Biochemical Engineering Journal 236 (2026) 110349

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Short communication

High-throughput determination of volumetric oxygen transfer coefficients (k_La) in microtiter plates using the BioLector system

Lukas Hartmann, Anke Neumann *

Karlsruhe Institute of Technology, Kaiserstr. 12, Karlsruhe 76131, Germany

ARTICLE INFO

Key words:
k_La
Oxygen transfer
Microtiter plate
High-throughput
BioLector

ABSTRACT

Characterization of cultivation parameters at screening scale is essential for transfer to production systems, particularly with respect to gas transfer efficiency, expressed as the volumetric oxygen transfer coefficient (k_La). In this work, the O_2 downregulation mode of the commercially available high-throughput screening platform BioLector was repurposed and shown to be suitable for determining k_La using the physical gassing-out method. As an example, the influence of liquid filling volume in microtiter plates, shaking frequency and aeration rate on k_La was investigated, resulting in values of up to 6 h^{-1} under the tested conditions. Overall, this semi-automated approach allows k_La characterization with little manual effort and supports process transfer between cultivation systems by enabling oxygen transfer conditions in BioLector experiments to be considered during scale translation.

1. Introduction

Process transfer between screening and production platforms is a routine task in bioprocess engineering. Achieving comparable process performance across scales, from shaken to stirred bioreactors, requires consideration of various factors such as power input and gas-liquid mass transfer [1,2]. In particular, for cultivations with specific demand for gaseous substrates, such as oxygen, knowledge and maintenance of gas transfer efficiency, described by the volumetric gas transfer coefficient k_La, are essential to ensure transferability of process performance from screening to production platforms, yet this parameter is frequently overlooked in screening experiments conducted in microtiter plates (MTP) and shake flasks [3–6].

Among the available approaches, the dynamic gassing-out method is one of the most widely used methods for k_La determination [2,7]. This biological approach determines k_La under oxygen-consuming conditions by temporarily interrupting aeration to estimate the cellular oxygen uptake rate and evaluating the dissolved oxygen tension (DOT) increase during re-aeration [8]. In contrast, the physical gassing-out method determines k_La starting from oxygen-free conditions by prior deoxygenation of the liquid, typically using an inert gas, and derives k_La from the DOT increase during reoxygenation [9]. This approach has previously been demonstrated in MTP systems by Fisher et al. [10]. The time efficiency of performing the physical gassing-out method depends on the rate of de- and reoxygenation and on whether the transition between oxygen-containing and inert gas is performed manually or automatically.

Real-time monitoring of microbial cultivations in shaken MTP was enabled by an approach originally introduced by Samorski et al., which was later translated into the commercially available BioLector (m2p-labs, Aachen, Germany; now part of Beckman Coulter Life Sciences, Brea, CA, USA) [11]. This screening platform allows online monitoring of biomass via backscatter, fluorescence, pH, and DOT during incubation. In addition, multiple gas supply modes designed for biological process control, including air supply for standard aerobic cultivations, anaerobic operation with N_2 for anaerobic cultivations, CO_2 upregulation for CO_2-dependent metabolisms, O_2 upregulation for high-oxygen-demand microorganisms and O_2 downregulation via air and N_2 mixing to establish microaerobic environments, are provided.

Here, we demonstrate that the gas supply mode originally intended for O_2 downregulation can be repurposed for k_La determination by applying the physical gassing-out method. By programming time-dependent changes in the oxygen fraction via the BioLector control software, deoxygenation and reoxygenation can be executed in an automated and reproducible manner. This repurposing enables semi-automated k_La determination in shaken MTP, while allowing dynamic variation of temperature, aeration rate and shaking frequency within a single structured protocol, as well as adjustment of filling volumes

* Corresponding author.
E-mail addresses: lukas.hartmann@kit.edu (L. Hartmann), anke.neumann@kit.edu (A. Neumann).

https://doi.org/10.1016/j.bej.2026.110349
Received 30 April 2026; Received in revised form 23 July 2026; Accepted 4 August 2026
Available online 5 August 2026

across the MTP.

