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Quantification of Oxygen Transfer in Test Tubes by Integrated Optical Sensing

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Abstract Immobilized sensor spots were applied for online measurement of dissolved O_2 in test tubes. Oxygen transport was quantified at varied shaking frequency and filling volumes. The k_La increased with increasing shaking frequency and decreasing filling volume. In non-baffled tubes the maximum k_La value was 70 h^{-1}, equivalent to a maximum O_2 transfer capacity of 15 mM h^{-1}. Monitoring of the hydrodynamic profile revealed that the liquid bulk rotated inside the tube with an inclined liquid surface, whereby the angle between the surface and tube wall increased with increasing shaking frequency. The k_La clearly correlated to the surface area. Placement of four baffles into the tubes improved the oxygen transfer up to 3-fold. The highest increase in k_La was observed at high filling volume and high shaking frequency. The maximum k_La in baffled tubes was 100 h^{-1}.

Key words: Gas-liquid mass transfer, optical sensor, screening, k_La, minireactor

Test tubes are very useful for developing inocula for small-scale fermentation and screening various types of strains in primary experiments [7]. However, these systems have not been characterized in great detail, which limits their potential. The supply of O_2, one of the major issues in the cultivation of aerobic organisms, has not been investigated so far. For cultures in small devices such as test tubes, sufficient O_2 supply is usually assumed, but is not measured because of a lack of methods for monitoring of dissolved O_2 [9]. A promising approach for online O_2 sensing is provided by fluorogenic compounds exhibiting quantitative dependence of quenching or luminescent decay time on O_2 concentration [6]. Immobilization of such fluorogenic compounds was successfully applied for accurate monitoring of dissolved O_2 in microtiter plates [5], and shake flasks [10]. The available fluorophores are not affected by autoclaving, allowing multiple use of sensor-equipped culture vessels. The used sensor system showed high precision over the whole range from 0 to 100% air saturation. Therefore, in contrast to previously presented chemo-optical methods [4, 9], the whole measured curve could be used to calculate the oxygen transfer rate. Furthermore, it does not need any recalibration after autoclaving. In the present work, such fluorophores-based sensors were applied to quantify oxygen transport in baffled and non-baffled test tubes at various shaking frequencies and filling volumes. This provides important knowledge for their routine application in various types of quantitative screening approaches.

MATERIALS AND METHODS

Optical System for O_2 Sensing

The O_2 sensing system consisted of a sensor tube with an immobilized optical sensor spot, an optical fiber, and a module for data processing (Fig. 1A). The tubes with round-shaped bottom were made of Duran glass (200 mm height × 30 mm diameter, Schott, Mainz, Germany). In selected experiments, the tubes were instrumented with four baffles, each of 5 mm width, which were glued into the test tubes. At the bottom, the baffles were round-shaped to fit into the curve of the tube bottom. A thin sensor spot of 10 mm diameter and thickness below 100 μm was created in the center of the tube bottom by dropping 30 μl of liquid cocktail solution with Sensor PST3 (PreSens GmbH, Regensburg, Germany) onto the bottom of a tube and subsequent evaporation. Four tubes with online optical oxygen sensing were run in parallel. The design of the optical coasters and the fibers placed has been described previously [10]. The coasters were connected to a four-channel oxygen meter (PreSens GmbH, Regensburg, Germany) for determination of the luminescent decay time of the O_2 sensor, which is related to the actual dissolved O_2 [6]. The module was further connected to a PC via a serial

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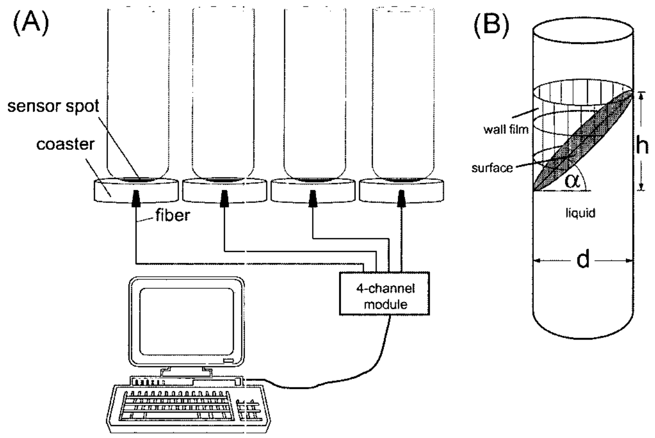

