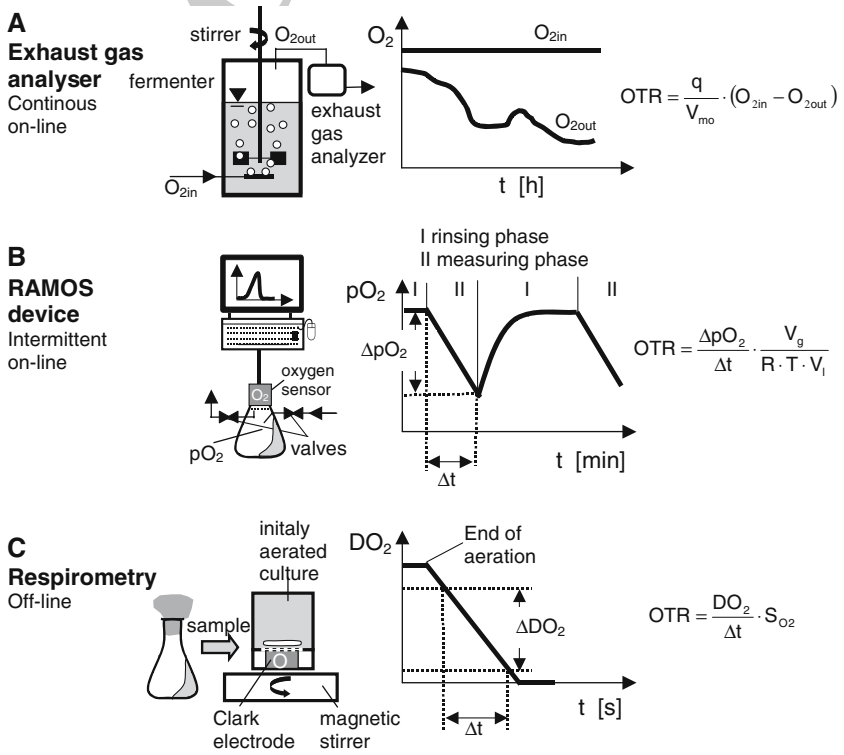

Fig. 1 Schematic depiction of different analytical procedures to determine the oxygen transfer rate (OTR). Nomenclature: **a** q specific aeration rate, V_{mo} molar gas volume at standard conditions, O_{2in} oxygen concentration of the inlet gas stream, O_{2out} oxygen concentration of the outlet gas stream; **b** pO_2 partial oxygen pressure in the headspace of the shake flask, Δt time interval of the measuring phase, V_g gas volume of the headspace of the shake flask, R gas constant, T temperature, V_l liquid filling volume; **c** DO_2 dissolved oxygen, Δt time interval of the measurement, S_{O_2} oxygen solubility

Journal : 10295 | Dispatch : 12-9-2006 | Pages : 8
Article No. : 176 | LE | TYPESET
MS Code : 176 | CP | DISK

over time (Δt) is measured. The OTR can be calculated, using the oxygen solubility (S_{O_2}).

A respirometer with an electro-chemical oxygen electrode (Rank Brothers, Cambridge, England) was used to measure the OTR of complex medium samples each drawn from an individual 250 ml standard shake flask with cotton plug or of minimal medium samples drawn from a fermenter. A sample of 5 ml culture broth was directly transferred to the measuring chamber. The aeration was performed with a silicone tube (ID. 3 mm) that produced large bubbles to avoid the formation of micro bubbles, which might otherwise act as an oxygen source during the measurement. DO_2 gradients were avoided by stirring the measuring chamber with a magnetic stirrer. After aeration, the measuring chamber was quickly closed. The time interval between a DO_2 of 80 and 20% was recorded with a stop watch. All manipulations were carried out in a thermo-constant room at 30°C. The respirometer was calibrated prior to the measurement with air and nitrogen.

The solubility (complex media 0.0011 mol/l/bar, minimal media 0.001 mol/l/bar) was calculated according to [21–23]. The effect of lactic acid on S_{O_2} was assumed to be equal to that of acetate. For lactic acid no data was available. However, the error can be assumed to be small, because organic acids have only a small influence on the solubility.

