



Animal Cell Culture in Stirred Bioreactors: Observations on Scale-up

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The scale-up of stirred tank bioreactors from 0.02 m³ to a 0.3 m³ commercial plant is discussed for hybridoma suspension cultures. Schemes for dissolved oxygen control with sparged air in serum containing media are described, as well as mechanical breakage of foam in small and large bioreactors. Porous metal spargers (180–200 × 10⁻⁶ m) were found to produce foams which were hard to control. Aeration with larger (≥ 0.001 m) multihole spargers is recommended.

Combined cell damage due to foam formation and control, and possible damage at mechanical seals or submerged bearings, were found to have no measurable effect on cell growth relative to roller bottle production. Hybridomas are shown to withstand significant impeller tip speed (> 1 m s⁻¹) and fluid turbulence as evidenced by impeller Reynolds numbers in excess of 10⁵. The size of the energy-dissipating terminal eddies was calculated to be greater than ten-fold that of the hybridoma cells. The specific fluid turnover rate was employed as the scale-up criterion.

NOTATION

C	Clearance of impeller from bottom of tank (m)
D	Diameter of tank (m)
d_i	Diameter of impeller (m)
$k_L a_L$	Overall gas–liquid volumetric mass transfer coefficient (s ⁻¹)
L	Static liquid height (m)
l	Length of terminal eddies (m)
N	Rotational speed of impeller (s ⁻¹)
P	Power (W)
P_o	Power number
Q	Volume flow of impeller (m ³ s ⁻¹)
Re_i	Impeller Reynolds number given by eqn (1)
T	Tip speed of impeller (m s ⁻¹)

V Volume of bioreactor (m³)

Greek Symbols

ϵ	Specific energy dissipation rate (W kg ⁻¹)
μ	Viscosity of liquid (Pa s)
ρ	Density of liquid (kg m ⁻³)

Subscripts

1	0.02 m ³ bioreactor
2	0.3 m ³ bioreactor

Abbreviations

DO	Dissolved oxygen (kg m ⁻³)
RO	Reverse osmosis

INTRODUCTION

Suspension cultures of animal cells are extensively used for the production of monoclonal antibodies, recombinant and non-recombinant proteins (tPA, erythropoietin, interferons, etc.) and vaccines

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based on viral antigens. Mainly stirred-tank^{1,2} and airlift bioreactors³⁻⁶ are employed in industrial cell culture. Significant literature on cell-culture technology is available^{1-3,7} and some of it contains qualitative details on large-scale bioreactors, particularly airlift systems. Fundamentals of stirred-tank bioreactor design have also been described^{8,9} but, little engineering data has been published on the scale-up of animal cell-culture processes.

This paper details the scale-up of a hybridoma suspension culture to a 0.3 m³ production plant for monoclonal antibodies. Operational considerations relating to aeration and foam formation in large-scale systems are discussed.

THE PRODUCTION PROCESS

A monoclonal antibody production process was designed to produce several different blood-typing antibodies using different murine hybridomas all of which could be grown as freely suspended cells in submerged culture. The requirements of process reliability, rapid turn around between various products, and speed of establishment in response to market demands, led to the choice of batch operation as the preferred production method.

A train of three bioreactors, 0.02, 0.075 and 0.3 m³ in total volume, was employed. Roller bottles were used to inoculate the 0.02 m³ bioreactor, which in turn provided inoculum for the 0.075 m³ unit which fed the 0.3 m³ device. This type of production scheme is commonly used in microbial fermentations and also in industrial cell culture.⁴ The working volume of the reactors was approximately 75% of the total volume and the inoculum size at each stage was roughly 25% (v/v). As soon as a reactor became free, it could be washed, sterilized and started with the next batch. Cleaning, sterilization and transfer between reactors were automated.¹⁰

In suspension culture of animal cells, the basic airlift and stirred-tank reactor configurations are perhaps the two competitive designs. Although the airlift bioreactors have been proven for hybridoma production at scales in excess of 2 m³,^{4,5} and some design information is now available,¹¹ a decision was made to use the stirred-tank concept for development and production. The need for similar reactors at various scales, and the height

restrictions in an existing building for the larger reactor, were the deciding factors.