2. Methods

2.1. Media

The composition of the incubation medium, developed as a production medium for malic acid with *Aspergillus oryzae*, was previously described by Kövilein et al., modified by Hartmann et al. and consisted of 120 g L^{-1} glucose monohydrate, 1.2 g L^{-1} $(NH_4)_2SO_4$, 0.1 g L^{-1} KH_2PO_4, 0.17 g L^{-1} K_2HPO_4, 0.1 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.1 g L^{-1} $CaCl_2{\cdot}2H_2O$, 5 mg L^{-1} NaCl, 60 mg L^{-1} $FeSO_4{\cdot}7H_2O$, 44 mg L^{-1} $ZnSO_4{\cdot}7H_2O$, and 20 µg L^{-1} biotin [12,13]. All components were prepared using demineralized water. Glucose monohydrate as well as the salt solution containing $(NH_4)_2SO_4$, KH_2PO_4, K_2HPO_4, NaCl and the antifoaming agent Contraspum A 4050 HAc (Zschimmer & Schwarz GmbH, Lahnstein, Germany) were sterilized separately by autoclaving at 121 °C, 1 bar for 20 min. The antifoam agent was added to the salt solution prior to autoclaving, corresponding to 100 µL for a total medium volume of 1.4 L. Stock solutions of $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}2H_2O$, $FeSO_4{\cdot}7H_2O$, $ZnSO_4{\cdot}7H_2O$ and biotin were prepared separately, sterilized by filtration with a pore diameter of 0.2 µm and added aseptically after cooling. The $FeSO_4{\cdot}7H_2O$ stock solution was prepared by dissolving it in 0.1 M H_2SO_4. The pH of the complete medium was adjusted to 7.00 ± 0.05 using NaOH and H_3PO_4 at 35 °C prior to dispensing. Depending on the experimental condition shown in Fig. 1 and Fig. 2, different liquid filling volumes were dispensed into the MTP wells.

2.2. BioLector set-up and operating conditions

Experiments were performed using a BioLector system (BioLector XT with Microfluidic XT), with protocols defined via the BioLection software (V. 5.7.1.0). The 48-well FlowerPlate MTP (M2P-MTP−48-BOH2) was aseptically filled and sealed according to the manufacturer's instructions using a gas-permeable foil (M2P-F-GP−10) and a silicone sealing foil (M2P-F-RSXT−10). Humidity control was enabled during incubation, while temperature was maintained constant at 35 °C, the orbital diameter was 3 mm and both shaking frequency and aeration rate during O_2 downregulation gassing were varied according to the experimental protocol. DOT was measured with optodes and expressed as a percentage, where 100% corresponds to saturation with pure oxygen in the gas phase. Accordingly, air saturation corresponds to approximately 21% of this value.

2.3. Automated deoxygenation-reoxygenation protocol

At the beginning of the automated protocol, as shown in Table 1, the O_2 downregulation (air:N_2) gassing mode was selected. Subsequently, shaking frequency and aeration rate were adjusted to the maximum values permitted for the respective filling volumes to ensure that air saturation was reached within 2 h. The oxygen fraction was then set to the minimum achievable value of 1% while maintaining maximum shaking frequency and aeration rate to achieve rapid deoxygenation. Thereafter, shaking frequency and aeration rate were set to the values for which k_La was to be determined, and the oxygen fraction was reset to 21% to initiate reoxygenation. Subsequently, deoxygenation and reoxygenation phases were alternated, with deoxygenation performed for 1 h under the previously described conditions followed by 1 h of reoxygenation.

Since the minimum oxygen fraction achievable in the BioLector O_2 downregulation mode was 1%, complete deoxygenation was not achieved prior to reoxygenation; however, k_La was determined from the slope of the linearized reoxygenation curve and was therefore independent of the initial DOT level.

2.4. Methodological considerations

Gas exchange occurs via the gas-permeable sealing foil covering the microtiter plate, while the wells are not directly sparged, resulting in an aeration principle comparable to shake flask cultivation. The gas atmosphere is distributed across the plate chamber, and potential mass transfer resistance introduced by the sealing foil is inherently included in the determined k_La values.

