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RESEARCH ARTICLE

Non-invasive and time-resolved measurement of the respiration activity of Chinese hamster ovary cells enables prediction of key culture parameters in shake flasks

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Abstract

Background: Shake flasks are frequently used for mammalian cell suspension cultures. For process development and routine culture monitoring, information on culture behavior is needed early on.

Main methods and major results: Here, cell-specific oxygen uptake rates (qO_2) of two CHO cell lines were determined from shake flask experiments by simultaneous measurement of oxygen transfer rates (OTR) and viable cell concentrations (VCC). For cell line one, qO_2 decreased from $2.38 \cdot 10^{-10}$ to $1.02 \cdot 10^{-10}$ mmol cell^{-1} h^{-1} during batch growth. For cell line two, qO_2 was constant ($1.90 \cdot 10^{-10}$ mmol h^{-1}). Determined qO_2 values were used to calculate the VCC from OTR data. Cumulated oxygen consumption and glucose consumption were correlated for both cell lines and enabled calculation of glucose concentrations from OTR data. IgG producing cell line one had an oxygen demand of ~15 $mmol_{oxygen}\ g_{glucose}^{-1}$, cell line two consumed ~5 $mmol_{oxygen}\ g_{glucose}^{-1}$. The established correlations for determination of VCC and glucose were successfully transferred to subsequent cultivations for both cell lines. Combined measurement of the OTR and the carbon dioxide transfer rate enabled quantitative determination of the lactate concentration (production and consumption) without sampling.

Conclusions and implications: Taken together, non-invasive measurement of the respiration activity enabled time-resolved determination of key culture parameters for increased process understanding in shake flasks.

KEYWORDS
carbon dioxide transfer rate, Chinese hamster ovary cells, oxygen transfer rate, respiratory quotient, shake flasks

1 | INTRODUCTION

Chinese hamster ovary (CHO) cells are the most important mammalian cells used for the stable production of therapeutic proteins.[1] Even though traditionally applied for microbial cultivations, shake flasks are nowadays also used to cultivate mammalian cells in suspension.[2,3] Moreover, fed-batch cultivation with bolus addition of culture medium is frequently carried out in shake flasks[4,5] for early-stage process development. However, even though they might be used for process development, the limited capability for instrumentation was cited as

disadvantageous for sufficient data quality and quantity from shake flask experiments.[6]

1.1 | Advantages of time-resolved monitoring of process parameters

During early process development, time-resolved monitoring provides process insights. Thus, it can determine process robustness and aid in process improvement.[7] Combined with modelling approaches, a mechanistic understanding of the process can be provided.[7,8] Further, time-resolved monitoring is superior to the traditional offline analysis of process parameters as the information is usually available without sample preparation[7], in real time. This is especially advantageous, when frequent sampling would be needed as frequent sampling is undesirable, especially when the overall culture volume is low. In addition, the number of passages was demonstrated to affect growth rates, product formation, and nutrient consumption of CHO cells.[9] Consequently, routine monitoring of the culture status during cell passaging is desirable.

Knowledge about certain process variables is needed for process optimization and control. The glucose concentration is important during batch cultivation as it is the main carbon source. Additionally, the glucose concentration usually is the parameter that is strictly regulated and fed to the culture in fed-batch cultivations. The oxygen uptake rate (OUR) can be used to determine the required oxygen mass transfer coefficient (k_La),[10] the k_La can then be used as a parameter for transfer of the cultivation process into larger (or smaller) bioreactor scales.[11] As lactate production results in acidification of the culture medium, time-resolved monitoring of the lactate concentration is desirable to take corrective actions during fermentation. Moreover, excessive lactate accumulation results in lower maximum cell concentrations and affects productivity.[12,13] In addition, lactate is routinely monitored in industrial mammalian cell culture processes[14] and it was demonstrated that lactate consumption was a key parameter for final antibody titers in industrial process runs.[15] Knowledge about the viable cell concentration (VCC) is also important, because it gives general information on the culture status[16] and enables calculation of growth rates, cell-specific yields, and productivities.

1.2 | Relevance of OUR and CER for mammalian cells

As the space for sensor integration in shake flasks is much more limited than in classical bioreactors, the number of sensors that can be integrated in a single shake flask is finite. Thus, to gather as much information as possible from experiments carried out in shake flasks, parameters providing the most insights into the culture status need to be identified and measured. Preferably, measurements are carried out non-invasively.

The OUR has been cited as one of the parameters with a huge variety of different applications for mammalian cells.[17] It provides general information on the culture status and is more informative than VCC measurement.[18] Combined measurement of OUR and VCC enables the calculation of the cell-specific oxygen uptake rate (qO_2). Moreover, information on the oxygen demand of the culture enable choosing correct cultivation parameters to meet the expected oxygen demand and to avoid oxygen limitation. In addition, a linear correlation between the amount of oxygen and glucose consumed has been described for different CHO cell lines during fed-batch operation.[19] This correlation enabled the adaptation of feeding strategies.[19] Moreover, a linear relationship between glutamine and oxygen consumption was reported for hybridoma cells.[20]

Next to OUR measurement, the carbon dioxide evolution rate (CER) is considered an important parameter, but its measurement remains challenging.[17] The presence of bicarbonate for pH maintenance complicates CER measurements.[17] As a result of the bicarbonate buffer system, carbon dioxide (CO_2) released during cultivation will give a mixed signal between CO_2 produced by the cells and CO_2 originating from the bicarbonate buffer.[21] Compared to ambient air, the concentration of CO_2 is usually increased to 5% to10% in the inlet gas. Thus, very small changes in the CO_2 concentration resulting from cell respiration are measured against a high absolute background signal. Detecting these small changes requires a high sensitivity and resolution of the sensors, and it makes the measurement of the CER additionally challenging.[22]

If the simultaneous measurement of OUR and CER is possible with high accuracy, calculating the respiratory quotient (RQ) is possible.[23,24] The RQ indicates the change from lactate production to lactate consumption for CHO cells.[24,25] In addition, it was proposed to be a useful parameter to follow the culture status of mammalian cells[21,22,25] and enable process control.[26]

1.3 | Monitoring of OUR and CER in shake flasks

Amongst others, direct monitoring of the OUR has been realized in shake flasks by the respiration activity monitoring system (RAMOS). Here, multiple flasks can be monitored in parallel. RAMOS uses electrochemical sensors to determine the oxygen transfer rate (OTR) of a culture.[27,28] The decrease of the oxygen partial pressure is measured in the headspace of the shake flask and a measurement and gas flow phase are alternated. During the measurement phase, the inlet and outlet valves of the flask are closed and the decrease in the oxygen partial pressure caused by respiration is measured. A short high-flow phase follows the measurement phase to bring the gas composition back to the level before the measurement phase. As the change of the oxygen concentration in the liquid can be assumed to be very small compared to OUR and OTR, the measured OTR directly reflects the OUR.[29,30] The validity of this assumption for CHO cultures is discussed in detail elsewhere.[29] As demonstrated recently by monitoring the OTR for an industrial CHO cell line, measurement times can be adapted flexibly to allow for the detection of very small changes in the oxygen partial pressure.[29] RAMOS also enables monitoring of the carbon dioxide transfer rate (CTR).[28] Here, the increase of the carbon dioxide partial

