Development of a mathematical model for the calculation of the Carbon Dioxide Evolution Rate in a RAMOS device

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Shake flasks are an inexpensive and effective way of reproducibly performing industrially relevant cell cultivations, and thus are widely used in research. The RAMOS device (Respiratory Activity Monitoring System) allows the monitoring of the Oxygen and Carbon Dioxide Transfer Rates (OTR and CTR) of cell cultures in shake flasks, providing more detailed process knowledge. Another important parameter in respirometry is the Carbon Dioxide Evolution Rate (CER), the rate at which CO\(_2\) is produced and excreted to the medium by a cell culture. Due to the high solubility of CO\(_2\) and the pH-dependent reactions in which it is involved, a discrepancy between CER and CTR can arise. In this work, a model for the calculation of the CER in a RAMOS device was developed, with pH and CO\(_2\) in the headspace as input variables. Validation of the model could not be performed; however, the model was able to predict a reasonable RQ (in comparison with typical values) for the cultivation of \(E.\) coli pRS7 eYFP-IL6 in TB and TB-glucose media, indicating the model’s potential for further development.

Keywords: Respirometry, RAMOS, Carbon Dioxide Evolution Rate, Shake flask, Bioprocess monitoring.

1. Introduction

1.1. Respirometry of fermentation processes

Microorganisms in aerobic fermentations consume oxygen and produce carbon dioxide. The measuring of this respiratory activity, or respirometry, is a powerful tool for monitoring and controlling industrial fermentation processes \([1][2]\). The most commonly measured variables in respirometry are the Oxygen Transfer Rate (OTR) and the Carbon Dioxide Transfer Rate (CTR). OTR is the rate at which O\(_2\) is transferred from the gas phase to the liquid phase, and the CTR is the rate of exchange of CO\(_2\) between the liquid and the gas phases. These rates can be readily calculated from in- and outlet gas stream analysis. The actual rate at which O\(_2\) is consumed by the microorganisms is called the Oxygen Uptake Rate (OUR). Due to the low solubility of oxygen, the OTR and OUR can be considered equal in most cases \([1]\). The rate at which CO\(_2\) is produced and excreted by the microorganisms is called the Carbon Dioxide Evolution Rate (CER). While the OUR can usually be approximated to the OTR, the same cannot be assumed for the CTR and the CER. Carbon dioxide is highly soluble, and although its solubility does not depend on the pH \([3]\), its reaction with water to form carbonic acid and its subsequent dissociation into bicarbonate and hydrogen ions further contributes to the accumulation of carbon dioxide in both species. At pH \(>6.5\), the amount of total dissolved CO\(_2\) (in both carbon dioxide and bicarbonate forms) in fermentation broths can be one to two orders of magnitude greater than that of O\(_2\). The rate of change in this concentration can be such that a significant difference between CER and CTR values arises \([1]\). For example, at pH 6.3, half of the total dissolved carbon dioxide is in the form of bicarbonate. An increase of 0.2 pH units makes more than 10% of the dissolved CO\(_2\) convert to bicarbonate. Changes in pH (such as those caused by pH control) can have a great effect on the amount of CO\(_2\) transferred to the gas phase,
which can lead to misunderstanding metabolic activity if the effect of pH shifts is not taken into account [2].

1.2. Interactions of CO₂

Carbon dioxide diffuses through the cell membrane and into the liquid medium. Afterwards, it can react with water to form carbonic acid, quickly dissociating to bicarbonate and hydrogen ions (eq. (1)).

\[
\frac{k_1}{k_{-1}} \quad \text{CO}_2 \rightleftharpoons H_2\text{CO}_3 \rightleftharpoons H\text{CO}_3^- + H^+ \quad (1)
\]

\(k_1\) and \(k_{-1}\) are the rates of the indicated reactions in h⁻¹ and \(K_{a1}\) is the first deprotonation constant of carbonic acid in mol.L⁻¹. The time-scale of changes in fermentation processes is long enough for the deprotonation of carbonic acid to be in equilibrium [1].