Results and discussion

Biological experiments were conducted to evaluate the ability of the EGA, the RAMOS device and the respirometer to determine the OTR. With a first set of experiments the accuracy of each individual measuring technique was determined independently, using defined minimal medium cultures. In a second set of experiments the measuring techniques were compared using minimal and complex medium cultures. Finally, based on theoretical considerations and literature data quantitation limit and range of the devices were compared. All validation characteristics in this work agree with the definitions of the Q2B Validation of Analytical Procedures [24].

Accuracy

The accuracy of the measuring techniques was evaluated by determining the specific growth rate (μ), which reflects the effect of environmental conditions on the activity of microorganisms. μ of an exponentially growing culture can be deduced from the OTR using the slope of a regression function (Eq. 1), t0 being the start of the exponential growth phase. If the culture growth is assumed to be ideally exponential, according to Eq. 1, the coefficient of determination (r^2) reflects the accuracy of the measuring set-up.

$$OTR_t = OTR_{t0} \cdot e^{\mu t} \quad (1)$$

The specific growth rate of each experiment was determined by fitting the measuring data with Eq. 1, using the least square method. For each fit the coefficient of determination (r^2) was calculated. Replicates were compared by calculating the average specific growth rate ($AV\mu$) and its coefficient of variation (CV_μ). Figure 2a depicts the OTR over the fermentation time of independent not pH controlled minimal medium laboratory fermenter cultures with EGA (three replicates). Two different exponential growth phases could be described. At fermentation times between 4 and 9.5 h the organism grew with an $AV\mu$ of 0.27 h^{-1} (solid line), which decreased to 0.21 h^{-1} (doted line) between 9.5 and 11 h. Figure 2b depicts independent not pH controlled minimal medium shake flask cultures with RAMOS device (six replicates). These cultures also show two different exponential growth phases. The average growth rates of the shake flask cultures are similar to the not pH controlled fermenter cultures (4–9.5 h, $AV\mu = 0.31\ h^{-1}$, solid line; 9.5–11 h, $AV\mu = 0.23\ h^{-1}$, doted line). Figure 2c depicts the OTR over fermentation time of pH controlled minimal medium laboratory fermenter and 50 l fermenter cultures with EGA (three replicates). The pH controlled cultures showed only a single exponential growth phase. The average growth rate of the pH controlled cultures (4–10.5 h, $AV\mu = 0.35\ h^{-1}$, solid line) was higher than $AV\mu$ of the not pH controlled cultures. The different fermenter scales had no influence on the culture characteristics. For all experiments depicted in Fig. 2 the coefficient of variation of the average growth rate (CV_μ) was below 7% and the coefficient of determination (r^2) always above 0.99.

The comparison of the three measuring techniques is depicted in Fig. 3 (a, minimal medium; b, complex medium). To set equal culture conditions in fermenters and shake flasks, the pH was not controlled and a single inoculated medium was prepared, which was distributed to fermenters and shake flasks. With all three measuring techniques it was possible to observe the general culture characteristics (Fig. 3). The slope of the OTR curves, the maximum OTR and the cultivation time were comparable. The OTR curves of the minimal medium cultures (Fig. 3a) recorded with EGA and RAMOS are very similar to the ones depicted in

Journal : 10295 Dispatch : 12-9-2006 Pages : 8
Article No. : 176 LE TYPESET
MS Code : 176 CP DISK