pressure in the gas phase is used. In addition to the RAMOS system, the so-called Transfer–Rate Online Monitoring ("TOM") device is commercially available from Kuhner AG, Birsfelden, Switzerland. Like the RAMOS system, it enables determination of the OTR in shake flasks using electrochemical sensors. However, while RAMOS utilizes difference pressure sensors for CTR determination, the TOM device relies on non-dispersive infrared sensors for CTR measurement. For determination of the CER from CTR measurements, it is assumed that the CO_2 concentration in the liquid does not change during the measurement phase (see Supplementary data). This is analogue to the assumption that the OTR will equal the OUR, if the oxygen concentration in the liquid does not change during measurement.[29] Only under this assumption, CER from cell respiration will equal the CTR. From the ratio of OTR and CTR the transfer quotient (TQ) can be calculated. If CTR equals CER and OTR equals OUR, the TQ equals the respiratory quotient (RQ). It should also be noted that the CTR will only directly reflect the CER over the course of the cultivation, if changes in the CO_2 concentration are only caused by cell respiration. In addition, the CO_2 concentration might be influenced by changes in temperature, ionic strength or pH value.[31] Hence, if any of these parameters change, the CTR will reflect the sum of CER and any additional effects not caused directly by cellular respiration. RQ measurement in shake flask has for example been applied to determine the metabolic activity of the fungus *Ustilago maydis* on complex substrates[32] and to understand cultivations of *Bacillus licheniformis* at microaerobic conditions.[33] Simultaneous measurement of OTR and CTR in shake flasks was used to provide insights for the cultivation of HEK293 cells.[34]

In this study, non-invasive, parallelized OTR and CTR measurements in shake flasks were used to follow the course of key culture parameters for CHO cells in real-time. Two different suspension cell lines were used in different media. The cell-specific oxygen uptake rates (qO_2) were determined to characterize each cell line. Afterwards, VCCs were calculated from the OTR and compared to VCC data from manual sampling. Next, the correlation between oxygen and glucose consumption was determined for both cell lines and glucose concentrations during cultivation were calculated from the OTR. Lastly, the fact that lactate formation results in CO_2 release from bicarbonate buffered culture media was exploited to determine the lactate concentration during cultivation. Taken together, it was demonstrated, how non-invasive and time-resolved measurement of the OTR and CTR can significantly increase the information content during early-stage process development and cultivation for mammalian cell cultures in shake flasks.

2 | MATERIAL AND METHODS

2.1 | Cell lines and culture media

Two different cell lines were used in this study. Cell line one is a CHO suspension cell line developed by Rentschler Biopharma SE producing an IgG 1 monoclonal antibody.[35] Cell line two is a CHO suspension cell line obtained from CLS Cell Lines Service GmbH, Germany as a producer cell line for different applications.

Cell line one was cultivated in serum-free, chemically defined culture medium (PowerCHO 2 with HEPES buffer and Pluronic F68) (Lonza AG, Switzerland) obtained as liquid formulation. The medium was supplemented with 6 mM L-glutamine (Gibco Life Sciences, Thermo Fisher Scientific, USA) and 30 µg mL^{-1} kanamycin (Carl Roth, Germany) or 1% (v/v) PenStrep (stock with 10,000 Units mL^{-1} penicillin and 10 g L^{-1} streptomycin) (Sigma-Aldrich, USA) as indicated in the experiment overview (Table 1). Cell line two was cultured in serum-free, chemically defined culture medium (sciNX w/o L-glutamine) (CLS Cell Lines Service GmbH, Germany). The medium was supplemented with 5 mM glutamine and 1% (v/v) PenStrep. Media were stored at 4°C until use. Directly before cultivation, glutamine and PenStrep or kanamycin were added to the media in the respective concentrations. All stock solutions were stored at -20°C in aliquots and thawed as needed. Before use, media and supplements were pre-heated to the cultivation temperature for about 30 min in a water bath (VWB2 12, VWR, USA).

2.2 | Monitoring of the OTR and CTR rate in shake flasks

The OTR was monitored with two different devices termed 'RAMOS' (short for Respiration Activity Monitoring System) and 'TOM' (short for Transfer-Rate Online Measurement). Key differences in both device types are described in Supplementary Table 1. Monitoring of the CTR was only carried out with the TOM device as the resolution of the differential pressure sensors utilized in the RAMOS device was too low for accurate measurement (data not shown). Adjustment of RAMOS for utilization of CHO cultures was described recently.[29] As the TOM device was integrated into an incubator where flasks were run in parallel for manual sampling, measurement values were affected by opening the incubator hood for sampling. As a result, OTR and CTR measurement values were correlated with sampling points and values that deviated more than 30% from the previous measurement value were considered outliers and excluded. For CHO cell line one, cultivation was carried out in glass and single-use plastic shake flasks in parallel using RAMOS as described by.[29] Cultivation of CHO cell line two was carried out in glass flasks using RAMOS and TOM.

2.3 | Cryopreservation, thawing, and cell passaging

Working cell banks for both cell lines were stored in the vapor phase of liquid nitrogen until use. All passages were carried out in 250 mL Corning plastic flasks closed with a vent-cap (Sigma-Aldrich, USA) with a filling volume of 50 mL at a temperature of 36.5°C and a shaking frequency of 140 rpm. The shaking diameter was set to 50 mm. An incubator with CO_2 and humidity control (ISF1-X, Kühner AG, Switzerland) was used and set to 5% and 70%, respectively. For cell line one, a working cell bank previously established was used for cultivation. Cryopreservation and cell passaging were carried out as described previously.[29]

TABLE 1 Overview of experiments presented in this study

	Experiment 1	Experiment 2	Experiment 3
Figures	Figure 1, 2, Supplementary Figure 1, 2, 3	Figure 3	Figure 4, 5, 6, Supplementary Figure 4, 5, 6, 7
Cell line	CHO cell line 1	CHO cell line 1	CHO cell line 2
Medium	Medium 1 PowerCHO™ 2	Medium 1 PowerCHO™ 2	Medium 2 sciNX
Media Supplements	6 mM glutamine, 30 µg mL^{-1} kanamycin	6 mM glutamine, 1% PenStrep	5 mM glutamine, 1% PenStrep
Initial viable cell concentration	$0.2 \cdot 10^6$ mL^{-1}	$0.2 \cdot 10^6$ mL^{-1}	$0.5 \cdot 10^6$ mL^{-1} $0.25 \cdot 10^6$ mL^{-1}
Flask type	glass and plastic	glass	glass
Device type	RAMOS	RAMOS	RAMOS and TOM
Number of passages after thawing	2	14	3
ID of cryogenic vial	#1	#1	#2

Cell line two was obtained as a 50 mL growing culture by CLS Cell Lines Service GmbH, Germany and cells were grown as described above until a cell density of about $2.5 \cdot 10^6$ mL^{-1} was reached. Cells were then centrifuged (8 min, 300 × *g*, room temperature) (Heraeus Multifuge X3R , Thermo Fisher Scientific, USA) and the VCC set to $1.2 \cdot 10^7$ mL^{-1} in a mixture of culture medium used for cultivation (45% v/v), fresh culture medium with supplements added (45% v/v) and dimethylsulfoxide (DMSO) (10% v/v). Aliquots of 1 mL of this mixture were transferred to 2 mL cryovials and frozen at a controlled cooling rate of -1°C min^{-1} using a Nalgene Mr. Frosty freezing container (Thermo Fisher Scientific, USA). After 24 h, the cryovials were transferred into a liquid nitrogen storage tank and stored in the vapor phase of liquid nitrogen. One vial was rapidly thawed for cultivation and its content resuspended in 9 mL fresh culture medium with supplements added. Afterwards, the cells were centrifuged for 5 min at 175 × *g* at room temperature using a Heraeus Multifuge X3R centrifuge (Thermo Fisher Scientific, USA). The supernatant was then discarded and the remaining cell pellet was resuspended in 3 mL fresh culture medium with supplements added. Afterwards, the volume was filled up to 20 mL using fresh culture medium with supplements added to cultivate the first passage. Cells were grown until a cell density of about $2 \cdot 10^6$ mL^{-1} was reached. The seed cell concentration for subsequent passages was set to $0.25 \cdot 10^6$ mL^{-1} in a volume of 20 mL.

