Scalability in Laboratory Bioreactors Based on Constant Volumetric Mass Transfer Coefficient (k_La)

De Jong, T. F.[1,2], Fernandes, C.[2], D'Ambruoso, A.[3], Koechlein, D.[3]

[1]Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft [2]Applikon Biotechnology B.V., Heertjeslaan 2, 2629 JG Delft, The Netherlands [3]Applikon Biotechnology Inc. 1180 Chess Dr. , Foster City , CA 94404

Scope

- This work studies the scalability between 3 different volumes of lab scale bioreactors, 500 mL, 3 L and 15 L.
- The scale-up method of constant k_La is investigated.
- Impeller tip speed (V_{tip}) or gassed power input of the stirrer per volume of liquid in the bioreactor (P_g/V_L) are used to make k_La constant between bioreactors.
- The scalability is validated by applying the findings from the k_La study, to a *K. lactis* aerobic cultivation.

Scale-up in bioreactors

- Oxygen plays an important role in aerobic bioprocesses and it is often the limiting factor of the bioreactor.
- k_La is a gas transfer coefficient, a measurement of the capacity of the bioreactor to transfer oxygen into the cultures.
- A common scaling-up strategy for aerobic bioprocess is keeping the k_La constant within the different scales.
- A constant k_La ensures equal oxygen transfer independent of physical differences between each scale.

Volumetric oxygen transfer coefficient

- k_La is influenced by factors such as bioreactor geometry, gas flow, superficial gas velocity, impeller type and speed, and power input for mixing per unit reaction volume.
- Several empirical relationships exist to calculate the effect of these main factors in order to estimate the k_La in a bioreactor.
- Recognized degrees of freedom in the scale-up process include the impeller tip speed (V_{tip}), the volumetric gas flow rate (F_g) and the ratio impeller to reactor diameter (D/Tv).
- The impeller is the main gas dispersing tool in a stirred bioreactor and its configuration and tip speed have a significant effect on the k_La.
- The gas flow rate per unit of bioreactor volume (F_g) also has an important effect on the k_La, as increasing the F_g increases the gas holdup in the bioreactor which leads to a higher surface area between gas and liquid phases, which in turn increases the k_La.
- Equation 1 can be used to estimate the k_La based on the V_{tip} and F_g.

$$k_La = \alpha\left(\frac{P_g}{V_L}\right)^b * (v_{gs})^c$$

Eq. 1 (P_g gassed power input of the stirrer; V_L the liquid volume of the reactor; v_{gs} the superficial gas velocity and α, b and c are constants)

Table 1 | Aeration and agitation range used to determine k_La.

Bioreactor	Aeration [vvm]	Agitation [m/s]
500 mL miniBio	1 – 2.0	1.5 – 2.9
3 L bioreactor	1 – 2.0	1.5 – 4.8
15 L bioreactor	1 – 1.7	1.7 – 4.5

Table 2 | The aeration and agitation settings for each bioreactor to achieve k_La of 576 h^{-1}.

Bioreactor	Aeration [L/min]	[vvm]	Agitation [rpm]	[m/s]
500 mL miniBio	0.5	1	1760	2.6
3 L bioreactor	3	1	1860	4.5
15 L bioreactor	20	1.7	1000	4.5