BIOREACTOR DEVELOPMENT

Calculations showed that surface aeration, even at the 0.02 m³ scale, could not sustain the anticipated cell densities of up to 2×10^{12} cells m⁻³. Sparger aeration was unavoidable: other complex systems, such as the caged aeration¹² and the silicone tube aeration,¹³ were ruled out. Sparger aeration is known to be successful with airlifts where it is essential to reactor operation. The sparger aeration concept needed to be tested with the cell lines of interest, especially in the serum-containing media that were necessary.

A bacterial stirred fermenter (0.02 m³) was modified for preliminary work. A liquid height aspect ratio of 1.2 was used in a 0.25-m (*D*) diameter flat-bottomed tank. The baffles were removed, a 20° pitch marine-type impeller (*d_i/D* = 0.35), located about a half-impeller diameter above the base of the tank, was arranged to pump upwards. The working volume was ~0.015 m³. A four-hole sparger (5×10^{-3} m hole diameter) was positioned half way between the propeller and the bottom of the tank; the holes were just inside the circle described by the tip of the propeller blades. The sparger holes could be covered by a sintered metal disc (180–200 $\times 10^{-6}$ m pore size) if desired.

Experiments were conducted with six different hybridoma cell lines to demonstrate the operational concepts (agitation, aeration, pH and dissolved oxygen control and foam control). All discussion here pertains to just one cell line; the results were similar for others.

Dulbecco's modified Eagle's medium (Catalog no. D 2902, Sigma Chemical Co., St. Louis, MO) supplemented with additional glucose to a total concentration of 4.5 kg m⁻³ was used. Sodium bicarbonate (3.7 kg m⁻³) and fetal calf serum (5% (v/v)) were added to the medium. Antibiotics were not used beyond the roller bottle stage (0.03 kg m⁻³ gentamycin sulfate was added to roller bottles). The media were made in reverse osmosis (RO) water (mean conductivity and standard deviation = $0.046 \pm 0.029 \times 10^{-6}$ S at 25–26°C), but tap water (conductivity = $0.234 \pm 0.009 \times 10^{-6}$ S at 25–26°C) could also be successfully used. The osmolality of the media was $310 \pm 16 \times 10^{-6}$

kmol kg^{-1} . Cultures were carried out at 25–35% (of air saturation) dissolved oxygen (DO), $37.0 \pm 0.3^\circ\text{C}$, and $\text{pH } 7.0 \pm 0.1$. Cell viability was determined using the trypan blue dye exclusion technique; a hemacytometer was employed for the cell counts.

The culture was aerated with a nitrogen/air mixture (1:0.43 molar ratio) so that even with no consumption of oxygen, the dissolved oxygen level could not rise above 30%. A constant total gas flow ($2.5 \times 10^{-6} \text{ N m}^3 \text{ s}^{-1}$) was used. Carbon dioxide and oxygen were added to the gas mixture (without alteration in total flow rate) in response to the pH and the DO controllers. Sodium hydroxide (0.2 kmol m^{-3}) was added to correct the low pH deviations from the set point. Foaming was unavoidable, mechanical foam control (Fundafilm[®], Chemap AG, Volketswil, Switzerland) was used because chemical antifoams were not acceptable (for downstream processing and product end-use considerations).