2.4 | Main culture cultivation conditions

After an appropriate number of passages (see Table 1 for details for each experiment), main culture cultivations were started. All cultivations were carried out at a temperature of 36.5°C with a shaking diameter of 50 mm and a shaking frequency of 140 rpm. An initial filling volume of 50 mL was used. Flasks for manual sampling were cultured in a humidified incubator (ISF1-X, Kühner AG, Switzerland) at 70% relative humidity and 5% CO_2. For manual sampling, glass flasks (Duran, Schott AG, Germany) closed with a cellulose plug (Rotilabo culture plug, Roth, Germany) and Corning plastic flasks closed with a vent-cap (Sigma-Aldrich, USA) were used. At each sampling point, 1 to 1.5 mL of culture volume were withdrawn from the flask under sterile conditions. Afterwards, the flask was put back to the incubator and sampled again at the next sampling point.

For monitoring of the respiration activity in glass flasks, modified glass flasks were used. However, these flasks were only modified in the upper part of the flask to enable active gassing needed for measurement. TOM flasks were cultured under the same conditions and in the same incubator as the flasks used for manual sampling. RAMOS flasks were cultured in parallel to the flasks for manual sampling in an incubator without CO_2 and humidity control (ISF1-X, Kühner AG, Switzerland). For gassing of the monitored RAMOS flasks, a gas mixture of 5% CO_2 in synthetic air was used. Monitoring of the OTR in single-use plastic flasks was carried out in a RAMOS device using adapters as described previously.[29] It was previously demonstrated that culture behavior in online monitored and offline sampled glass and plastic flasks from the RAMOS device is comparable.[29] In all cases, the same types of flasks (either glass or single-use plastic) were used for online monitoring and offline sampling.

2.5 | Determination of offline parameters

Parameters determined from manual sampling with relevance to this study are described in the following sections. VCC analysis was carried out immediately after sampling using culture broth. For further analysis, 1 mL of culture broth was transferred into a 1.5 mL tube and centrifuged in a table centrifuge (mini centrifuge Rotilabo, Carl Roth, Germany) for 3 to 5 min at a rotation rate of 6000 rpm at room temperature. The supernatant was used for the determination of glucose and lactate concentration and stored at -20°C until use.

2.5.1 | Determination of VCC

Determination of the VCC was carried out by manual counting using a hemocytometer (Counting chamber C-Chip Neubauer improved, Carl Roth, Germany) and a vital exclusion stain. If needed, the culture broth was diluted with medium. Afterwards, 10 µL of appropriately diluted culture broth was mixed with 10 µL of trypan blue (cell line one) or 10 µL of erythrosin B solution (Merck KGaA, Germany) (cell line two). Finally, 10 µL of the mixture were added to the chamber. For each sample, the cell numbers in four quadrants were manually counted, averaged and corrected by the dilution factor given by the manufacturer.

2.5.2 | Glucose and lactate measurement

The glucose and lactate concentrations in the culture supernatant were determined by high-pressure liquid chromatography (HPLC) analysis. Before analysis, the samples were filtered and transferred into a vial. An organic acid resin column (Rezex ROA-Organic Acid H+ (8%), 300 × 7.8 mm, Phenomenex Inc, USA) was used for separation. As HPLC system, a Dionex Ultimate 3000 system (Thermo Scientific, USA) equipped with a refractive index detector (RefractoMax 520, Shodex, Germany) was used. As mobile phase 5 mM H_2SO_4 was used at a flow rate of 0.8 mL min^{-1}. For medium one, separation was carried out at a temperature of 40°C. For medium two, a temperature of 60°C was used.

3 | RESULTS AND DISCUSSION

3.1 | Determination of cell-specific oxygen uptake rates (qO2) utilizing measurement of the oxygen transfer rate (OTR) in shake flasks

A data set previously published and described in detail[29] was exploited further in this study. From the published data set, the measured OTR, VCC and glucose concentration were used. The course of these data over the cultivation time is depicted in Supplementary Figure 1. From the measured OTR and VCC data, calculation of the cell-specific oxygen uptake rate (qO_2) was performed. Assuming that the OTR equals the OUR, the OTR can be defined as the product of VCC and qO_2 (Equation 1).

$$OUR = OTR = qO_2 \cdot VCC \quad (1)$$

As growth was shown to be similar in glass and plastic flasks, both data sets were used for determination of qO_2 (Figure 1).

Figure 1 depicts the correlation between the VCC measured with the hemocytometer from manual sampling and the measured OTR at the respective sampling point until 144 h. Later data points were not considered, since the VCC started to decrease (Supplementary Figure 1B, closed and open blue triangles). The correlation revealed a two-phase behavior (Figure 1, solid green and dashed orange line, respectively). The intersection of the linear regression lines (at VCC = $1.73 \cdot 10^9$ L^{-1} and OTR = 0.44 mmol L^{-1} h^{-1}) was defined as the transition from the first phase to the second phase. The obtained qO_2 values (qO_2 of $2.38 \cdot 10^{-10}$ mmol h^{-1} in the first phase and $1.02 \cdot 10^{-10}$ mmol h^{-1} in the second phase) agree well with the literature. Seidel, Maschke, Werner, Jossen, and Eibl[36] recently analyzed 46 data sets from twenty different publications to determine an average qO_2 value for CHO cells. A mean value of $2.07 \cdot 10^{-10}$ mmol h^{-1} was reported.[36]

