Oxygen and Carbon Dioxide Mass Transfer and the Aerobic, Autotrophic Cultivation of Moderate and Extreme Thermophiles: A Case Study Related to the Microbial Desulfurization of Coal

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Mass transfers of O₂, CO₂, and water vapor are among the key processes in the aerobic, autotrophic cultivation of moderate and extreme thermophiles. The dynamics and kinetics of these processes are, in addition to the obvious microbial kinetics, of crucial importance for the industrial desulfurization of high-pyritic coal by such thermophiles. To evaluate the role of the temperature on the gas mass transfer, kₐ measurements have been used to supplement the existing published data. Oxygen mass transfer from gas (air) to liquid (5 mM H₂SO₄ in water) phase as a function of the temperature has been studied in a laboratory-scale fermentor. At 15, 30, 45, and 70°C, (kₐ) values (for oxygen) were determined under three different energy input conditions by the dynamic gassing in/out method. The (kₐ) value was shown to increase under these conditions with increasing temperature, and straight lines were obtained when the logarithm of (kₐ) was plotted against the temperature. By multiplying the equilibrium concentration of O₂ in water with (kₐ) maximal, O₂ transfer capacities were calculated. It appeared that in spite of a decreased solubility of O₂ at elevated temperature in mechanically mixed fermentors the calculated transfer capacities showed only minor changes for the range between 15 and 70°C. However, in an air-mixed fermentor the transfer capacity for O₂ decreased slowly but steadily.

Carbon dioxide mass transfer was predicted by calculations on the basis of the data for oxygen transfer. The maximal CO₂ transfer capacity, calculated as the product of the equilibrium CO₂ concentration times (kₐ), decreased slowly as the temperature increased over the range 15–70°C under all three energy input conditions. Subsequent process design calculations showed that for aerobic, autotrophic cultures, CO₂ limitation is more likely to occur than O₂ limitation.

INTRODUCTION

In 1986 a Dutch feasibility study on the microbial desulfurization of low-sulfur coal (<1% pyritic sulfur) was completed. Although the slow growth of the biomass and the pyrite (FeS₂) oxidation kinetics demand huge reactors, cost analysis indicated that the process might be economically feasible. A regime analysis, based on characteristic time constants, was used to identify the rate-limiting processes. The mass transfer of oxygen and carbon dioxide from gas into the liquid phase appeared to be the rate-limiting process. Simple suspension reactors, such as cascades of Pachuca tanks, appeared to be appropriate to fulfill the requirements for adequate aeration and bulk mixing and to prevent sedimentation in the bottom of the reactors. Recently an investigation into the potential of this process design for the treatment of high-sulfur coals has started. This study is part of an EC project on microbial desulfurization of coal in which laboratories from Italy (University of Cagliari: bacterial removal of organic S), the United Kingdom (Warren Spring Laboratory: removal of pyrite by extremely thermophilic bacteria), the Federal Republic of Germany (Bergbau Forschung GmbH: characterization of coal before and after desulfurization), and the Netherlands (Delft University of Technology: removal of pyrite by moderately thermophilic bacteria and reactor design and scale-up) co-operate.

Because the oxidation of pyrite by oxygen is a highly exothermic reaction (ΔH° = −1481 kJ/mol FeS₂, microbial desulfurization of high-pyritic coal results in an in-
crease of the temperature in the reactor such that the growth of, and pyrite oxidation by, mesophilic bacteria would be partially or completely inhibited if the reactor was not cooled. In this case, thermophilic, pyrite-oxidizing bacteria might be more appropriately used. However, operation at higher temperatures has several important consequences for the leaching process which should be considered. One of these is the mass transfer of oxygen and carbon dioxide from gas to the liquid phase. The actual transfer rate can be approximated by the following equations for O₂ and CO₂:

\[
\text{OTR} = (k_{L}a)_{o} \cdot [C_{o,L} - C_{o,L}] \quad \text{(1a)}
\]

\[
\text{CTR} = (k_{L}a)_{c} \cdot [C_{o,L}^* - C_{o,L}] \quad \text{(1b)}
\]

Expressed in words, the oxygen or carbon dioxide transfer rate is the product of three terms, i.e., the liquid phase mass transfer coefficient \([k_{L}a]_{o}\) or \([k_{L}a]_{c}\), the gas–liquid interfacial area per unit volume of reactor (a), and the difference between the equilibrium \((C_{o,L}^*)\) and the actual \((C_{o,L})\) concentration of O₂ or CO₂ in the liquid phase.

The aim of this study was to gain a better insight into the effect of increasing temperature on oxygen and carbon dioxide mass transfer. The results are of importance for the use of moderately thermophilic (growth range 40–60°C) and extremely thermophilic (growth range 60–80°C) bacteria in large-scale (e.g., microbial desulfurization of coal) and small-scale (e.g., chemostat cultures) cultivation. Although the study was aimed at microbial desulfurization, the basic data have been acquired in such a way that they should have general validity for other thermophilic systems. This article presents experimental data on the maximum mass transfer of O₂ in a laboratory-scale fermentor as a function of the temperature under three different energy input conditions. Furthermore, the maximum mass transfer of CO₂ has been calculated for the same conditions on the basis of \((k_{L}a)_{o}\) values for O₂.

