Supplementary Material (ESI) for Lab on a Chip

Electronic Supplementary Information:

Microbioreactor arrays with integrated mixers and fluid injectors for high-throughput experimentation with pH and dissolved oxygen control

Harry L. T. Lee, Paolo Boccazzi, Rajeev J. Ram, and Anthony J. Sinskey

Measurement Techniques

Optical density (OD) and cell density measurement. Online optical density measurements were taken using two 1mm diameter PMMA optical fibers (Industrial Fiber Optics, IF-C-U1000). Excitation was provided by 650nm LEDs square wave modulated at 4kHz. The magnitude of the transmitted light was measured using the same phase sensitive detection algorithm used for the fluorescence sensors. The numerical aperture of the collection fibers were reduced by two CNC machined 500µm pinholes approximately 1cm apart, inserted into the fiber mounts constructed using 1mm i.d. hypodermic tubing (Small parts, HTX-17R). This minimized the detection of scattered light, which would cause a deviation from the linear Lambert-Beer relationship between density of scatterers (cells) and optical density.[1] With the pinholes, the optical density, scaled to a 1cm path length was found to be linear up to an OD of 40.

Because the devices were made entirely from PDMS, face sealing to the actuation manifold compressed the growth wells slightly, and caused variations in the optical path length. For this reason, optical density measurements were calibrated with an endpoint measurement from samples removed at the end of the bioreactions. The endpoint samples were diluted 40:1 and the optical density measured using a collimated 650nm diode laser and photodetector and a cuvette with a 1cm path length.

Calibration between optical density and dry cell weight (dcw) was performed by taking four 50mL samples from a 4L bioreactor, measuring the optical densities, and then centrifuging and resuspending the cells in water three times. After drying more than 24 hours in a vacuum oven, the dishes were weighed giving a conversion factor of 0.33±0.006 g-dcw/OD.

$$\mu = \frac{1}{OD}\frac{dOD}{dt} \tag{1}$$

Growth rate calculation. The growth rate was calculated from the optical density through

The optical density data was first low-pass filtered with an FIR filter[2] to reduce the noise. The derivative of the optical density curve was estimated with a four point discrete time approximation to the derivative. The doubling time was calculated as the reciprocal of the growth rate multiplied by the natural logarithm of two. The group delay of the digital filters was accounted for to produce the appropriate alignment with the time axis.

Dissolved oxygen measurement. Dissolved oxygen sensors were fabricated[3] using platinum-octaethyl-porphyrine in polystyrene. 5mm diameter glass coverslips (Warner Instruments, CS-5R) were first etched with glass etchant (Armour, 15-0150) to roughen the surface. A 1:1 mixture of a solution of 3% (w/w) polystyrene (Sigma Aldrich, 430102-1KG) in toluene (Sigma Aldrich, 244511-100ML) and a solution of saturated PtOEP (Frontier Scientific, PtO534) in toluene was the sensor cocktail. 3µL of the cocktail was deposited on each roughened coverslip and the solvent was allowed to evaporated, completing the sensor fabrication.

Fluorescence quenching was measured by monitoring the phase shift[4] of the fluorescence (650nm) at 5kHz with respect to the excitation (380nm). Calibration curves capturing the nonlinear relationship between the phase shift and dissolved oxygen concentration were generated by measuring the phase shift for known dissolved oxygen concentrations, generated by mixing different ratios of aerated and deaerated water. Calibration of the sensors in the bioreactor devices were performed using a two point calibration at 100% saturation in air at the beginning of the cell culture, and 0% at the end of the cell culture by mixing with nitrogen. The response time of the sensor was less than one second.

Oxygen transfer coefficient measurement. Oxygen transfer coefficient measurements were performed using a dynamic

$$\frac{dC}{dt} = k_L a(C^* - C) \tag{2}$$

gassing procedure[5]. This technique is based on measuring the time response of the dissolved oxygen due to step changes in the oxygen concentration of the aeration gas which is modeled by where C is the dissolved oxygen concentration and C^* is the oxygen saturation concentration in equilibrium with the aeration gas. The mixing devices were mixed with nitrogen as the actuation gas until the dissolved oxygen concentration in all of the wells approached zero, at which point the actuation gas was switched to air. The dissolved oxygen concentration was sampled every 3 seconds and the resulting data fit to an exponential function to estimate the oxygen transfer coefficient.

