

Recommendations for process engineering characterisation of single-use bioreactors and mixing systems by using experimental methods

Supplement to the 2nd edition: Volumetric mass transfer coefficient of CO₂

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IMPRINT

Publisher



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Publication date: October 2021

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1 BACKGROUND

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1.1 Introduction

Since the mid-2000s, the use of single-use bioreactors (SUB) and single-use mixing systems (SUM) in biopharmaceutical research and production has increased enormously in terms of scope and diversity. This means that single-use technology (SUT) in all process steps – especially in laboratory and pilot scale – is now of considerable importance for biopharmaceuticals and biosimilars manufacturing. SUB and SUM are mainly used in mammalian cell culture processes for the production of biotherapeutics such as proteins, cell-based therapeutics or gene transfer vectors. In addition, SUT is also used for the cultivation of plant cell cultures, microorganisms, and algae as well as for special products in the food and cosmetics sector [DECHEMA 2011], [Lehmann et al. 2014], [Eibl and Eibl 2019].

As early as 2011, a status paper systematised and classified conventional stainless steel or glass bioreactors according to their characterisation methods [DECHEMA 2011]. In 2016, the first edition of “Recommendations for process characterisation of single-use bioreactors and mixing systems by using experimental methods” was published [DECHEMA 2016]. The authors are members of the DECHEMA expert group “Single-Use Technology in Biopharmaceutical Manufacturing”, in which experts from industry and academia collaborate to provide knowledge-based guidelines to the community. In 2020, a completely revised 2nd edition of these recommendations was published which includes a number of improvements [DECHEMA 2020]. The most important improvement was in the method for determining the volumetric mass transfer coefficient of oxygen ($k_{La}(O_2)$ value). To assist users of single-use devices, the DECHEMA expert group also provides a user-friendly Microsoft Excel-based evaluation tool for determining the $k_{La}(O_2)$ value, which can be downloaded from the DECHEMA homepage [DECHEMA 2020].

Aim of this supplement “Volumetric mass transfer coefficient of CO₂” to the “Recommendations for process characterisation of single-use bioreactors and mixing systems by using experimental methods (2nd edition)”.

In many cell cultivation and fermentation processes both oxygen and carbon dioxide play an important role. With detailed recommendations [DECHEMA 2020 and the present supplement] the DECHEMA expert group addresses the mass transfer of these two gases between the gas phase and the liquid phase. The authors aim to provide – besides an outline of the theoretical background – validated and standardised methods for the determination of the volumetric mass transfer coefficients of oxygen and of carbon dioxide.

In aerobic cultivations or fermentations, the oxygen supply to the cells or microorganisms usually determines the maximum cell density that can be reached and is, therefore, directly connected to the productivity of the process. Therefore, the determination and optimisation of oxygen supply is critical for most biopharmaceutical processes and is usually well characterised during process development. Since the solubility of oxygen in water is quite low the interfacial surface area of the gas bubbles and the boundary layer thicknesses in the liquid become limiting at high cell densities. The decisive parameter here is the volumetric mass transfer coefficient of oxygen ($k_{La}(O_2)$) [DECHEMA 2020]. Fac-

tors that can be optimised in order to increase the interfacial area include the gassing device and the gassing rate as well as the type and speed of the agitator since the latter play an important role in the dispersion and distribution of the gas bubbles.

Besides oxygen, carbon dioxide is also an important factor for most cell cultivation bioprocesses. It is produced by the cells, serves as pH buffer and nutrient but is toxic in high concentrations. Due to the production of carbon dioxide by the cells, it needs to be stripped from the bioreactor. The transfer of carbon dioxide from the liquid phase to the gas bubbles can be described analogously to oxygen by the volumetric mass transfer coefficient of carbon dioxide ($k_L a(\text{CO}_2)$). The experimental determination of the $k_L a(\text{CO}_2)$ value is somewhat more difficult than in the case of $k_L a(\text{O}_2)$ due to the prevailing carbonate buffer system.

However, the higher solubility of carbon dioxide in water compared to oxygen leads to earlier attainment of Henry equilibrium. Hence, most bubbles leave the reactor surface completely saturated and the gassing volume and not the interfacial area becomes limiting for carbon dioxide removal. A deeper insight into this topic is provided by [Sieblist et al. 2011]. This difference in transfer kinetics and the resulting saturation effects lead to apparently lower $k_L a(\text{CO}_2)$ values, although the values of the mass transfer coefficients in the liquid (k_L) in their derivation from Ficks' law are very similar for oxygen and carbon dioxide, and the surface-to-volume ratio (a) is identical. For large-scale bioreactors, this fact must be taken into account. However, for SUB, which belong to small-scale bioreactors, the distinction between the apparent $k_L a(\text{CO}_2)$ value and the real $k_L a(\text{CO}_2)$ value is not essential. The apparent $k_L a(\text{CO}_2)$ value can be used here to characterise the bioreactor and to calculate the concentration of dissolved carbon dioxide at steady state.

As a consequence of the previously described effects the transfers of oxygen and carbon dioxide need to be well balanced for each process and the knowledge of $k_L a(\text{CO}_2)$ value is a valuable tool to estimate and compare the dissolved carbon dioxide concentrations in different bioreactors.

Since this aspect has often been neglected so far and some issues arise in the experimental determination of the $k_L a(\text{CO}_2)$ value, the DECHEMA expert group "Single-Use Technology in Biopharmaceutical Manufacturing" prepared this supplement to the "Recommendations for process characterisation of single-use bioreactors and mixing systems by using experimental methods (2nd edition)". In addition to a theoretical consideration, this supplement provides manufacturers and operators of SUB with a uniform method for determining the volumetric mass transfer coefficient of carbon dioxide by means of a validated standard operating procedure (SOP). Furthermore, this guideline can also be used for reusable systems.

Warning:

It must be noted that the method described here can only be used for single-use bioreactors that have sufficient material durability to low pH values (down to $\text{pH} \approx 4$). We ask for appropriate verification and do not assume any responsibility.

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As in the case of the method for determining the $k_L a(\text{O}_2)$ value, the DECHEMA expert group has also developed a Microsoft Excel-based evaluation tool for determining the $k_L a(\text{CO}_2)$ value to assist users of SUB in applying the experimental method (see section 2.9).

1.2 Determination method of the volumetric mass transfer coefficient of carbon dioxide

The mass transfer of carbon dioxide molecules from gas phase to liquid phase (or vice versa) can be quantified by the carbon dioxide transfer rate (CTR). The two-film theory of Lewis and Whitman as described in [DECHEMA 2020] can also be used here. By analogy with oxygen, CO_2 mass transfer can be viewed as a first-order kinetic process in which the gradient between the current CO_2 concentration ($[\text{CO}_{2,d}]$) and the CO_2 saturation concentration according to Henry's law ($[\text{CO}_{2,d}]_{\text{sat}}$) is the driving force. For a mass transfer of carbon dioxide from the liquid phase to the gas phase to take place, a decreasing concentration of dissolved carbon dioxide in the direction of the interphase is required in the liquid-side boundary layer. This means that more carbon dioxide must be dissolved in the medium than corresponds to the Henry equilibrium at the interphase (see Eq. 1).

$$-\frac{d[\text{CO}_{2,d}]}{dt} = \text{CTR} = k_L a(\text{CO}_2) \cdot ([\text{CO}_{2,d}] - [\text{CO}_{2,d}]_{\text{sat}}) \quad \text{Eq. 1}$$

As in the case of oxygen, the value of the mass transfer coefficient ($k_L a(\text{CO}_2)$) includes the mass transport residences in both laminar boundary layers (i.e., actually two carbon dioxide mass transfer coefficients), the Henry volatility constant of carbon dioxide ($K_H(\text{CO}_2)$), and the interfacial surface area per liquid volume (a), which depends on the size and number of bubbles dispersed in the continuous liquid phase. From the ratio of the diffusion coefficients of oxygen ($D(\text{O}_2) = 2.10 \cdot 10^{-9} \text{ m}^2/\text{s}$ at 25 °C) and carbon dioxide ($D(\text{CO}_2) = 2.03 \cdot 10^{-9} \text{ m}^2/\text{s}$ at 25 °C) it can be concluded that the mass transfer rate of both species is very similar. It should be noted, however, that the solubilities in water (i.e., the Henry volatility constants) are significantly different and that an approximately 30 times higher amount of CO_2 can be dissolved in water. Under steady-state conditions, the dissolved CO_2 saturation concentration is in equilibrium with the CO_2 partial pressure in the gaseous phase, as given by Henry's law (see Eq. 2).

$$[\text{CO}_{2,d}]_{\text{sat}} = \frac{p(\text{CO}_2)}{K_H(\text{CO}_2)} \quad \text{Eq. 2}$$

Note 1:

It should be noted that the name "Henry constant" is not consistently defined in the literature. The authors therefore recommend using the term "Henry constant" and the abbreviations K_H or H only if the constant is given as "Henry volatility constant" in the unit "Pa·L·mol⁻¹" (or units derived therefrom). The reciprocal of this constant with the unit "mol·L⁻¹·Pa⁻¹" (or units derived therefrom), on the other hand, should be referred to as "Henry solubility constant" or "solubility constant" for short, and not also as "Henry constant". Accordingly, a different symbol (such as S_H or Λ) should be used for the solubility constant to avoid confusion.