The general course of a cell culture run is shown in Fig. 1. The culture peaked around 45–50 h, a cell concentration of almost $2 \times 10^{12} \text{ cells m}^{-3}$ was attained (at a constant impeller speed of 2 s^{-1}) and a fairly high and stable cell viability ($> 80\%$) was maintained until the decline phase of the batch. The results of Fig. 1 agreed with the roller-bottle culture data. The results demonstrated that mechanical agitation under the conditions employed had no detrimental effect on cells; furthermore, the theoretical possibility of cell damage at the mechanical seal on the bottom-entering impeller shaft was shown to be of little concern. The reactor hydrodynamics were very satisfactory at the agitation level used: good bulk mixing, absence of stagnant zones, complete

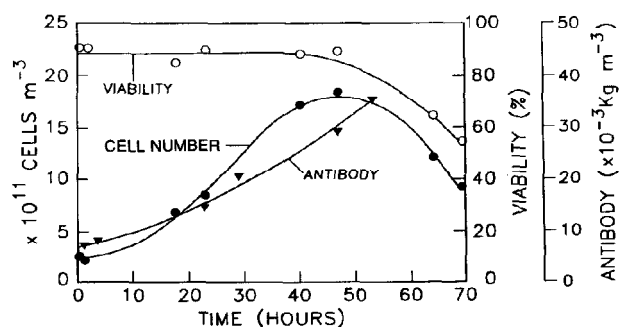


Fig. 1. Typical cell growth, viability and antibody production profiles in the 0.02 m^3 bioreactor with marine-type impeller (speed = 2 s^{-1}).

suspension of cells and good DO, pH and temperature control were obtained.

Mechanical control of foam caused no problems. Excessive cell debris was not observed in the culture fluid. Any cells which rose with the foam probably perished and did not contribute to the productivity of the culture. Mechanical breakage of foam almost certainly destroyed the cells in the foam, and the resulting debris could be seen attached to the wall of the vessel above the level of liquid; however, the effect was so insignificant that it had no measurable impact on cell growth, viability or antibody productivity.

The four-hole sparger produced relatively large ($\sim 0.01\text{--}0.02 \text{ m}$ diameter) bubbles which rapidly rose to the surface where most of them collapsed. The bubbles which persisted formed an easy to control foam with a large foam cell structure. The porous-metal sparger, on the other hand, gave rise to finer bubbles of $\sim 0.002\text{--}0.003 \text{ m}$ diameter (and a much higher $k_L a_L$ as was evidenced by the shorter DO electrode calibration time with this sparger compared to the four-hole device) which formed a difficult to control, highly densely packed foam in the serum-containing media. The small bubbles were stable at the surface; the self-collapse mechanism of the larger bubbles did not operate for the smaller ones. The four-hole sparger was used in all subsequent work reported here.

A large number of test runs were performed, mostly to evaluate the product and other operations. Cell growth data from eight separate runs, representing 41 data points, are shown in Fig. 2. The best-fit growth profile (solid line) is shown with lines representing $\pm 20\%$ of the best-fit

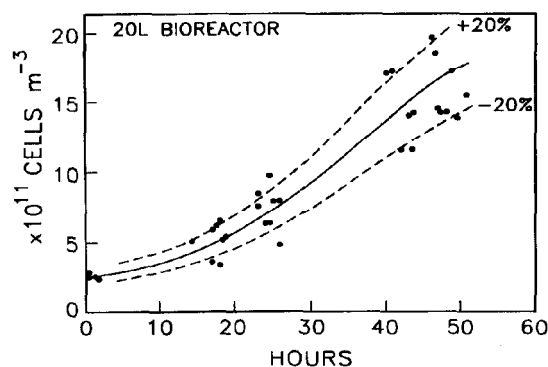


Fig. 2. Cell concentration versus time. Data from eight different runs are shown. The solid line represents the best-fit average growth profile.

curve. The figure shows the expected level of reproducibility of culture growth; the information is useful for assessment of performance of the larger scale bioreactors.