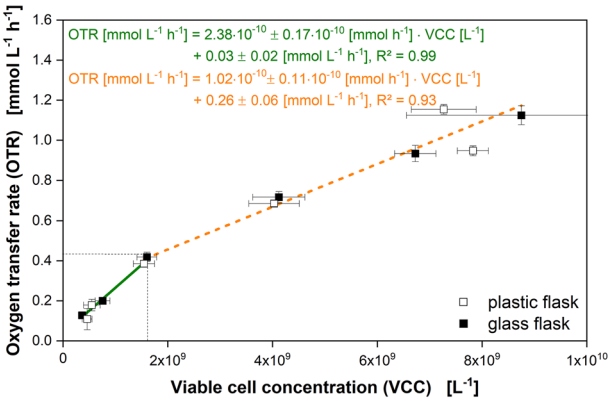

FIGURE 1 Determination of cell-specific oxygen uptake rates (qO_2) for CHO cell line one. The correlation between the OTR and the VCC was determined until 120 h using a data set published previously (Supplementary Figure 1).[29] For determination of VCC using a hemocytometer, four quadrants from one sample were counted and averaged. Open squares are data from single-use plastic flasks, closed squares are data from glass flasks. Data from experiment 1. Solid green line shows a linear fit for VCCs up to $1.6 \cdot 10^9$ L^{-1} resulting in a qO_2 of $2.38 \cdot 10^{-10}$ mmol h^{-1}. Dashed orange line shows a linear fit for VCCs from $1.6 \cdot 10^9$ to $8.75 \cdot 10^9$ L^{-1} resulting in a qO_2 of $1.02 \cdot 10^{-10}$ mmol h^{-1}. The intersection of both regression curves (dotted lines) (VCC = $1.73 \cdot 10^9$ L^{-1}, OTR = 0.44·mmol L^{-1} h^{-1}) was used to define the transition between the different qO_2 values

In general, changes in qO_2 values can be caused by several different culture parameters like cell density or cell age.[17] Deshpande and Heinzle reported a decrease in qO_2 from $3.2 \cdot 10^{-10}$ to $1.8 \cdot 10^{-10}$ mmol h^{-1} for a CHO cell line and concluded that this was caused by the so-called 'crowding phenomenon'.[37] The crowding phenomenon originally described a decrease in qO_2 with increasing cell concentrations for lymphocytes.[38] Pappenreiter, Sissolak, Sommeregger, and Striedner[39] reported qO_2 values of about $1.6 \cdot 10^{-10}$ mmol h^{-1} during cultivation in a 15 L bioreactor. A linear correlation between VCC and OUR was found up to cell concentrations of $1 \cdot 10^7$ mL^{-1} in their study. In addition, they reported two different slopes for the OUR in dependence of the packed cell volume (PCV).[39] A decrease in qO_2 was also reported for hybridoma cells upon depletion of glutamine.[40] Further, varying qO_2 values have been reported in batch cultures for CHO cells, however at lower initial glucose concentrations.[41] Taken together, the calculated qO_2 values from our study compare well with data from the literature, especially considering that differences in absolute values for qO_2 are reasonable as determination is affected by the measurement conditions.[16]

At a given set of cultivation parameters, the maximum OTR that can be physically reached (OTR_{max}), can be calculated according to Meier et al. assuming that gas-liquid mass transfer limits oxygen transfer.[42] Calculation of the OTR_{max} under the experimental conditions applied in this study results in an OTR_{max} of 7.5 mmol L^{-1} h^{-1}. Inserting this value and the average qO_2 value of 2.07 $\cdot 10^{-10}$ mmol h^{-1} for CHO cells[36] into Equation 1 indicates that about 36 million cells per mL can sufficiently be supplied with oxygen in shake flasks at the chosen conditions. However, restrictions can occur at changing cultivation conditions, as the OTR_{max} decreases with increasing filling volume. Consequently, attention needs to be paid if the filling volume is changed during cultivation. Rising filling volumes are typically the case during fed-batch cultures. In CHO fed-batch cultures cell densities of more than 25 million cells per mL have been reported in shake flasks.[43] Therefore, these cultures might be close to or even run into an oxygen limitation during cultivation. To prevent an oxygen limitation, the shaking frequency might have to be increased or the initial filling volume needs to be lowered. With (semi)-perfusion cultures, even higher VCCs might be reached already in shake flasks.[44] Here sufficient oxygen supply will become even more critical. These exemplary considerations underline the importance of paying attention to oxygen supply, especially at changing conditions (e.g., filling volume) during cultivation.

3.2 | Calculation of the glucose concentration from the OTR obtained in shake flasks

Fed-batch operation is often realized in small-scale cultivations by bolus feeding of media components after an initial batch phase. The composition of the feed is usually designed to not become limiting. To determine the optimal time point for feed addition, the glucose concentration in the culture broth needs to be known. Thus, we determined the correlation between oxygen consumption and glucose consumption to estimate the residual concentration of glucose based on the OTR measurement during cultivation. The cumulated oxygen consumption is reflected by the integral of the OTR (Int_{OTR}) over time (Supplementary Figure 2). Therefore, the Int_{OTR} was plotted against the amount of glucose consumed (Figure 2), that is until the last data point, where glucose was still detected in the culture broth. Again, data points for glass and single-use plastic flasks from a previously published data set (Supplementary Figure 1) were used to establish the correlation. Fitting of the data points using Equation 2 gave very good accuracy between the consumed amount of glucose and the oxygen integral (Figure 2, red line).

$$\text{Glucose}_{\text{consumed}}\left[\text{g L}^{-1}\right] = \frac{13.511 \pm 1.694\left[\text{g L}^{-1}\right] \cdot \text{Int}_{\text{OTR}}\left[\text{mmol L}^{-1}\right]}{199.7 \pm 38.1\left[\text{mmol L}^{-1}\right] + \text{Int}_{\text{OTR}}\left[\text{mmol L}^{-1}\right]} \quad (2)$$

A linear correlation between the cumulated oxygen consumption and the glucose consumption in a stirred tank reactor was previously reported in fed-batch cultivations.[19] Fitting by using Equation 2

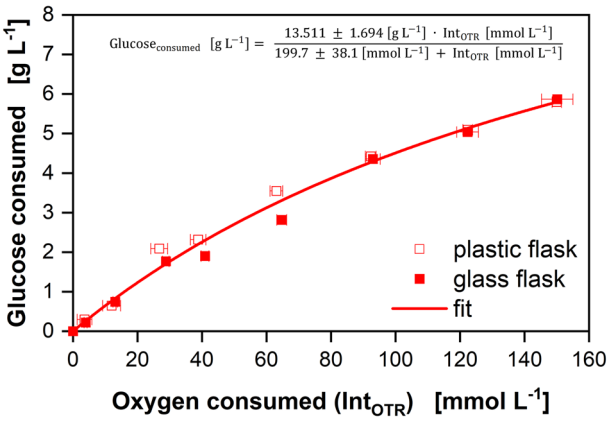

FIGURE 2 Correlation between consumed amount of oxygen (Int_{OTR}) and consumed amount of glucose for CHO cell line one. The integral of the oxygen transfer rate (OTR) was calculated and plotted against the amount of glucose consumed. The OTR was determined for glass and single-use plastic flasks each in biological triplicates. The glucose concentration was determined by HPLC measurement from one glass and one single-use plastic flask run in parallel. Data from experiment 1 that was published previously.[29]

assumes that the stoichiometry is linear at low oxygen demand. Consequently, the relationship between Int_{OTR} and glucose consumed is linear at low Int_{OTR}. Higher glucose concentrations are present in the first part of the batch cultivation (left part of Figure 2). Thus, a linear relationship between consumed amounts of glucose and oxygen is also observed in the beginning of batch fermentations and is well reflected by the fit equation. However, as the glucose concentration decreases in batch mode, more oxygen is consumed per amount of glucose consumed and, consequently, a deviation from linear behavior is observed (right part in Figure 2). The deviation from linear behavior occurs when about 5 g L^{-1} of glucose are still left in the medium. This deviation from linear behavior is also accounted for in the fit equation. A higher oxygen consumption per glucose consumed indicates that glucose is not used for growth and, thus, biomass generation, but oxidized for energy metabolism. An increase in oxidative metabolism and, consequently, an increasing amount of oxygen consumed per glucose consumed was also reported for hybridoma cells.[45] The change in glucose metabolization is most likely also connected to the change in qO_2 observed (Figure 1) and points to general changes in cell metabolism. A decrease in glucose consumption with decreasing glucose concentration has previously been reported for CHO cells and was associated with the change from exponential growth to late exponential growth phase.[46] The observation that glucose is not used for biomass generation is also in excellent agreement with the course of the VCC (Supplementary Figure 1). Even at comparably high glucose concentrations of about 3 g L^{-1} (after 144 h of cultivation), the VCC does not increase further, but enters a plateau phase (Supplementary Figure 1, blue triangles).