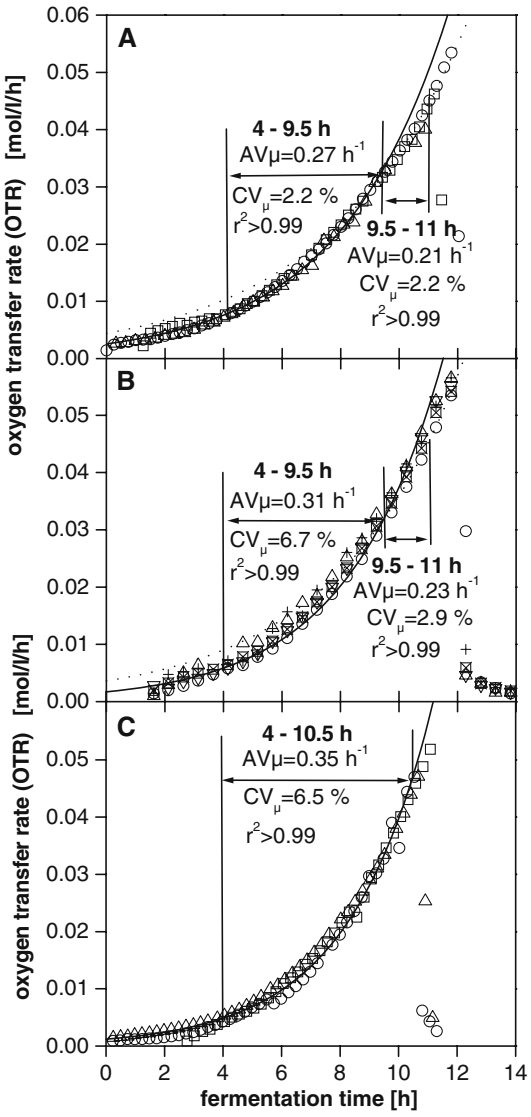

Fig. 2 Accuracy of *C. glutamicum* ATCC 13032 minimal medium fermenter and shake flask cultures with exhaust gas analyzer (*EGA*) and RAMOS device. **a** Laboratory fermenter with EGA, pH not controlled *open square*, *open circle*, *open triangle*. **b** Shake flasks with RAMOS device, pH not controlled *open square*, *open circle*, *open triangle*, *plus symbol*, *cross symbol*, *open inverted triangle*. **c** Laboratory fermenter with EGA, pH controlled *open square*; 50 l fermenter, pH controlled *open circle*, *open triangle*. Exponential fits *solid line*, *dotted line*. Culture conditions laboratory fermenter, Biostat M, Braun Biotech: total capacity 1.5 l, working volume 1 l, specific aeration rate 2 vvm, rushton turbine (four blades, diameter 47 mm, blade height 9 mm). Culture conditions 50 l fermenter LP351, Bioengineering AG: total capacity 50 l, working volume 15 l, specific aeration rate 0.5 vvm, three rushton turbines of that one submersed (six blades, diameter 120 mm, blade height 25 mm), four baffles (height 600 mm, width 30 mm). The dissolved oxygen (DO_2) of the fermenter cultures was maintained above 30% by adjusting the stirrer speed. Culture conditions shake flasks, RAMOS device: unbaffled 250 ml measuring flasks [2], shaking diameter 50 mm, shaking frequency 300 rpm, filling volume 10 ml

Fig. 2a, b. With the average of the growth rates of Fig. 2a, b (μ = 0.29 h^{-1}, dashed line, μ = 0.22 h^{-1}, dotted line) the measuring data of both devices can be fitted with a r^2 higher than 0.99. The complex medium cultures (Fig. 3b) have a higher growth rate than the minimal medium cultures. The measuring values of EGA and RAMOS device can be fitted with a μ of 0.66 h^{-1} (0–4.5 h, dashed line) resulting for both devices in a r^2 higher than 0.97. The accuracy of the respirometer was found to be noticeably lower than the accuracy of the EGA and the RAMOS device. This observation did not depend on the fermentation device (fermenter, shake flask). The minimal medium samples (Fig. 3a) were taken from a single fermenter culture (two replicates per fermentation time). Each complex medium sample was taken from an individual shake flask (two replicates per fermentation time). With the respirometer it was not possible to differentiate the two exponential growth phases on minimal medium. The lower accuracy is reflected by the in comparison to the EGA and the RAMOS device lower coefficient of determination (minimal medium 4–9.5 h r^2 = 0.96, complex medium 0–4.5 h r^2 = 0.94). The specific growth rates observed in this study are in good agreement with the data of Cocaign et al. [25, 26] who found for *C. glutamicum* ATCC 17965 batch cultures a maximum growth rate of 0.35 h^{-1} on lactate and of 0.6 h^{-1} on glucose. In conclusion, the high accuracy of EGA and RAMOS device allows to describe the respiration and culture characteristics of microorganisms in detail. Thus, both are methods to be applied for scale-up purposes. With the respirometer it is possible to describe the general culture characteristics of a biological culture. However, the accuracy is too low for a detailed analysis of the culture characteristics.