Measurements were performed on mixing devices fabricated with the bottom side entirely of glass. This was important otherwise the dissolved oxygen concentration did not follow a single exponential time dependence due to oxygen diffusion and absorption in the bulk of the PDMS device, which generated a long tail in the response. In this case, the k_La measured by dynamic gassing would not be well defined and would not be representative of the maximum oxygen uptake rate that could be supported.

pH measurement. The pH was measured using commercial fluorescent sensor spots (Presens, HP5). These sensors have a linear range between 5.5 and 7.5 and are usable from pH 5 and 8. The excitation LEDs (470nm) were modulated at 44 kHz and the relative phase shift of the fluorescence (530nm-700nm) for different pH was calibrated using a series of buffer solutions. Calibration curves were transferable to different sensor spots from the same lot and therefore no individual calibrations were carried out in the bioreactor devices.

Oxygen Transfer Coefficient Calculation

$$\frac{dC}{dt} = k_L a(C^* - C) - OUR \tag{3}$$

The definition of the oxygen transfer coefficient assumes a perfectly mixed bulk liquid and a liquid film model for air bubbles circulating in the medium. The dissolved oxygen concentration, C, is modeled with the following equation.
Where the k_La captures the liquid film thickness, diffusion coefficient, and total surface area of bubbles per unit volume, C* is the saturation concentration in the liquid for a given oxygen partial pressure, and OUR is the oxygen uptake rate. From Eq. (3), we can see that measurement of the k_La using a dynamic gassing method[5] also indicates the maximum oxygen uptake rate, $OUR_{max} = k_LaC^*$, that can be supported in steady state before oxygen limitation (C≈0), which is the biologically

$$C(t) = C^*(1 - e^{-k_L a_{dg} t}) \tag{4}$$

relevant parameter.

$$k_L a_{ss} = \frac{OUR_{max}}{C^*} \tag{5}$$

The equivalence between the k_La measured using a dynamic gassing method, k_La_{dg}, through fitting the measured dissolved oxygen for a step change in C* to a function of the form

$$\frac{\delta C}{\delta t} = \frac{\delta}{\delta z}\left(D(z)\frac{\delta C}{\delta z}\right) - OUR \tag{6}$$

and the k_La as defined by solving Eq. (3) in steady state,
is not generally true for flat form factor bioreactors, where the oxygen concentration is not uniform inside the liquid. In this case, the appropriate physical model is
Where D(z) is the diffusion coefficient of PDMS (-d<z<0) or water (0<z<L).

The k_La extracted from a dynamic gassing measurement, k_La_{dg}, can be calculated by reducing Eq. (6) into Eq. (3) through

$$k_L a_{dg} = D_w \lambda^2 \tag{7}$$

an eigenmode expansion, keeping only the lowest order mode. For a PDMS membrane of thickness d, a water layer of

$$C_i(z) = \begin{cases} a_{i1}\cos(\lambda_i z) + a_{i2}\sin(\lambda_i z) & 0 < z < L \quad (8a) \\ b_{i1}\cos(\sqrt{\frac{D_w}{D_p}}\lambda_i z) + b_{i2}\sin(\sqrt{\frac{D_w}{D_p}}\lambda_i z) & -d < z < 0 \quad (8b) \end{cases}$$

thickness L, and a glass, oxygen impermeable base (Fig. 1), we

$$\sqrt{\frac{D_w}{D_p}} K \tan(\lambda L)\tan(\sqrt{\frac{D_w}{D_p}}\lambda d) = 1 \tag{9}$$

have

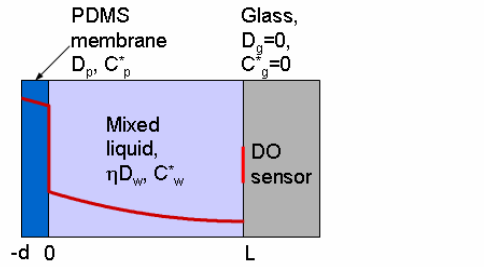

Fig. 1 Flat bioreactor model with PDMS membrane, growth well., and oxygen impermeable base.