To determine the $K_a(\text{CO}_2)$ value, the total CO_2 concentration in the medium must be considered. This is because CO_2 dissolved in water forms a carbonate buffer system. This consists of four species: dissolved CO_2 ($\text{CO}_{2,d}$), carbonic acid (H_2CO_3), hydrogen carbonate (HCO_3^-), and carbonate (CO_3^{2-}) (see Fig. 1).

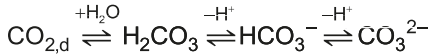


Figure 1: Species formed from CO_2 in aqueous solutions.

Therefore, the conversion of dissolved CO_2 into the other three carbonate species needs to be taken into account. The ratio of dissolved CO_2 to carbonic acid at room temperature (25 °C) is 99.76 to 0.24 and it is independent of the pH value and the ion concentration in the medium [Musvoto et al. 2000]. Hence, the formation of carbonic acid can be neglected and both species are combined into a single component “ $\text{CO}_{2,\text{fl}}$.” (see Eq. 3). This is referred to as dissolved CO_2 in the following and the resulting error is neglected [Contreras 2007]. Consequently only the conversion of “ $\text{CO}_{2,\text{fl}}$.” to the remaining species hydrogen carbonate and carbonate are taken into account.

$$[\text{CO}_{2,\text{fl}}] = [\text{CO}_{2,d}] + [\text{H}_2\text{CO}_3] \approx [\text{CO}_{2,d}] \quad \text{Eq. 3}$$

The CO_2 dissolved in the medium is in acid-base equilibria with the anions hydrogen carbonate and carbonate (see Eq. 4 and Eq. 5).



Here, the constants K_1 and K_2 are the respective equilibrium constants or dissociation constants (see Eq. 6 and Eq. 7).

$$K_1 = \frac{[\text{HCO}_3^-] \cdot [\text{H}^+]}{[\text{CO}_{2,\text{fl}}]} \quad \text{Eq. 6}$$

$$K_2 = \frac{[\text{CO}_3^{2-}] \cdot [\text{H}^+]}{[\text{HCO}_3^-]} \quad \text{Eq. 7}$$

The concentrations of hydrogen carbonate and carbonate can therefore be calculated from the constants K_1 , K_2 , and the pH value present (see Eq. 8 and Eq. 9).

$$[\text{HCO}_3^-] = \frac{K_1 \cdot [\text{CO}_{2,\text{fl}}]}{10^{-\text{pH}}} \quad \text{Eq. 8}$$

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$$[\text{CO}_3^{2-}] = \frac{K_2 \cdot [\text{HCO}_3^-]}{10^{-\text{pH}}} = \frac{K_2 \cdot K_1 \cdot [\text{CO}_{2,\text{fl.}}]}{(10^{-\text{pH}})^2} \quad \text{Eq. 9}$$

The dissociation constants K_1 and K_2 are temperature-dependent and can be calculated according to [Minkevich and Neubert 1984] for water with Eq. 10 and Eq. 11.

$$K_1 = e^{\left(-11.582 - \frac{918.9\text{K}}{T}\right)} \frac{\text{mol}}{\text{L}} \stackrel{25^\circ\text{C}}{=} 4.281 \cdot 10^{-7} \frac{\text{mol}}{\text{L}} \quad \text{Eq. 10}$$

$$K_2 = e^{\left(-17.790 - \frac{1789.1\text{K}}{T}\right)} \frac{\text{mol}}{\text{L}} \stackrel{25^\circ\text{C}}{=} 4.654 \cdot 10^{-11} \frac{\text{mol}}{\text{L}} \quad \text{Eq. 11}$$

As mentioned above, the total CO_2 concentration in the medium ($[\text{CO}_{2,\text{tot}}]$) is composed of the concentrations of dissolved CO_2 (incl. carbonic acid), hydrogen carbonate, and carbonate (see Eq. 12).

$$[\text{CO}_{2,\text{tot}}] = [\text{CO}_{2,\text{fl.}}] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \quad \text{Eq. 12}$$

By inserting Eq. 8 and Eq. 9 into Eq. 12, Eq. 13 and subsequently Eq. 14 are obtained for the total CO_2 concentration in the medium.

$$[\text{CO}_{2,\text{tot}}] = [\text{CO}_{2,\text{fl.}}] + \frac{K_1 \cdot [\text{CO}_{2,\text{fl.}}]}{10^{-\text{pH}}} + \frac{K_2 \cdot K_1 \cdot [\text{CO}_{2,\text{fl.}}]}{(10^{-\text{pH}})^2} \quad \text{Eq. 13}$$

$$[\text{CO}_{2,\text{tot}}] = \frac{(10^{-\text{pH}})^2 \cdot [\text{CO}_{2,\text{fl.}}] + 10^{-\text{pH}} \cdot K_1 \cdot [\text{CO}_{2,\text{fl.}}] + K_2 \cdot K_1 \cdot [\text{CO}_{2,\text{fl.}}]}{(10^{-\text{pH}})^2} \quad \text{Eq. 14}$$

By transforming Eq. 14, the concentration of dissolved CO_2 can then be calculated (see Eq. 15). The expression outlined with a blue dashed line including the dissociation constants K_1 and K_2 can be summarized as the pH-dependent molar fraction α_1 of dissolved CO_2 in the total carbon dioxide concentration.

$$[\text{CO}_{2,\text{fl.}}] = \frac{(10^{-\text{pH}})^2}{(10^{-\text{pH}})^2 + 10^{-\text{pH}} \cdot K_1 + K_2 \cdot K_1} [\text{CO}_{2,\text{tot}}] = \alpha_1 [\text{CO}_{2,\text{tot}}] \quad \text{Eq. 15}$$

By inserting Eq. 15 into Eq. 8 the concentration of hydrogen carbonate can also be expressed as molar fraction α_2 (outlined with red line) of hydrogen carbonate in the total carbon dioxide concentration (see Eq. 16 and Eq. 17).

$$[\text{HCO}_3^-] = \frac{K_1 \cdot [\text{CO}_{2,\text{fl.}}]}{10^{-\text{pH}}} = \frac{K_1 \cdot (10^{-\text{pH}})^2}{10^{-\text{pH}} \cdot \left((10^{-\text{pH}})^2 + 10^{-\text{pH}} \cdot K_1 + K_2 \cdot K_1 \right)} \cdot [\text{CO}_{2,\text{tot}}] \quad \text{Eq. 16}$$

$$[\text{HCO}_3^-] = \frac{K_1 \cdot 10^{-\text{pH}}}{(10^{-\text{pH}})^2 + 10^{-\text{pH}} \cdot K_1 + K_2 \cdot K_1} \cdot [\text{CO}_{2,\text{tot}}] = \alpha_2 \cdot [\text{CO}_{2,\text{tot}}] \quad \text{Eq. 17}$$

Similarly, the concentration of carbonate can be expressed as the molar fraction α_3 (outlined with green dotted line) of carbonate in the total carbon dioxide concentration by inserting Eq. 15 into Eq. 9 (see Eq. 18 and Eq. 19).

$$[\text{CO}_3^{2-}] = \frac{K_2 \cdot K_1 \cdot [\text{CO}_{2,\text{fl.}}]}{(10^{-\text{pH}})^2} = \frac{K_2 \cdot K_1 \cdot (10^{-\text{pH}})^2}{(10^{-\text{pH}})^2 \cdot \left((10^{-\text{pH}})^2 + 10^{-\text{pH}} \cdot K_1 + K_2 \cdot K_1 \right)} \cdot [\text{CO}_{2,\text{tot}}] \quad \text{Eq. 18}$$

$$[\text{CO}_3^{2-}] = \frac{K_2 \cdot K_1}{(10^{-\text{pH}})^2 + 10^{-\text{pH}} \cdot K_1 + K_2 \cdot K_1} \cdot [\text{CO}_{2,\text{tot}}] = \alpha_3 \cdot [\text{CO}_{2,\text{tot}}] \quad \text{Eq. 19}$$

Equations 15, 17, and 19 show that the composition of the total CO_2 concentration in the medium is pH-dependent due to dissociation equilibria (see Fig. 2).

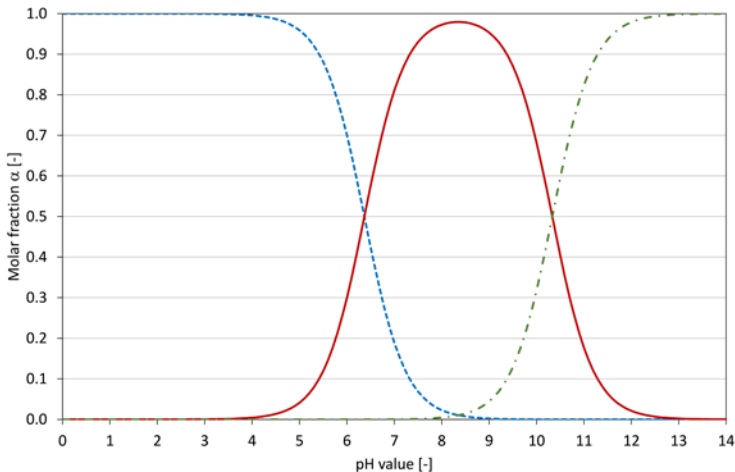


Figure 2: Molar fractions of dissolved CO_2 (blue dashed line), hydrogen carbonate (red line), and carbonate (green dotted line) in water as a function of the pH value.

