Bioprocess Performance Characterization of a Mini-scale Bioreactor for Recombinant Mammalian Cells

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The need for scale down models

With the increasing number of potential therapeutic molecules coming through from discovery, bioprocess scientists and engineers who have to guide process design and development have had to establish new technologies and methodologies for rapidly screening, defining, optimizing and characterizing process options. For these technologies and methodologies to be truly successful, they need to be scalable from operations at milliliter quantities in the lab to commercial scale operations. Scale down models (SDMs) need to provide assurance that product quality and process performance attributes are maintained between and within scales. Factors that contribute to this challenge include the need for qualification, validation, regulatory requirements, the pressure to reduce cost and development time and resource requirements.

SDM equipment and operation

3l Applikon Bioreactor : WV : 1.5l

Applikon MiniBio 500 : WV = 250ml

	Maximum	Center	Minimum
P/V	10.39 W/m^3	6.83 W/m^3	3.49 W/m^3
500ml	280 RPM	243 RPM	194 RPM
3l	231 RPM	200 RPM	160 RPM

Temp: 37°C | pH: 7 | C_L= 40%sat | Cell line: CHO DG44 | Seed D: 0.3x10^6 cell/ml | Media: PROCHO5 (supplemented) Complete media | Impeller: single marine impeller | Sparger: Micro-sparger: Aeration | DO control (maximum 0.007 VVM)

SDM fundamentals

A Q-b-D approach to SDM of mammalian cell culture bioreactor

In this study we applied Q-b-D approach to test and compare the performance of a new miniature bioreactor (Applikon Minibio 500ml) with a standard 3l-scale Applikon Bioreactor. Oxygen is sparingly soluble in culture media. For example, compared to glucose, oxygen is nearly 16000 times less soluble making it the only nutrient that needs to be supplied continuously. This makes oxygen transfer rate (OTR) a major driver in design and scale up of bioreactors. In a mammalian cell culture bioreactor, the dissolved oxygen (DO) concentration (C_L) typically is maintained between 20% and 40% corresponding to approximately 1.0-20mg of O_2 per liter of media (mg/l) depending on temperature and media composition. The maximum DO concentration (C*) at 100% saturation is also function of temperature and media composition and can vary between 5-7mg/l. OTR can be calculated using the simple relationship [1]:

$$OTR = k_La\ (C^*-C_L) \qquad [1]$$

where k_La is known as the volumetric oxygen transfer rate and is known to be a strong function of power (P) input per unit of the cell culture volume (V) in the bioreactor (P/V), which is also known as the impeller energy dissipation rate (Watts of power per cubic meter of cell culture volume (W/m^3). With C* and C_L effectively fixed by temperature and buffer composition and for a constant aeration rate expressed as volume of air supplied per volume of cell culture (vvm), P/V becomes the only parameter that can be used to increase k_La. This makes P/V an important criterion and one that has been used for decades in biological and non-biological two-phase (gas-liquid) operations. In turbulent flow, which is the condition in most mammalian bioreactors, the power value (P) for the impeller is given [2]:

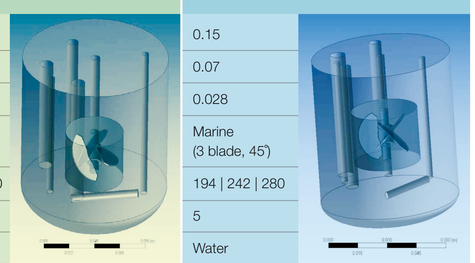
$$P = k_pPN^3D^5 \qquad [2]$$

Where D is the impeller diameter, N is the rotational speed (revolution per seconds), *P* is the media density and k_p is the impeller power number that is related to impeller type and bioreactor geometry. Maintaining geometrical similarities during scale up and scale down means that k_p remains constant. The parameter k_p can be determined experimentally or obtained using computational fluid dynamics (CFD).