Fig. 1. Experimental setup of online oxygen monitoring in shaken test tubes (A); Hydrodynamic profile of the liquid inside the test tube during orbital shaking with liquid surface (grey area), liquid wall film (streaked area), tube diameter (d), and height of cylinder mantle between the upper and lower ends of the inclined surface (h) (B).

interface. The whole setup was controlled by graphically orientated software (PreSens GmbH, Regensburg, Germany). All experiments were carried out at 30°C in an incubator with orbital shaking at Ø=25 mm (Multitron II, Infors AG, Bottmingen, Switzerland). Hereby, four sensor tubes and the corresponding four coasters were kept in position by a plastic holder. For oxygen quantification, a two-point calibration with N_2 and air, respectively, was applied.

Quantification of Volumetric Gas-Liquid Mass Transfer Coefficient (k_La)

The volumetric gas-liquid mass transfer coefficient (k_La, given in h^{-1}) was determined in duplicate with N_2. To this end, water-filled sensor flasks were initially calibrated and subsequently purged with N_2 gas. After the DO was constant at 0% for a few minutes, the N_2 supply was stopped. Additionally, immediate replacement of the N_2 gas phase by air was performed. From the resulting increase of DO, k_La was calculated via nonlinear curve fit using the software Origin 6.0 (Microcal, Northampton, MA, U.S.A.).

Quantification of Liquid Film Mass Transfer Coefficient (k_L)

The liquid film mass transfer coefficient (k_L, given in m s^{-1}) was determined from k_La as follows. Photos of shaken tubes were taken with a digital camera at different shaking frequencies and filling volumes. The liquid surface was observed as rotating inclined ellipse inside the tube (Fig. 1B). From the digital pictures, the surface angle (α) of the rotating liquid against the tube wall corresponding to the adjusted conditions was read out manually. The liquid surface area ($A_{surface}$) was calculated via α and the tube diameter (d) using Equation 1.

$$A_{surface}=\frac{d}{2}\cdot\frac{d}{\cos(\alpha)\cdot 2}\cdot\pi \qquad \text{(Equation 1)}$$

The liquid film mass transfer coefficient (k_L, given in m s^{-1}) was determined from k_La via Equation 2, whereby the liquid volume (V) and the liquid surface area ($A_{surface}$) were considered.

$$k_L=k_La\cdot\frac{V}{A_{surface}} \qquad \text{(Equation 2)}$$

By rotation of the liquid inside the tube, a thin liquid film on the tube wall opposite to the liquid bulk is formed. The wall film area (A_{film}) is that fraction of the inner tube wall between the upper and the lower ends of the inclined liquid surface that is not directly covered with the liquid bulk (Fig. 1B). It was calculated as half of the corresponding cylinder mantle area (Eq. 3).

$$A_{film}=\frac{d\cdot h\cdot\pi}{2} \qquad \text{(Equation 3)}$$

In further calculations of k_L, this wall film was assumed to contribute, in addition to the liquid surface, to oxygen transport (Eq. 4).

$$k_L=k_La\cdot\frac{V}{A_{surface}+A_{film}} \qquad \text{(Equation 4)}$$

Chemicals

Chemicals were supplied from Sigma (Deisenhofen, Germany) and were of analytical grade.