1 BACKGROUND

The problem is now to determine the value of the total CO_2 concentration in the medium. For this purpose, a charge balance can be established for the liquid phase due to its charge neutrality (see Eq. 20).

$$[\text{H}^+] + c_Z = [\text{OH}^-] + [\text{HCO}_3^-] + 2 \cdot [\text{CO}_3^{2-}] \quad \text{Eq. 20}$$

Here, c_Z is the excess concentration of positive charges of inert ions (hereinafter referred to as “ECPC”), which, according to Eq. 21, results from the concentrations of the inert cations (c_i^+) and the inert anions (c_j^-) as well as the respective ionic valences (q_i^+ and q_j^-). The term “inert ions” implies that only ion species with negligible influence on the carbonate buffer system may be involved. In particular, it should be noted that in the experimental method for $k_1a(\text{CO}_2)$ determination presented below, no additional buffer system (such as a phosphate buffer system) is permitted to be present in the medium.

$$c_Z = \sum_i (q_i^+ \cdot c_i^+) - \sum_j (q_j^- \cdot c_j^-) \quad \text{Eq. 21}$$

In a medium, the ECPC is caused by the added salts. The fact that ECPC (formed e.g. by Na^+ ions) is present even in deionized water is due to the equilibrium setting of the lime-carbonic acid equilibrium in the potable water in connection with the respective water treatment technology (e.g. via ion exchanger).

By inserting Eq. 17 and Eq. 19 into Eq. 20, the total CO_2 concentration can now be determined with the help of the charge balance and the ion product of water K_W (see Eq. 22 and Eq. 23).

$$[\text{H}^+] + c_Z = \frac{K_W}{[\text{H}^+]} + (\alpha_2 + 2 \cdot \alpha_3) \cdot [\text{CO}_{2,\text{tot}}] \quad \text{Eq. 22}$$

$$[\text{CO}_{2,\text{tot}}] = \frac{10^{-\text{pH}} + c_Z - \frac{K_W}{10^{-\text{pH}}}}{\alpha_2 + 2 \cdot \alpha_3} \quad \text{Eq. 23}$$

Values for the temperature-dependent ion product of water K_W are tabulated in the literature for specific temperatures. In order to calculate the ion product of water for a certain desired temperature, a temperature-dependent function is needed. To provide the reader with such an appropriate function, the values for the ion product of water given in [D’Ans and Lax 1992] were fitted with a 4th degree polynomial (see Eq. 24). Equation 24 is given as a function of temperature in degrees Celsius due to minor rounding errors.

$$K_W = \left(4.979 \cdot 10^{-7} \cdot \frac{\text{T}^4}{\text{°C}^4} - 1.634 \cdot 10^{-6} \cdot \frac{\text{T}^3}{\text{°C}^3} + 7.894 \cdot 10^{-4} \cdot \frac{\text{T}^2}{\text{°C}^2} + 9.294 \cdot 10^{-3} \cdot \frac{\text{T}}{\text{°C}} + 0.1154 \right) \cdot 10^{-14} \frac{\text{mol}^2}{\text{L}^2}$$

$$\text{Eq. 24}$$

By inserting Eq. 23 into Eq. 15, the concentration of dissolved CO₂ can be calculated (see Eq. 25).

$$[\text{CO}_{2,\text{fl}}] = \alpha_1 \cdot [\text{CO}_{2,\text{tot}}] = \frac{\alpha_1}{\alpha_2 + 2 \cdot \alpha_3} \cdot \left(10^{-\text{pH}} + c_z - \frac{K_W}{10^{-\text{pH}}} \right) \quad \text{Eq. 25}$$

Unfortunately, the ECPC (c_z) is not known, so that the formula cannot be applied directly. However, in the experimental method for determining the $k_{\text{La}}(\text{CO}_2)$ value presented below, this concentration does not change during the course of the experiment, since only CO₂ or air is alternately supplied during the measurement. Therefore, it is possible here to experimentally determine the ECPC at a time (here denoted by the index “eq.”) when the solution has a constant pH value. This is the case when the solution and the gas phase are in equilibrium according to Henry’s law (see Eq. 26).

$$c_z = \left[\frac{K_W}{\text{H}^+} \right]_{\text{eq.}} - [\text{H}^+]_{\text{eq.}} + (\alpha_{2,\text{eq.}} + 2 \cdot \alpha_{3,\text{eq.}}) \cdot [\text{CO}_{2,\text{tot}}]_{\text{eq.}} \quad \text{Eq. 26}$$

Such an equilibrium exists, for example, in the time period in which the solution is gassed with CO₂ at constant pressure and the pH value does not change (anymore). During this period, the lower pH equilibrium value is present in the course of the experiment. The total CO₂ concentration in this equilibrium is obtained from the CO₂ pressure in the reactor headspace during gassing with CO₂ using Eq. 15 in combination with Eq. 2 (see Eq. 27).

$$[\text{CO}_{2,\text{tot}}]_{\text{eq.}} = \frac{[\text{CO}_{2,\text{fl}}]_{\text{eq.}}}{\alpha_{1,\text{eq.}}} \approx \frac{[\text{CO}_{2,\text{d}}]_{\text{eq.}}}{\alpha_{1,\text{eq.}}} = \frac{p(\text{CO}_2)_{\text{eq.}}}{K_{\text{H}}(\text{CO}_2) \cdot \alpha_{1,\text{eq.}}} \quad \text{Eq. 27}$$

The ECPC can now be calculated from the CO₂ partial pressure and the pH value at equilibrium using Eq. 28, which is obtained by inserting Eq. 27 into Eq. 26.

$$c_z = \left[\frac{K_W}{\text{H}^+} \right]_{\text{eq.}} - [\text{H}^+]_{\text{eq.}} + (\alpha_{2,\text{eq.}} + 2 \cdot \alpha_{3,\text{eq.}}) \cdot \frac{p(\text{CO}_2)_{\text{eq.}}}{K_{\text{H}}(\text{CO}_2) \cdot \alpha_{1,\text{eq.}}} \quad \text{Eq. 28}$$

The temperature-dependent Henry constant contained therein can be calculated according to [Royce and Thornhill 1991] with Eq. 29.

$$K_{\text{H}}(\text{CO}_2) = 1000 \cdot e^{\left(11.25 - \frac{395.9 \text{ K}}{T - 175.9 \text{ K}} \right)} \frac{\text{Pa} \cdot \text{L}}{\text{mol}} \stackrel{25^\circ\text{C}}{=} 30.156 \cdot 10^5 \frac{\text{Pa} \cdot \text{L}}{\text{mol}} \quad \text{Eq. 29}$$

Alternatively, the determination of ECPC can also be carried out at the upper pH equilibrium value in the course of the experiment. That is, at the maximum pH value, which occurs when gassing is carried out with air and equilibrium is reached with the partial pressure of CO₂ in air. In this case, the partial pressure of CO₂ can be determined with the help of the CO₂ molar fraction and the pressure in the reactor headspace. If the CO₂ molar fraction in the reactor headspace cannot be measured, it can be estimated using the average molar fraction of 0.04 % CO₂ in air, which corresponds to 40.52 Pa at atmospheric pressure.

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Now that ECPC is known, the concentration of dissolved CO_2 at each time point can be calculated from the measured pH by combining Eq. 25 and Eq. 28 (see Eq. 30 and Eq. 31).

$$[\text{CO}_{2,\text{fl.}}] = \frac{\alpha_1}{\alpha_2 + 2 \cdot \alpha_3} \cdot \left(10^{-\text{pH}} + c_z - \frac{K_W}{10^{-\text{pH}}} \right) \quad \text{Eq. 30}$$

$$[\text{CO}_{2,\text{fl.}}] = \frac{\alpha_1}{\alpha_2 + 2 \cdot \alpha_3} \cdot \left(10^{-\text{pH}} + \left(\frac{K_W}{10^{-\text{pH}_{\text{eq.}}}} - 10^{-\text{pH}_{\text{eq.}}} + (\alpha_{2,\text{eq.}} + 2 \cdot \alpha_{3,\text{eq.}}) \cdot \frac{p(\text{CO}_2)_{\text{eq.}}}{K_H(\text{CO}_2) \cdot \alpha_{1,\text{eq.}}} \right) - \frac{K_W}{10^{-\text{pH}}} \right) \quad \text{Eq. 31}$$

From the time course of the dissolved CO_2 concentration (when CO_2 is stripped from the medium with air), the $k_L a(\text{CO}_2)$ value can be determined using a differential equation according to Eq. 1 (see Eq. 32).

$$-\frac{d[\text{CO}_{2,\text{fl.}}]}{dt} = k_L a(\text{CO}_2) \cdot ([\text{CO}_{2,\text{fl.}}] - [\text{CO}_{2,\text{fl.}}]_{\text{sat}}) \quad \text{Eq. 32}$$

Note II:

If a reaction also takes place in parallel to the mass transport in the laminar boundary layer, then this can change the mass transport. This is due to the changes in the concentration gradient caused by the reaction. In section 2.9.3, an estimation of the effect of the reaction on mass transport is performed. Based on this estimation, it becomes clear that the reaction has only a negligible effect on mass transport in the present case.

The integration of Eq. 32 results in a logarithmic equation (see Eq. 33 and Eq. 34).

$$\int_{[\text{CO}_{2,\text{fl.}}]_0}^{[\text{CO}_{2,\text{fl.}}]} \frac{d[\text{CO}_{2,\text{fl.}}]}{[\text{CO}_{2,\text{fl.}}] - [\text{CO}_{2,\text{fl.}}]_{\text{sat}}} = \int_0^t -k_L a(\text{CO}_2) \cdot dt \quad \text{Eq. 33}$$

$$\ln \left(\frac{[\text{CO}_{2,\text{fl.}}] - [\text{CO}_{2,\text{fl.}}]_{\text{sat}}}{[\text{CO}_{2,\text{fl.}}]_0 - [\text{CO}_{2,\text{fl.}}]_{\text{sat}}} \right) = -k_L a(\text{CO}_2) \cdot (t - t_0) \quad \text{Eq. 34}$$

Plotting the left hand side of Eq. 34 against time gives the $k_L a(\text{CO}_2)$ value as the negative value of the slope of a linear regression.