Materials and methods

k_La as function of impeller tip speed and gassed power input over liquid volume

- k_La was determined for a 500 mL miniBio, 3 L and 15 L bioreactors (Applikon Biotechnology, Delft).
- The 500 mL bioreactor was assembled with an Applisens classic pH sensor, Lumisense optical DO sensor, L-type gas sparger, 2 rushton impellers, sampling pipe, 0.39 ratio impeller to reactor diameter (D/Tv) (all material from Applikon Biotechnology, Delft). Parameters were controlled with the my-Control controller (Applikon Biotechnology, Delft). The 3 L bioreactor had the same configuration as the 500 mL except 3 baffles were added and the controller used was the ez-Control (Applikon Biotechnology, Delft). The ratio of impeller to reactor diameter (D/Tv) is 0.35 for this vessel. The 15 L bioreactor used the same configuration as the 3 L. This vessel has a ratio of impeller to reactor of 0.39 (Applikon Biotechnology, Delft).
- The static method of gassing out was used to determine the k_La. Briefly, demi-water was sparged with nitrogen to strip oxygen from the bioreactors. The bioreactors were then sparged with air and the oxygen increase was registered as a function of time. When liquid and gas phase are assumed ideally mixed, the k_La is calculated by plotting the logarithmic change of oxygen concentration as a function of time.
- The k_La was determined for the three bioreactors at varying aeration and agitation ranges as described in Table 1.

Scale-up validation

- The yeast *Kluyveromyces lactis* (obtained from ATCC distributed by LGC standards, Wesel, Germany) was cultured in the 500 mL, 3 L and 15 L bioreactors.
- Yeast grew in minimal medium described by Verduyn *et al*. (1992) (nicotinic acid was increased to 5 mg/L, Kiers *et al*. (1998)).
- The cultivations were controlled at 30 °C and pH 5 by automatic addition of 4 M KOH.
- The O_2 and CO_2 in the exhaust gas were measured with BlueSense off-gas sensors (BlueSense - Applikon Biotechnology, Netherlands).
- Biomass was determined by measuring OD_{600} in a spectrophotometer and DCW at different time points during cultivation.
- The correlations found in this study were used to determine the V_{tip} and P_g/V_L for each bioreactor (Table 2) that allows for the same k_La value in the 3 bioreactors.
- The k_La was fixed to 576 h^{-1} in the 3 bioreactors (maximum value measured for the 15 L bioreactor). To achieve this k_La value, the V_{tip} and P_g/V_L for the 500 mL miniBio, were set to 2.6 m/s and 5.6 KW/m^3, respectively; in the 3 L and the 15 L those were set to 4.5 m/s and 9 KW/m^3.

Results and discussion

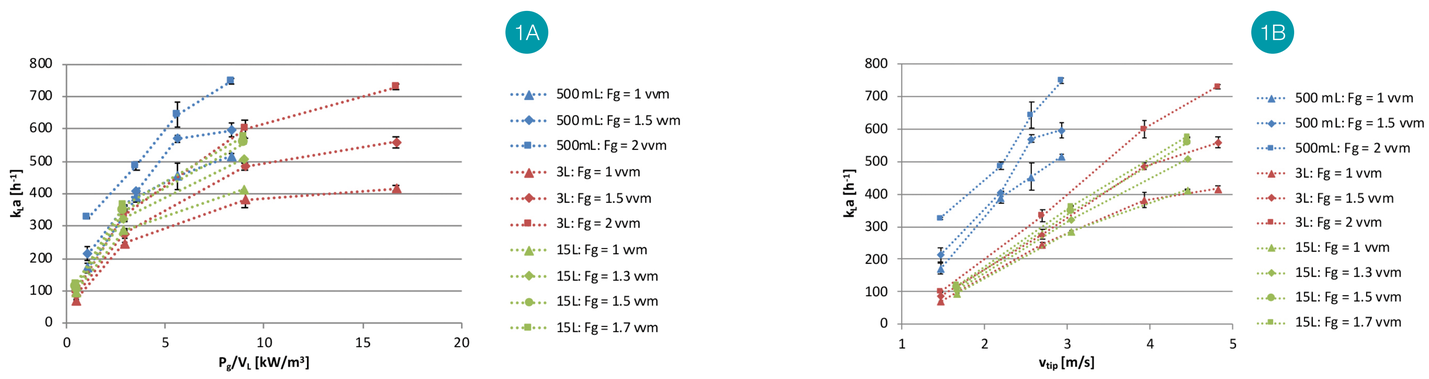

Figure 1 | (A) The k_La values for the 500 mL (blue), the 3 L (red) and the 15 L (green) bioreactors as a function of gassed power input over liquid volume at varying air flow ; (B) The k_La values for the 500 mL (blue), the 3L (red) and the 15 L (green) bioreactors as a function of the impeller tip speed at varying air flow.