Precision

The precision of the measuring devices was compared with the cumulative consumed oxygen (c_{O_2}), which is independent of the biological kinetics, and depends on the amount of limiting substrate. c_{O_2} is calculated by integrating the OTR according to Eq. 2. The time of the maximum oxygen transfer rate (OTR_{max}) was used as the upper boundary.

$$c_{O_2}(t) = \int_0^{t_{OTR\,max}} OTR\,dt \qquad (2)$$

The c_{O_2} values were derived from the data presented in Fig. 3. The use of a single inoculated medium

Journal : 10295 Dispatch : 12-9-2006 Pages : 8
Article No. : 176 LE TYPESET
MS Code : 176 CP DISK

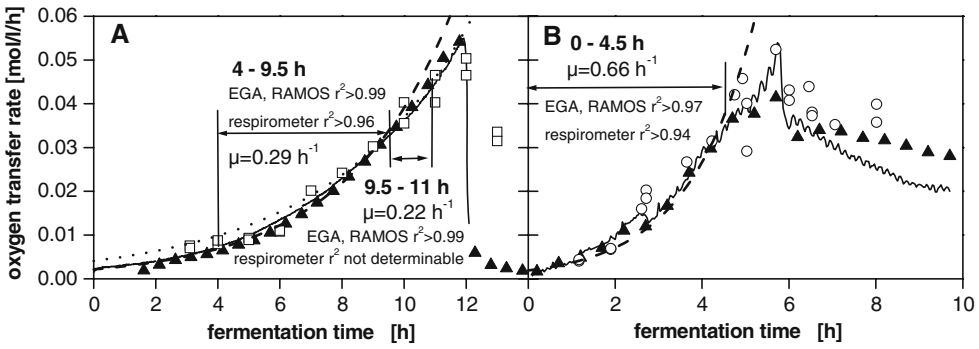

Fig. 3 Comparison of different respiration measurement techniques. Oxygen transfer rate of *C. glutamicum* ATCC 13032 over fermentation time. **a** Minimal medium with lactic acid, **b** complex medium with glucose. Fermenter with exhaust gas analyzer (*EGA*) *solid line*; shake flask with RAMOS device *filled triangle*; fermenter with respirometer *open square*; shake flask with respirometer *open circle*. Exponential fits *dashed line*, *dotted line*. Culture conditions laboratory fermenter: Biostat M Braun Biotech, total capacity 1.5 l, working volume 1 l, specific aeration rate 2 vvm, rushton turbine (four blades, diameter 47 mm, blade height 9 mm). Culture conditions shake flasks: unbaffled 250 ml (standard flasks, or RAMOS measuring flasks [2]), shaking diameter 50 mm, shaking frequency 300 rpm, filling volume 10 ml

assured identical substrate concentrations for all measuring devices. c_{O_2} of the minimal media cultures was 0.22 mol/l with a deviation of less then 2% between EGA and RAMOS. The scatter of the respirometer did not allow a determination of c_{O_2}. The complex media cultures had a c_{O_2} of 0.11 mol/l with a deviation of less then 5% EGA and RAMOS device. The precision of EGA and RAMOS has been found to be equivalently high. The measuring values of the respirometer are largely dependant on personal experience of the operator resulting in a low precision and robustness.

Quantitation limit and range

EGA, RAMOS device and respirometer were compared considering the minimal (V_{min}) and maximal (V_{max}) reaction volume, minimal OTR_{min}, and maximal OTR_{max} oxygen transfer rate, and online measurement possibilities. All results are summarized in Table 1. The measuring set-ups consist of three components: an oxygen measuring device (EGA, RAMOS, respirometer), a fermentation vessel (fermenter, shake flask), and a fermentation environment (e.g. temperature and pH control, thermo-constant room). All three components and their interaction influence the quantitation limit and range of the measuring set-ups, making it difficult to precisely determine quantitation limits and ranges. Particularly, the characteristics of stirred tank fermentations depend more on the fermenter size, which can range from 250 ml to 500 m^2, and the mode of operation than on the EGA. The measurement of low OTR values can be influenced by the quality of the temperature control. Considering these constrains the values in Table 1 are only intended to give a general orientation. In addition to literature data simple theoretical considerations were used to determine quantitation limit and range of the EGA. The values given in Table 1 for the RAMOS device and the respirometer were experimentally (not all data shown) confirmed by recording OTR over time curves under the given conditions.