Analogous to the removal of N_2 from the headspace of the bioreactor in the context of $k_L a(\text{O}_2)$ determination as described in [DECHEMA 2020], the CO_2 must be removed from the headspace before gassing with air can be started. If the CO_2 is not removed completely, deviations from the exponential equations can occur due to modified gas composition in the headspace. As a result, the driving force and thus the saturation concentration is modified, resulting in a lower $k_L a(\text{CO}_2)$ value. Hence, it is necessary to flush the headspace with air (agitation switched off) prior to the actual measurement until exhaust gas analysis ensures 21 % of oxygen and thus atmospheric conditions in the headspace. If exhaust gas analysis is not available, then the headspace volume should be exchanged at least three times. The associated extension in time for the measurement must be considered when planning experiments.

References

- Contreras, E. M., Carbon dioxide stripping in bubbled columns, *Ind. Eng. Chem. Res.* 2007, **46**, 6332–6337. DOI: 10.1021/ieo61350i
- D'Ans, J., Lax, E., Taschenbuch für Chemiker und Physiker, Volume 1, 4th re-edited and revised edition, Springer-Verlag, Berlin, 1992
- DECHEMA, Statuspapier des temporären Arbeitskreises „Single-Use-Technologie in der biopharmazeutischen Produktion“, 2011
- DECHEMA, Recommendations for process engineering characterisation of single-use bioreactors and mixing systems by using experimental methods, 2016
- DECHEMA, Recommendations for process engineering characterisation of single-use bioreactors and mixing systems by using experimental methods, 2nd completely revised edition, 2020
- Eibl, R., Eibl, D. (Eds.): *Single-Use Technology in Biopharmaceutical Manufacture*, Second Edition, John Wiley & Sons, Hoboken, 2019
- Lehmann et al., Disposable Bioreactors for the Cultivation of Plant Cell Cultures, in: *Production of Biomass and Bioactive Compounds using Bioreactor Technology*, Springer Berlin, 2014, 17-46
- Minkevich, I.G., Neubert, M., Influence of carbon dioxide solubility on the accuracy of measurements of carbon dioxide production rate by gas balance technique, *Acta Biotechnol.* 1985, **5**(2), 137-143
- Musvoto, E., Wentzel, M., Loewenthal, R., Ekama, G., Integrated chemical - physical processes modelling I. Development of a kinetic-based model for mixed weak acid/base systems, *Wat. Res.* 2000, **34**, 1857-1867
- Royce, P.N., Thornhill, N.F., Estimation of dissolved carbon dioxide concentrations in aerobic fermentations, *AIChE J.* 1991, **37**(11), 1680-1686, DOI: 10.1002/aic.690371111
- Sieblist, C. et al., Insights into large-scale cell-culture reactors: II. Gas-phase mixing and CO₂ stripping, *Biotechnol. J.* 2011, **6**, 1547–1556

Abbreviations

ECPC	Excess concentration of positive charges of inert ions
SOP	Standard operating procedure
SUB	Single-use bioreactors
SUM	Single-use mixing systems
SUT	Single-use technology

1 BACKGROUND

Symbols used

a	Interfacial surface area per liquid volume [m^{-2}]	$[\text{CO}_3^{2-}]$	Concentration of carbonate [$\text{mol}\cdot\text{L}^{-1}$]
α_1	Mole fraction of dissolved CO_2 [-]	CTR	Carbon dioxide transfer rate [$\text{mol}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$]
$\alpha_{1,\text{eq.}}$	Mole fraction of dissolved CO_2 at equilibrium for ECPC determination [-]	c_z	Excess concentration of positive charges of inert ions (ECPC) [$\text{mol}\cdot\text{L}^{-1}$]
α_2	Mole fraction of hydrogen carbonate [-]	δ	Thickness of boundary layer [m]
$\alpha_{2,\text{eq.}}$	Mole fraction of hydrogen carbonate at equilibrium for ECPC determination [-]	$[\text{H}^+]$	Concentration of hydrogen ions [$\text{mol}\cdot\text{L}^{-1}$]
α_3	Mole fraction of carbonate [-]	$[\text{H}^+]_{\text{eq.}}$	Concentration of hydrogen ions at equilibrium for ECPC determination [$\text{mol}\cdot\text{L}^{-1}$]
$\alpha_{3,\text{eq.}}$	Mole fraction of carbonate at equilibrium for ECPC determination [-]	$[\text{HCO}_3^-]$	Concentration of hydrogen carbonate [$\text{mol}\cdot\text{L}^{-1}$]
$\beta(\text{air})$	Air supply expressed in volume air per volume of liquid and per minute [min^{-1}]	$[\text{H}_2\text{CO}_3]$	Concentration of carbonic acid [$\text{mol}\cdot\text{L}^{-1}$]
$\beta(\text{CO}_2)$	CO_2 supply expressed in volume CO_2 per volume of liquid and per minute [min^{-1}]	K_1	Dissociation constant of H_2CO_3 [$\text{mol}\cdot\text{L}^{-1}$]
c_i^+	Concentrations of inert cations of species i [$\text{mol}\cdot\text{L}^{-1}$]	k_1	Reaction rate constant of a 1 st order reaction [s^{-1}]
c_j^-	Concentration of inert anions of species j [$\text{mol}\cdot\text{L}^{-1}$]	K_2	Dissociation constant of HCO_3^- [$\text{mol}\cdot\text{L}^{-1}$]
$[\text{CO}_{2,\text{d}}]$	Concentration of dissolved CO_2 (excl. carbonic acid) [$\text{mol}\cdot\text{L}^{-1}$]	k_2	Reaction rate constant of a 1 st order reaction [s^{-1}]
$[\text{CO}_{2,\text{d}}]_{\text{eq.}}$	Concentration of dissolved CO_2 (excl. carbonic acid) at equilibrium for ECPC determination [$\text{mol}\cdot\text{L}^{-1}$]	$K_{\text{H}}(\text{CO}_2)$	Henry constant of CO_2 [$\text{Pa}\cdot\text{L}\cdot\text{mol}^{-1}$]
$[\text{CO}_{2,\text{d}}]_{\text{sat}}$	Saturation concentration of dissolved CO_2 (excl. carbonic acid) [$\text{mol}\cdot\text{L}^{-1}$]	$k_{\text{L}}a(\text{CO}_2)$	Volumetric mass transfer coefficient of CO_2 [h^{-1}]
$[\text{CO}_{2,\text{fl.}}$	Concentrations of dissolved CO_2 (incl. carbonic acid) [$\text{mol}\cdot\text{L}^{-1}$]	K_{w}	Ion product of water [$\text{mol}^2\cdot\text{L}^{-2}$]
$[\text{CO}_{2,\text{fl.}}]_0$	Concentration of dissolved CO_2 (incl. carbonic acid) at the beginning of the experiment [$\text{mol}\cdot\text{L}^{-1}$]	$[\text{OH}^-]$	Concentration of hydroxide ions [$\text{mol}\cdot\text{L}^{-1}$]
$[\text{CO}_{2,\text{fl.}}]_{\text{eq.}}$	Concentration of dissolved CO_2 (incl. carbonic acid) at equilibrium for ECPC determination [$\text{mol}\cdot\text{L}^{-1}$]	$p(\text{CO}_2)$	Partial pressure of CO_2 [Pa]
$[\text{CO}_{2,\text{fl.}}]_{\text{sat}}$	Saturation concentration of dissolved CO_2 (incl. carbonic acid) [$\text{mol}\cdot\text{L}^{-1}$]	$p(\text{CO}_2)_{\text{eq.}}$	Partial pressure of CO_2 at equilibrium for ECPC determination [Pa]
$[\text{CO}_{2,\text{tot}}]$	Total concentration of CO_2 [$\text{mol}\cdot\text{L}^{-1}$]	q_i^+	Ionic valences of inert cations of species i [-]
$[\text{CO}_{2,\text{tot}}]_{\text{eq.}}$	Total concentration of CO_2 at equilibrium for ECPC determination [$\text{mol}\cdot\text{L}^{-1}$]	q_j^-	Ionic valences of inert anions of species j [-]
		T	Temperature [K or °C]
		t	Time [h]
		t_0	Time at the beginning of the experiment [h]
		u_{tip}	Stirrer tip speed [$\text{m}\cdot\text{s}^{-1}$]

2 Guideline – Experimental determination of the volumetric mass transfer coefficient of CO₂

The volumetric mass transfer coefficient of carbon dioxide ($k_L a(\text{CO}_2)$) is a key parameter in bioprocess engineering and specifies the transfer of carbon dioxide between gaseous and liquid phases. It is comprised of the diffusion coefficients of CO₂ in air and water, the thicknesses of the two laminar boundary layers, and the interfacial area per volume of liquid. The experimental determination of the $k_L a(\text{CO}_2)$ values recommended by the DECHEMA expert group is based on the dynamic CO₂ saturation and stripping from the liquid media. Preferably, the measurement is performed during the strip-out process, i.e., when the pH value increases. First, carbon dioxide is sparged into the media which results in a drop of the pH value. After switching off the CO₂ gassing a headspace exchange should be performed to reduce the residual CO₂ concentration in the gassing phase. This results in a more precise measurement of the carbon dioxide transfer. Subsequently, the carbon dioxide is removed from the liquid media by aeration and the pH value increases.

