Culture scale-up studies as seen from the viewpoint of oxygen supply and dissolved carbon dioxide stripping

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Oxygen supply and dissolved carbon dioxide (dCO2) stripping are two of the most important control parameters in cell culture. In this study, we investigated the effect of scale-up on the volumetric gas transfer coefficient with bioreactors of different sizes (working volume: 80 L, 500 L, 2000 L, and 10,000 L; aspect ratio: 1.0–1.6). Sparging air into water increased the volumetric oxygen transfer coefficient (kLa), an index of oxygen supply efficiency, by scale-up roughly in proportion to the depth of the water. A corresponding increase in kLa was found in a real cell culture of Chinese hamster ovary cells. dCO2 stripping efficiency was evaluated in water tests using changes in kLa, an index defined in relation to kLa. kLa increased following surface aeration, but the rate of increase was reduced by scale-up, which was attributed to a decrease in the liquid surface-to-volume ratio. A similar decrease in efficiency was observed in a 2000 L bioreactor by increasing the liquid volume at constant liquid surface area. The observed scale-up effects are discussed based on a simple theoretical consideration.

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Research, development, and commercialization of antibody medicines have recently been conducted within the pharmaceutical industry. Various kinds of antibody medicines are generated from cultures of animal cells such as Chinese hamster ovary (CHO) cells. Of all the animal cells, CHO cells are preferred for the mass production of recombinant proteins, since they are ideal hosts with respect to rapid growth rate, high stability and efficient foreign gene expression (1). Over the last decade, many studies promoting cell growth or an increase in the productivity of proteins have been conducted (2–5).

In general, antibody medicines have the advantage of low toxicity; however, they require large dosages to be effective. Therefore, it is necessary to establish a high-density culture system in large-scale bioreactors. The scale-up of culture starts from a few liters in the laboratory and goes through many hundreds of liters, up to several thousand according to the stage of development. The culture volume may finally reach the order of a 10,000 L scale at the commercial manufacturing stage.

One of the most important factors in operating cell culture bioreactors is mass transfer, which includes oxygen supply and dissolved carbon dioxide (dCO2) stripping. In the scale-up of industrial cell culture, dCO2 buildup is one of the most serious problems. The accumulation of dCO2 is implicated in reduced growth and productivity, in addition to adverse effects on glycosylation (6–8). Therefore, it is very important to remove dCO2 in large-scale cultures. Another important parameter is oxygen supply. Oxygen supply and dCO2 stripping, the key operations for maintaining the metabolism of cultured cells, must be properly controlled. Conventionally, oxygen supply and dCO2 stripping are performed by gas sparging and agitation in large-scale cultures, because of their large mass transfer rate and operational simplicity. However, both gas sparging and agitation rates are restricted to low levels, because animal cells are very sensitive to shear stress and are easily damaged (9–12). These restrictions result in dCO2 accumulation in large bioreactors. In addition, the liquid surface-to-volume ratio decreases gradually as the bioreactor enlarges. This also induces dCO2 accumulation in large-scale cultures. Thus, it is difficult to control gas transfer in bioreactors, because the effects of scale-up must be matched.

Many studies on oxygen supply, such as the scale-up strategy to maintain constant kLa have been actively conducted to date. However, oxygen transfer and dCO2 stripping over a wide range of volumes from pilot to plant scale have not been rigorously examined using some real bioreactors, except by process modeling and simulations (13–15).

In this study, we experimentally examined the effect of scale-up on oxygen transfer and dCO2 stripping with different working-volume bioreactors (80 L, 500 L, 2000 L, and 10,000 L). The results are
discussed in relation to the theoretical considerations. We obtained some important findings about scale-up strategies.

MATERIALS AND METHODS

Bioreactors  All data in this paper were obtained using 80 L, 500 L, 2000 L, and 10,000 L bioreactors and a 2000 L agitation test tank, which have impellers, baffles, and spargers, as shown in Fig. 1. Here, the volume of the bioreactors is expressed as the basic working volume. The 80 L and 500 L bioreactors did not have a middle impeller, and the 2000 L agitation test tank had an impeller not inclined near the bottom, and an impeller inclined at the middle. All bioreactors and the 2000 L agitation tank have four baffles. The aspect ratios are from 1.0 to 1.6. The liquid depths are 520 mm (80 L), 905 mm (500 L), 1530 mm (2000 L reactors), and 2285 mm (10,000 L reactors).