With the developed correlations, the VCC and the glucose concentration can be calculated from the time-resolved OTR data, resulting

in a time-wise resolution of one data point per hour (Supplementary Figure 3). As the same data were used to establish the correlations, the calculated and measured VCC data agreed well for both flask types (Supplementary Figure 3, blue symbols) until the point when the VCC started to decrease. Besides the VCC, the glucose concentration was in very good agreement with the offline measured glucose concentration (Supplementary Figure 3, red symbols). However, after depletion of glucose and lactate, cell respiration continued for another 24 h (compare Supplementary Figure 1). Consequently, the oxygen integral increased further (Supplementary Figure 2), resulting in negative calculated glucose concentrations. As mentioned previously,[29] the oxygen consumption after depletion of glucose and lactate was most likely attributed to the consumption of another carbon source that was not accounted for in the correlation. Since the media composition is proprietary, it is not possible to specify the exact compound metabolized. However, as discussed previously,[29] this 'shoulder' in the OTR was observed for hybridoma cells after glutamine depletion, indicating oxidation of alternative substrates (e.g., glucose, other amino acids or fatty acids).[40] In addition, metabolization of galactose might be reasonable. Addition of galactose was demonstrated to enable metabolization of lactate in CHO cell cultures and was consumed after depletion of glucose.[47]

3.3 | Transfer of established correlations for measurement of VCC and glucose to a subsequent cultivation

The transferability of the established correlations for estimation of VCC and glucose to a new data set was investigated by a second experiment (Table 1). The data are presented in Figure 3. The OTR data of this data set were published previously,[29] but not used for further calculations. The course of the OTR from the first experiment (Table 1, Supplementary Figure 1) is depicted for comparison (Figure 3A, closed orange squares). Compared to the first experiment (Figure 3A, closed orange squares), an increase in the lag-phase of 20 h was observed for the second experiment (Figure 3A, closed black stars).

The determined correlations for calculation of the VCC (Figure 1) and the glucose concentration (Figure 2) from the OTR and Int_{OTR}, respectively, were used to directly calculate the VCC (Figure 3B, open blue triangles) and the glucose concentration (Figure 3B, open red squares) from the measured OTR (Figure 3A, closed black stars) for experiment 2. The estimated VCC and glucose concentration from the time-resolved OTR was compared to offline data for VCC and glucose concentrations obtained from manual sampling (Figure 3B, closed blue triangles and closed red squares, respectively). The VCC was predicted precisely from the OTR until the VCC started to decrease (after around 168 h). A precise prediction until the VCC started to decline is reasonable as the correlation was established using the experimental data until the VCC started to decrease. Moreover, calculating the VCC from the OTR is only possible, if the qO_2 remains constant during cultivation. Otherwise, changes in qO_2 need to be determined and

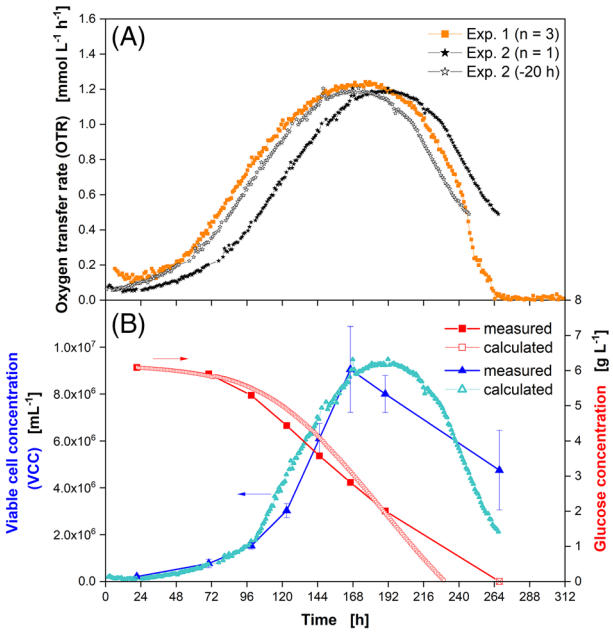

FIGURE 3 Reproducibility of culture behaviour for CHO cell line one and prediction of viable cell concentration (VCC) and glucose concentration during cultivation. (A) The OTR is shown as the average of a triplicate determination (closed orange squares, experiment 1), and as a single determination (experiment 2, closed black stars). The OTR data depicted in Figure 3A were originally published as Figure 6 in Ihling et al.[29] (B) The glucose concentration (open red squares) was calculated from the oxygen consumption (Int_{OTR}) using the correlation established in Figure 2 using data from experiment 1. Data are compared to data from manual sampling (closed red squares) obtained in experiment 2 (see Table 1). The VCC (open blue triangles) was calculated from the OTR using the correlation established in Figure 1 using data from experiment 1. Data are compared to data from manual sampling (closed blue triangles) from experiment 2 (see Table 1). CHO cells (initial VCC = $0.2 \cdot 10^6$ mL^{-1}) were cultured at 36.5°C in serum-free chemically defined medium supplemented with 6 mM glutamine (30 µg mL^{-1} kanamycin in experiment 1, 1% PenStrep in experiment 2). Cultivation conditions: 250 mL glass shake flasks, 50 mL filling volume, 140 rpm shaking speed, 50 mm shaking diameter. Flasks for determination of OTR were gassed with 5% CO_2 in synthetic air at a rate of 6.25 mL min^{-1}. Flasks for manual sampling were closed with a cellulose plug and cultured with 5% CO_2 in air at 70% r.h

taken into account. Here, it might be plausible that qO_2 changed once the cells entered the stationary phase. In addition, it was demonstrated for CHO fed-batch cultures, that a deviation between VCC and OUR was observed after the peak VCC was reached.[48] The course of the glucose concentration was also well reflected by the estimation based on the Int_{OTR}. The good agreement between data determined from manual sampling and concentrations predicted from non-invasive measurement of the OTR demonstrated that the established correlations could be applied to a subsequent cultivation without manual sampling. An accurate prediction using the OTR was even possible, although the duration of the lag-phase differed between the cultivation utilized to establish the correlations.