The $k_L a(\text{CO}_2)$ value can be obtained from the rate of the pH increase. This is possible because the concentration of dissolved CO₂ can be calculated from a pH measurement if the excess concentration of positive charges of inert ions (ECPC) is known and remains constant during the experiment. The advantage of this method is that only a pH sensor is required for the measurement. pH sensors are ideally suited for the dynamic determination of $k_L a(\text{CO}_2)$, since the measurement signals react promptly to a change in pH value in the medium and are available on-line. For the data evaluation, the ECPC is required and must therefore be determined individually for each experiment.

During the stripping process, the pH values must be recorded continuously. Computer-aided data acquisition with a sampling rate between 5 and 10 s is required to obtain accurate results. The data is then evaluated according to Eq. 34. It should be noted that during the stripping process the current CO₂ concentration in the liquid ($[\text{CO}_{2,\text{fl}}]$) is always greater than (or equal to) the saturation concentration at the interface in equilibrium with the gaseous phase ($[\text{CO}_{2,\text{fl}}]_{\text{sat}}$). Therefore, the time derivative of the concentration, i.e., the change in concentration, is negative (concentration decrease).

The determination of $k_L a(\text{CO}_2)$ is carried out in the bioreactor with the respective desired standard configuration with regard to gassing system, stirrer, electrodes, other internals, and the liquid level. Only a pH sensor is required for the measurement. The use of a sodium chloride solution (8.8 g·L⁻¹ NaCl in deionized water) and a temperature of 25 °C are suggested for comparative studies. In particular, no additional buffer system (such as a phosphate buffer system) is permitted to be present in the medium.

2 GUIDELINE – EXPERIMENTAL DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT OF CO₂

2.1 Materials

- » Bioreactor system
- » Control unit
- » Computer-aided data acquisition
- » Carbon dioxide supply
- » Air supply
- » 2 Beakers or graduated flasks
- » 2 pH buffers (pH = 4 and pH = 7) if necessary
- » Sodium chloride solution (8.8 g·L⁻¹ NaCl in deionized water) for comparative studies or medium (without a second buffer system)
- » pH sensor

Note III:

Since this method is based on a dynamic measurement of pH value, the time behaviour of the pH sensor must be considered. When using unsuitable sensors (very long response time), considerable errors may occur with the determination of the $k_L a(\text{CO}_2)$ values. A detailed discussion of the response time of sensors ($t_{63\%,\text{crit}}$) can be found in [DECHEMA 2020]. There, the experimental determination of the response time is also shown on an example with a dissolved oxygen sensor. Here, a maximum response time of the pH sensor have to be significant lower than the used sampling time.

2.2 Calibration of the pH sensor (two-point calibration)

The two-point calibration for multi-use pH sensors is performed with two pH buffers. When using pre-installed single-use sensors, initialisation and recalibration may be required after a defined wetting time and after reaching the process temperature. The initialisation parameters should be entered manually. Since the calibration procedure depends on the control unit, the control unit user guide should be consulted. In most cases, a predefined automated calibration procedure is provided.

1. Set up the bioreactor, the control unit and the data acquisition software.
2. In the case of multi-use pH sensor go to the calibration menu of the control unit and start the calibration of the pH sensor with the pH = 7 buffer at the desired process temperature (25 °C ±0.5 °C is suggested for comparative studies).
3. Proceed for multi-use pH sensor with the pH = 4 buffer at the desired process temperature till calibration is completed (25 °C ±0.5 °C is suggested for comparative studies).

2.3 Experimental setup

4. In the case of multi-use pH sensor mount the pH sensor in the bioreactor.
5. Fill up the bioreactor system to the desired working volume with sodium chloride solution.
6. Set the desired agitation parameters.
7. Adjust the sodium chloride solution to the desired process temperature (25 °C ±0.5 °C is suggested for comparative studies).

2.4 Determination of the excess concentration of positive charges of inert ions

It is recommended to perform the determination of the ECPC at the lower pH equilibrium value. Slight variations in gas composition or headspace pressure cause only a small error here. Alternatively, the ECPC can also be determined at the upper equilibrium value. In this case, the required values for the calculation of c_z are generated in section 2.5 and you can proceed with step 11.

8. Introduce CO₂ in the bioreactor until a constant pH value is measured.
9. Document the constant pH value (lower pH equilibrium value).
10. Document the corresponding partial pressure $p(\text{CO}_2)$ in the headspace of the bioreactor.

2.5 Determination of dissolved CO₂ saturation concentration

The dissolved CO₂ saturation concentration is needed to determine the $k_a(\text{CO}_2)$ value according to Eq. 34.

11. Set the aeration parameters to be tested (with the partial pressure of CO₂ in the gas supply).
12. Introduce air in the bioreactor until a constant pH value is measured.
13. Document the constant pH value (upper pH equilibrium value).
14. Document the corresponding molar fraction of CO₂ and the pressure in the headspace of the bioreactor.
15. Calculate c_z either with the data generated in steps 8 – 10 or with the data generated in steps 12 – 14 using Eq. 35.

$$c_z = \frac{K_W}{[\text{H}^+]_{\text{eq}}} - [\text{H}^+]_{\text{eq}} + (\alpha_{2,\text{eq}} + 2 \cdot \alpha_{3,\text{eq}}) \cdot \frac{p(\text{CO}_2)_{\text{eq}}}{K_H(\text{CO}_2) \cdot \alpha_{1,\text{eq}}} \quad \text{Eq. 35}$$

16. Calculate $[\text{CO}_{2,\text{fl}}]_{\text{sat}}$ with the data generated in steps 12 - 14 using Eq. 36.

$$[\text{CO}_{2,\text{fl}}]_{\text{sat}} = \frac{\alpha_{1,\text{sat}}}{\alpha_{2,\text{sat}} + 2 \cdot \alpha_{3,\text{sat}}} \cdot \left(10^{-\text{pH}_{\text{sat}}} + c_z - \frac{K_W}{10^{-\text{pH}_{\text{sat}}}} \right) \quad \text{Eq. 36}$$

2 GUIDELINE – EXPERIMENTAL DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT OF CO₂

2.6 Measurement procedure

The determination of $k_L a(\text{CO}_2)$ values is based on the alternate elimination and subsequent accumulation of dissolved carbon dioxide in a sodium chloride solution in the bioreactor system. CO₂ or air are introduced alternately into the solution via a gassing device (sparger, dip tube, open tube, etc.) or the liquid surface. The $k_L a(\text{CO}_2)$ determination is performed as a function of the gassing rate and the stirrer speed. During the experiments, the measurement data should be recorded at an interval of 5 - 10 s.

17. Start data acquisition for pH measurement (if not already active).
18. Stop the air supply (if still active from previous steps).
19. Introduce CO₂ in the bioreactor until a pH value below pH = 4.5 is measured.
20. Stop the CO₂ supply.
21. Stop agitation and wait until the liquid is no longer in motion; start the air supply to flush the headspace until exhaust gas analysis ensures 21 % of oxygen or until the headspace volume is exchanged at least three times.

Details about flushing the headspace and its time requirements can be found in [DECHEMA 2020].

22. Set the aeration and the agitation parameters to be tested and start agitation again. For active gassing systems overlay aeration should be set at 0.05 vvm.
23. Start aeration.
24. Strip-out the CO₂ from the liquid with air until a pH value above pH = 5.5 is measured.
25. To determine the $k_L a(\text{CO}_2)$ value, the investigations should be performed at least three times, but preferably up to five times, by repeating steps 18 to 24.

2.7 Evaluation

26. For evaluation purposes, pH values between pH = 4.5 and pH = 5.5 are used.
27. Calculate the dissolved CO₂ concentrations at each time point using Eq. 37.

$$[\text{CO}_{2,\text{fl.}}] = \frac{\alpha_1}{\alpha_2 + 2 \cdot \alpha_3} \cdot \left(10^{-\text{pH}} + c_z - \frac{K_W}{10^{-\text{pH}}} \right) \quad \text{Eq. 37}$$

28. Determine $[\text{CO}_{2,\text{fl.}}]_0$ at the beginning of each strip-out process from the calculated dissolved CO₂ concentrations.
29. Calculate the logarithmic expression of the left-hand side of Eq. 38 for each strip-out process.

$$\ln \left(\frac{[\text{CO}_{2,\text{fl.}}] - [\text{CO}_{2,\text{fl.}}]_{\text{sat}}}{[\text{CO}_{2,\text{fl.}}]_0 - [\text{CO}_{2,\text{fl.}}]_{\text{sat}}} \right) = -k_L a(\text{CO}_2) \cdot (t - t_0) \quad \text{Eq. 38}$$

2 GUIDELINE – EXPERIMENTAL DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT OF CO₂

30. Plot the left-hand side of Eq. 38 as a function of time for an evaluation range between pH = 4.5 and pH = 5.5 with at least seven values (see Fig. 3).

Non-linear slopes may be caused by secondary effects superimposed to the first-order kinetics of the carbon dioxide mass transfer. This may include, among others, mixing inefficiencies at low agitation and/or in large bioreactors.

31. Insert a linear trend line with the corresponding trend line equation.
 32. The negative value of the slope of the trend line represents the $k_L a(\text{CO}_2)$ value (see Fig. 3).