where λ is the smallest eigenvalue[6] corresponding to eigenfunction solutions of the form
Satisfying the boundary conditions determines λ, through
where D_w (2.19×10^{-5} cm^2/s)[7] is the diffusion coefficient of oxygen in water, D_p (2.15×10^{-5} cm^2/s)[8,9] is the diffusion coefficient of oxygen in PDMS, and K (0.3) is the partition

$$k_L a_{dg} \approx \left(\frac{\pi}{2}\right)^2 \frac{D_w}{L^2} \tag{10}$$

coefficient, or ratio of oxygen saturation concentrations in

$$k_L a_{dg} \approx \frac{(D_p/K)}{Ld} \tag{11}$$

water, C^*, (0.27mM)[10] and PDMS, C^*_p, (0.9mM)[11] in an oxygen partial pressure of 0.21atm.

Two limiting cases are amenable to an analytical solution. When diffusion through water (L>>d) is dominant we have

$$k_L a_{ss} = \frac{1}{\frac{Ld}{(D_p/K)} + \frac{L^2}{2D_w}} \tag{12}$$

and for perfect, instantaneous mixing ($D_w \rightarrow \infty$)

To calculate k_La_{ss} as defined in Eq. (5) where in this case,

$$k_L a_{\underset{\text{mem}}{ss}} = \frac{(D_p/K)}{Ld} \tag{13}$$

OUR_{max} is the OUR where the minimum steady state oxygen

$$k_L a_{\underset{\text{diff}}{ss}} = 2\frac{D_w}{L^2} \tag{14}$$

concentration equals zero (z=L) we solve Eq. (6) in steady state resulting in
which can be interpreted as the parallel sum of the k_La_{ss} due to the membrane
and diffusion
which agrees with the limiting cases from Eq. (10) and Eq. (11). This is important because it indicates that k_La measurements using dynamic gassing are representative of the k_La derived under steady state conditions, which indicate the maximum oxygen uptake rate that can be supported.

Supplementary Material (ESI) for Lab on a Chip

Notes and references

1 N. L. Swanson, B. D. Billard, and T. L. Gennaro, *Appl. Opt.,* 1999, **38**, 5887-5893.

2 A. V. Oppenheim, R. W. Schafer, *Discrete-Time Signal Processing*, Prentice Hall, Upper Saddle River, 2nd edn., 1999.

3 D. B. Papkovsky, *Sens. Actuators, B,* 1995, **29**, 213-218.

4 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Kluwer Academic, Boston, 2nd edn., 1999, ch. 5, pp. 141-184.

5 V. Linek, P. Benes, and V. Vacek, *Chem. Eng. Technol.*, 1989, **12**, 213-217.

6 Successive eigenvalues were larger by a factor of (2n+1).

7 C. E. St. Denis, and C. J. D. Fell, *Can. J. Chem. Eng.*, 1971, **49**, 885.

8 X. Lu, I. Manners, and M. A. Winnik, *Macromolecules*, 2001, **34**, 1917-1927.

9 D_p was measured independently for Sylgard 184 by fitting the time response of the oxygen concentration at the bottom of 660μm and 1525μm thick slabs of Sylgard 184 to finite difference calculated responses. $D_p = 2.1 \times 10^{-5} cm^2/s$ was the best fit.

10 E. Douglas, *J. Phys. Chem.*, 1964, **68**, 169-174.

11 No measurements of C^*_p for Sylgard 184, a vinyl terminated, silica filled PDMS, were available. The best fit to the data $C^*_p = 9 \times 10^{-4} M$ at 0.21atm O_2, was consistent with reduced O_2 saturation in silica filled PDMS[8] and measured values for pure PDMS[12].

12 T. C. Merkel, V. I. Bondar, K. Nagal, B. D. Freeman, I. Pinnau, *J Polym. Sc. Pt. B-Polym Phys.*, 2000, **38**, 415-434.