**MATERIALS AND METHODS**

**Measurement of \((k_{L}a)_{o}\)**

A 1.5-L fermentor (internal diameter 0.096 m; Applikon, the Netherlands) was filled with 1 L of 5 mM sulfuric acid (pH 2). The temperature was maintained at 15, 30, 45, or 70°C. Anaerobiosis was obtained by sparging nitrogen or argon through the fluid. The contents of the fermentor were stirred by two four-bladed (height 0.012 m) turbines (diameter 0.045 m), which were attached to the central impeller shaft at a distance of 0.05 m from each other. Subsequently air was sparged through the system. Three different combinations of stirring rate and air flow were employed: (1) 700 rpm, 7.5 L air/h; (2) 0 rpm, 75 L air/h; (3) 200 rpm, 7.5 L air/h. Under condition 2 the impeller shaft was left in place. These conditions correspond to energy inputs of about 900, 30, and 20 W/m³, respectively, and were selected as model systems for mechanically well-mixed, gas-mixed, and mechanically moderately mixed reactors, respectively.

In the dynamic gassing in/out method, it is assumed that the rate of O₂ transfer can be described by Eq. (1a). Via integration, Eq. (2) can be obtained:

\[
\log \left( \frac{C_{o,L}^* - C_{o,L}}{C_{o,L}^* - C_{o,L}} \right) = \frac{v_{o}}{(k_{L}a)_{o}} \cdot \left( t' - t \right)
\]

where \(C_{o,L}^*\) is the oxygen concentration in water that is in equilibrium with air at a given temperature; \(C_{o,L}^*\) and \(C_{o,L}^*\) are the actual oxygen concentrations present at \(t\) and \(t'\) minutes; and \(C_{o,L}\), \(C_{o,L}^*\), and \(C_{o,L}^*\) were calculated at the time the concentration of O₂ in the liquid phase had increased to 20–30% and 60–80% of the air-saturated concentration \((C_{o,L}^*)\), respectively. Calculation of \((k_{L}a)_{o}\) using Eq. (2) is justified when the following conditions are met: The liquid phase mixing time (I), the response time of the O₂ electrode (II), and the gas phase residence time (III) must be lower than the time constant for O₂ transfer \((1/(k_{L}a)_{o})\). (I): The mixing times in the fermentor were estimated to be 1–4 s (ref. 4), 13 s (ref. 5), and 5–14 s (R. G. J. M. van der Lans, personal communication, 1989), respectively. These mixing times are at least one order of magnitude lower than the time constants for oxygen transfer (see Table 1). (II): The response time of the galvanic electrode (1–3 s) when equipped with a high-sensitivity Teflon membrane (Tamsen, Zoetermeer, the Netherlands) was much smaller than the highest measured time constant for O₂ transfer (23 s; see Table 1). (III): The gas phase residence time was at least one order of magnitude lower than the time constant for O₂ transfer for conditions 2 and 3. With condition 1, these time constants were similar and consequently, \((k_{L}a)_{o}\) will be underestimated. However, it can be shown [steady-state calculations assuming \(C_{o,L}^*\) is continuously zero; see Eq. (4)] that \((k_{L}a)_{o}\) is maximally underrated by 20%. Moreover, \((k_{L}a)_{o}\) is probably underestimated by a lower percentage because \(C_{o,L}^*\) increases from zero to \(C_{o,L}^*\) during the actual measurement. Summarizing, at this initial stage of investigation, the simplified calculation of \((k_{L}a)_{o}\) using

### Table 1. Values of \((k_{L}a)_{o}\) for oxygen at different temperatures and different mixing conditions in a laboratory-scale fermentor.

<table>
<thead>
<tr>
<th>(T(°C))</th>
<th>Condition 1</th>
<th>Condition 2</th>
<th>Condition 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.92 ± 0.09 (9)</td>
<td>0.193 ± 0.009 (5)</td>
<td>0.064 ± 0.003 (5)</td>
</tr>
<tr>
<td>30</td>
<td>1.29 ± 0.14 (9)</td>
<td>0.222 ± 0.004 (5)</td>
<td>0.084 ± 0.003 (5)</td>
</tr>
<tr>
<td>45</td>
<td>1.69 ± 0.16 (10)</td>
<td>0.267 ± 0.015 (5)</td>
<td>0.101 ± 0.005 (4)</td>
</tr>
<tr>
<td>70</td>
<td>2.05 ± 0.12 (12)</td>
<td>0.385 ± 0.009 (5)</td>
<td>0.153 ± 0.005 (4)</td>
</tr>
</tbody>
</table>