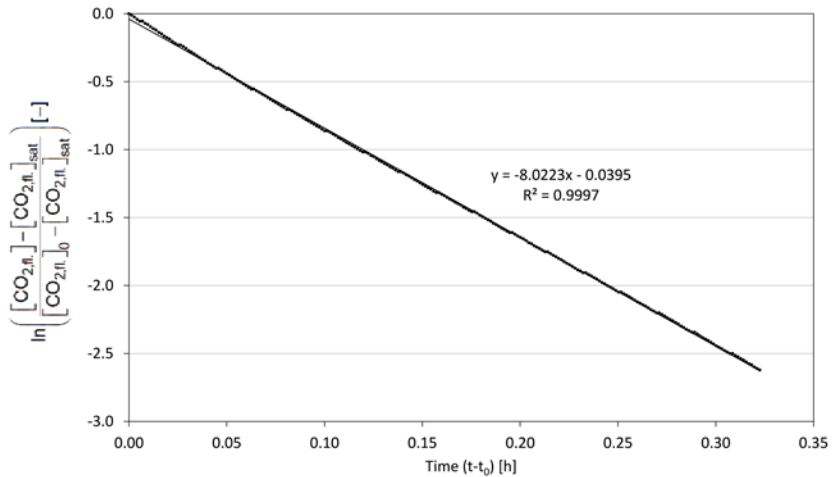


Figure 3: Determining the $k_L a(\text{CO}_2)$ value by plotting the left hand side of Eq. 38 as a function of time; the negative value of the slope of the trend line represents the $k_L a(\text{CO}_2)$ value.

If the $k_L a(\text{CO}_2)$ measurements for defined agitation rate and aeration rate have been performed at least 5 times, the standard deviation should be below the range of 10 %.

33. The $k_L a(\text{CO}_2)$ values ($k_L a(\text{CO}_2)_1, k_L a(\text{CO}_2)_2, \dots, k_L a(\text{CO}_2)_j$ with $j = \text{count}$) should be averaged by the arithmetic mean (see Eq. 39).

$$k_L a(\text{CO}_2) = \frac{1}{j} \cdot \sum_{i=1}^j k_L a(\text{CO}_2)_i = \frac{k_L a(\text{CO}_2)_1 + k_L a(\text{CO}_2)_2 + \dots + k_L a(\text{CO}_2)_j}{j} \quad \text{Eq. 39}$$

2 GUIDELINE – EXPERIMENTAL DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT OF CO₂

Note IV:

The experimental technique is based on the assumption that the mass transfer rate of carbon dioxide can be described with a first-order kinetics following Eq. 1. Thus, it is assumed that:

- » The bioreactor content (liquid phase and gas phase) is well mixed and the temperature inside the vessel is homogenous.
- » The locally measured pH value is representative for the complete vessel (i.e., no dead zones are present and the pressure gradients are also negligible).
- » The response time of the pH sensor is negligible compared to the carbon dioxide mass transfer rate (see [DECHEMA 2020] for analogue details of oxygen measurement).

2.8 Calculator sheet for $k_L a(\text{CO}_2)$

For an easier and faster calculation of the $k_L a(\text{CO}_2)$ values according to the method described in this publication the authors developed a calculation tool. The Excel-based tool is available for download at www.dechema.de/studien.

2.9 Appendix

2.9.1 Preparation of sodium chloride solution

- » Weigh out 8.8 g NaCl in a weighing dish and transfer it to a 1 L measuring beaker.
- » Add approx. 700 mL deionized water (room temperature).
- » Add a magnetic stirrer bar and dissolve the solution by stirring.
- » Remove the magnetic stirrer bar and transfer the solution in an 1 L measuring flask.
- » Rinse out the beaker with approx. 100 mL deionized water and transfer this liquid also to the 1 L measuring flask.
- » Fill-up the measuring flask to the 1 L mark with deionized water (room temperature).
- » For sterile applications, the sodium chloride solution should be autoclaved (121 °C, 20 min).

The sodium chloride solution can be stored at room temperature. Minimum shelf-life: 1 year.

2.9.2 Example

Determination of $k_L a(\text{CO}_2)$ value for a 2 L stirred bioreactor system. The bioreactor was equipped with a ring sparger, a multi-use pH electrode, and a two-stage segment blade stirrer. The following parameters were used in the subsequent steps:

- $u_{\text{Tip}} = 1.2 \text{ m}\cdot\text{s}^{-1}$,
- $T = 25 \text{ }^\circ\text{C}$,
- $\beta(\text{air}) = 0.1 \text{ vvm}$ (sparger aeration).

- » The reactor was prepared corresponding to steps 4 - 7.
- » The ECPC was determined corresponding to steps 8 - 10 (see Tab. 1). Table 1 additionally contains the ECPC calculation at the upper pH equilibrium value to give an impression of the error that arises from this calculation.

Table 1: Experimental and calculated data for the ECPC determination (*The deviation in ECPC of about 10 % at the upper pH equilibrium value is due to the error in the CO₂ partial pressure in air).

Parameter		Value at lower pH equilibrium	Value at upper pH equilibrium*
pH _{eq.}	[-]	4.15	7.31
K _W	[mol ² ·L ⁻²]	1.00·10 ⁻¹⁴	
α _{1,eq.}	[-]	0.99399	0.10257
α _{2,eq.}	[-]	6.010·10 ⁻³	0.89657
α _{3,eq.}	[-]	3.951·10 ⁻⁹	8.519·10 ⁻⁴
p(CO ₂) _{eq.}	[Pa]	1.013·10 ⁵	40.53
K _H (CO ₂)	[Pa·L·mol ⁻¹]	30.156·10 ⁵	
c _Z	[mol·L ⁻¹]	1.3318·10 ⁻⁴	1.1786·10 ⁻⁴

- » The dissolved CO₂ saturation concentration was determined corresponding to steps 11 - 16; the determined value for [CO_{2,fl.}]_{sat} is given in Table 2.
- » The measurement process for determining the $k_L a(\text{CO}_2)$ value corresponding to steps 17 - 25 was performed in triplicate (see Fig. 4).
- » Dissolved CO₂ concentrations were calculated according to steps 26 - 27 (see Tab. 3).
- » The dissolved CO₂ start concentration ([CO_{2,fl.}]₀) was determined corresponding to step 28 from the calculated data (see Tab. 2).

2 GUIDELINE – EXPERIMENTAL DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT OF CO₂

Table 2: Experimental and calculated data at the beginning of the strip-out process and at equilibrium with air.

Parameter		Value
pH ₀	[-]	4.50
[CO _{2,fl.}] ₀	[mol·L ⁻¹]	0.012
pH _{sat}	[-]	7.31
[CO _{2,fl.}] _{sat}	[mol·L ⁻¹]	1.315·10 ⁻⁴

- » Based on the experimental raw data, it is possible to determine the $k_L a(\text{CO}_2)$ value corresponding to steps 29 - 32 (see Tab. 3 and Fig. 5).
- » The determined $k_L a(\text{CO}_2)$ values were averaged corresponding to step 33 (see Eq. 40).

$$k_L a(\text{CO}_2) = \frac{7.9829 \frac{1}{\text{h}} + 8.0223 \frac{1}{\text{h}} + 8.0749 \frac{1}{\text{h}}}{3} = 8.03 \frac{1}{\text{h}} \quad \text{Eq. 40}$$

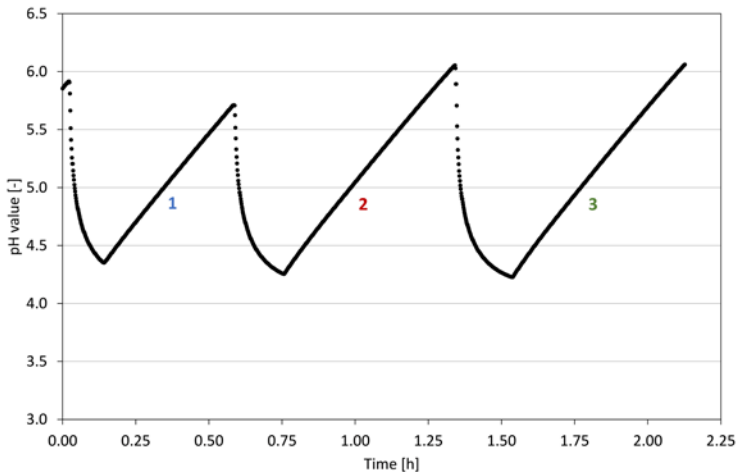


Figure 4: Time course of the measured pH values during a triplicate determination (marked with blue 1, red 2 and green 3).

2 GUIDELINE – EXPERIMENTAL DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT OF CO₂

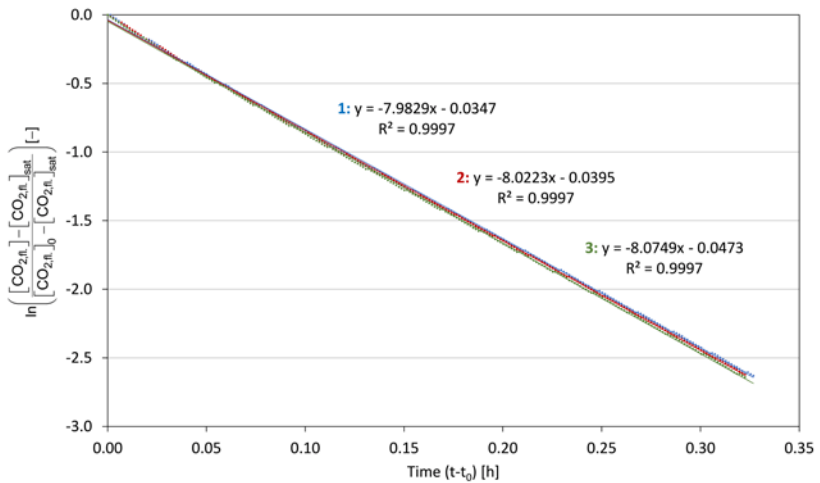


Figure 5: The $k_L a(\text{CO}_2)$ value is determined by plotting the left hand side of Eq. 38 as a function of time; the negative values of the slopes of the trend lines represent the $k_L a(\text{CO}_2)$ values for the triplicate determination of Figure 4 (marked with blue 1, red 2 and green 3).