A potential source of systematic deviation arises from the upstream water bottle used for gas humidification, which can act as an additional oxygen buffer during deoxygenation and reoxygenation. This effect was assessed by comparing k_La measurements obtained with dry and humidified air under identical operating conditions. At an aeration rate of 100 mL min^{-1} and a shaking frequency of 900 rpm, k_La values determined with dry air were approximately 5% higher than those obtained

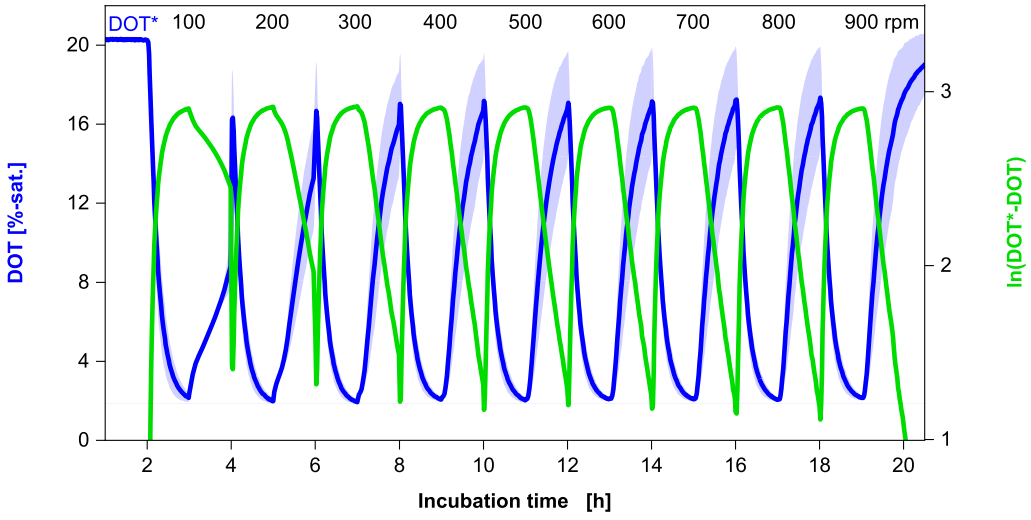

Fig. 1. Automated alternating deoxygenation and reoxygenation sequence used for k_La determination at successive shaking frequencies. Incubation in 48-well FlowerPlate MTP with 0.6 mL, pH 7.00, 35 °C and an orbital diameter of 3 mm, with 25 mL min^{-1} air during 1 h oxygenation at the indicated shaking frequencies and 100 mL min^{-1} air:N_2 mixture containing 1% O_2 at 1300 rpm during 1 h deoxygenation. Humidity control was enabled. k_La was determined from the linear phase of ln(DOT*-DOT) following the switch to air. Data represent means ± standard deviations of 5 replicate wells. Standard deviations are illustrated only for DOT measurements and are shown as blue shaded areas.