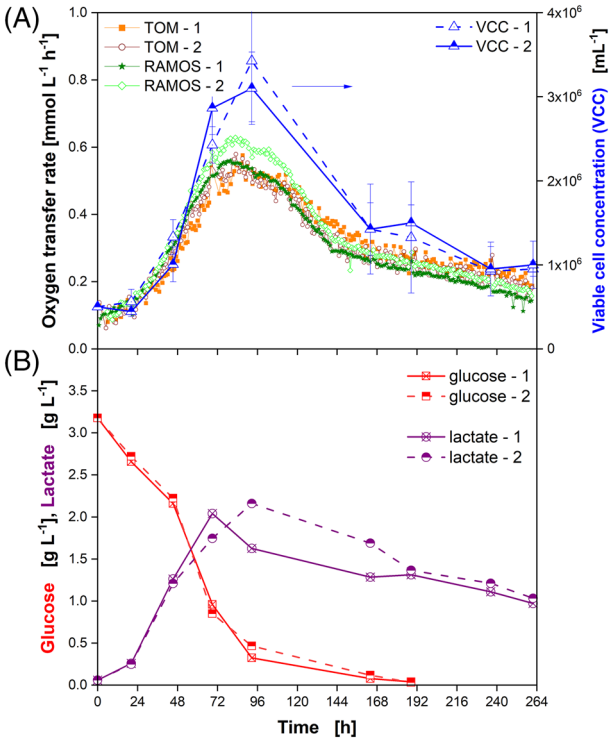

FIGURE 4 Cultivation of Chinese hamster ovary (CHO) cell line two with time-resolved monitoring of the oxygen transfer rate (OTR) and manual sampling. (A) OTR values in duplicate for cultivation in RAMOS device (closed green stars and open green diamonds) and TOM device (closed orange squares and open purple circles) are depicted over the cultivation time. Viable cell concentration (VCC) (blue triangles) determined from manual sampling is depicted in duplicate. (B) Glucose concentration (red squares) and lactate concentration (purple circles) determined from manual sampling over the cultivation time. Data from experiment 3 (see Table 1). CHO cells (initial VCC = $0.5 \cdot 10^6$ mL^{-1}) were cultured at 36.5°C in serum-free chemically defined medium supplemented with 5 mM glutamine and 1% PenStrep. Flasks were gassed with 5% CO_2 in synthetic air at a rate of 6.25 mL min^{-1} (RAMOS) or with 5% CO_2 in air at 70% r.h. at a rate of 11 mL min^{-1} (TOM). Flasks for manual sampling were closed with a cellulose plug and cultured with 5% CO_2 in air at 70% r.h. Cultivation conditions: 250 mL glass shake flasks, 50 mL filling volume, 140 rpm shaking speed, 50 mm shaking diameter

3.4 | Transfer of calculation of VCC and glucose concentration from OTR data in shake flasks to a second CHO cell line

To demonstrate that the presented approach for calculating VCC and glucose concentrations from measurement of the OTR is broadly applicable, the methodology was applied to a second CHO cell line that was cultured in a different medium ("medium 2"). Here, two different device options (RAMOS and TOM) were used in parallel (see Supplementary Table 1 for device specifications).

The course of the OTR (n = 4) and VCC (n = 2) is depicted in Figure 4A. The maximum OTR reached, and the shape of the OTR curve differed significantly from the behavior of the first cell line (Supplementary Figure 1). However, the measured OTR between the two different devices was very similar indicating comparable culture behavior in both devices.

The course of the VCC (Figure 4A, blue triangles) from flasks manually sampled and ran in parallel to the monitored flasks was well reflected in the shape of the OTR curve. Glucose was consumed in the first part of the cultivation and probably consumed after around 120 h (Figure 4B red squares). Lactate was initially formed but consumed as the glucose concentration decreased (Figure 4B, purple circles). After glucose was depleted, lactate was still left in the medium and consumed slowly for a considerable time. Prolonged glucose consumption indicated non-optimal growth conditions and some improvement potential of the medium. Media optimization was, however, not part of this study.

Calculation of qO_2 from simultaneous measurement of VCC and OTR resulted in a constant qO_2 during the exponential phase (until 69 h) (Supplementary Figure 4A). The obtained qO_2 of $1.90 \cdot 10^{-10}$ mmol h^{-1} is in the range of qO_2 values reported previously (see above), but the overall VCCs reached were lower than for the first cell line (compare Figure 1). Thus, it seems reasonable, that a constant qO_2 was observed, as the crowding phenomenon,[38] for example, might only occur at higher VCCs. Another reason for the different qO_2 values determined for both cell lines could be attributed to differences in phenotypes and genotypes of the two CHO cell lines. CHO cells are known to be very diverse in genotypes and phenotypes.[49–52] As the CHO cell lines used in this study originated from different sources, it is reasonable that a different cell behavior is observed. Additionally, CHO cell line one produces a monoclonal antibody (mAb). Differences in mAb producing and native CHO cell lines regarding growth and substrate consumption have also been reported.[46] Like the first cell line, applying the determined qO_2 value to the online data set used for calibration enabled retrospective calculation of the VCC with a higher temporal resolution (Supplementary Figure 4B). As can be seen, the standard deviation of the VCC calculated from the OTR was relatively low compared to the error of manual cell counting (Supplementary Figure 4B). In addition, the course of the VCC was also estimated quite accurately from the OTR in later stages of the cultivation. Accurate determination of the VCC in later stages of the cultivation indicates that the qO_2 did not change as soon as glucose was depleted and lactate was consumed.

The correlation between glucose and oxygen consumption was determined next (Supplementary Figure 5A). Again, a fit function (Supplementary Figure 5A) with linear dependency at low oxygen consumption was found to describe the experimental data best. Data until 96 h were considered, as the glucose concentration after 168 h (Figure 4, red squares) was very low (< 0.1 g L^{-1}). The correlation gave good accuracy when applied to the data set used for calibration (Supplementary Figure 5B).

At low oxygen consumption, the ratio of the linear relationship between glucose and oxygen consumption is obtained by division of the constants obtained from Equation 2. The reciprocal of the constants represents the amount of oxygen per glucose consumed. Consequently, data from Figure 2 (denominator = 199.7 mmol L^{-1}, nominator = 13.511 g L^{-1}) and Supplementary Figure 5 (denomina-

tor = 26.7 mmol L^{-1}, nominator = 5.468 g L^{-1}) were used for cell line one and cell line two, respectively. The first cell line exhibited a much higher oxygen consumption per glucose (~15 $mmol_{oxygen}$ $g_{glucose}^{-1}$) than the second cell line (~5 $mmol_{oxygen}$ $g_{glucose}^{-1}$). The obtained values are in the same order of magnitude as reported for hybridoma cells[45] and CHO cells in fed-batch cultivation.[19] As discussed above, the observed deviation might be caused by phenotypic and genotypic differences in the cell lines. Another reason for the deviation between the cell lines might be differences in the culture media (glucose concentration). The deviation might also result from a different cell metabolism caused by monoclonal antibody production in cell line one, as antibody production requires additional metabolic resources. That differences in the ratio are caused by monoclonal antibody production might be most plausible, as cell metabolism was found to change drastically between the transition from peak growth to peak antibody production for a single CHO cell line.[53] A highly oxidative state of metabolism, that is, increased flux into the oxidative tri-carboxylic acid cycle and oxidative pentose phosphate pathway, also corresponds to increased antibody production.[53] This could explain the significantly higher oxygen demand per glucose consumed for cell line one. While the demonstrated methodology is suitable to derive VCC and glucose concentrations from OTR measurement, it is not possible to determine the reasons for the observed differences in cell line behavior.