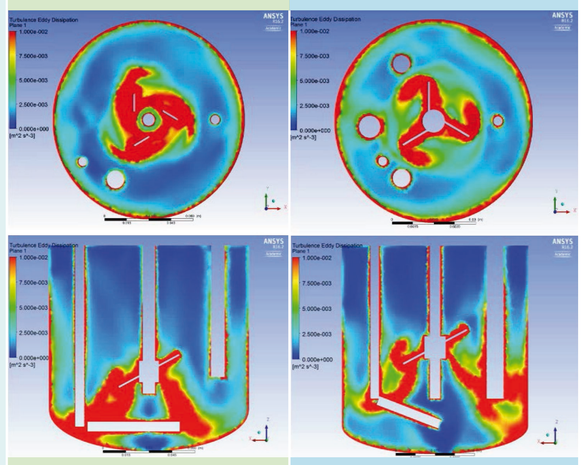
Doran, Pauline M. Bioprocess Engineering Principles. London: Academic Press, 1995.
Shuler, Michael L., and Fikret. Kargi. Bioprocess Engineering : Basic Concepts. 2nd ed. Upper Saddle River, NJ: Prentice Hall, 2002.

SDM characterization using CFD

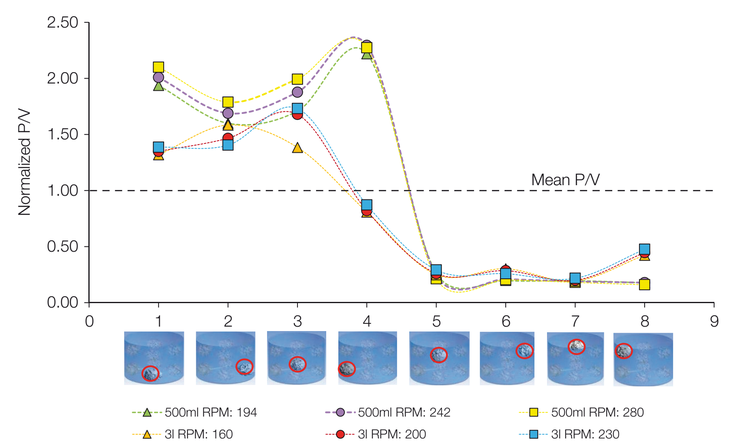
Design Parameters	3l Bioreactor	MiniBio 500ml
Height (m)	0.25	0.15
Diameter (m)	0.13	0.07
Impeller diameter (m)	0.045	0.028
Impeller type	Marine (3 blade, 45°)	Marine (3 blade, 45°)
Impeller speed (RPM)	160 | 200 | 230	194 | 242 | 280
Number of probes	5	5
Fluid (single phase)	Water	Water



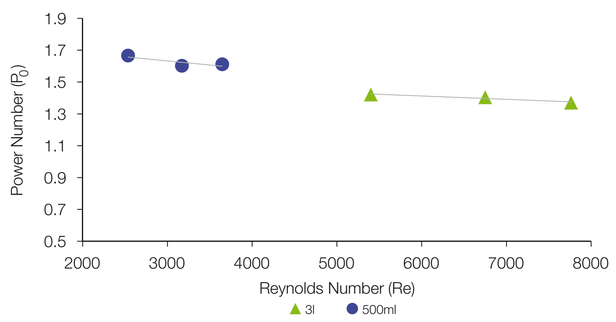
Energy dissipation rates for center-point P/V



Energy dissipation rates for center-point P/V predicted using ANSYS. Minor differences between the two scales, e.g. kp values, are likely caused by the differences between the two geometries, e.g. number, size and position of inserts as shown.



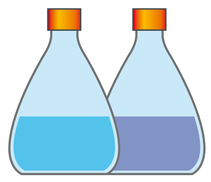
CFD generated power-curves for the 3l and the 500ml SDM Bioreactor. Local P/V relative to the mean value for the 3l and the 500ml SDM Bioreactor (profiles generated by CFD locations are shown by red circles).



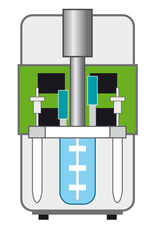
Correlation between power number and Reynolds number for marine impeller without sparging using CFD generated P/V.

Empirical comparison of 3l to 500ml SDM bioreactor

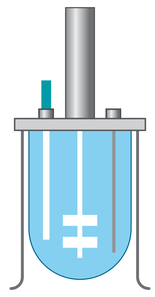
Thaw
CHO DG44 Producing a-IL8, Adapted to ProCHO5, passaged 3-4 times



Shake flask
ProCHO5 Complete Media (supplemented with L-glutamine, HT, Pen Strep, insulin, lipids) 8% CO_2, 37°C, 3 days in 135ml SF then transferred to 250ml SF