*Note: The \(k_{L}a\) for O₂ was measured at four temperatures (15, 30, 45, and 70°C) and three experimental conditions in a 1.5-L laboratory-scale fermentor. The values are expressed as mean values ± standard deviation (S.D.) for n number of observations. Experimental conditions:*

Condition 1: stirring speed = 700 rpm; air flow rate = 7.5 L/h
Condition 2: stirring speed = 0 rpm; air flow rate = 75 L/h
Condition 3: stirring speed = 200 rpm; air flow rate = 7.5 L/h

The three conditions were used as models for a mechanically well-mixed, a gas-mixed, and a mechanically moderately mixed reactor, respectively.
Eq. (2) appears to be justified under all three sets of experimental conditions. The rate \((k_Oa)_O\) (min\(^{-1}\)) is presented as the mean ± S.D. \((n)\), i.e., the mean value of \(n\) determinations from \(n\) resaturation curves at the specified conditions, together with the standard deviation (S.D.).

The increase in oxygen concentration was followed with an oxygen electrode (either galvanic or Clark electrode). The 100% signal of the galvanic electrode in air-saturated 5 mM \(\text{H}_2\text{SO}_4\) solution varied in proportion to the temperature of that solution: 12 mV at 15°C, 27 mV at 30°C, 35 mV at 45°C, and 53 mV at 70°C. Since the solubility of oxygen decreases at higher temperatures (see Fig. 1a), the sensitivity of the electrode clearly increases with increasing temperature: 0.038 mV · L/μmol \(\text{O}_2\) at 15°C, 0.115 at 30°C, 0.187 at 45°C, and 0.434 at 70°C. For comparison a few experiments were carried out with a Clark-type electrode. This showed a lower 100% air saturation signal and a smaller dependence on the temperature: 6 mV at 15°C, 9 mV at 45°C, and 10 mV at 70°C.

Values of \((k_Oa)_O\) measured at 30°C [1.19 ± 0.07 (3)] or 70°C [2.67 ± 0.10 (3)] with the galvanic electrode did not differ significantly from those measured with the Clark electrode, which were, respectively, 1.12 ± 0.17 (3) and 2.53 ± 0.10 (3). In this case argon was used to create anaerobic conditions in the fermentor, which was resaturation according to experimental condition 1.

In the first instance, nitrogen and argon were used to replace the air in the solution. However, it was noticed that at 30°C slightly lower \((k_Oa)_O\) values were found with argon than with nitrogen. The ratio of the \(k_Oa\) for \(\text{O}_2\) in the presence of nitrogen or argon was 1.12 ± 0.07 (5). At 70°C, however, this difference could no longer be detected [ratio = 1.00 ± 0.07 (3)]. All the \((k_Oa)_O\) values presented in the results section were obtained using nitrogen.

**RESULTS**

**Oxygen and Carbon Dioxide Solubility in Acid Water**

At equilibrium between dry air \((C_{ao}^a = 0.20946\) atm and \(C_{ao}^\text{atm} = 0.00033\) atm at 1 atm air pressure\(^6\)) and the liquid phase, the concentration of oxygen in the liquid phase can be calculated as a function of the temperature with the aid of Henry’s law \([C_{ao}^a\text{dry air}] = 55.55 \times C_{ao}^\text{atm}/H_o\) (mol \(\text{O}_2/\text{L})\) and Henry’s constants \((H_o)\).\(^7\) Calculation of the equilibrium concentrations of \(\text{O}_2\) in this way (Fig. 1a; upper line) results in solubility data consistent with the recent data presented by Schumpe et al.\(^2\) for the temperature range between 0 and 50°C and by Wilhelm et al.\(^7\) for the range between 0 and 80°C. However, this concentration must be corrected for the equilibrium water vapor pressure \((C_{ao}^\text{eq}\text{in atm})\), which becomes increasingly important at higher temperatures,\(^10\) in order to obtain the solubility of \(\text{O}_2\) for water-saturated air at 1 atm:

\[
C_{ao}^\text{eq} = 55.55 \times C_{ao}^\text{atm} \times (1 - C_{ao}^\text{atm})/H_o
\]

The corrected concentrations of dissolved \(\text{O}_2\) are also shown as a function of the temperature (Fig. 1a; lower line).