Instead of transferring the correlations for the second cell line to a subsequent culture run under the same conditions, prediction of VCC and glucose concentration from OTR data for a culture inoculated with a smaller initial cell density ($2.5{\cdot}10^5$ mL^{-1}) was investigated. The course of the OTR was different compared to the culture inoculated with an initial VCC of $5{\cdot}10^5$ mL^{-1} (Supplementary Figure 6). Transfer of the correlations established for an initial VCC of $5{\cdot}10^5$ mL^{-1} to an initial VCC of $2.5{\cdot}10^5$ mL^{-1} gave a good prediction of the VCC over the whole cultivation time (Figure 5, blue curves) and an acceptable prediction of the glucose concentration (Figure 5, red curves).

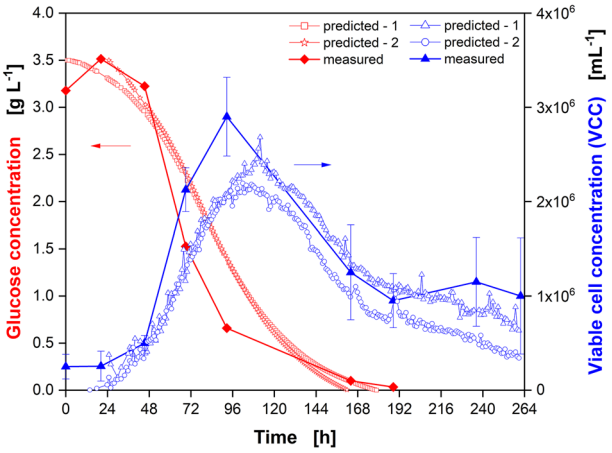

FIGURE 5 Prediction of glucose concentration and viable cell concentration (VCC) for cell line two with a different inoculation density. The glucose concentration for an inoculation density of $0.25{\cdot}10^6$ mL^{-1} (open red squares and stars) was calculated from the oxygen consumption (Int_{OTR}) using the correlation established in Supplementary Figure 5 for an inoculation density of $0.5{\cdot}10^6$ mL^{-1}. Data are compared to data from manual sampling (closed red diamonds). The VCC (open blue triangles and circles) was calculated from the OTR using the correlation established in Supplementary Figure 4 for an inoculation density of $0.5{\cdot}10^6$ mL^{-1}. Data are compared to data from manual sampling (closed blue triangles). Data from experiment 3 (Table 1). CHO cells (initial VCC = $0.25{\cdot}10^6$ mL^{-1}) were cultured at 36.5°C in serum-free chemically defined medium supplemented with 5 mM glutamine and 1% PenStrep). Flasks were gassed with 5% CO_2 in synthetic air at a rate of 6.25 mL min^{-1} (RAMOS). Flasks for manual sampling were closed with a cellulose plug and cultured with 5% CO_2 in air at 70% r.h. Cultivation conditions: 250 mL glass shake flasks, 50 mL filling volume, 140 rpm shaking speed, 50 mm shaking diameter

3.5 | Additional insights into culture behavior and calculation of the lactate concentration by measurement of the CTR and the TQ in shake flasks

For the cultivation of cell line two, the CTR was measured in parallel to the OTR in the same flask in the TOM device. The measurement setup described here is advantageous for accurate CTR determination as only the change in the carbon dioxide partial pressure in the gas phase is measured. Consequently, the exact inlet and outlet concentrations of CO_2 are irrelevant for measurement. As the liquid concentration of CO_2 is assumed to be in equilibrium with the gas phase, the CO_2 concentration in the liquid does not have to be known for calculation of the CTR.

The change in carbon dioxide partial pressure during measurement, which was used to calculate the CTR, gave quite accurate values and enabled a reasonable observation of culture progression (Figure 6A, closed cyan triangles). The combined knowledge of OTR and CTR enabled the non-invasive calculation of the transfer quotient (TQ) (Figure 6A, closed black circles). In analogy, the ratio of OUR and CER is defined as the respiratory quotient (RQ) (Equation 3).

$$TQ = \frac{CTR}{OTR};\ RQ = \frac{CER}{OUR} \tag{3}$$

RQ values for mammalian cells are dependent on medium composition and cultivation conditions and might vary quite strongly.[26] For CHO cells, RQ values slightly above 1 were observed during lactate production from glucose, while RQ values lower than 1 were observed during subsequent lactate consumption.[24,25] In case that the CTR equalled the CER and the OTR equalled the OUR (see below), the measured TQ corresponds to the RQ. Consequently, a switch from lactate production to lactate consumption was expected at around 90 h judging from the course of the TQ value (Figure 6A, closed black circles). At about this time, the TQ decreased below 1 (Figure 6A, intercept of dashed black lines). This observation is in good agreement with the lactate concentration determined from two manually sampled flasks (Figure 6B, purple circles). Consequently, time-resolved TQ values obtained without sampling in shake flasks were suited to distinguish between lactate production and lactate consumption.

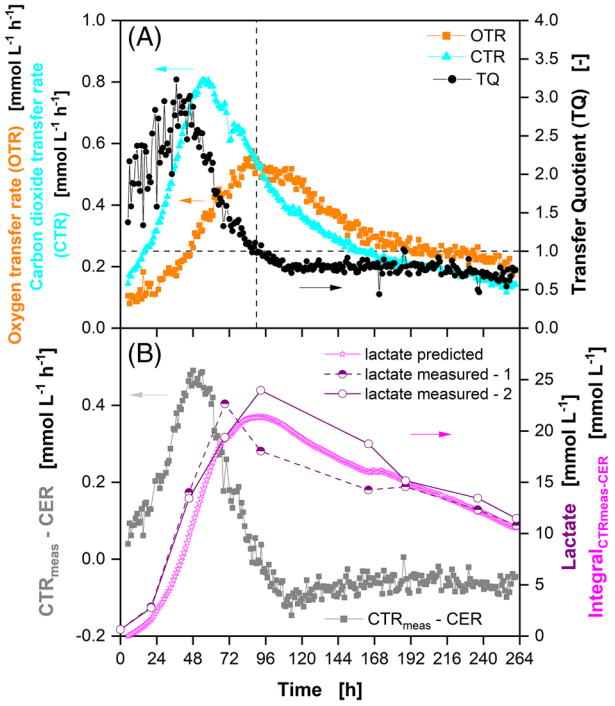

FIGURE 6 Non-invasive determination of the oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR) and transfer quotient (TQ) and prediction of lactate concentration for cell line two. (A) Course of the OTR (closed orange squares), CTR (closed cyan triangles) and TQ (closed black circles) over the cultivation time using the TOM device. Dashed horizontal line marks a TQ of 1. Dashed vertical line depicts time-point at which the TQ decreased from above 1 to below 1. (B) Difference between CTR_{meas} and CER (closed grey squares) during cultivation. The integral of the difference between CTR_{meas} and CER is presented as pink open stars and equals the amount of lactate in the culture medium (in mmol L^{-1}). Lactate concentration measured by HPLC from flasks manually sampled and run in parallel to the monitored flasks is depicted as purple circles (double determination). Data from experiment 3 (see Table 1). CHO cells (initial VCC = $0.5 \cdot 10^6$ mL^{-1}) were cultured at 36.5°C in serum-free chemically defined medium supplemented with 5 mM glutamine and 1% PenStrep. Flasks were gassed with 5% CO_2 in air at 70% r.h. at a rate of 11 mL min^{-1} (TOM). Flasks for manual sampling were closed with a cellulose plug and cultured with 5% CO_2 in air at 70% r.h. in the same incubator. Cultivation conditions: 250 mL glass shake flasks, 50 mL filling volume, 140 rpm shaking speed, 50 mm shaking diameter