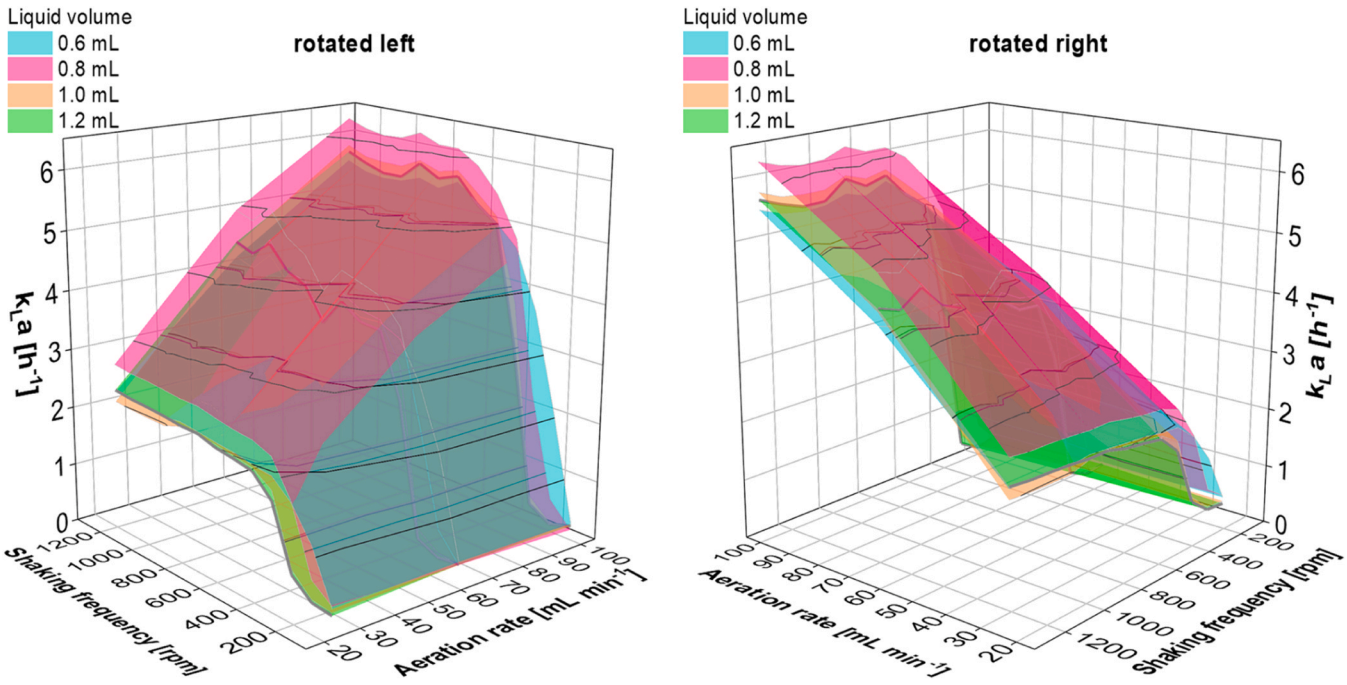

Fig. 2. k_La as a function of shaking frequency and aeration rate for different filling volumes determined using the O_2 downregulation method in 48-well FlowerPlate MTP. Incubation with pH 7.00, 35 °C, with an orbital diameter of 3 mm, with air during 1 h oxygenation at the indicated shaking frequencies and aeration rates and 100 mL min^{-1} air:N_2 mixture containing 1% O_2 at 1300 rpm during 1 h deoxygenation. Humidity control was enabled. 13 shaking frequencies ranging from 100 to 1300 rpm were tested in combination with 4 filling volumes and 3 aeration rates of 25, 50 and 100 mL min^{-1} air, resulting in a total of 156 conditions with 5–6 replicates per condition. Both graphs show the same dataset rotated to enhance visualization of the multidimensional parameter space. Data represent means of replicates.

Table 1
Schematic representation of the automated deoxygenation-reoxygenation protocol. Within the BioLection software, humidity control was enabled and temperature was set to a constant value. For the gassing mode, O_2 downregulation using an air:N_2 mixture was selected. Shaking frequency and O_2 concentration in the inlet gas were set as variable values. The protocol shown corresponds to the measurements presented in Fig. 1.

Time of shaking [h]	Shaking frequency [rpm]	Time of aeration [h]	Aeration rate [mL min^{-1}]	Concentration of O_2 [%]
0	1300	0	100	21
2	1300	2	100	1
3	100	3	25	21
4	1300	4	100	1
5	200	5	25	21
6	1300	6	100	1
7	300	7	25	21
8	1300	8	100	1
9	400	9	25	21
10	1300	10	100	1
11	500	11	25	21
12	1300	12	100	1
13	600	13	25	21
14	1300	14	100	1
15	700	15	25	21
16	1300	16	100	1
17	800	17	25	21
18	1300	18	100	1
19	900	19	25	21

with humidified air, a deviation that was accepted in the following measurements to minimize evaporation.

2.5. Calculation of k_La

The calculation of k_La from the increase in DOT was performed during the reoxygenation phase according to the description provided by Hartmann et al. [14]. DOT*, as shown in Eq. 1, represents the DOT value at air saturation and is determined at the end of the initial 2 h oxygenation phase. Accordingly, plotting ln(DOT* − DOT) versus time t during reoxygenation results in a linear relationship with a slope corresponding to $-k_La$.

$$\ln(DOT^* - DOT) = -k_L a\, t + \ln(DOT^*) \quad (1)$$

All data processing was performed using Microsoft Excel (V. 2108, Microsoft Corporation, Redmond, WA, USA).