\[
[H_2\text{CO}_3] = \frac{10^{-pH}[H\text{CO}_3^-]}{K_{a1}} \quad (2)
\]

At high pH values, CO₂ can also react with hydroxide ions to yield bicarbonate ions [2]:

\[
\frac{k_2}{k_{-2}} \quad \text{CO}_2 + OH^- \rightleftharpoons H\text{CO}_3^- \quad (3)
\]

\(k_2\) and \(k_{-2}\) are the rates of the indicated reactions, in M⁻¹.h⁻¹ and h⁻¹ respectively. Further dissociation of bicarbonate and CO₂ complexation with amine groups from proteins can be considered negligible for the usual pH range at which fermentations take place (pH 4-8)[1]. The transfer of CO₂ from the liquid phase to the gas phase is a purely physical process, limited on the liquid film. The CTR can be expressed by eq. (4).

\[
CTR = K_{l\alpha CO_2}([\text{CO}_2] - p_{\text{CO}_2} h_{\text{CO}_2}) \quad (4)
\]

\(K_{l\alpha CO_2}\) is the volumetric mass transfer coefficient for CO₂, \([\text{CO}_2]\) is the concentration of dissolved CO₂ (mM), \(p_{\text{CO}_2}\) is the partial pressure of CO₂ (atm) and \(h_{\text{CO}_2}\) is the Henry’s constant for CO₂ (mM.atm⁻¹). The solubility of CO₂ is affected by ions present in the liquid. Corrections for the solubility are available in the literature [4].

The complex net of reactions and interactions in which carbon dioxide is involved makes this a difficult parameter to measure. Several models for its determination can be found in the literature [1][2][5].

1.3. Shake flasks and the RAMOS device

Shake flasks are used in an estimated 90% of all cell culture experiments at some point [6]. With volumes ranging from 25 mL to 6 liters, the simplicity and low cost of these fermentation vessels make them an outstanding tool for the initial stages of process development, when intensive screening of strains, media and culture has to be performed. However, this simplicity comes at a cost: little information regarding culture conditions can be obtained. If the wrong decision regarding strain, media or process conditions is made during the initial stages of process development, it can be very costly and difficult to revert the negative outcome in later stages of the development. Insufficient process information could even hide the potential of better candidates. The use of non-invasive sensors can greatly increase the amount of information that can be obtained [7]. There are already devices that allow the monitoring of important physiological variables such as pH [8], dissolved oxygen [9] or dissolved CO₂ [10], for example. The RAMOS device
(Respiratory Activity Monitoring System), developed by Anderlei et al. [11] at the chair of Bioprocesses from RWTH Aachen, allows the online monitoring of the Oxygen Transfer Rate (OTR) and the Carbon Dioxide Transfer Rate (CTR), two important fermentation parameters. This device has been used for several applications, such as determining oxygen limitation in shake flasks, screening of microorganisms, optimizing media, investigating secondary substrate limitations, process development and optimization, and monitoring of pre-cultures for fermentations in stirred reactors [12]. Up to eight modified shake flasks can be connected to a RAMOS device. Each of these flasks is adapted with a gas inlet, a gas outlet, a feed inlet, and a modified top for lodging an electrochemical O₂ sensor. These vessels are secured onto a base that is then latched onto an orbital shaker. Throughout a RAMOS measurement, a cycle consisting of three phases with different lengths is repeated. In the first phase (rinse phase), air is flushed into the flask, with such a flow that gas concentrations and hydrodynamics inside the flask closely resemble those of a normal Erlenmeyer flask with a sterile barrier (cotton plug, for example). In this way, results obtained with the device can be transferred to normal shake flasks. During the second phase (stop-phase), the flask is closed, air tight. The microbial activity causes the decrease of the partial pressure of O₂ (pO₂) and the increase of the partial pressure of CO₂ (pCO₂). The partial pressures are monitored with an electrochemical O₂ sensor and a differential pressure sensor. The OTR is calculated from the decrease of pO₂ in the headspace of the flask (eq. (5)); the CTR is calculated as the sum of the OTR and the pressure differential (eq. (6)).

\[
\text{OTR} = \frac{V_g}{V_l \cdot R \cdot T} \frac{dpO_2}{dt} \quad (5)
\]

\[
\text{CTR} = \frac{V_g}{V_l \cdot R \cdot T} \frac{dp_{\text{headspace}}}{dt} + \text{OTR} \quad (6)
\]

\(V_g\) and \(V_l\) are the headspace and liquid volume, \(R\) is the ideal gas constant, \(T\) is temperature and \(p_{\text{headspace}}\) is the headspace pressure.