RESULTS AND DISCUSSION

Coverage of Sensor Spot

Complete coverage of the sensor with liquid is crucial for accurate quantification of dissolved O_2. The influence of shaking rate (150, 200, 250, 300 rpm) and filling volume (1, 2, 3, 4, 5, 10 ml) on the coverage of the sensor was investigated in non-baffled tubes filled with a solution containing 2% sodium dithionite and 100 mM sodium carbonate as described previously [5]. Due to oxygen depletion in the solution by the reaction with dithionite, a signal of 0% for dissolved oxygen only results, if the sensor is continuously covered. For 1 ml filling volume, the sensor was completely covered with liquid at 150 rpm. This was not the case at higher shaking frequencies. Under these conditions, the liquid probably forms a thin film only at the glass wall of the tube, whereas the tube bottom containing the sensor spot is not completely covered. With increasing filling volume, the maximal shaking rate at complete sensor coverage increased to 200 rpm (2 ml), 250 rpm (3 ml), and 300 rpm (4, 5, 10 ml), respectively. According to the results, the following measurements of dissolved oxygen were carried out under conditions of

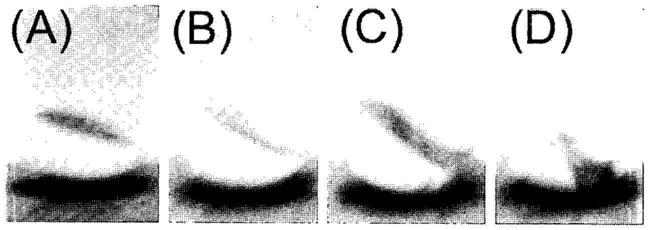

Fig. 2. Hydrodynamic profile of non-baffled test tubes (200x30 mm) filled with 4 ml water at shaking frequencies of 150 rpm (A), 200 rpm (B), 250 rpm (C), and 300 rpm (D). Pictures were taken by a digital camera.

complete coverage of the sensor. In baffled test tubes the turbulent mixing resulted in complete sensor coverage under all conditions tested.

Hydrodynamic Profiles in the Tubes

The hydrodynamic profile in non-baffled test tubes, was monitored using a digital camera. Figure 2 (A-D) shows the distribution of the liquid inside the tube for different shaking frequencies at a filling volume of 4 ml. During shaking, the liquid bulk rotated with an inclined surface, whereby the angle between tube wall and liquid significantly increased with increasing shaking rate. At 4 ml filling volume, the surface angle was determined as 17° (150 rpm), 27° (200 rpm), 38° (250 rpm), and 59° (300 rpm), respectively. The resulting surface angle at 5 ml was 21° (150 rpm), 26° (200 rpm), 35° (250 rpm), and 51° (300 rpm). Rather similar values resulted for 10 ml with 17° (150 rpm), 25° (200 rpm), 36° (250 rpm), and 54° (300 rpm). For 3 ml volume, the liquid angle was 21°, 34°, 45°, and 64° at the corresponding shaking rates between 150 and 300 rpm.

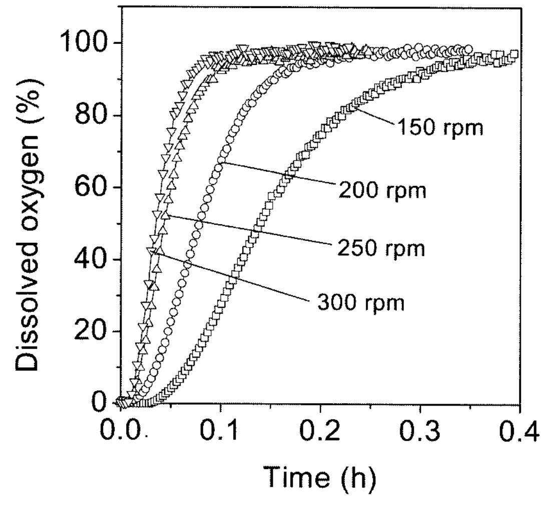

Fig. 3. Quantification of gas-liquid mass transfer in water-filled sensor test tubes via dynamic experiments with optical sensing of dissolved O_2: Time profile of dissolved O_2 at varied shaking with N_2 purging in the initial phase.

