Electronic Supplementary Material (ESI) for RSC Advances.

# Supplementary material to

# Insufficient oxygen diffusion leads to distortions of microbial growth parameters assessed by isothermal microcalorimetry

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## 1. General applicable $Y_{O/B}$

In the main paper the ratio of mole oxygen consumed per mole biomass formed $Y_{O/B}$ is of crucial importance to simulate the influence of oxygen bioavailability on calorimetric signals. Bacterial growth on any carbon substrate with the composition of $CH_{SH}O_{SO}$ can be described by a simple stoichiometric equation (S1).

$$Y_{S/B}\, CH_{SH}O_{SO} + 0.25\, NH_3 + Y_{O/B}\, O_2 \rightarrow CH_{1.70}O_{0.42}N_{0.25} + Y_{H2O/B}\, H_2O + Y_{CO2/B}\, CO_2 \qquad \text{(S1)}$$

$Y_{i/B}$ are yield coefficients describing the number of moles of the substances $i$ consumed (negative sign) or formed (positive sign) per one mole of formed biomass. $SH$, $SO$ describe the number of hydrogen and oxygen atoms per carbon atom in the carbon-substrate molecule $CH_{SH}O_{SO}$. $CH_{1.70}O_{0.42}N_{0.25}$ is a general equation describing bacterial biomass [1]. The yield coefficients are not independent on each other, as they have to fulfill the elementary balances. Taking this into consideration, $Y_{O/B}$ depends only on $Y_{S/B}$ (eq. S2).

$$Y_{O/B} = Y_{S/B}\left(\frac{SH}{4} - \frac{SO}{2} + 1\right) - 1.0275 \qquad \text{(S2)}$$

$Y_{S/B}$ is determined by the energy content of the substrate [2-5] ,which in turn depends on the relative degree of reduction of the substrate $\gamma_S$ [6]. Equation S3 describes the simplest relation between $\gamma_S$ and $Y_{S/B}$ [7].

$$\begin{aligned} Y_{S/B} &= \frac{7.69}{\gamma_S} \quad if \quad \gamma_S \leq 4.67 \\ Y_{S/B} &= 1.67 \quad if \quad \gamma_S > 4.67 \end{aligned} \qquad \text{with } \gamma_S = 4 + SH - 2\,SO \qquad \text{(S3)}$$

A general valid $Y_{O/B}$ can be obtained (eq. S4) by combining eqs. S2 and S3:

$$\begin{aligned} Y_{O/B} &= 0.897 && if \quad \gamma_S \leq 4.67 \\ Y_{O/B} &= 0.417\, SH - 0.835\, SO + 0.642 && if \quad \gamma_S > 4.67 \end{aligned} \qquad \text{(S4)}$$

This means that $Y_{O/B}$ is independent on the carbon-substrate and the considered strain for all carbon-substrates with $\gamma_S \leq 4.67$. This holds true for carbohydrates, 70% of amino acids, many proteins, many organic acids etc. The most calorimetric measurements discussed in the main paper follow growth on carbon-substrates with $\gamma_S \leq 4.67$. Thus, it is justified to apply $Y_{O/B} = 0.897$ for simulating and discussing the influence of oxygen bioavailability on calorimetric measurements in closed ampoules. Note, for degradation experiments of PHAs' of oil contaminants or growth experiments on simple alcohols or $CH_4$ the second case $Y_{O/B} = 0.417\, SH - 0.835\, SO + 0.642$ has to be considered.

## 2. Analytical solutions of the oxygen balance

The bacterial oxygen consumption rate can be described by Monod-type kinetics (eq. S5)

$$R_O = Y_{O/B}\, B\, \mu_{\max} \frac{O}{k_0 + O} \qquad \text{(S5)}$$

$R_O$, $Y_{O/B}$, $\mu_{max}$, $k_O$, $O$ are the oxygen consumption rate, yield coefficient, maximum specific growth rate, half saturation constant and oxygen concentration, respectively. The oxygen consumption rate becomes independent of the oxygen concentration and therefore of oxygen diffusion, if $k_O$ approaches to very small values ($k_O << O$). As a consequence, the respiratory bacterial growth is independent of oxygen diffusion. The test of this hypothesis using numerical solutions of mathematical models describing the oxygen balance often fails due to the very small values of $k_O$ in comparison to $O$. Analytical solutions of the mass balance equation could potentially overcome that limitation.