In the third phase, the gas in- and outlets are reopened, and air is flushed at a higher flow rate to quickly reestablish the pO₂. Each cycle usually lasts 30 minutes.

2. CER calculation in the RAMOS device

In the present work, a novel method for the calculation of the CER in a RAMOS device is presented, based on the CTR measuring method developed by Hansen, which uses Non-Dispersive Infra Red (NDIR) CO₂ sensors [13]. An estimate for the CER in each stop-phase is obtained with a mathematical model, taking as inputs the evolution of CO₂ in the headspace during this phase and the corresponding pH value, which is measured offline by sampling normal shake flasks running in parallel. Online measurement of pH in a RAMOS device has been done with direct measurement with an H⁺ electrode in a specially adapted flask [14], and also with pH sensitive sensor spots [8]. In the work developed by Scheidle et al. [8], offline pH measurements were made in parallel normal shake flasks to test the accuracy of the online measurements made with the sensor spots, presenting a maximum difference of ± 0.05 pH units, showing that good estimates for the pH profile in a RAMOS flask can be obtained from offline measurements.
2.1. Model equations

The evolution of \( p_{\text{CO}_2} \) in the headspace during the stop phase is described by a second order polynomial (eq. (7)):

\[
p_{\text{CO}_2}(t) = a \cdot t^2 + b \cdot t + c \tag{7}
\]

As demonstrated by Hansen [13]

\[
a = \frac{1}{2} \frac{dp_{\text{CO}_2, \text{rinse}}}{dt} \frac{F_{\text{out}}}{V_g} \tag{8}
\]

where \( p_{\text{CO}_2, \text{rinse}} \) is the \( \text{CO}_2 \) partial pressure during the rinse phase (atm) and \( F_{\text{out}} \) is the flow of gas out of the flask (L.h\(^{-1}\)) during the rinse phase. The differential equation for \( p_{\text{CO}_2} \) then becomes

\[
\frac{dp_{\text{CO}_2}}{dt} = \frac{dp_{\text{CO}_2, \text{rinse}}}{dt} \frac{F_{\text{out}}}{V_g}, t + b \tag{9}
\]

with \( c \) as the initial value. The CTR can be expressed by eq. (10).

\[
CTR = \frac{dp_{\text{CO}_2}}{dt} \frac{V_g}{V_L \cdot R \cdot T} \tag{10}
\]

Equaling equations (4) and (10), deriving and combining the result with eq. (9) yields a differential equation for \([\text{CO}_2]\).

\[
\frac{d[\text{CO}_2]}{dt} = \frac{dp_{\text{CO}_2}}{dt} \cdot h_{\text{CO}_2} + \frac{dp_{\text{CO}_2, \text{rinse}}}{dt} \frac{F_{\text{out}}}{V_g \cdot R \cdot T \cdot K_L a_{\text{CO}_2}} \tag{11}
\]

The initial value is obtained simply by substituting \( \frac{dp_{\text{CO}_2}}{dt} \) by \( b \). The differential equation for bicarbonate is given by eq. (12).

\[
\frac{d[\text{HCO}_3^-]}{dt} = (k_1 + k_2 \cdot 10^{P_{\text{H}_2} - 14})[\text{CO}_2] - (k_{-1} \cdot 10^{-P_{\text{H}_2} + k_{-2}})[\text{HCO}_3^-] \tag{12}
\]