It is important to note that since microbial desulfurization occurs below pH 3, only the solubility of \(\text{CO}_2\) and not of \(\text{HCO}_3^-\) or \(\text{CO}_3^{2-}\) must be considered.\(^1\) Furthermore, the amount of \(\text{CO}_2\) present as \(\text{H}_2\text{CO}_3\) can also be neglected.\(^1\) The solubility of \(\text{CO}_2\) at 1 atm dry air \([C_{ao}^\text{atm}\text{dry air}] = 55.55 \times C_{ao}^\text{atm}/H_c\) (mol \(\text{CO}_2/\text{L})\) as a function of the temperature can be calculated with the aid of Henry’s constants for \(\text{CO}_2\) \((H_c)\).\(^7\) The resulting equilibrium concentrations (Fig. 1b; upper line) are in agreement with the data of

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**Figure 1.** Oxygen and carbon dioxide solubility in acid water as a function of the temperature. The concentrations (μmol/L) of \(\text{O}_2\) (a) and \(\text{CO}_2\) (b) in acid water in equilibrium with 1 atm dry air \((C_{ao}^a = 0.20946\) atm; \(C_{ao}^\text{atm} = 0.00033\) atm) are shown as a function of the temperature (°C), with (●) and without (×) correction for the water vapor pressure.
Schumpe et al.\textsuperscript{9} for the temperature range between 0 and 50°C and of Wilhelm et al.\textsuperscript{2} for the range between 0 and 80°C. Again, these equilibrium concentrations must be corrected for the water vapor pressure:

$$C_{eq}^* = \frac{55.55 \cdot C_{eq}^{\star} \cdot (1 - C_{eq}^{\star})}{H_{eq}} \quad (3b)$$

The concentrations of CO\textsubscript{2} in acid water in equilibrium with 1 atm of water-saturated air are given in Fig. 1b (lower line) as a function of the temperature. Comparison of the corrected equilibrium concentrations of O\textsubscript{2} and CO\textsubscript{2} in water, which both show a sigmoidal dependence on the temperature, reveals that the CO\textsubscript{2} concentration is 21–38 times lower than the O\textsubscript{2} concentration for the range between 15 and 70°C when air is used as the gas phase. Furthermore, it is clear that omission of the correction for the presence of water vapor in the air leads to serious overestimation of the O\textsubscript{2} and CO\textsubscript{2} liquid equilibrium concentrations.

**Oxygen Mass Transfer as a Function of the Temperature**

In Table 1, \((k_La)_0\) values are presented for the three different aeration conditions and four temperatures. It can be seen that for each of the three experimental conditions, the \((k_La)_0\) increased with increasing temperature. It is also clear that \((k_La)_0\) had the lowest values when air was introduced at a moderate flow and the fermentor was stirred at a moderate speed (condition 3). A 10-fold increase in air flow (condition 2), without any stirring at all, resulted in values of \((k_La)_0\) 2.5–3.0 times higher than for condition 3. However, the highest values were found with a moderate air flow and rapid stirring (condition 1).

From Figure 2 it can be seen that semi-log plots of \((k_La)_0\) versus the temperature apparently resulted in straight lines for all three experimental conditions. Hence, \((k_La)_0\) values can be estimated from these plots for any temperature between 15 and 70°C. With this knowledge, it is possible to calculate the maximal capacity of oxygen transfer (on the assumption that \(C_{eq} = 0\)) from gas to the water phase by multiplying \((k_La)_0\) by \(C_{eq}^*\) at temperatures between 15 and 70°C. In Figure 3 (open symbols), the maximal capacity of oxygen supply is given as a function of the temperature for the three experimental conditions (see Table 1). It is evident that with condition 1, the maximal oxygen transfer capacity decreased slightly between 15 and 25°C, subsequently increased from 25 to 60°C, and decreased again from 60 to 70°C. However, the variation in the rate of transfer was relatively small, and the lowest capacity (at 25°C) was still 90% of the highest capacity of transfer (at 60°C). With condition 3, the capacity of transfer also showed only minor changes in the temperature range between 15 and 70°C. The lowest value (at 70°C) was still 92% of the highest value (at 15°C). However, the oxygen transfer capacity with condition 2 declined more or less gradually from 58.3 at 15°C to 43.2 \(\mu\)mol O\textsubscript{2}/L \cdot min at 70°C.

![Figure 2](image)

**Figure 2.** Log\((k_La)_0\) as a function of the temperature for three different mixing conditions in a laboratory-scale fermentor. The logarithm of \((k_La)_0\) (min\(^{-1}\)) is given as a function of the temperature \(T\) in °C. Experimental conditions 1, 2, and 3 are the same as indicated in Table 1. The linear curves can be described by the following equations:

- Condition 1 (•): \(\log(k_La)_0 = 0.0081T - 0.144\) (corr. coeff. = 0.9978)
- Condition 2 (♦): \(\log(k_La)_0 = 0.0055T - 0.809\) (corr. coeff. = 0.9953)
- Condition 3 (▲): \(\log(k_La)_0 = 0.0068T - 1.290\) (corr. coeff. = 0.9983)