Table 1. Volumetric gas-liquid mass transfer coefficient (k_La) in non-baffled test tubes (200x30 mm) at varied shaking frequency and filling volume. The k_La is given in h^{-1} and was determined by dynamic experiments with optical sensing of dissolved oxygen.

	3 ml	4 ml	5 ml	10 ml
150 rpm	16±2	12±2	10±2	5±1
200 rpm	36±2	23±2	19±0	12±1
250 rpm	50±2	38±1	34±4	16±2
300 rpm	73±3	57±0	44±2	32±1

Quantification of Oxygen Transport

Figure 3 depicts time curves of dissolved O_2 signals during dynamic k_La experiments with 5 ml filling volume in non-baffled tubes at different shaking rates. Due to the high

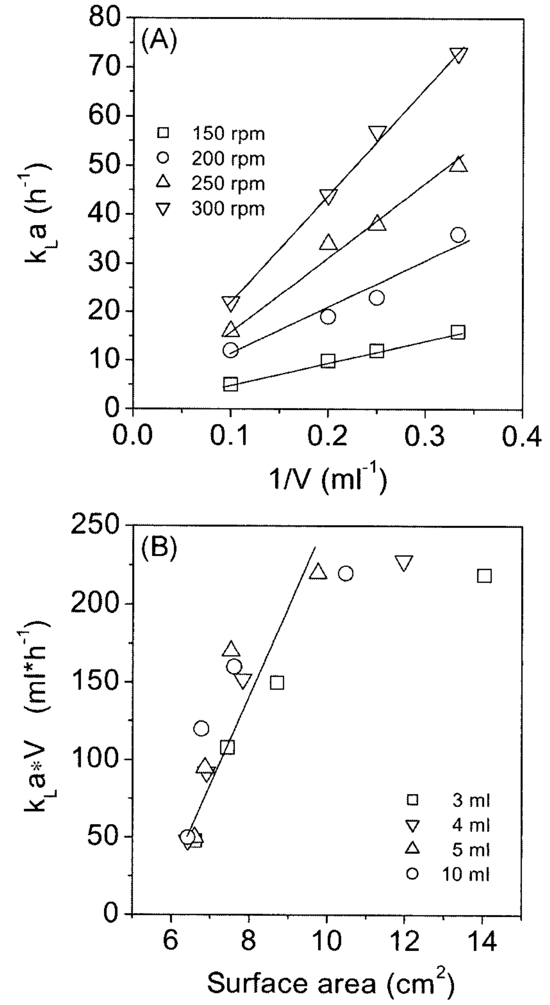

Fig. 4. Dependency of the gas-liquid mass transfer coefficient for O_2 (k_La) on operational parameters in non-baffled test tubes (200x30 mm).

Correlation of k_La with volume (V) at various shaking frequencies and filling volumes (A). Correlation of k_LA with the surface area (A) at various shaking frequencies and filling volumes (B).