It is assumed that the bicarbonate is in equilibrium with the dissolved \( \text{CO}_2 \) in the beginning of the stop-phase. Therefore, \( \frac{d[\text{HCO}_3^-]}{dt} \approx 0 \) and the initial value is obtained the initial value for \([\text{CO}_2]\) by

\[
\frac{(k_1 + k_2 \cdot 10^{P_{\text{H}_2} - 14})}{(k_{-1} \cdot 10^{-P_{\text{H}_2} + k_{-2}})} \]

The same will not be assumed for the stop-phase, since here no \( \text{CO}_2 \) is removed from the flask, and as such its concentration in the liquid will increase at a higher rate. To take into account the time constant of the sensors, \( \tau \), eq. (13) is used to fit the experimental data, with \( b \) and \( c \) from eq. (7) as fitting parameters.

\[
\frac{dp_{\text{CO}_2, \text{sensor}}}{dt} = \frac{p_{\text{CO}_2} - p_{\text{CO}_2, \text{sensor}}}{\tau} \tag{13}
\]

Finally, the CER is calculated with eq. (14).

\[
\text{CER} = CTR + \frac{\Delta[\text{CO}_2] + \Delta[\text{HCO}_3^-]}{t_{sp}} \tag{14}
\]

\( \Delta[\text{CO}_2] \) and \( \Delta[\text{HCO}_3^-] \) are the difference between the final and initial \( \text{CO}_2 \) and bicarbonate concentrations, and \( t_{sp} \) is the length of the stop-phase.

The volumetric mass transfer coefficient for \( \text{O}_2 \) is calculated with the correlation given by Maier [15] for low viscosity media in shake flasks (eq. (15)).

\[
K_L a_{\text{O}_2} = 6.67 \times 10^{-6} \cdot N^{1.16} \cdot V_L^{0.83} \cdot d_0^{0.38} \cdot d_1^{1.92} \tag{15}
\]

\( N \) is the shaking frequency (min\(^{-1}\)), \( V_L \) is the liquid volume (mL), \( d_0 \) is the shaking diameter (cm) and \( d \) is the flask’s largest diameter (cm). The ratio of mass transfer coefficients for \( \text{O}_2 \) and \( \text{CO}_2 \) is proportional to the ratio of their diffusivities [16].

\[
\frac{K_L a_{\text{CO}_2}}{K_L a_{\text{O}_2}} = \left( \frac{D_L a_{\text{CO}_2}}{D_L a_{\text{O}_2}} \right)^2 = 0.89 \Leftrightarrow K_L a_{\text{CO}_2} = 0.89 K_L a_{\text{O}_2} \tag{16}
\]

Even though the diffusivities are dependent on the medium composition, their ratio is not [16]. The Henry’s law coefficient for \( \text{CO}_2 \) in pure water (in
mM.atm\(^{-1}\)) is calculated with the correlation given by Zheng et al [17].

\[
h_{CO_2} = 36 \times \exp \left[ 2200 \times \left( \frac{1}{T} - \frac{1}{298.15} \right) \right] \quad (17)
\]

The values of the model parameters are found in table 1.

Table 1 - Parameters used in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>310 K</td>
</tr>
<tr>
<td>(\tau)</td>
<td>0.0237h</td>
</tr>
<tr>
<td>(F_{out})</td>
<td>0.6 L.h(^{-1})</td>
</tr>
<tr>
<td>N</td>
<td>340 rpm</td>
</tr>
<tr>
<td>(d_0)</td>
<td>5 cm</td>
</tr>
<tr>
<td>(d)</td>
<td>8 cm</td>
</tr>
<tr>
<td>(V_i)</td>
<td>10 mL</td>
</tr>
<tr>
<td>(V_g)</td>
<td>Varies with flask</td>
</tr>
<tr>
<td>(K_aCO_2)</td>
<td>273 h(^{-1})</td>
</tr>
<tr>
<td>(h_{CO_2})</td>
<td>27.2 mM.atm(^{-1}) [17]</td>
</tr>
<tr>
<td>(k_1)</td>
<td>5.030 \times 10^{-2} h(^{-1}) [18]</td>
</tr>
<tr>
<td>(k_2)</td>
<td>6.346 h(^{-1}) [13]</td>
</tr>
<tr>
<td>(k_3)</td>
<td>1.117 \times 10^{8} M(^{-1})h(^{-1}) [18]</td>
</tr>
<tr>
<td>(K_{31})</td>
<td>10(^{-3.581}) M [18]</td>
</tr>
</tbody>
</table>