However, these maximal capacities of O\textsubscript{2} transfer are calculated on the basis of the assumption that the partial pressure of O\textsubscript{2} in water-saturated air is the same for air flowing in and out of the fermentor. This is not necessarily the case if the mass transfer of O\textsubscript{2} to the bulk liquid leads to a significant decrease of the partial O\textsubscript{2} pressure in the air leaving the fermentor. In order to check the validity of this assumption, a steady-state mass transfer balance for oxygen which includes the air flow rate (the gas phase is assumed to be ideally mixed) has been made. At a total pressure of 1 atm the partial pressures of O\textsubscript{2}, CO\textsubscript{2}, and H\textsubscript{2}O are numerically equal to their respective mole fractions. Hence, the transfer of O\textsubscript{2} (mol/h) is given by the difference between \(\Phi_{eq}^G \times C_{eq}^*/V_o\) and \(\Phi_{eq}^O \times C_{eq}^O/V_o\). A good estimate of \(\Phi_{eq}^G\) can be obtained from the equation \(\Phi_{eq}^G = \Phi_{eq}^G \times C_{eq}^G \times C_{eq}^O\), in which \(\Phi_{eq}^G\) is the gas flow rate corrected for thermal expansion. The parameter \(C_{eq}^O\) can be approximated by the value of \(C_{eq}^O\) for dry air. Insertion of these parameters results in the following steady-state mass transfer balance:

$$\frac{\Phi_G}{V_o} \cdot \left\{C_{eq}^* - \frac{C_{eq}^O}{1 - C_{eq}^O} \right\} = V_o \cdot (k_La)_0 \cdot \frac{55.55 \cdot C_{eq}^O}{H_o} \quad (4)$$

The parameters \(\Phi_G\) and \(V_o\) represent the volumetric gas flow rate and the molar volume of O\textsubscript{2}, both calculated at 20°C; the parameter \(C_{eq}^O\) is the oxygen partial pressure in the water-saturated air flowing out of the fermentor under
Figure 3. Maximal capacity of oxygen transfer as a function of the temperature in a laboratory-scale fermentor. The maximal capacity of transfer of O₂ (μmol/L · min) from gas to the liquid phase is given as a function of the temperature (°C) for the three experimental conditions specified in Table 1. Open symbols (1, □, 2, ○, 3, △) denote the product ([kₐα]₀ × C₀ argue). Closed symbols (1, ■) indicate the maximal capacity of oxygen transfer, corrected for the decrease of C_e × (1 - C_w) due to steady-state transfer of oxygen from gas to the liquid phase. For conditions 2 and 3, these corrected maximal transfer capacities nearly coincide with the uncorrected figures. Therefore these figures are not shown in the graph.

steady-state conditions; and V_L is the liquid volume. Rearrangement of Eq. (4) leads to

\[
C_{\text{O₂}}^{\text{om}} = \frac{\Phi_0 \cdot C_{\text{O₂}}^* \cdot H_0 \cdot (1 - C_{\text{wO₂}}^*)}{1333 \cdot V_L \cdot (k_{\alpha a})_0 \cdot (1 - C_{\text{wO₂}}^*) + \Phi_0 \cdot H_0}
\]

(5a)

The maximal capacity of O₂ transfer will change accordingly and is equal to

\[
\text{OTR} = \frac{(k_{\alpha a})_0 \cdot 55.55 \cdot C_{\text{O₂}}^{\text{om}}}{H_0}
\]

(6a)

Calculation of C_{\text{O₂}}^{\text{om}} and OTR revealed that for conditions 2 and 3, the change in the amount of O₂ in the air was insignificant. However, for condition 1, C_{\text{O₂}}^{\text{om}} was calculated to be about 76–79% of the value for C_{\text{O₂}}^* × (1 - C_{\text{wO₂}}^*). As a consequence OTR is reduced in proportion, as can be seen in Figure 3 (■).

CALCULATION

Carbon Dioxide Mass Transfer as a Function of the Temperature

The rate of transfer of carbon dioxide can be estimated on the basis of the measured k_{\alpha a} values for oxygen. On the reasonable assumption that the penetration theory can be used to describe mass transfer from the air bubbles (the diameter of which were at least greater than 0.002 m) present in the fermentor, the relationship between the k_{\alpha} for O₂ and the k_{\alpha} for CO₂ can be expressed as follows:\footnote{BOOGERD ET AL.: MASS TRANSFER AND CULTIVATION OF THERMOPHILES}

\[
(k_{\alpha})_{\text{C}} = (k_{\alpha})_0 \cdot \left[\frac{(D_{\text{C}})_{\text{C}}}{(D_{\text{O₂}})_{\text{O₂}}}\right]^{0.5}
\]

(7)