Table 3: Experimental raw data and calculated data for the $k_L a(\text{CO}_2)$ determination.

Time (t-t ₀) [h]	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38
0.000	4.502	0.012	0.000	4.500	0.012	0.000	4.501	0.012	0.000
0.003	4.508	0.012	-0.017	4.509	0.012	-0.025	4.511	0.012	-0.028
0.006	4.518	0.012	-0.044	4.520	0.012	-0.055	4.521	0.011	-0.055
0.008	4.526	0.011	-0.066	4.526	0.011	-0.072	4.532	0.011	-0.086
0.011	4.537	0.011	-0.097	4.535	0.011	-0.097	4.542	0.011	-0.113
0.014	4.547	0.011	-0.124	4.544	0.011	-0.121	4.551	0.011	-0.138
0.017	4.556	0.010	-0.149	4.554	0.011	-0.149	4.561	0.010	-0.165
0.019	4.567	0.010	-0.179	4.564	0.010	-0.176	4.566	0.010	-0.179
0.022	4.571	0.010	-0.190	4.574	0.010	-0.203	4.576	0.010	-0.206
0.025	4.581	0.010	-0.217	4.584	0.010	-0.231	4.586	0.010	-0.233
0.028	4.591	0.010	-0.244	4.588	0.010	-0.241	4.595	0.009	-0.258
0.031	4.599	0.009	-0.266	4.599	0.009	-0.271	4.606	0.009	-0.287
0.033	4.610	0.009	-0.295	4.608	0.009	-0.296	4.616	0.009	-0.314

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Time (t-t ₀) [h]	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38
0.036	4.620	0.009	-0.322	4.618	0.009	-0.323	4.621	0.009	-0.328
0.039	4.629	0.009	-0.347	4.627	0.009	-0.347	4.630	0.009	-0.352
0.042	4.633	0.009	-0.357	4.637	0.008	-0.374	4.640	0.008	-0.379
0.044	4.644	0.008	-0.387	4.647	0.008	-0.400	4.650	0.008	-0.406
0.047	4.653	0.008	-0.411	4.652	0.008	-0.414	4.659	0.008	-0.430
0.050	4.661	0.008	-0.432	4.662	0.008	-0.441	4.669	0.008	-0.456
0.053	4.671	0.008	-0.459	4.671	0.008	-0.465	4.678	0.008	-0.480
0.056	4.681	0.008	-0.486	4.681	0.008	-0.491	4.683	0.007	-0.494
0.058	4.690	0.007	-0.510	4.690	0.007	-0.515	4.692	0.007	-0.518
0.061	4.694	0.007	-0.520	4.700	0.007	-0.542	4.701	0.007	-0.542
0.064	4.705	0.007	-0.549	4.705	0.007	-0.555	4.712	0.007	-0.571
0.067	4.714	0.007	-0.573	4.714	0.007	-0.579	4.720	0.007	-0.592
0.070	4.722	0.007	-0.594	4.723	0.007	-0.603	4.730	0.007	-0.618
0.072	4.732	0.007	-0.621	4.733	0.007	-0.629	4.740	0.006	-0.645
0.075	4.742	0.006	-0.647	4.742	0.006	-0.653	4.745	0.006	-0.658
0.078	4.746	0.006	-0.658	4.752	0.006	-0.679	4.754	0.006	-0.681
0.081	4.756	0.006	-0.684	4.762	0.006	-0.705	4.764	0.006	-0.708
0.083	4.766	0.006	-0.710	4.766	0.006	-0.716	4.773	0.006	-0.731
0.086	4.775	0.006	-0.734	4.776	0.006	-0.742	4.783	0.006	-0.757
0.089	4.785	0.006	-0.760	4.785	0.006	-0.765	4.792	0.006	-0.781
0.092	4.794	0.006	-0.783	4.794	0.006	-0.789	4.802	0.005	-0.807
0.095	4.802	0.005	-0.804	4.803	0.005	-0.812	4.807	0.005	-0.820
0.097	4.807	0.005	-0.817	4.812	0.005	-0.836	4.815	0.005	-0.841
0.100	4.817	0.005	-0.843	4.822	0.005	-0.862	4.825	0.005	-0.867
0.103	4.826	0.005	-0.867	4.826	0.005	-0.872	4.835	0.005	-0.893
0.106	4.835	0.005	-0.890	4.835	0.005	-0.896	4.844	0.005	-0.916
0.108	4.845	0.005	-0.916	4.845	0.005	-0.922	4.853	0.005	-0.940
0.111	4.854	0.005	-0.940	4.855	0.005	-0.948	4.862	0.005	-0.963
0.114	4.864	0.005	-0.965	4.864	0.005	-0.971	4.868	0.005	-0.979
0.117	4.867	0.005	-0.973	4.873	0.005	-0.994	4.877	0.005	-1.002

2 GUIDELINE – EXPERIMENTAL DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT OF CO₂

Time (t-t ₀) [h]	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38
0.120	4.877	0.005	-0.999	4.882	0.004	-1.018	4.886	0.004	-1.025
0.122	4.886	0.004	-1.022	4.886	0.004	-1.028	4.895	0.004	-1.048
0.125	4.896	0.004	-1.048	4.895	0.004	-1.051	4.905	0.004	-1.074
0.128	4.905	0.004	-1.071	4.906	0.004	-1.080	4.914	0.004	-1.097
0.131	4.914	0.004	-1.095	4.915	0.004	-1.103	4.924	0.004	-1.123
0.133	4.923	0.004	-1.118	4.924	0.004	-1.126	4.929	0.004	-1.136
0.136	4.928	0.004	-1.131	4.933	0.004	-1.149	4.938	0.004	-1.159
0.139	4.937	0.004	-1.154	4.938	0.004	-1.162	4.947	0.004	-1.182
0.142	4.946	0.004	-1.177	4.947	0.004	-1.185	4.956	0.004	-1.205
0.145	4.956	0.004	-1.203	4.956	0.004	-1.208	4.965	0.004	-1.229
0.147	4.965	0.004	-1.226	4.965	0.004	-1.231	4.975	0.004	-1.254
0.150	4.973	0.004	-1.246	4.975	0.004	-1.257	4.984	0.003	-1.277
0.153	4.984	0.003	-1.275	4.984	0.003	-1.280	4.989	0.003	-1.290
0.156	4.988	0.003	-1.285	4.994	0.003	-1.306	4.998	0.003	-1.313
0.159	4.996	0.003	-1.305	4.998	0.003	-1.316	5.007	0.003	-1.336
0.161	5.006	0.003	-1.331	5.007	0.003	-1.339	5.017	0.003	-1.362
0.164	5.016	0.003	-1.357	5.017	0.003	-1.365	5.026	0.003	-1.385
0.167	5.025	0.003	-1.380	5.026	0.003	-1.388	5.035	0.003	-1.408
0.170	5.034	0.003	-1.403	5.034	0.003	-1.408	5.039	0.003	-1.418
0.172	5.038	0.003	-1.413	5.044	0.003	-1.434	5.049	0.003	-1.444
0.175	5.048	0.003	-1.439	5.053	0.003	-1.457	5.058	0.003	-1.467
0.178	5.057	0.003	-1.462	5.057	0.003	-1.467	5.067	0.003	-1.490
0.181	5.067	0.003	-1.487	5.067	0.003	-1.493	5.077	0.003	-1.516
0.184	5.075	0.003	-1.508	5.076	0.003	-1.516	5.085	0.003	-1.536
0.186	5.085	0.003	-1.533	5.086	0.003	-1.542	5.095	0.003	-1.562
0.189	5.094	0.003	-1.556	5.095	0.003	-1.565	5.100	0.003	-1.575
0.192	5.098	0.003	-1.567	5.104	0.003	-1.588	5.108	0.003	-1.595
0.195	5.107	0.003	-1.590	5.113	0.003	-1.611	5.118	0.003	-1.621
0.197	5.117	0.003	-1.615	5.118	0.003	-1.623	5.128	0.002	-1.646
0.200	5.125	0.002	-1.636	5.127	0.002	-1.647	5.137	0.002	-1.669