Measurement of $k_\text{La}$ using water tests  $k_\text{La}$ was measured as follows. The bioreactors were filled with water to the basic working volume, and nitrogen was sparged into the water from the bottom until $[\text{DO}]_{\text{w}}$ reached about 20%.

RESULTS AND DISCUSSION

In general, cell culture systems are very complex. The flow profiles of gas and liquid are complicated, and affected by culture tank geometry, agitation, sparging etc. Therefore, it is laborious to analyze...
all effects of the control parameters to scale-up the system properly. In chemical engineering, it is important to find a methodology for determining the working equation for each system. It is also important to clarify the effects of scale-up on key factors in the culture system. In this study, we focused on the effect of scale-up on \( k_{La} \) and \( k_{Laco2} \) and examined these experimentally using bioreactors with a wide range of working volumes.

**Evaluation of \( k_{La} \) and \( k_{Laco2} \) by sparging using water tests**  
Fig. 4a-c shows \( k_{La} \) as a function of sparging gas volume, per liquid volume, per min (vvm) at various tip speeds and bioreactor volumes. \( k_{La} \) clearly increased in proportion to vvm, except that it tended to saturate at low and middle tip speed conditions (0.5 m/s and 0.75 m/s, respectively) in large bioreactors. \( k_{La} \) also increased with scale-up and tip speed.

Fig. 5a-c shows the effect of tip speed, vvm of sparged aeration, and bioreactor volume on \( k_{Laco2} \) with no aeration of the liquid surface. \( k_{Laco2} \) increased with increasing vvm and increasing bioreactor volume at all tip speeds; \( k_{Laco2} \) increased roughly in proportion to vvm at a low tip speed of 0.5 m/s, but less effectively at high vvm at middle and high tip speeds of 0.75 and 1.0 m/s.

As shown in Figs. 4 and 5, \( k_{La} \) and \( k_{Laco2} \) increased roughly linearly in proportion to vvm, although saturation points were found in large bioreactors (2000 L and 10,000 L). To determine the characteristic points, we approximated the plots in Fig. 4a-c and Fig. 5a-c to straight lines, and defined the slopes as \( \phi_{O2} \) and \( \phi_{CO2} \), respectively. As shown in Fig. 6, \( \phi_{O2} \) increased linearly in proportion to liquid depth in 80 L–10,000 L bioreactors, except for the largest bioreactor (10,000 L) at low tip speed. On the other hand, \( \phi_{CO2} \) increased linearly in proportion to liquid depth only in a narrow range for depths less than 1000 mm (80 L–500 L bioreactors) and saturated at a roughly constant level, even at high tip speed. The difference in the behavior of \( \phi_{O2} \) and \( \phi_{CO2} \)
indicates that it is not always valid to adjust the culture conditions for 
\( dCO_2 \) stripping based only on the constant \( k_0a \), which is conventional 
in real cultures.

As shown in the Appendix, in the case that the rate of gas transfer 
between a bubble and the liquid is slow, the relationship between the 
volumetric mass transfer coefficient \( K \) and sparging volume per liquid 
volume per minute \( vvm = Q/V \) is derived as

\[
K = \frac{dQ}{V} = \frac{aQ}{V} \frac{\tau}{\Omega} \frac{r}{r_f}
\]

where \( V \) is the liquid volume; \( Q \) is the bubble volume sparged per unit 
time to the bioreactor; \( \tau \) is the lifetime of a bubble; \( L \) is the liquid depth; 
\( \Omega \) is defined as the ratio of the diffusion constant of the gas 
and the length of the boundary layer surrounding the bubble; and \( r \) is 
the radius of a bubble. \( \Omega \) is defined as \( Q/V (= vvm) \). \( \phi_{CO2} \) and \( \phi_{CO2} \) are 
estimated from \( K/\Omega \). If \( k, r, \) and \( \Omega \) can be approximated as constants, the 
proportional increase of \( \phi \) versus depth is derived as shown in Eq. A12. 
Therefore, the behavior of \( \phi_{CO2} \) observed in Fig. 6a is roughly explained by 
the above theoretical considerations, though a deviation was found at 
the upper end of the bioreactor size range. On the other hand, the 
increase in \( \phi_{CO2} \) was clearly suppressed by scale-up, and \( \phi_{CO2} \) saturation 
occurs at a much lower depth of \( \sim 1000 \) mm, as shown in Fig. 6b. 