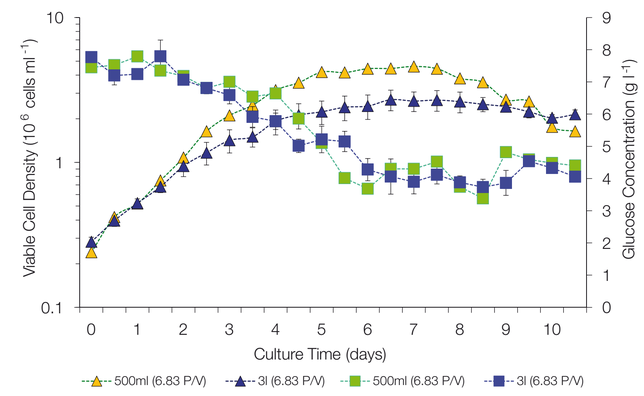


500ml Applikon MiniBio

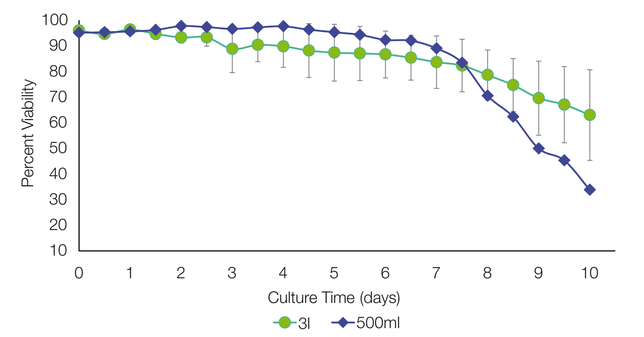


3l Applikon bioreactor

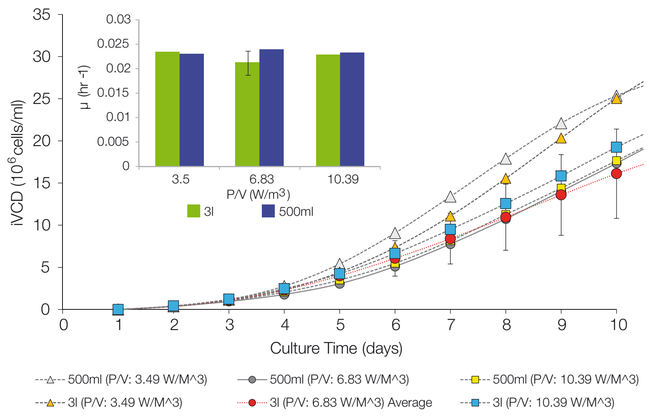
Inoculation
VCD at 0.3 (106 cells/ml) VCD, >90% viability. Fed-batch with glucose and glutamine maintained between 3-6g/l



VCD (triangles) and glucose concentration (squares) of 500ml and 3l bioreactors run at center point P/V conditions. Three 3l bioreactors and one 500ml bioreactor were run at center point for 10-day fed-batch processes. Error bars are one SD for the 3l runs.



Cell culture viability of 500ml and 3l bioreactors run at center point P/V conditions. Three 3l bioreactors and one 500ml bioreactor were run at center point in 10-day fed-batch processes. Error bars are one SD for the 3l runs.



Plots of integral viable cell density calculated using the trapezoidal rule for integration. Plots show the cumulative viable cells (iVCD, million cells/ml) over the culture period (days). Error bars denote one standard deviation for the 3l runs.

Conclusions and recommendations

In this study we show how computational fluid dynamics (CFD) can be used as a powerful tool in the process of characterizing and developing SDMs. The simulation's results demonstrate that power dissipation is distributed similarly across both scales at each condition examined. The results also show that energy dissipation is not homogeneous at either scale; ranging from ¼ of the mean P/V at zones furthest from the impeller to twice the mean P/V in the impeller zone. These results show the value of the information gathered from accurate model simulations which can guide scientists and engineers by providing spatial and temporal data on the physical phenomena inside the bioreactor. We also demonstrate scalability of the 500ml MiniBio system by comparing its performance with that of a 3l bioreactor in terms of iVCD and specific growth rate at equal P/V value. Each of the iVCD profiles of the 500ml runs fall within three standard deviations of the 3l scale center point data, demonstrating comparability within the tested parameters. Our results support the applicability of CFD and constant P/V in providing the basis for a Q-b-D approach to SDM validation. Additional work is planned to validate the SDM bioreactor using 30l and larger scales. Future work will also include comparison of k_La between SDM and larger scales as well as a comparison of titer and CQAs, e.g. PTMs, between the 500ml MiniBio SDM and larger scale bioreactors.

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