The dependence of the diffusion coefficients of O₂ and CO₂ on the temperature can be estimated by the method of Wilke and Chang\footnote{BOOGERD ET AL.: MASS TRANSFER AND CULTIVATION OF THERMOPHILES} for dilute solutions in water. According to these authors, the ratio (D_C)/C_{\text{O₂}} is independent of the temperature and equal to [V_{\text{O₂}}/V_{\text{C}}]^{0.4}. After introduction of the values 25.6 (ref. 13) and 37.2 (ref. 14) for the molecular volumes of O₂ and CO₂, the k_{\alpha} for CO₂ can be calculated with Eq. (7) to be 0.893 times the k_{\alpha} for O₂. When the transfers of CO₂ and O₂ are compared under the same experimental conditions, it seems valid to assume that the k_{\alpha} for carbon dioxide equals 0.893 times the k_{\alpha} for oxygen. Consequently, k_{\alpha} values for CO₂ can be calculated as a function of the temperature for the aforementioned three experimental conditions (see Table 1). By multiplying the calculated k_{\alpha} values for CO₂ by C_{\text{O₂}} at temperatures between 15 and 70°C, the maximal capacity of carbon dioxide transfer is obtained as a function of the temperature. This dependence is graphically presented in Figures 4a,b (open symbols). It is obvious that for all three conditions the supply of CO₂ gradually decreased between 15 and 70°C. However, it is assumed that the CO₂ partial pressure in the air flow does not change upon passage through the fermentor. The mass transfer balance for CO₂ can be represented by Eq. (4) if every oxygen is changed to a carbon. The following equations, which are similar to Eqs. (5a) and (6a), give C_{\text{CO₂}}^{\text{om}} and CTR:

\[
C_{\text{CO₂}}^{\text{om}} = \frac{\Phi_0 \cdot C_{\text{CO₂}}^* \cdot H_{\text{C}} \cdot (1 - C_{\text{wCO₂}}^*)}{1327 \cdot V_L \cdot (k_{\alpha a})_0 \cdot (1 - C_{\text{wCO₂}}^*) + \Phi_0 \cdot H_{\text{C}}}
\]

(5b)

\[
\text{CTR} = \frac{(k_{\alpha a})_0 \cdot 55.5 \cdot C_{\text{CO₂}}^{\text{om}}}{H_{\text{C}}}
\]

(6b)

It appeared that in the temperature range between 15 and 70°C, C_{\text{CO₂}}^{\text{om}} was reduced to 12–18, 88–95, or 67–80% of the value for water-saturated air [C_{\text{CO₂}}^* × (1 - C_{\text{wCO₂}}^*)] for conditions 1, 2, and 3, respectively. Consequently, the same reduction was observed for CTR (Figs. 4a,b; closed symbols). Furthermore, for condition 1 a change in the shape of the curve occurs in addition to the rather drastic decrease in the capacity of CO₂ transfer. Moreover, the decrease of CTR with temperature is much more pronounced than observed for OTR. For condition 1, CTR slowly decreased, whereas OTR even increased in the range from 15 to 70°C. For conditions 2 and 3 the lowest capacities of CO₂ transfer at 70°C were, respectively, 46 and 63% of the highest value (at 15°C). The lowest capacities of O₂ transfer for conditions 2 and 3 are 74 and 92%, respectively, of the highest observed value in the temperature range from 15 to 70°C.
It should be noticed that the maximum capacities of gas transfer presented in this article were calculated on the assumption that the actual concentration of gas in the aqueous phase is zero. In the case of O₂, this simplified calculation seems to be justified because, in general, bacterial Monod saturation constants (Kₘ) for O₂ are much lower than the equilibrium concentrations of O₂ in water (315–122 μmol/L) in the range from 15 to 70°C. With respect to CO₂, the situation is more complicated. The Michaelis–Menten constants (Kₘ) of purified ribulose-1,5-bisphosphate carboxylases from Thioacillus intermedius, Thioacillus neapolitanus, and Thioacillus ferrooxidans for CO₂ have been reported to be 750, 850, and 28 μmol/L, respectively. Furthermore, in the neutrophilic T. neapolitanus, uptake of CO₂ was found to be energy dependent and accumulation levels of 500–1500-fold were observed. Hence it is very conceivable that acidophilic bacteria also contain active transport systems for CO₂, which scavenge CO₂ from the extracellular aqueous phase. Consequently, the actual concentration of CO₂ will be low. If, however, the Monod saturation constant (Kₘ) for CO₂ was in the same order of magnitude as the equilibrium concentrations of CO₂ in acid water (15–3 μmol/L), the actual CTR will be less than the maximum predicted CTR.

In this study, it has been shown that, especially at elevated temperature, the decrease of C_CO₂ and C_CO₂ due to water evaporation cannot be neglected in calculations on mass transfer. Furthermore, the results indicate that for mechanically mixed fermentors (Fig. 3; conditions 1 and 3) the decrease in the equilibrium concentration of O₂ in air-saturated, dilute aqueous sulfuric acid solution with increasing temperature (from 315 μM at 15°C to 122 μM at 70°C) is largely compensated for by the increase of the k_a for O₂. Thus the maximal capacity of carbon dioxide transfer from gas to the liquid phase shows only minor changes in the range from 15 to 70°C. Without mechanically stirring but with a 10-fold higher flow rate of air, the transfer capacities lie between those for conditions 1 and 3, and a slow but steady decrease with increasing temperature appears.