3. Materials and Methods

3.1. Sensor calibration and step-response

The monitoring of pCO\(_2\) in the flask headspace was done with NDIR CO\(_2\) sensors of the model MSH-P-CO\(_2\), produced by Dynament (Derbyshire, UK). The sensors work in the range of 0-5% of volume of CO\(_2\), producing an output signal in the range of 0.4-2 V. In the RAMOS device used in this work, the sensors are plugged into a circuit board that, besides supplying the 5 volts needed to power them, also transmits the signal to an ADAM module that in turn transmits the signal to the computer. For unknown reasons, possibly inherent to the circuit board, the sensors showed different offset values, and so it was necessary to determine the calibration curve for each sensor. This calibration curve is needed to convert the raw signal registered by the RAMOS software to pCO\(_2\). Since pCO\(_2\) and the output signal were found to be linearly proportional, a good estimate for the calibration curves can be obtained from two points. To obtain calibration curves and to study the step response of the sensors, a special device was assembled. In order to eliminate the mixing effects that can occur if a step increase of pCO\(_2\) is applied to a flask, each sensor was attached to a support with a very small volume, through which the gas sample is flushed. An O-ring between the sensor and the sampled gas ensures that no external air enters the sensor. The supports were fixated onto an aluminum base. The gas sample flows through a valve and its flow is then adjusted to 80 mL.min\(^{-1}\) by a mass flow controller. Afterwards, it flows through an eight-way splitter, directing the gas to eight capillaries of the same length that feed it to the supports holding the sensors. These are connected to the same electronic parts as in a RAMOS device, which allow the collection of data. A step input of 4.892 \times 10^{-2} atm of pCO\(_2\) was used.(the pCO\(_2\) is increased from 3.8 \times 10^{-4} atm to 4.93 \times 10^{-2} atm, the step size is given by the difference). A function of the form given by eq. (13) was fitted to each sensor’s step response with the Solver from Microsoft Excel, using the time constant \(\tau\) as the fitting parameter.

3.2. Fermentation examples

E. coli BL21 pRSet eYFP-IL6 and E. coli JM109 were used to study the applicability of the model.
3.2.1 Growth media

*E. coli* BL21 pRSet eYFP-IL6 was grown on phosphate-buffered TB and TB-glucose media. TB and TB-Glucose were prepared with 24 g.L\(^{-1}\) of yeast extract, 12 g.L\(^{-1}\) of tryptone, 12.54 g.L\(^{-1}\) of KH\(_2\)PO\(_4\), and 5 g.L\(^{-1}\) of glycerol or glucose, respectively. All reagents were manufactured by Carl Roth GmbH, Karlsruhe, Germany. The formulations for these media were obtained from the work of Losen [14].

The media were autoclaved for 20 minutes at 121\(^\circ\)C. To avoid darkening the medium due to Maillard reactions during autoclaving, two different solutions were prepared for TB-glucose, one with 10 g/L glucose and one with double the concentration of all the other reagents. For TB and TB-glucose, the pH was close to 7 without any adjustments.

3.2.2 Culture conditions

The fermentations were carried out at 310 K, on orbital shakers (Kühner AG, Birsfelden, Switzerland) with a shaking diameter of 5 cm. In RAMOS experiments, the flasks were flushed with air at a flow of 0.6 L.min\(^{-1}\) per flask during the rinsing phases, and with 3.6 mL.min\(^{-1}\) during the high flow phases. The length of each phase is in Table 2.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Length (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-Flow Phase (Phase 1)</td>
<td>54</td>
</tr>
<tr>
<td>Low-Flow Phase (Phase 2)</td>
<td>1500</td>
</tr>
<tr>
<td>Valve-stop Phase (Phase 3)</td>
<td>60</td>
</tr>
<tr>
<td>Stop-flow phase (Phase 4)</td>
<td>300</td>
</tr>
</tbody>
</table>

It should be noted that these time intervals are defined by the user in the RAMOS software. The stop-phase described before is actually composed by phases 3 and 4, with the defined length of phase 4 being the total length of the stop-phase.