3. Results & Discussion

Fig. 1 illustrates the determination of k_La as an example for a specific set of operating conditions. During the first 2 h, the system was operated at maximum shaking frequency and aeration rate to ensure that the DOT value at air saturation DOT* was reached within the predefined time frame. Subsequently, alternating deoxygenation and reoxygenation cycles with a duration of 1 h each were applied. At low shaking frequencies of 100 and 200 rpm, the DOT initially increased only slowly before exhibiting a sudden rise. This behavior is likely caused by insufficient bulk mixing at low shaking frequencies, resulting in diffusion-dominated oxygen transport rather than convective mass transfer. Since the wells were not directly sparged and oxygen transfer occurred via surface aeration from the well headspace, an oxygen concentration gradient likely developed within the liquid column under these conditions. The delayed increase in the measured DOT likely reflects the time required for this diffusion front to reach the sensor location at the bottom of the well.

Fig. 2 shows the multidimensional dependence of k_La on shaking frequency, aeration rate and liquid filling volume in a 48-well MTP.

With respect to shaking frequency, k_La was very low at low shaking frequencies, indicating diffusion-dominated oxygen transfer due to limited liquid motion. A pronounced increase in k_La occurred above 500 rpm, followed by a plateau at higher shaking frequencies. This behavior suggests that the effective gas-liquid interfacial area reaches a saturation limit at high shaking frequencies, rendering further increases in shaking frequency ineffective for enhancing oxygen transfer. Another plausible explanation is that the observed plateau is influenced by hydrodynamic effects related to out-of-phase operating conditions, as originally described by Büchs et al., in which the liquid no longer follows the shaker motion in phase, resulting in a reduced effective transfer of mechanical energy to the liquid [15]. Such conditions have been reported to occur at higher shaking frequencies, which correspond to the regime in which the k_La plateau was observed in the present study, in combination with small shaking diameters, as used in the BioLector system, baffle-like vessel geometries, as mimicked by the FlowerPlate well design, and increased liquid viscosity, which is plausible due to the high glucose concentration of the medium. In contrast to the plateau in k_La observed at higher shaking frequencies, k_La increased with aeration rate across the entire investigated range without reaching a plateau at the maximum aeration settings of the device. With respect to liquid filling volume, overall differences in k_La were small across the investigated parameter space. At low shaking frequencies, however, the smallest filling volume of 0.6 mL exhibited slightly higher k_La values than larger volumes, likely because even low shaking frequencies were sufficient to induce slow liquid motion at smaller volumes, whereas larger filling volumes remained largely diffusion-dominated.

Previous studies report k_La values covering a wide numerical range, including approximately 4–88 h^{-1} reported by Fisher et al. and 10–40 h^{-1} reported by John et al., with which the values determined in the present study partially overlap, supporting the plausibility of the obtained k_La range; however, their data were obtained using different cultivation devices and determination methods and are therefore not directly comparable [10,16–19].

4. Conclusion

This study presents a rapid approach for k_La determination in the BioLector screening platform that requires only minimal operational effort. By repurposing the O_2 downregulation gassing mode, k_La can be determined within a semi-automated workflow without additional hardware or complex experimental procedures. The method enables systematic and high-throughput characterization of oxygen transfer across multiple operating parameters, facilitating improved comparability between screening and larger-scale cultivation platforms.

CRediT authorship contribution statement

Lukas Hartmann: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Anke Neumann:** Writing – review & editing, Supervision, Conceptualization.

Ethics statement

The authors have nothing to report.

Patient consent statement

Not applicable.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (OpenAI, San Francisco, California, United States) in order to improve language quality and clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Permission to reproduce material from other sources

Not applicable.

Clinical trial registration

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors received no external funding.

Data Availability

Data will be made available on request.