Table 1 Summary of the different oxygen transfer rate measuring devices: analytical procedure, quantitation limit, range, precision

The values given in this table are intended to give a general orientation. However, values vary with the measuring set-up and the device used

Name	Exhaust gas analyzer	RAMOS	Respirometer
Reaction vessel	Fermenter	Shake flask	Any
Common O_2 sensor type	Magneto-mechanical	Electro-chemical	Electro-chemical
Number of parallel fermentation vessels	1–(5)	6–12	(1)
Online monitoring	Yes	Yes	No
Measuring interval	Continuous	10–30 min	Limited by manual handling
Reaction volume (l)	>0.25	0.005–0.1	For the device used 0.001–0.007
OTR, OUR_{max} (mol/l/h)	0.2–0.6	0.08	0.05
OTR, OUR_{min} (mol/l/h)	1×10^{-4}	1×10^{-4}	$>1 \times 10^{-5}$

Journal : 10295 Dispatch : 12-9-2006 Pages : 8
Article No. : 176 LE TYPESET
MS Code : 176 CP DISK

Exhaust gas analyzer

The minimal volumetric flow of ~0.5 l/min to keep the magneto-mechanical oxygen sensor working determines V_{min}. A standard fermenter q (~2 vvm) therefore has a V_{min} of ~0.25 l. It can be reduced by changing to electro-chemical oxygen electrodes or to mass spectrometry. V_{max} is determined by the size of the fermenter. Power input and the size of the fermenter determine OTR_{max}. A standard stirred tank fermenter has a OTR_{max} of 0.2–0.6 mol/l/h [28]. If the minimal specific aeration rate is considered to be 0.01 vvm, a standard value for cell cultures, and the minimal oxygen concentration difference 1×10^{-4} mol/mol, OTR_{min} is 1×10^{-4} mol/l/h. With multiplexing up to five fermenters can be operated with one EGA, if the measuring interval is increased to 30 min. Continuous online monitoring is possible.

RAMOS

To reduce the measuring error due to evaporation, V_{min} should be higher than 5 ml. V_{max} depends on the oxygen requirements of the microorganisms and the size of the shake flask. A bacteria culture in a 250 ml shake flask requires a V_{max} of less then 25 ml [20]. For low respiring cell cultures V_{min} can reach up to 150 ml in 250 ml shake flasks. OTR_{min} of the standard RAMOS device is ~1×10^{-3} mol/l/h and can be decreased to 1×10^{-4} mol/l/h by further reducing the influence of ambient conditions especially of temperature fluctuations. OTR_{max} is determined by the culture conditions [20]. For a standard shaker (n_{max} = 350 rpm) and a reaction volume of 10 ml, OTR_{max} is ~0.065 mol/l/h and can be raised to 0.1 mol/l/h by increasing the shaking frequency up to 500 rpm or using baffled shake flasks. The OTR is measured intermittently with a measuring interval of 10–30 min.

Respirometer

The minimal sample size (~1 ml) needed to wet the oxygen electrode determines V_{min}. Smaller samples can be processed in respirometers with needle type electro-chemical or optical oxygen sensors. The air tightness of the measuring chamber and the oxygen consumption of the electrochemical oxygen electrode determine OTR_{min}. Theoretically, even the respiration of single organisms can be measured. With an optical oxygen sensor we could determine OTRs lower than 1×10^{-5} mol/l/h (data not shown). OTR_{max} is ~0.05 mol/l/h, at higher respiration rates the oxygen consumption is faster than the mass transfer of the aeration. The mass transfer of the culture vessel has no influence on the measurement, impeding the detection of oxygen limitations due to insufficient culture conditions. Online monitoring is not possible (manual sample injection).