Since glucose and glycerol have different typical respiratory quotients (1.0 and 0.75 respectively) [19], this parameter will be used as an indicator of the suitability of the model. Two RAMOS flasks with a total volume of 250 mL were used for making the pre-cultures. The flasks were filled with 10 mL of TB or TB-glucose medium, supplemented with 100 µg.L\(^{-1}\) of ampicillin. Each flask was then inoculated with 100 µL of a cryoculture of *E. coli* BL21 pRSet eYFP-IL6 that had been grown on TB medium. The flasks were shaken at 350 rpm, and the oxygen transfer rate of the pre-cultures was monitored with an in-house built RAMOS device. The cultures were stopped after the OTR reached around 40 mM.h\(^{-1}\). Then, suitable volumes of TB and TB-glucose supplemented with 100 µg.L\(^{-1}\) of ampicillin were inoculated with the corresponding pre-culture, at an inoculum rate of 1%. A total of eight RAMOS flasks and twenty normal 250 mL shake flasks were filled with 10 mL of inoculated broth each, half of the flasks with TB and the other half with TB-glucose. The fermentation was performed for 10 hours at a shaking frequency of 340 rpm, 10 rpm slower than the pre-culture, in order to obtain a smoother signal from the CO\(_2\) sensors. Monitoring of the OTR and logging of the CO\(_2\) sensors signal was done with a RAMOS device. The logging frequency was set to 60 measurements per minute. The pH values were obtained by offline measurement with a pH electrode in the normal shake flasks, with each flask being used for a single measurement.
4. Results and discussion

4.1. Sensor calibration and step-response

A set of eight sensors was exposed to a step increase of $pCO_2$ from $3.8 \times 10^{-4}$ to $4.93 \times 10^{-2}$ atm, showing different responses in the produced output signal, as can be seen in fig. 1.

![Fig. 1 - Step-response of eight sensors. Step size: 0.049 atm of pCO2; T=310K; Flow=0.6 L.h\(^{-1}\).](image)

The time constant $\tau$ was in average 0.0237 h, with a standard deviation of 0.0015 h (fig. 2).

![Fig. 2 - Step-response of a CO2 sensor.](image)

4.2. Constant and shifting pH simulation

To understand the effect of the pH on the CER predicted by the model, the bicarbonate concentration during a stop-phase was simulated for constant pH values ranging from 6 to 8. The effect of a shifting pH was also evaluated. The stop-phase used in this example is from the cultivation of E. coli pRS5 eYFP-IL6 in TB-glucose medium, 3 hours into the fermentation.

The effect of different constant pH values in the CER is represented in fig. 4.

![Fig. 4 - Predicted CER for different pH values. E. coli BL21 pRS5 eYFP-IL6 in TB-glucose medium, time=3 hours, T=310 K, N=340 rpm, $d_0=5$ cm, $V_L=0.010$ L, $V_g=0.281$ L.](image)

The effect of different constant rates of change in the pH is shown in fig. 5, for a starting pH of 7.5. As demonstrated, the pH greatly affects the predicted CER. Many factors can affect the pH of the medium, and as such it is difficult to predict how the pH behaves during a stop-phase solely from offline measurements. A way to overcome this issue would be to use pH sensitive sensor spots, as have Scheidle et al. [8] for example.
Fig. 5 - Predicted CER for constant rates of change in pH. *E. coli* BL21 pRSet eYFP-IL6 in TB-glucose medium. Initial pH=7.5, time=3 hours, T=310 K, N=340 rpm, \(d_0=5\) cm, \(V_L=0.010\) L, \(V_g=0.281\) L.