Jackson and Shen proposed the following correlation between (k_a)₀ and temperature (10–30°C): (k_a)₀ = (k_a)₀° × Θ^π°-20 (T in °C). The temperature coefficient Θ can vary from 1.016 to 1.037 for an impeller–sparger combination. On the assumption that this equation also holds for temperatures between 15 and 70°C, Θ values of 1.019, 1.013, and 1.016 can be calculated for conditions 1, 2, and 3, respectively. Because (k_a)₀ is likely to increase with increasing temperature in the same way under all three conditions, the difference in Θ possibly indicates that the total surface area (a) of the air bubbles in the fermentor increases differently with increasing temperature for the three conditions. Presumably an increase in temperature affects the residence time of the air bubbles in the fermentor progressively, from condition 1 to condition 3 to condition 2.

With respect to CO₂ transfer, one important conclusion is that at the relatively low gas flow rates used in this study, depletion of available CO₂ in air occurs, especially at high

**DISCUSSION**

The rates of mass transfer of oxygen and carbon dioxide are of crucial importance for any small- or large-scale cultivation of aerobic, autotrophic microorganisms. Because we are interested in desulfurization of high-pyritic coal by thermophilic bacteria, we have, as a first approach, studied the influence of the temperature on O₂ and CO₂ mass transfer in a laboratory-scale fermentor.
values of \((k_A a)_c\) (condition 1). Consequently, maximal transfer capacities are lower than the products \((k_A a)_c \times C_O^2\) indicate (Figs. 4a, b). For all three conditions, a gradual decrease of the capacity of CO₂ transfer is observed at increasing temperature.

The implications of our results for physiological studies on aerobic, autotrophic bacteria can be demonstrated by a theoretical example: *T. ferrooxidans*—like bacteria growing in a medium containing ferrous iron or tetrahionate in a chemostat at a dilution rate \(D\) of 0.05 h⁻¹ and temperatures between 20 and 70°C. With the aid of some published data on growth parameters, the volumetric rates of bacterial O₂ \(r_O\) and CO₂ \(r_C\) consumption have been calculated. The concentrations of Fe²⁺ and S₄O₄²⁻ in the medium reservoir were chosen such that \(r_O\) was equal for both continuous cultures. The ratios OTR/\(r_O\) and CTR/\(r_C\) are indicative for gas mass transfer limitation at values less than 1. These ratios are presented in Figures 5a–c as a function of the temperature. It can be seen that OTR/\(r_O\) becomes lower than 1 only for condition 3. Hence, in this case, O₂ can be the limiting factor. The ratio CTR/\(r_C\) for Fe²⁺ cultures indicates that CO₂ limitation is only expected to occur under condition 3. The ratio CTR/\(r_C\) for S₄O₄²⁻ cultures predicts that these cultures will be limited by CO₂ under all three conditions. This means that for condition 3, oxygen as well as carbon dioxide may limit bacterial growth. Because CTR/\(r_C\) is still smaller than OTR/\(r_O\), it can be reasoned that both the ferrous iron and tetrahionate cultures are more likely to be limited by CO₂ than by O₂. However, the difference between these ratios is small, and the temperature difference of 23°C probably determines whether O₂ or CO₂ becomes limiting in this case.

Previously, Hazeu et al. observed that the yields of *T. ferrooxidans*, growing as a continuous culture on tetraionate or thiosulfate in a mechanically well-mixed fermentor, increased during aeration with CO₂-enriched air. Similar observations were made by Eccleston and Kelly. Moreover, in a gas-mixed batch culture of *T. ferrooxidans* an increase of the specific growth rate and the yield on ferrous iron was observed during aeration with CO₂-enriched air. On the assumption that the actual CTR approaches the maximum CTR in these cultures, a comparison of the data given by the authors on gas–liquid mixing with the data presented in this article reveals that growth in all these cultures was probably not limited by CO₂ mass transfer. Thus, the CO₂-effect must be due to another factor. A possible explanation can be found in the amount of energy needed for the uptake of CO₂. If CO₂ must be accumulated intracellularly in order to saturate the ribulose-1,5-bisphosphate carboxylase enzymes to a sufficient degree, the formed concentration gradient drives CO₂ out of the cell via passive diffusion. However, when CO₂-enriched air is supplied, the actual concentration of CO₂ will be higher, leakage of CO₂ will be smaller, and the amount of energy needed for the reuptake of lost CO₂ will be less.

Consequently, a higher yield is observed in the chemostat. In a batch culture, one part of the gain in energy can also be used to grow at a higher specific growth rate by increasing the intracellular concentration of inorganic carbon. Alternatively, the \(K_i\) value of *T. ferrooxidans* for CO₂ may be so high that the actual CTR is much lower than the maximal CTR. In that case the cultures are likely to be limited by CO₂ mass transfer.