THE LARGE-SCALE BIOREACTORS

The two larger reactors (0.075 m³ and 0.3 m³ total volumes) were geometrically similar to each other, but somewhat different to the modified 0.02 m³ device used to obtain the developmental data. A schematic of the reactors is shown in Fig. 3. The vessels were designed with a profiled, almost hemispherical (Fig. 3) bottom, and the impeller was arranged for downward pumping. These modifications were done to achieve good drainage, smoother fluid flow at the bottom,¹⁴ and an enhanced ability to suspend the cells even at lower impeller pumping rates. Table 1 compares the relevant geometric ratios of the 0.02 m³ and the 0.3 m³ reactors.

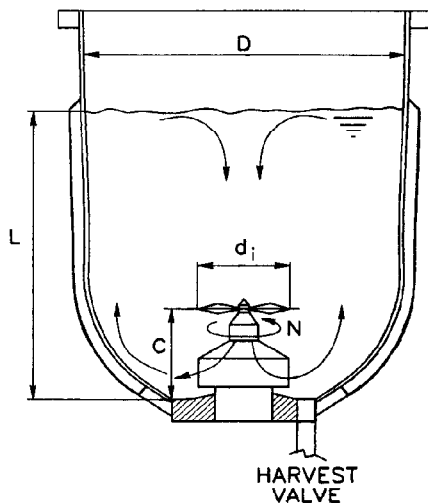


Fig. 3. The stirred bioreactor geometry.

Table 1. Geometric ratios of the bioreactors

Reactor size (m ³)	d_i (m)	d_i/D	L/D	C/d_i
0.02	0.088	0.352	~1.2	0.477
0.30	0.210	~0.29	~1	1.13

The 0.075 m³ reactor was geometrically similar to the 0.3 m³ unit.

On the larger reactors, the aspect ratio was reduced to 1.0 for better bulk mixing with the single impeller. Agitation through mechanical seals was considered risky; hence magnetically coupled agitators were used. To accommodate the mechanical design of the coupling, the impeller clearance from the bottom of the tank needed to be higher: the C/d_i of 1.13 was 2.4-fold that employed on the developmental scale. The downward flow from the propeller, combined with the profiled bottom, compensated for any small deterioration in performance due to the higher location of the stirrer on the large bioreactors. At $d_i/D \sim 0.29$, the impellers on the larger vessels were comparable to that on the 0.02 m³ device.

The cell growth results obtained in an unoptimized test run (impeller speed = 1.67 s⁻¹) are shown in Fig. 4. For comparison, the best-fit growth curve determined in the 0.02 m³ developmental reactor (see Fig. 2) is also shown in Fig. 4. To assess the results, some of the bioreactor geometry associated operational parameters need to be evaluated. Table 2 presents the impeller Reynolds number which was calculated using

$$Re_i = \frac{\rho N d_i^2}{\mu} \quad (1)$$

with an assumed $\rho = 1000 \text{ kg m}^{-3}$, and $\mu = 10^{-3} \text{ Pa s}$ for the medium. The assumption was reasonable because actual measurements in similar media, e.g. TNM-FH with 10% (v/v) fetal bovine serum, have displayed almost water-like viscosity at $1.2 \times 10^{-3} \text{ Pa s}$.¹⁵ Based on the Re_i , the two reactors operated in a turbulent regime. The other parameters listed in Table 2 relate to impeller tip speed (T), the power input per unit volume (P/V)

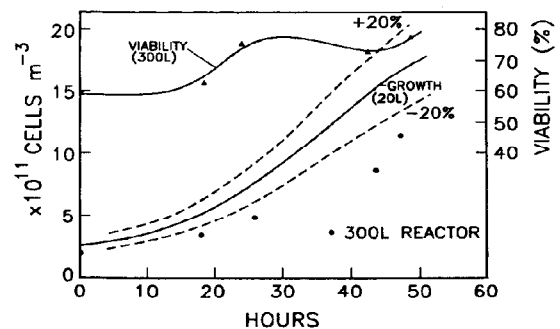


Fig. 4. Cell growth profile. Comparison of the average growth curve of Fig. 2 with data from the 0.3 m³ reactor. The 0.02 m³ vessel used the modified impeller of Fig. 5. The cell viability data for the 0.3 m³ reactor are also shown.