Although it is very difficult to identify the reason for this difference in gas 
exchange behavior, the time required for the gas transfer for carbon 
dioxide may be smaller than that for oxygen because the solubility of 
carbon dioxide in water is larger than that of oxygen. Therefore, we could 
attribute the saturation of \( \phi_{CO2} \) to this effect as shown by Eq. A14 in 
the case of fast gas transfer. However, we cannot specify a reason 
for saturation at this stage, since the parameters \( k, r, \) and \( \Omega \), which we 
estimated as constant, could be changed by operations such as agitation 
through the parameters of the bioreactor (e.g., tip speed, or \( vvm \)). In 
addition, the sparging velocity of bubbles could be increased by scale-up 
even if \( vvm \) remains the same, as shown in Fig. 2. This tendency could 
also affect the gas transfer efficiency through the parameters.

**CHO cell culture** Table 1 shows the total oxygen supply volume 
per culture volume in each tank (80, 500, and 2000 L) during CHO cell 
culturing. These cultures started with the same initial viable cell 
density, \( 2.0 \times 10^5 \) cells/mL, and DO was controlled at 100 ± 10% during 
the cultures. The cell growth rate was almost the same, irrespective 
of the tank volume. As shown in Table 1, the total oxygen supply volume 
per culture volume during culture decreased according to the scale-up 
of the bioreactor. This behavior is consistent with the behavior of \( k_0a \) 
during water tests.

**Evaluation of dCO2 stripping from the liquid surface using water tests** Fig. 8 shows the relationship between aeration to liquid 
surface and \( dCO2 \) accumulation by scale-up at a fixed tip speed of 
0.75 m/s. Where there was no surface aeration, the sparging aeration 
volume was controlled, so that \( k_0a^{dCO2} \) was within 0.15–0.20. The 
sparging aeration volumes were 0.5 L min\(^{-1}\) (80 L), 1.5 L min\(^{-1}\) 
(500 L), 3.0 L min\(^{-1}\) (2000 L) and 15 L min\(^{-1}\) (10,000 L), which 
correspond to the value of \( vvm \) at 0.00825, 0.003, 0.0015 and 0.0015 L 
min\(^{-1}\) \( vvm \), respectively. Ten and twenty times surface aeration of 
the sparging aeration in volume was then added while sparging aeration 
was maintained. As shown in Fig. 8, the efficiency of \( dCO2 \) stripping by 
surface aeration in 80 and 500 L bioreactors was much greater than 
that in 2000 L and 10,000 L bioreactors. \( k_0a^{dCO2} \) increased with surface 
aeration roughly proportionally in 80 L and 500 L bioreactors in the 
experimental range of surface aeration (20 times surface aeration of 
the sparging aeration corresponds to the value of \( vvm \) at 0.125 and 
0.06 L min\(^{-1}\) \( vvm \), respectively, for 80 L and 500 L bioreactors). On the 
other hand, the rate of the increase of \( k_0a^{dCO2} \) with surface aeration 
decreased and was saturated at the higher surface aeration in 2000 L 
and 10,000 L bioreactors (20 times surface aeration of the sparging 
aeration in volume corresponds to the value of \( vvm \) at 0.03 L 
min\(^{-1}\) \( vvm \) for both 2000 L and 10,000 L bioreactors). This is 
attributed to the decrease in liquid surface-to-volume ratios and 
increasing bioreactor volumes.

**Table 1.** Total oxygen supply volume per culture volume during CHO cell culturing in 
80 L, 500 L, and 2000 L bioreactors

<table>
<thead>
<tr>
<th>Tank scale</th>
<th>Culture volume (L)</th>
<th>Total oxygen supply/culture volume (L/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 L</td>
<td>71</td>
<td>647</td>
</tr>
<tr>
<td>500 L</td>
<td>403</td>
<td>468</td>
</tr>
<tr>
<td>2000 L</td>
<td>1896</td>
<td>297</td>
</tr>
</tbody>
</table>
Fig. 9 shows the effect of liquid depth on dCO₂ stripping, observed using the same 2000 L agitation test tank. kₐdCO₂ decreased with the increasing liquid surface-to-volume ratio. In typical cultures of animal cells in 2000 L fed-batch reactions, the liquid volume starts at 1500 L and reaches 2000 L at the end of culture. Fig. 9 indicates that this increase in volume in the real culture also reduced the liquid surface-to-volume ratio, resulting in a decrease in kₐdCO₂. On the other hand, the sparger pore size had no significant effect on kₐdCO₂.