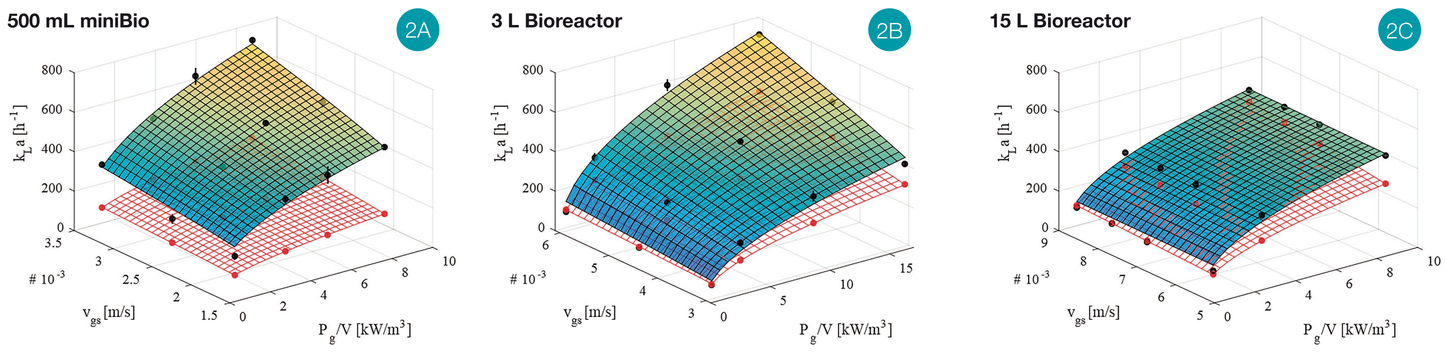

Figure 2 | The theoretical k_La values calculated using Eq. 1, found in literature (red dots) and the experimentally determined k_La values (black dots) including standard deviation. The optimal k_La correlations (colored grid) and theoretical k_La correlation (red grid) are also indicated.

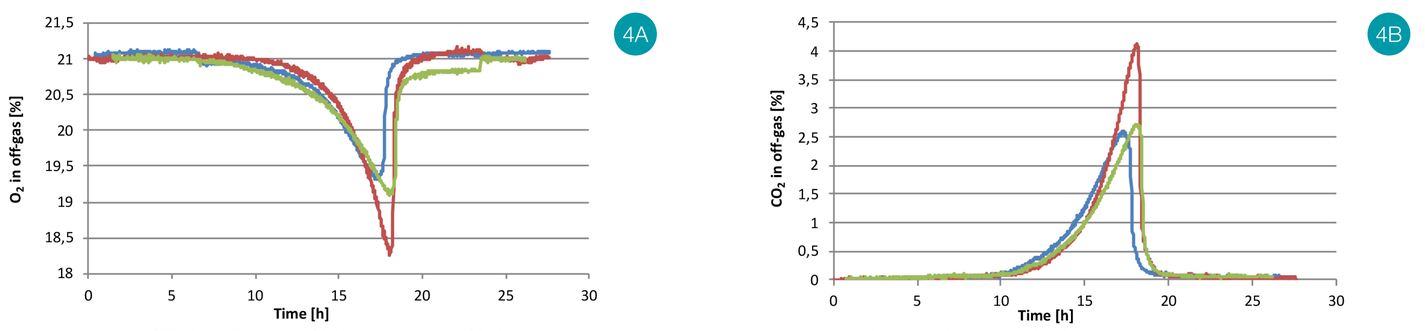

Figure 4 | (A) O_2 off-gas profile during *K. lactis* cultivation; (4B) CO_2 off-gas profile during *K. lactis* cultivation.