References

[1] J. Büchs, et al., Power consumption in shaking flasks on rotary shaking machines: I. Power consumption measurement in unbaffled flasks at low liquid viscosity, Biotechnol. Bioeng. 68 (6) (2000) 589–593, https://doi.org/10.1002/(SICI)1097-0290(20000620)68:63.0.CO;2-J.
[2] F. Garcia-Ochoa, E. Gomez, Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview, Biotechnol. Adv. 27 (2) (2009) 153–176, https://doi.org/10.1016/j.biotechadv.2008.10.006.
[3] J. Büchs, Introduction to advantages and problems of shaken cultures, Biochem. Eng. J. 7 (2) (2001) 91–98, https://doi.org/10.1016/S1369-703X(00)00106-6.
[4] H.F. Zimmermann, et al., Oxygen limitation is a pitfall during screening for industrial strains, Appl. Microbiol. Biotechnol. 72 (6) (2006) 1157–1160, https://doi.org/10.1007/s00253-006-0414-6.
[5] W.A. Duetz, et al., Methods for intense aeration, growth, storage, and replication of bacterial strains in microtiter plates, Appl. Environ. Microbiol. 66 (6) (2000) 2641–2646, https://doi.org/10.1128/AEM.66.6.2641-2646.2000.
[6] W. Klöckner, J. Büchs, Advances in shaking technologies, Trends Biotechnol. 30 (6) (2012) 307–314, https://doi.org/10.1016/j.tibtech.2012.03.001.
[7] A.L. Damiani, M.H. Kim, J. Wang, An improved dynamic method to measure k_La in bioreactors, Biotechnol. Bioeng. 111 (10) (2014) 2120–2125, https://doi.org/10.1002/bit.25258.
[8] G. Rao, Dynamic measurement of the volumetric oxygen transfer coefficient in fermentation systems, Biotechnol. Bioeng. 104 (5) (2009) 841–853, https://doi.org/10.1002/bit.22566.
[9] W.S. Wise, The measurement of the aeration of culture media, J. Gen. Microbiol. 5 (1) (1951) 167–177, https://doi.org/10.1099/00221287-5-1-167.
[10] J.T. Fisher, et al., Mixing and oxygen transfer characteristics of a microplate bioreactor with surface-attached microposts, Biotechnol. J. 16 (5) (2021) e2000257, https://doi.org/10.1002/biot.202000257.
[11] M. Samorski, G. Müller-Newen, J. Büchs, Quasi-continuous combined scattered light and fluorescence measurements: a novel measurement technique for shaken microtiter plates, Biotechnol. Bioeng. 92 (1) (2005) 61–68, https://doi.org/10.1002/bit.20573.
[12] A. Kövilein, et al., Immobilization of *Aspergillus oryzae* DSM 1863 for L-Malic Acid Production, Fermentation 8 (1) (2022) 26, https://doi.org/10.3390/fermentation8010026.
[13] L. Hartmann, et al., Advancing malic acid production of *Aspergillus oryzae*: combined process optimization and application of various substrates, Biotechnol. Bioeng. 123 (6) (2026) 1634–1647, https://doi.org/10.1002/bit.70201.
[14] L. Hartmann, et al., Influence of Zn^{2+} and oxygen supply on malic acid production and growth of *Aspergillus oryzae*, Biotechnol. Bioeng. 123 (5) (2026) 1123–1138, https://doi.org/10.1002/bit.70160.
[15] J. Büchs, S. Lotter, C. Milbradt, Out-of-phase operating conditions, a hitherto unknown phenomenon in shaking bioreactors, Biochem. Eng. J. 7 (2) (2001) 135–141, https://doi.org/10.1016/s1369-703x(00)00113-3.
[16] G.T. John, et al., Integrated optical sensing of dissolved oxygen in microtiter plates: a novel tool for microbial cultivation, Biotechnol. Bioeng. 81 (7) (2003) 829–836, https://doi.org/10.1002/bit.10534.

[17] M. Funke, et al., The baffled microtiter plate: increased oxygen transfer and improved online monitoring in small scale fermentations, Biotechnol. Bioeng. 103 (6) (2009) 1118–1128, https://doi.org/10.1002/bit.22341.
[18] F. Kensy, C. Engelbrecht, J. Büchs, Scale-up from microtiter plate to laboratory fermenter: evaluation by online monitoring techniques of growth and protein expression in Escherichia coli and Hansenula polymorpha fermentations, Microb. Cell. Fact. 8 (2009) 68, https://doi.org/10.1186/1475-2859-8-68.
[19] F. Kensy, et al., Oxygen transfer phenomena in 48-well microtiter plates: determination by optical monitoring of sulfite oxidation and verification by real-time measurement during microbial growth, Biotechnol. Bioeng. 89 (6) (2005) 698–708, https://doi.org/10.1002/bit.20373.