Summary and overall conclusion

To design an industrial fermentation a large number of experiments, according to [3, Büchs] in large companies up to several hundred thousand individual experiments per year, are necessary, to select a strain, to improve the medium and to characterize the productivity at different culture conditions. These screening tasks are normally performed in shake flasks or laboratory fermenters. Because of their simplicity and inexpensiveness, according to [29], the only practical way is to perform the major part of these experiments in shake flasks. However, shake flasks lack the possibility to monitor the culture during the experiment. This limits their application to simple standard tasks and may result in unexpected scale-up problems. To overcome these limitations [1, 4] introduced the RAMOS device, which allows the online monitoring of the respiration of microbial cultures in shake flasks.

The focus of this study was to compare and evaluate three different respiration measurement techniques exhaust gas analyzer, RAMOS device, and respirometer. By choosing an appropriate biological model system it was possible to compare the different techniques independent of their analytical procedure or the reaction vessel used for cultivation. Exhaust gas analyzer and RAMOS device resulted in very similar culture characteristics. Accuracy, and precision of both devices was high. Respirometry is a simple and cost efficient tool to check the activity of a biological culture. But accuracy and precision have been found too low for screening purposes. The results show, that the respiration measured in shake flasks and fermenters can be very similar. This allows, to increase the productivity by performing screening experiments in online monitored shake flasks which traditionally are performed in fermenters. Additionally, the online monitoring increases the knowledge gained from a single shake flask experiment and facilitates the identification of parameters critical for scale-up (Table 1).

Acknowledgments We thank Dipl.-Ing. Karen Otten and Prof. Dr.-Ing. Horst R. Maier, Institute for Ceramic Components in Mechanical Engineering, RWTH Aachen University and Silvia Denter for their help and advice. This work was supported by a grant from the German Research Foundation (DFG).

Journal : 10295 Dispatch : 12-9-2006 Pages : 8
Article No. : 176 LE TYPESET
MS Code : 176 CP DISK