Table 2. Operational parameters of the bioreactors

Reactor size (m ³)	<i>N</i> (s ⁻¹)	<i>Re</i> _i	<i>Nd</i> _i (m s ⁻¹)	<i>N</i> ³ <i>d</i> _i ⁵ / <i>V</i> × 10 ³ (m ² s ⁻³)	<i>Nd</i> _i ³ / <i>V</i> (s ⁻¹)
0.020	2.00	15 488	0.176	2.1	0.068
0.300	1.67	73 500	0.350	6.3	0.051

and the specific pumping rate of the impeller (Q/V):

$$T \propto Nd_i \quad (2)$$

$$\frac{P}{V} \propto \frac{N^3 d_i^5}{V} \quad (3)$$

$$\frac{Q}{V} \propto \frac{Nd_i^3}{V} \quad (4)$$

The scale ratios of the operational parameters are shown in Table 3. In view of the observations from small-scale experiments, neither the impeller tip speed nor the Reynolds number were considered to be the critical scale-up parameters as long as certain limitations were not reached. Thus, in initial experiments the larger bioreactors were operated at almost twice the tip speed (Table 2) of the smaller device. Similarly, the impeller Reynolds number on the larger unit was almost five-fold that on the 0.02 m³ vessel, although a sufficiently developed turbulent regime existed at both scales.

As oxygen transfer was not a limiting factor, the main scale-up consideration was to maintain a similar fluid turnover (i.e. impeller pumping rate per unit bioreactor volume) at the two scales. Because the bulk fluid movement at the small scale was higher than necessary, the larger reactor was operated at 75% of the specific pumping rate of the smaller device. This constraint led to a specific power input which was three-fold higher than on the smaller reactor; however, the power input was still quite low, < 3 W m⁻³ at the 0.3 m³ scale (estimated with a constant assumed power number of 0.4). An alternative approach to scale-up could have been to relax the geometric similarity criteria to possible advantage. A larger impeller (e.g. $d_i/D=0.5$) could have been used on the larger reactor to provide the same Q/V as on the smaller scale unit, but with lower N , tip speed and impeller Reynolds number.

In animal cell bioreactors, aeration rates are kept low. Bubbling occurs in a very small volume

Table 3. Scale-up ratios for some operational parameters for the 0.02 m³ (subscript 1) and the 0.3 m³ (subscript 2) reactors

Parameter	Value
Tip speed ratio, T_2/T_1	1.99
Reynolds number ratio, Re_2/Re_1	4.75
Specific power input ratio, $(P_2/V_2)/(P_1/V_1)$	3.0
Specific pumping capacity ratio, $(Q_2/V_2)/(Q_1/V_1)$	0.75

of the reactor. Breakup of gas bubbles with an impeller is neither desired nor necessary. In this situation, the transfer of oxygen takes place in a small portion of the reactor; bulk flow of fluid then becomes the main vehicle for attainment of a homogenous distribution of the transferred oxygen. This is the rationale for scale-up based on bulk flow.

The growth data in Fig. 4 show that the cells experienced a longer lag, and somewhat slower growth, in the 0.3 m³ bioreactor compared to the curve obtained at the smaller scale. The cell viability data (Fig. 4) revealed, however, that the larger scale batch was inoculated with a significantly less vigorous inoculum (< 60% viable) than was used in the developmental runs (> 80% viable; see, e.g., Fig. 1). Despite this, the cell viability did increase to ~ 75% within 25 h of inoculation of the 0.3 m³ reactor (Fig. 4). The difference in the inoculum viability was a strong explanation for the difference in growth results at the two scales of operation.