![Figure 5](image-url)

Figure 5. Carbon dioxide or oxygen limitation in continuous cultures of *T. ferrooxidans*—like bacteria. The ratios OTR/\(r_O\) and CTR/\(r_C\) are given as a function of the temperature for the three conditions specified in Table 1. (a), (b), (c) show these ratios for conditions 1, 2, and 3, respectively. Symbols: ×, OTR/\(r_O\) for Fe²⁺ and S₄O₄²⁻ cultures; ▽, CTR/\(r_C\) for Fe²⁺ cultures; ●, CTR/\(r_C\) for S₄O₄²⁻ cultures. Growth conditions: continuous culture of *T. ferrooxidans*—like bacteria; \(D = 0.05\) h⁻¹, \(T = 20–70°C\), pH 3.0. Medium: 140 mM Fe²⁺ or 10 mM S₄O₄²⁻. Air supply and mixing: see conditions 1, 2, and 3 of Table 1. Growth parameters: \(Y_{X/S} = 0.2\) g D.W./mol (Hazeu et al.); \(Y_{X/S} = 9.8\) g D.W./mol (Hazeu et al.); Eccleston and Kelly; C content (% of biomass) = 48% (Hazeu et al.); \(r_O = 29\) µmol O₂/L · min (Fe²⁺ and S₄O₄²⁻); \(r_C = 0.9\) (Fe²⁺) or 3.3 (S₄O₄²⁻) µmol CO₂/L · min.
CONCLUSIONS

1. For equilibrium concentrations of O\textsubscript{2} and CO\textsubscript{2} in acid water, a significant correction results from taking water vapor pressure at high temperature into consideration.

2. The volumetric mass transfer coefficients of O\textsubscript{2} and CO\textsubscript{2} are weakly dependent on the temperature in the range 15–70°C.

3. The maximal capacities of O\textsubscript{2} transfer for the air-mixed and the mechanically moderately mixed fermentors are, to a good approximation, equal to the product $(k_a \alpha)_a \times C_{O_2}$ in the temperature range 15–70°C. However, such an approximation results in the overestimation of the maximum transfer rate for the mechanically well-mixed fermentor because there is significant depletion of the available O\textsubscript{2} in the gas phase. The maximal capacities for the well-mixed, the moderately mixed, and the gas-mixed fermentors were, respectively, 241 (mean value), 19 (mean value), and 58–43 μmol O\textsubscript{2}/L·min in the temperature range between 15 and 70°C.

4. The maximum capacity of CO\textsubscript{2} transfer for all three conditions cannot be appropriately estimated by the product $(k_a \alpha)_a \times C_{CO_2}$ due to depletion of the available CO\textsubscript{2} in the gas phase. Deposition occurs especially at low gas flow rates and high values of $(k_a \alpha)_a$.

Calculation of the maximum CO\textsubscript{2} transfer rates must be performed on the basis of the CO\textsubscript{2} partial pressure in the air leaving the fermentor. Furthermore, in the temperature range between 15 and 70°C the maximum capacities decrease from 1.5 to 1.4, 2.1 to 1.0, and 0.6 to 0.4 μmol CO\textsubscript{2}/L·min for conditions 1, 2, and 3, respectively.

5. By a theoretical example, it is shown that the growth of aerobic, autotrophic, and acidophilic bacteria in continuous cultures is more likely to be limited by CO\textsubscript{2} than by O\textsubscript{2}. These results are of consequence for physiological studies of chemolithotrophic growth and for process design incorporating these bacteria.

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NOMENCLATURE

- $k_a$: liquid phase mass transfer coefficient of O\textsubscript{2} or CO\textsubscript{2} (m/min)
- $\alpha$: gas–liquid interfacial area per unit volume of reactor (m\textsuperscript{2}/m\textsuperscript{3})
- $k_{l,a}$: volumetric mass transfer coefficient of O\textsubscript{2} or CO\textsubscript{2} (min\textsuperscript{-1})
- $\Delta H^*$: standard reaction enthalpy (kJ/mol)
- OTR: oxygen transfer rate (μmol/L·min)
- CTR: carbon dioxide transfer rate (μmol/L·min)
- $C_{E_{O_2}}$: equilibrium partial pressure of O\textsubscript{2} or CO\textsubscript{2} in dry air (atm)
- $C_{E_{CO_2}}$: equilibrium partial pressure of CO\textsubscript{2} in the liquid phase, corrected for water vapor pressure (μmol/L)
- $C_{L_{CO_2}}$: actual concentration of O\textsubscript{2} or CO\textsubscript{2} in the liquid phase (μmol/L)
- $C_{E_{CO_2}}$: equilibrium partial pressure of water in the gas phase (atm)
- $H_k$: Henry’s constant for O\textsubscript{2} or CO\textsubscript{2} (atm)
- $V_i$: volume of liquid in the fermentor (L)
- $D_L$: diffusion coefficient of O\textsubscript{2} or CO\textsubscript{2} in water (cm\textsuperscript{2}/s)
- $V_m$: molecular volume of O\textsubscript{2} or CO\textsubscript{2} at normal boiling point (cm\textsuperscript{3}/mol)
References