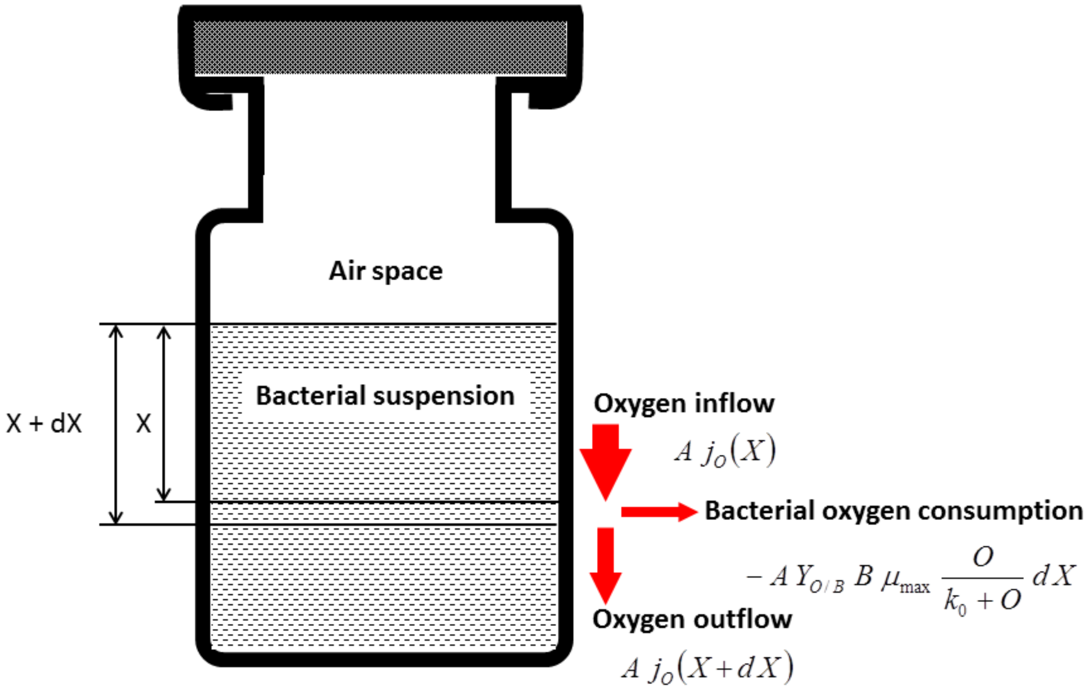

Figure S1: Oxygen balance for a slice of infinite small thickness $dX$ in the depth of $X$ in a calorimetric ampoule.

Figure S1 illustrates the oxygen balance for a slice of infinite small thickness. Eq. S6 is the mathematical description of this oxygen balance.

$$\dot{n}_O = A\left( j_O(X) - j_O(X+dX) + Y_{O/B}\, B\, \mu_{\max} \frac{O}{k_0 + O} dX \right) \qquad with \qquad j_O = -D\frac{dO}{dX} \tag{S6}$$

$\dot{n}_O$, *A, $j_O$, D* are the oxygen accumulation, the oxygen exchanging surface of the slice, the oxygen flow, and the diffusion constant, respectively. $\dot{n}_O = 0$ can be assumed at steady state and eq. S3 results.

$$\frac{\partial^2 O}{\partial X^2} = \frac{Y_{O/B}\, B\, \mu_{\max}}{D} \frac{O}{k_0 + O} \tag{S7}$$

This equation can be integrated analytically providing eq. S8.

$$X = \sqrt{\frac{2D}{Y_{O/B}\, B\, \mu_{\max}} \left( k_O \ln\left(\frac{O}{O_0}\right) + O - O_O \right)} \tag{S8}$$

$O_0$ is the oxygen concentration in liquid phase at the surface between the bacterial suspension and the air containing head space in the vial (X=0). This equation will be applied in the main paper to estimate the influence of $k_O$ on the heat signal. Figure S2 illustrates the influence of the oxygen affinity on the oxygen profile.

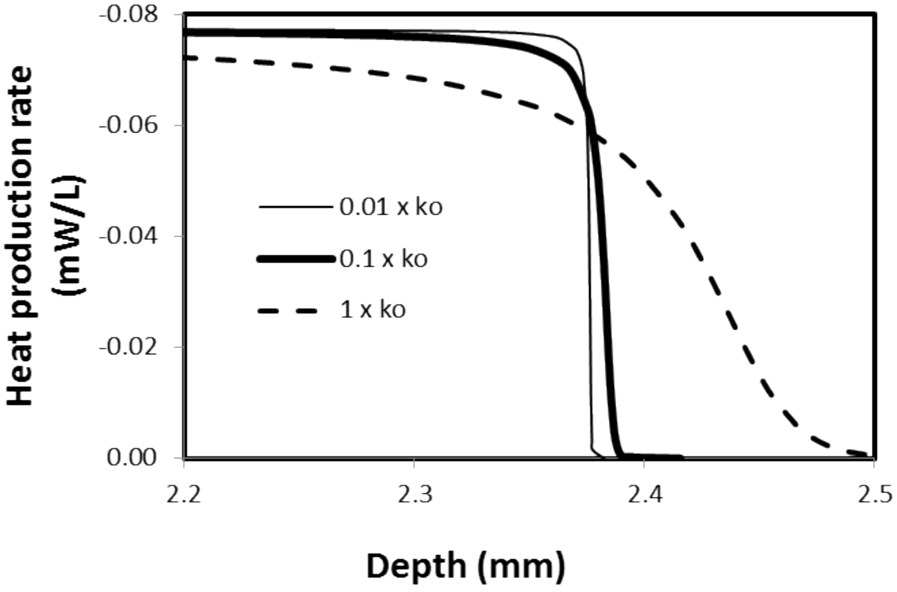

Figure S2: Influence of the oxygen half saturation ($k_O$) on the heat production rate (*B* = *3.55 mol* $m^{-3}$, $O_0$ = *0.206 mol* $m^{-3}$, $Y_{O/B}$ = *0.897 mol* $mol^{-1}$, $k_O$=*2.37x*$10^{-3}$ *mol* $m^{-3}$, $\mu_{MAX}$=*0.19* $h^{-1}$)

# References

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