2 GUIDELINE – EXPERIMENTAL DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT OF CO₂

Time (t-t ₀) [h]	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38
0.203	5.134	0.002	-1.659	5.136	0.002	-1.670	5.146	0.002	-1.692
0.206	5.143	0.002	-1.682	5.145	0.002	-1.693	5.155	0.002	-1.716
0.209	5.153	0.002	-1.708	5.155	0.002	-1.718	5.160	0.002	-1.728
0.211	5.157	0.002	-1.718	5.164	0.002	-1.741	5.170	0.002	-1.754
0.214	5.167	0.002	-1.744	5.168	0.002	-1.752	5.178	0.002	-1.775
0.217	5.176	0.002	-1.767	5.178	0.002	-1.777	5.188	0.002	-1.800
0.220	5.185	0.002	-1.790	5.187	0.002	-1.800	5.197	0.002	-1.823
0.222	5.194	0.002	-1.813	5.196	0.002	-1.824	5.207	0.002	-1.849
0.225	5.203	0.002	-1.836	5.205	0.002	-1.847	5.216	0.002	-1.872
0.228	5.212	0.002	-1.859	5.215	0.002	-1.872	5.220	0.002	-1.883
0.231	5.217	0.002	-1.872	5.223	0.002	-1.893	5.229	0.002	-1.906
0.234	5.226	0.002	-1.895	5.228	0.002	-1.906	5.240	0.002	-1.934
0.236	5.235	0.002	-1.918	5.237	0.002	-1.929	5.248	0.002	-1.955
0.239	5.245	0.002	-1.944	5.246	0.002	-1.952	5.257	0.002	-1.978
0.242	5.254	0.002	-1.967	5.256	0.002	-1.978	5.266	0.002	-2.001
0.245	5.262	0.002	-1.988	5.265	0.002	-2.001	5.275	0.002	-2.025
0.247	5.271	0.002	-2.011	5.273	0.002	-2.022	5.279	0.002	-2.035
0.250	5.276	0.002	-2.024	5.283	0.002	-2.048	5.289	0.002	-2.061
0.253	5.285	0.002	-2.048	5.288	0.002	-2.061	5.298	0.002	-2.084
0.256	5.294	0.002	-2.071	5.296	0.002	-2.082	5.307	0.002	-2.108
0.259	5.303	0.002	-2.094	5.306	0.002	-2.108	5.317	0.002	-2.134
0.261	5.313	0.002	-2.120	5.315	0.002	-2.131	5.325	0.002	-2.154
0.264	5.321	0.002	-2.141	5.323	0.002	-2.152	5.330	0.002	-2.167
0.267	5.331	0.002	-2.167	5.333	0.001	-2.178	5.340	0.001	-2.194
0.270	5.335	0.001	-2.178	5.342	0.001	-2.202	5.349	0.001	-2.217
0.272	5.344	0.001	-2.201	5.346	0.001	-2.212	5.357	0.001	-2.238
0.275	5.353	0.001	-2.225	5.356	0.001	-2.238	5.367	0.001	-2.264
0.278	5.362	0.001	-2.248	5.364	0.001	-2.259	5.376	0.001	-2.288
0.281	5.371	0.001	-2.272	5.373	0.001	-2.283	5.385	0.001	-2.312
0.284	5.380	0.001	-2.296	5.383	0.001	-2.309	5.390	0.001	-2.325

Time (t-t ₀) [h]	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38	pH [-]	[CO _{2,fl.}] [mol·L ⁻¹]	Left hand side of Eq. 38
0.286	5.385	0.001	-2.309	5.392	0.001	-2.333	5.398	0.001	-2.346
0.289	5.393	0.001	-2.330	5.396	0.001	-2.343	5.408	0.001	-2.372
0.292	5.403	0.001	-2.356	5.406	0.001	-2.370	5.418	0.001	-2.399
0.295	5.412	0.001	-2.380	5.415	0.001	-2.394	5.426	0.001	-2.420
0.298	5.421	0.001	-2.404	5.424	0.001	-2.417	5.435	0.001	-2.444
0.300	5.429	0.001	-2.425	5.434	0.001	-2.444	5.445	0.001	-2.471
0.303	5.439	0.001	-2.452	5.442	0.001	-2.465	5.448	0.001	-2.479
0.306	5.444	0.001	-2.465	5.451	0.001	-2.489	5.457	0.001	-2.503
0.309	5.451	0.001	-2.484	5.455	0.001	-2.500	5.467	0.001	-2.530
0.311	5.460	0.001	-2.508	5.464	0.001	-2.524	5.476	0.001	-2.554
0.314	5.469	0.001	-2.532	5.473	0.001	-2.548	5.485	0.001	-2.578
0.317	5.479	0.001	-2.559	5.483	0.001	-2.575	5.495	0.001	-2.605
0.320	5.487	0.001	-2.581	5.492	0.001	-2.600	5.499	0.001	-2.616
0.325	5.501	0.001	-2.618	5.500	0.001	-2.621	5.508	0.001	-2.640

2.9.3 Estimation of the influence of the reaction on mass transfer

If a reaction takes place in the boundary layer at the same time as mass transfer, then the concentration gradient can be changed and thus the mass transfer rate can be influenced. In the present case, therefore, the 1st order reaction of carbonic acid to carbon dioxide, which takes place in the boundary layer, has to be considered (see Eq. 41).



To estimate the influence of a reaction on the mass transfer, the dimensionless Hatta number was introduced in process engineering. This relates the maximum possible mole number change due to the reaction to the maximum possible mole number change due to mass transfer (see Eq. 42).

$$\text{Ha} = \sqrt{\frac{\left(\frac{dn_{\text{reac}}(\text{CO}_2)}{dt}\right)_{\text{max}}}{\left(\frac{dn_{\text{dif}}(\text{CO}_2)}{dt}\right)_{\text{max}}}} \quad \text{Eq. 42}$$

2 GUIDELINE – EXPERIMENTAL DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT OF CO₂

Usually, the Hatta number for a first order reaction parallel to a mass transfer from gas to liquid in an absorption process (with mass transfer resistance mainly in the liquid phase) is given by Eq. 43.

$$Ha = \sqrt{\frac{k_1 \cdot \delta}{k_L}} \quad \text{Eq. 43}$$

In the present case, however, the process is a desorption process and care must be taken to formulate the Hatta number correctly. The highest possible reaction rate for the formation of carbon dioxide according to Eq. 41 in the boundary layer can be formulated with the maximum carbonic acid concentration present. Since the reaction taking place in the boundary layer converts carbonic acid to carbon dioxide, the maximum carbonic acid concentration is in the bulk phase and thus also on the liquid side of the boundary layer. The maximum mass transfer occurs when the concentration at the interphase is zero. Taking these facts into account, the Hatta number can be formulated in this particular case according to Eq. 44, where the position $x = \delta$ denotes the liquid side of the boundary layer.

$$Ha = \sqrt{\frac{k_1 \cdot [\text{H}_2\text{CO}_3]_{x=\delta} \cdot V_{\text{film}}}{k_L a(\text{CO}_2) \cdot ([\text{CO}_{2,d}]_{x=\delta} - 0) \cdot V}} \quad \text{Eq. 44}$$

The volume of the boundary layer (V_{film}) can be calculated using equation 45.

$$V_{\text{film}} = A \cdot \delta = a \cdot V \cdot \delta \quad \text{Eq. 45}$$

Inserting Eq. 45 into Eq. 44 results in Eq. 46.

$$Ha = \sqrt{\frac{k_1 \cdot [\text{H}_2\text{CO}_3]_{x=\delta} \cdot a \cdot V \cdot \delta}{k_L a(\text{CO}_2) \cdot [\text{CO}_{2,d}]_{x=\delta} \cdot V}} = \sqrt{\frac{k_1 \cdot [\text{H}_2\text{CO}_3]_{x=\delta} \cdot \delta}{k_L (\text{CO}_2) \cdot [\text{CO}_{2,d}]_{x=\delta}}} \quad \text{Eq. 46}$$

It is assumed that all chemical reactions in the liquid phase are in equilibria. This is then also true for the considered reaction on the liquid side of the boundary layer at the position $x = \delta$ (see Eq. 47 and Eq. 48).

$$\left. \frac{d[\text{CO}_{2,d}]}{dt} \right|_{x=\delta} = 0 = k_1 \cdot [\text{H}_2\text{CO}_3]_{x=\delta} - k_2 \cdot [\text{CO}_{2,d}]_{x=\delta} \quad \text{Eq. 47}$$

$$[\text{H}_2\text{CO}_3]_{x=\delta} = \frac{k_2}{k_1} \cdot [\text{CO}_{2,d}]_{x=\delta} \quad \text{Eq. 48}$$

Inserting Eq. 48 into Eq. 46 results in Eq. 49.

$$\text{Ha} = \sqrt{\frac{k_1 \cdot \frac{k_2}{k_1} \cdot [\text{CO}_{2,d}]_{x=\delta} \cdot \delta}{k_L(\text{CO}_2) \cdot [\text{CO}_{2,d}]_{x=\delta}}} = \sqrt{\frac{k_2 \cdot \delta}{k_L(\text{CO}_2)}} \quad \text{Eq. 49}$$

By using the defining equation for the mass transfer coefficient, Eq. 50 follows from Eq.49.

$$\text{Ha} = \sqrt{\frac{k_2 \cdot \delta^2}{D(\text{CO}_2)}} \quad \text{Eq. 50}$$

The Hatta number can be calculated at 25 °C by inserting the appropriate values according to [Royce and Thornhill 1991] and assuming a boundary layer thickness of approximately 10 μm (see Eq. 51).

$$\text{Ha} = \sqrt{\frac{0.028 \frac{1}{\text{s}} \cdot (10 \cdot 10^{-6} \text{ m})^2}{2.03 \cdot 10^{-9} \frac{\text{m}^2}{\text{s}}}} = 0.037 \quad \text{Eq. 51}$$

The Hatta number is therefore far below the critical value of 0.3, and the influence of the reaction on mass transfer can be neglected. An influence of the reaction on the mass transfer is only to be expected if the boundary layer thickness is ≥ 80 μm.





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