- The results show a high reproducibility between bioreactors, with an average biomass concentration of 10.9 ± 0.2 g DW/L (AVG ± STDEV).
- The biomass yield on glucose obtained was of 0.54 ± 0.01 g DW/g glucose.
- The final OD_{660} obtained for the 500 mL, 3 L and 15 L was 69.8, 71.6 and 69.0 respectively.
- The off-gas analyses indicates (Figure 4A and 4B) similar growth profile in the three bioreactors.

k_La experimental and theoretical correlations

- Figure 1 depicts the experimentally determined results for the 3 bioreactors for varying conditions.
- The highest k_La values obtained were 748 h^{-1}, 730 h^{-1} and 576 h^{-1} for the 500 mL, 3 L and 15 L bioreactors respectively.
- These results indicate that scaling up between the 3 bioreactors using constant P_g/V_L or V_{tip} to keep constant k_La is limited to certain values.
- In Figure 2 are represented the experimental k_La values (black dots) and the theoretical k_La values (red dots) obtained from Eq. 1 at varying aeration and agitation.
- The results showed that the k_La values are underestimated by the theoretical correlation when the volume of the bioreactor decreases from 15 L to 500 mL.
- New k_La correlations were fitted to the experimental data, using the coefficients α, b and c as variables.
- Figure 3 (A) shows the prediction of the k_La when using the optimal correlation. It shows that newly calculated correlations (Figure 3 (B)) provides a more accurate prediction of the k_La.

$$k_La\,[h^{-1}] = 3600 \cdot 1.022^{10} \cdot \boldsymbol{\alpha} \cdot \left(\frac{P_g}{V_L}\right)^{\boldsymbol{b}} \cdot (v_{gs})^{\boldsymbol{c}}$$

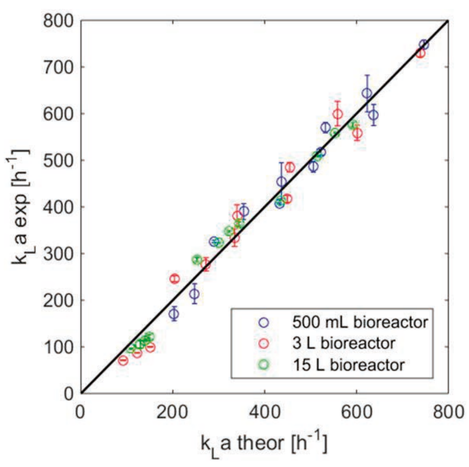

Figure 3 | (A) Comparison of experimental k_La data with the theoretical values. The black line shows the relation between experimental and theoretical data in case of a perfect prediction (3B). The coefficients required to optimal fit between k_La correlation and experimental k_La.

3B

Bioreactor	α	b	c	adj R^2	RMSE [h^{-1}]
500 mL miniBio	0.075	0.4	0.5	0.9644	32.24
3 L bioreactor	0.079	0.45	0.72	0.9672	38.60
15 L bioreactor	0.030	0.48	0.61	0.9805	24.91

Conclusions

- Maximal k_La values of 748 h^{-1}, 730 h^{-1} and 576 h^{-1} were obtained for 500 mL, 3 L and 15 L bioreactors respectively.
- The scaling- up method based on keeping k_La constant by varying V_{tip} or P_g/V_L proved to be limiting in this study. Only restricted V_{tip} and P_g/V_L can be used to achieve a matching k_La in the 3 bioreactors.
- The coefficients α, b and c for the general k_La relation were determined and k_La correlations based on the experimental results were obtained for each of the three bioreactors. These correlations showed a better prediction of the k_La values than the theoretical correlation.
- The final biomass concentration and final yield of biomass on glucose are calculated for each bioreactor. The resulting biomass concentrations and yields are nearly identical for all three bioreactors (10.9 ± 0.2 g DW/L, and 0.54 ± 0.01 g DW/g glucose). These values agree with the values found in literature (X_t = 9.8 g DW/L and $Y_{X/S}$ = 0.49 g DW/g glucose).
- Applikon lab-scale bioreactors 500 mL, 3 L and 15 L were successfully used for scaling-up *K. lactis* aerobic cultivation.

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