The turbulence within the fluid was unlikely to have caused any damage to the cells even at the high impeller Reynolds number which occurred in the 0.3 m³ reactor. This conclusion is based on the following analysis of the turbulent field: using an assumed power number of 0.4 under the turbulent conditions which prevailed in the reactor (75% full), the energy dissipation rate per unit mass of liquid (ϵ) was calculated as

$$\epsilon = \frac{PoN^3 d_i^5}{0.75 \times 0.3} \quad (5)$$

where Po is the power number. Kolmogoroff's isotropic turbulence theory was used to calculate the length l of the energy-dissipating terminal eddies:

$$l = \left(\frac{\mu^3}{\rho^3 \varepsilon} \right)^{1/4} \quad (6)$$

At an Re_i of 73 500, l was found to be 1.3×10^{-4} m, which was more than 10-fold greater than the diameter of the hybridoma cells. Note that the ratio of the size of the primary eddies (assumed to be that of the impeller generating the eddies) to that of the terminal eddies (l), i.e. the ratio d_i/l , was sufficiently large¹⁷ that isotropic turbulence must have existed in the reactor. Although, the scale of turbulence is some indicator of shear fields, a detailed characterization of shear rates in bioreactors is not possible at present;¹⁶ hence, the discussion has been limited to such parameters as impeller tip speeds, Reynolds numbers and the length scale of terminal eddies.

The 0.02 m³ reactor data shown in Fig. 4 were obtained on a reactor which was identical to the developmental unit, except that it had the magnetically coupled impeller ($d_i/D=0.46$) shown in Fig. 5. The larger impeller was run at 1 s^{-1} .

The 0.3 m³ and the 0.02 m³ magnetically stirred bioreactors differed from the research unit ('growth' line in Fig. 4) in two respects: they used (i) magnetically driven agitators,¹⁴ and (ii) a different DO and pH control strategy. Air alone was used to control the dissolved oxygen in the production units. The rate of flow of air and CO₂ varied, respectively, in response to the DO and

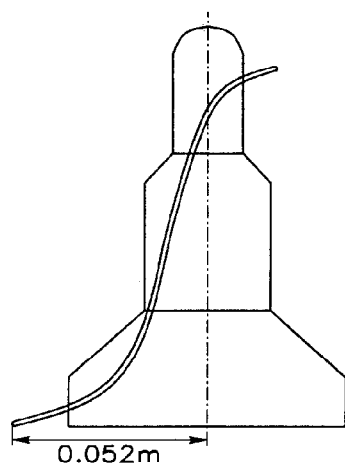


Fig. 5. The impeller geometry (only one blade shown).

the pH controllers. Oxygen and nitrogen were not used, nor was the total gas flow kept constant. These modifications led to simpler aeration systems, less expensive to install and operate. The growth results, particularly in the 0.02 m³ reactor (Fig. 4) compared well with the data of the developmental unit; modifications to the DO and pH control methods did not cause any deterioration in performance.

CONCLUSIONS

Bioreactor scale-up and operational considerations were examined for hybridoma suspension cultures in stirred-tank bioreactors. Dissolved oxygen control in serum-containing media could be achieved satisfactorily with sparger aeration. Sintered-metal or porous-plate spargers were not suitable for aeration in serum-containing media; larger hole spargers worked well. Air, without supplementation with nitrogen or oxygen, could be used at all scales of operation. The inevitable foam formation which accompanied aeration had no significant impact on the productivity of the culture. The foam could be controlled solely by mechanical means; no chemical agents were used. Antibiotics were dispensed with in the production plant.¹⁰ Hybridoma cells were found to be far more shear-tolerant than is generally believed. Fairly high impeller tip speeds, Reynolds numbers, submerged aeration, grinding action of the mechanical seals and support bearings of the magnetic agitators, did not cause sufficient damage to cells to affect the performance of stirred bioreactors up to 0.3 m³.

Scale-up based on maintaining a good bulk flow in the reactor was satisfactory; a low-pitch, marine-type impeller could be used in baffle-free tanks with profiled bottoms. Larger, slower impellers could also be employed.

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