measurement frequency with intervals of about 1 s, the time-dependent increase of dissolved O_2 after the stop of N_2 supply was very well resolved. It can be clearly seen that the velocity of the dissolved O_2 increase depended on the shaking rate, which indicates a higher O_2 transport capacity at elevated shaking. The sensor signal always converged to 100% O_2, underlining the consistency of the measurement. The determined k_La values are shown in Table 1. For all measurements, a good accordance between the two duplicates was achieved. The k_La values ranged between 5 h^{-1} for 10 ml and 150 rpm and 73 h^{-1} for 3 ml and 300 rpm. A linear increase of the O_2 transport capacity with increasing shaking speed could be identified. At 3 ml and 4 ml filling volumes, the k_La increased almost 5-fold in the range from 150 rpm to 300 rpm, whereas the increase for 5 ml and 10 ml was less pronounced. The k_La in the tubes was much less influenced by the shaking speed as compared to shake flasks, where k_La increased almost 30-fold between 100 and 300 rpm [10]. As observed for the shaking frequency, the filling volume also had an influence on oxygen transport. For each shaking frequency, the k_La value increased linearly with the reciprocal volume. This indicates a constant surface area for each shaking rate, which was independent on the volume in the tube (Fig. 4A). Obviously, the slope of the lines increased with the shaking frequency. This confirms visual observations of increased inclination of the liquid surface at higher shaking rate (Figs. 2A-D). To further describe the oxygen transport in the tubes, the surface area of the liquid $A_{surface}$ was calculated from the measured surface angle and the tube diameter (Eq. 1). The plot of the product $k_La * V = k_L * A_{surface}$ against $A_{surface}$ yielded a linear correlation for all experiments except at combinations of low volume and high shaking, where the assumption of an ellipse surface was no more fulfilled. Thus it can be concluded that, over a broad range, oxygen transfer in non-baffled shaken tubes is controlled by the size of surface area, which increases with increasing inclination of the surface. From the slope in Fig. 4B, the k_L-value can be calculated. If we assume that only $A_{surface}$ is contributing to mass transfer, an average value of about 4.5×10^{-5} m s^{-1} results. By rotation of the liquid bulk, a thin liquid film on the inner tube wall is formed. If we assume that this liquid film area on the tube wall (A_{film}) is also contributing to oxygen transfer, an average k_L-value of 2.1×10^{-5} m s^{-1} is obtained. The latter value is identical to the value reported for microtiter plates [5]. The higher value is closer to the value reported for surface aeration in mammalian cell cultivation, where a value of $k_L = 6.5 \times 10^{-5}$ m s^{-1} was found [3]. From these data we can, therefore, not draw clear conclusions whether the liquid film on the wall contributes to the oxygen transfer or not. The maximum transport coefficient k_La observed in non-baffled tubes was 70 h^{-1}. With an O_2 solubility of 235 µmol l^{-1} [8], maximum O_2 transfer capacity of about 15 mM h^{-1} can be calculated. For shaken devices, a low speed of rotation is recommended to avoid plug moistening [1]. Such a low rotation speed is clearly linked to significantly reduced O_2 transfer.

Table 2. Volumetric gas-liquid mass transfer coefficient (k_La) in four-fold baffled test tubes (200x30 mm) at varied shaking frequency and filling volume. The k_La is given in h^{-1} and was determined by dynamic experiments with optical sensing of dissolved oxygen.

	3 ml	4 ml	5 ml	10 ml
150 rpm	17±3	11±2	8±2	5±1
200 rpm	48±2	40±1	37±1	22±1
250 rpm	90±2	66±1	69±2	55±2
300 rpm	103±1	96±3	91±2	93±1

Table 3. Maximal volumetric gas-liquid mass transfer coefficients (k_La) in different small-scale cultivation devices.

Cultivation device	k_La (h^{-1})	Reference
Non-baffled test tube	70	This work
Baffled test tube	100	This work
Baffled shaken flask	150	[10]
96-well microtiter plate	130	[5]
Deep-well microplate	190	[2]

Improvement of Oxygen Transport

An increase of the oxygen transport capacity by an increase of the shaking frequency beyond 300 rpm seems not easily possible due to practical limitations of the available equipment. A further reduction of the liquid volume could lead to improved oxygen supply, but does not seem favorable due to a reduced volume for sampling. In order to improve oxygen transport in the tubes, additional experiments were carried out with baffled tubes (Table 2). The use of baffles resulted in 2- 3-fold larger values for k_La as compared to non-baffled tubes. Hereby, the highest benefit on k_La was found for high shaking rates and high filling volume. The maximum k_La of 103 h^{-1}, equal to a maximum O_2 transfer capacity of about 22 mM h^{-1}, was observed at 300 rpm and 3 ml volume.

The maximum transport coefficient k_La observed in non-baffled and baffled tubes of 70 h^{-1} and 100 h^{-1}, respectively, is below typical values observed in baffled shake flasks, 96-well microtiter plates, or deep-well microplates (Table 3).

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