Considering the stoichiometry for growth on glucose with simultaneous lactate formation and glutamine as the carbon source, the calculated RQ should only be slightly above 1 (~1.1). However, the measured TQ is largely above 1 in the first part of the cultivation (Figure 6A, closed black circles). This higher TQ is caused by a larger CTR compared to the OTR. Comparison with the expected biological RQ indicates that the TQ did not equal the RQ in this part. Consequently, higher CTR values were attributed to additional CO_2 release from the liquid into the gas phase due to lactate formation.

Under physiological conditions, the buffer reaction of the bicarbonate buffer can be simplified as shown in Equation 4,[24] because the amount of carbonic acid (H_2CO_3) in the liquid phase can be neglected around neutral pH. The ratio between lactate and proton formation during glycolytic metabolism is assumed to be 1:1.[54] Consequently, for each mole of lactate formed, one mole of carbon dioxide is released. Assuming that the ratio between hydrogen carbonate (HCO_3^-) and CO_2 is 1:1,[22] lactate formation led to a release of CO_2 from the liquid phase (Equation 4). Here it is implied that the CO_2 concentration in the liquid did not change for any other reason than lactate formation.

$$CO_2\,(g) \uparrow \Leftrightarrow CO_2\,(l) + H_2O \Leftrightarrow HCO_3^- + H^+ \tag{4}$$

Winckler et al.[24] observed TQ values decreasing from about 2.5 to about 1 for CHO cells grown on glucose with simultaneous lactate formation and calculated the biological RQ to be close to 1.[24] This is in agreement with the RQ expected from the stoichiometry (~1.1 during growth on glucose with simultaneous glutamine consumption and lactate formation). The difference between TQ and RQ can thus be attributed to the difference between measured CTR (CTR_{meas}) and biological CER. This difference equals the CO_2 released due to lactate formation (CTR_{buff}) (Equation 5).

$$CTR_{buff} = CTR_{meas} - CER \tag{5}$$

Assuming, highly simplified, that the RQ from cell respiration is 1 and that the OTR equals the OUR,[29] the CER equals the OTR (Equation 6). Together with the use of Equation 5, CTR_{buff} can then be calculated from measured OTR and CTR data (Figure 6B, closed grey squares).

$$RQ = \frac{CER}{OUR} = 1, \text{ with } OUR = OTR : \Rightarrow OTR = CER \tag{6}$$

By integration of CTR_{buff} over time, the lactate concentration was directly obtained. The calculated lactate concentration (Figure 6B, open pink stars) was in excellent agreement with the lactate concentration determined from manual sampling (Figure 6B, purple circles) during lactate production. The accuracy is especially striking considering the simplifications made for the estimation of the lactate concentration from the respiration activity data. In addition, subsequent lactate consumption was also predicted very well. The accurate prediction of lactate consumption is reasonable as lactate consumption will shift the equilibrium of CO_2 towards the liquid form (Equation 4). This results in less CO_2 release into the gas phase and consequently a lower TQ than expected. Again, the difference between TQ and RQ can be used to calculate the lactate consumption. It should be emphasized that the lactate concentration measured by HPLC was not used for calibration or calculation of the lactate concentration from the respiration activity data. Consequently, predicting the lactate concentration profile was possible completely without sampling and can therefore be regarded as an orthogonal and independent measurement to HPLC analysis.

Calculation of lactate formation from respiration activity is only possible, if bicarbonate is present in the culture medium as a direct correlation between the buffer system, CO_2 release and lactate formation would otherwise not be given. For media buffered with synthetic buffers, the CTR should equal the CER, if no non-biological CO_2 formation occurs. Thus, measurement of the CTR in parallel to the OTR might

be of interest to determine the biological RQ and investigate the correlation between changes in RQ and culture behavior.

4 | CONCLUSIONS

This study used simultaneous measurement of the OTR and VCC in shake flasks to determine qO_2 values for two different CHO cell lines. For CHO cell line one, a two-phase behavior was observed and the qO_2 decreased from $2.38 \cdot 10^{-10}$ to $1.02 \cdot 10^{-10}$ mmol h^{-1} during growth on glucose. For cell line two, the qO_2 was constant at $1.90 \cdot 10^{-10}$ mmol h^{-1}. Both values are in good agreement with literature data.

The determined qO_2 values were used to retrospectively calculate the VCC from the measured OTR with a 24-fold increased resolution compared to manual sampling. In subsequent experiments, determined qO_2 values were validated and used to calculate VCCs without sampling. A good correlation between measured and predicted VCCs was obtained for both cell lines until the maximum VCC was reached. For cell line two, the course of the VCC was also predicted very well during subsequent lactate consumption. Taken together, the presented approach can be used for process monitoring to spot deviations from expected culture behavior or to determine optimum time-points for passaging and culture termination without the need for manual sampling.

A linear correlation between the amounts of glucose and oxygen consumed was found for both cell lines. For cell line one, which produced a monoclonal antibody, oxygen consumption was ~15 $mmol_{oxygen}$ $g_{glucose}{}^{-1}$. For cell line two, which did not produce a monoclonal antibody, oxygen consumption was ~5 $mmol_{oxygen}$ $g_{glucose}{}^{-1}$. The established correlations enabled satisfactory calculation of the glucose concentration without sampling. Only at low glucose concentrations (< 1 g L^{-1}), prediction was less accurate. Time-resolved information on the current glucose concentration could be used to schedule time points for bolus feeding and, again, spot differences in culture behavior.

Measurement of the OTR was combined with measurement of the CTR to enable direct calculation of the TQ for cell line two in a bicarbonate buffered medium. The TQ was found to be above 1 during lactate production and decreased below 1 during lactate consumption. Thus, the switch from lactate production to lactate consumption could be determined in shake flasks without sampling. Additionally, the lactate concentration during cultivation was accurately calculated in a time-resolved manner from CTR and OTR data. Time-resolved calculation provides the option to take corrective actions during cultivation, if the lactate concentration gets too high and detrimental effects for the culture are expected.

In conclusion, our study demonstrates the benefits of non-invasive monitoring of the respiration activity to estimate key culture parameters during CHO cell cultivation in shake flasks. Our approach also underlines how monitoring the OTR can be utilized in combination with offline parameter measurement to determine critical differences in cell line behavior. In future, the methods presented might be used for process control in process analytical technology (PAT) applications or to compare data and culture behavior between different scales without sampling. In addition, combination with mechanistic models might be suited to follow and predict culture behavior during the process.

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COMPETING INTERESTS

Dietmar Lang, Britta Reichenbächer, and Marvin Kadisch are employed by Rentschler Biopharma SE.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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