4.3. Fermentation examples

At around 6.5 hours into the fermentation, the device had a problem and the measurement was resumed only ca. 1.5 hours later.

Although not so evident from the OTR profile, diauxic growth can be observed from the CTR and CER profiles, indicating a change in substrate, in conjunction with a change in pH evolution. This could be caused by what is known as the acetate metabolism overflow. High growth rate aerobic *E. coli* cultivations often result in the excretion of acetate, acidifying the medium. This phenomenon was found to be caused by carbon catabolite repression of the Acetil-CoA synthetase [20]. When the primary carbon source is exhausted, the cell starts to consume acetate if no other more readily metabolized substrates are available [13]. The consumption of acetate causes the medium pH to rise again. After completely exhausting carbohydrate substrates, catabolic degradation of aminoacids becomes predominant, with ammonia accumulating in the broth as a result, further increasing the pH [20]. In the work of Hansen [13], this particular strain was grown in Wilms-MOPS medium and this mechanism was observed. Another relevant feature that can be observed is the discrepancy between the two methods for CTR calculation. The IR sensor method seems much more sensitive to small changes; for example, at the 3.5 hour point in the TB-glucose medium, there is a very subtle drop in CTR-\(P\), while the CTR-\(S\) shows a very pronounced decrease. A large difference is also noticeable when the stationary phase is reaching the end, especially for the TB-glucose medium. For example, at 5.5h, CTR-\(P\) is 27 mM.h\(^{-1}\) and CTR-\(S\) is only 7 mM.h\(^{-1}\). The Respiratory (RQ) and Transfer Quotients, obtained with both pressure differential and IR sensor methods (TQ-\(S\) and TQ-\(P\)) were calculated (figs. 8 and 9). The RQ values do not correspond exactly to the typical values; however, in both situations there is a visible change in RQ around 3h and 4h for TB-
glucose and TB respectively, suggesting a change in carbon source. In the TB-glucose medium, the RQ is relatively constant around 1.4 decaying to 1.2 after the substrate shift at 3.5 hours, which is in agreement with the change from glucose to acetate, with typical RQ values of 1 and 0.8 respectively; in the medium containing glycerol, the RQ increases slightly from ca. 1 to 1.2 four hours in, which is also in accordance with the substrate shift from glycerol to acetate (typical RQ values of 0.75 and 0.80 respectively).

5. Conclusion and outlook

In this work, a mathematical model for the Carbon Dioxide Evolution Rate of microbial cultures in a RAMOS device was developed, with the use of NDIR sensors for pCO₂ measurement and offline pH measurements. The response of the model to simulated constant and shifting pH was studied, for a set of experimental data from a stop-phase. In either situation, the CER was significantly affected. Therefore, taking offline pH measurements and simply assuming a constant value throughout the stop-phase may produce a less accurate CER; online measurement with pH-sensitive sensor spots would tackle this problem and reduce uncertainty.

Validation of the model was not carried out; a possible strategy would be to somehow simulate a known CER by adding known amounts of bicarbonate to a flask or using a slow-release device, monitoring the pCO₂ in the headspace and using online pH measurement, as have Neeleman et al [21]. Another strategy would be to perform offline liquid sample analysis to establish a mass balance to carbon, as well as online monitoring of the pH.

The suitability of the model was tested by monitoring the growth of E. coli pRSet eYFP-IL6 in media containing either glucose or glycerol as the sole carbohydrate source. The differences in respiratory quotients indicated that the model was able to provide a good estimate of the CER. However, this estimate can only be as good as the CTR that can be calculated from the NDIR sensors, and this method showed some discrepancies with the state-of-the-art pressure differential method, unlike the good agreement between both methods.
that Hansen obtained [13]. There was also high variability between the CTR calculated with different sensors in the same experimental conditions. The high noise of the sensors might have contributed to this situation; also, the CTR calculation method may have to be ameliorated. Only with a proper CTR measuring method, coupled with online pH measurement, can the CER be accurately predicted.

6. References