References

1. Anderlei T, Büchs J (2001) Device for sterile online measurement of the oxygen transfer rate in shaking flasks. Biochem Eng J 7:157–162
2. Stöckmann C, Maier U, Anderlei T, Knocke C, Gellissen G, Buechs J (2003) The oxygen transfer rate as key parameter for the characterization of *Hansenula polymorpha* screening cultures. J Ind Microbiol Biotechnol 30:613–622
3. Büchs J, Maier U, Milbradt C, Zoels B (2000) Power consumption in shaking flasks on rotary shaking machines: II nondimensional description of specific power consumption and flow regimes in unbaffled flasks at elevated liquid viscosity. Biotechnol Bioeng 68:594–601
4. Anderlei T, Zang W, Papaspyrou M, Büchs J (2004) Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. Biochem Eng J 17:187–194
5. Silberbach M, Maier B, Zimmermann M, Büchs J (2003) Glucose oxidation by oxydans: characterization in shaking-flasks, scale-up and optimization of the pH profile. Appl Microbiol Biotechnol 62:92–98
6. Stöckmann C, Losen M, Dahlems U, Knocke C, Gellissen G, Büchs J (2003) Effect of oxygen supply on the passaging, stabilizing and screening of recombinant *Hansenula polymorpha* production strains in test tube cultures. FEMS Yeast Res 4:195–205
7. Raval KN, Hellwig S, Prakash G, Ramos-Plasencia A, Srivastava A, Büchs J (2003) Necessity of a two-stage process for the production of azadirachtin-related limonoids in suspension cultures of *Azadirachta indica*. J Biosci Bioeng 96:16–22
8. Wittmann C, Kim HM, John G, Heinzle E (2003) Characterization and application of an optical sensor for quantification of dissolved O_2 in shake-flasks. Biotechnol Lett 25:377–380
9. Boyles DT (1978) Specific growth rate measurement in an oxygen electrode chamber. Biotechnol Bioeng 20:1101–1104
10. Ridgway HF (1977) Source of energy for gliding motility in *Flexibacter polymorphus*: effects of metabolic and respiratory inhibitors on gliding movement. J Bacteriol 131:544–556
11. Wittmann C, Yang T, Kochems I, Heinzle E (2001) Dynamic respiratory measurements of *Corynebacterium glutamicum* using membrane mass spectrometry. J Microbiol Biotechnol 11:40–49
12. Montagnolli W, Zamboni A, Luvizotto-Santos R, Yunes JS (2004) Acute effects of *Microcystis aeruginosa* from patos lagoon estuary, southern Brazil, on the microcrustacean *Kalliapseudes schubartii* (Crustacea: Tanaidacea). Arch Environ Contam Toxicol 46:463–469
13. Frost MT, Wang Q, Moncada S, Singer M (2005) Hypoxia accelerates nitric oxide-dependent inhibition of mitochondrial complex I. In activated macrophages. Am J Physiol Regul Integr Comp Physiol 288:394–400
14. Lee YH, Tsao GT (1979) Dissolved oxygen electrodes. Adv Biochem Eng Biotechnol 13:35–86
15. Kohls O, Scheper TH (2000) Setup of a fiber optical oxygen multisensor-system and its application in biotechnology. Sens Actuators 70:121–130
16. Kinoshita S, Udaka S, Shimono M (1957) Amino acid fermentation. I. Production of L-glutamic acid by various microorganisms. J Gen Appl Microbiol 3:193–205
17. Chamsartra S, Hewitt CJ, Nienow AW (2005) The impact of fluid mechanical stress on *Corynebacterium glutamicum* during continuous cultivation in an agitated bioreactor. Biotechnol Lett 27:693–700
18. Seletzky JM, Noack U, Fricke J, Hahn S, Büchs J (2006) Metabolic activity of *Corynebacterium glutamicum* grown on L-lactic acid under stress. Appl Biotechnol Bioeng (published online). DOI:10.1007/s00253-006-0436-0
19. Maier B, Dietrich C, Büchs J (2001) Correct application of the sulphite oxidation methodology of measuring the volumetric mass transfer coefficient k_La under non-pressurized and pressurized conditions. Trans IChemE 79:Part C
20. Maier U, Büchs J (2001) Characterization of the gas–liquid mass transfer in shaking bioreactors. Biochem Eng J 7:99–106
21. Schumpe A, Deckwer WD (1979) Estimation of O_2 and CO_2 solubilities in fermentation media. Biotechnol Bioeng 21:1075–1078
22. Schumpe A, Quicker G, Deckwer WD (1982) Gas solubilities in microbial culture media. Adv Biochem Eng 48:1–38
23. Rischbieter E, Schumpe A (1996) Gas solubilities in aqueous solutions of organic substances. J Chem Eng Data 41:809–812
24. Int. Conf, Harmonization tech. Requirements reg. Pharmaceuticals Human use (ICH) (1996) Guidance for industry Q2B Validation of analytical procedures
25. Cocaign M, Monnet C, Lindley ND (1993) Batch kinetics of *Corynebacterium glutamicum* during growth on various carbon substrates: use of mixtures to localize metabolic bottlenecks. Appl Microbiol Biotechnol 40:526–530
26. Cocaign-Bousquet M, Guyonvarch A, Lindley ND (1996) Growth rate-dependent modulation of carbon flux through central metabolism and the kinetic consequences for glucose-limited chemostat cultures of *Corynebacterium glutamicum*. Appl Environ Microbiol 62:429–436
27. Cocaign-Bousquet M, Lindley ND (1995) Pyruvate overflow and carbon flux within the central metabolic pathways of *Corynebacterium glutamicum* during growth on lactate. Enzyme Microb Technol 17:260–267
28. Enfors SO, Mattiasson B (1983) Oxygenation of processes involving immobilized cells. In: Mattiasson B (ed) Immobilized cells and organelles. CRC Press, Boca Raton, FL, pp 41–60
29. Kennedy MJ, Reader SL, Davies J, Rhoades DA, Silby HW (1994) The scale up of myceleal shake flask fermentations: a case study of gamma linolenic acid production by *Mucor hiemalis* IRL 51. J Ind Microbiol 13:212–216

Journal : 10295 | Dispatch : 12-9-2006 | Pages : 8
Article No. : 176 | ☐ LE | ☐ TYPESET
MS Code : 176 | ☑ CP | ☑ DISK