**FIG. 8.** Effect of surface aeration on kₐdCO₂ in bioreactors of different sizes with a sparger pore size of 100 μm.

**FIG. 9.** kₐdCO₂ observed using the same 2000 L agitation test tank at the indicated sparger pore size, liquid depth, and liquid volume at sparged aeration volumes of 5.7 L min⁻¹ or 0 L min⁻¹ (no sparge) (a) and kₐdCO₂ as a function of liquid surface area/volume ratio at different sparging pore sizes (b).
CONCLUSION

In this study, the effect of scale-up on the volumetric gas transfer coefficient in bioreactors was measured for a wide range of volumes from 80 L to 10,000 L. Both kLa and kLa_{02} increased roughly in proportion to vvm, and the proportional constant of these values increased with the increasing depth of liquid at lower depths, which is consistent with a simple theoretical consideration, as shown in the Appendix. However, this linear relationship was not satisfied at greater depths, and saturation behavior was observed, especially for kLa_{02}. This result suggests that it is not always valid to adjust the culture conditions based on only the evidence observed in CHO cultures shows that the average vvm of sparged oxygen at constant DO during the culture, which decreased during the scale-up of bioreactors, is consistent with the roughly linear increase in kLa vs. vvm in the scale-up using water tests. This result suggests that test results are useful for evaluating oxygen supply in real cultures. It was also demonstrated that a decrease in the liquid surface-volume ratio by scale-up (or increasing liquid volume in bioreactors) affected kLa_{02} and resulted in CO_{2} accumulation.

APENDIX A. RELATIONSHIP BETWEEN VOLUMETRIC MASS TRANSFER COEFFICIENT AND SPARGING VOLUME PER LIQUID VOLUME PER UNIT TIME

The rate of change of the concentration in the medium by sparging with bubbles is expressed as

\[
\frac{dC}{dt} = k(C_f - C)
\]

Then, the change of gas concentration in the medium by sparging with bubbles is given as

\[
\frac{C_f - C(t)}{C_f - C_0} = e^{-k\tau} = e^{-\alpha t}
\]

where \(k\) is the volumetric mass transfer coefficient (e.g., \(k_{da}, k_{la_{02}}\)); \(C_f, C_0,\) and \(C(t)\) are the gas concentrations in saturated medium liquid, at the beginning of air sparging \((t = 0)\), and at sparging time \(t\).

We will define the average mass (oxygen or carbon dioxide in the present study) transfer from a bubble into the medium liquid during unit time at sparging time \(t\) as \(q(t)\). Where the rate of mass transfer is slow, this process is not completed during the lifetime \(\tau\) of the bubble in the tank. In this case, \(q(t)\) is expressed as

\[
q(t) = \frac{1}{\tau} \int_{0}^{\tau} q(t, t') dt'
\]

where \(q(t, t')\) is the rate of mass transfer between a bubble and the medium liquid at the elapsed time \(t'\) since the beginning of sparging \((0 < t' < \tau)\), and is given by

\[
q(t, t') = 4\pi r^2 k(C_i(t, t') - C(t))
\]

where \(k\) is the rate of mass transfer from a bubble to the medium liquid, defined as the ratio of the diffusion constant of the gas and the length of the boundary layer surrounding the bubble. \(C_i\) is the mass concentration at the gas–liquid interface, which depends on the mass concentration in the bubble and the solubility in the liquid, and is given by

\[
4\pi r^2 \frac{dC_i(t, t')}{dt'} = -q(t, t')
\]

When \(C(t)\) varies more slowly than \(C_i(t, t')\), Eqs. A4 and A5 give

\[
C_i(t, t') = C(t) + (C_f - C(t))e^{-\alpha t}
\]

The lifetime of a bubble, \(\tau\), is estimated as

\[
\tau = \frac{L}{v_i}
\]

where \(L\) is the liquid depth, \(v_i\) is the terminal velocity of bubbles, and \(\alpha\) is a coefficient due to agitation. Thus, combining Eqs. A1, A10 and A11, \(C_i(0) = C_f\) we have

\[
K = \frac{aQ\tau}{V} = \frac{aKL}{\Omega}
\]

where \(\Omega\) is defined by \(Q/V = vvm\). \(\phi_{O_2}\) and \(\phi_{CO_2}\) are defined in the text and are expressed as \(K/\Omega\).

In the case of time \(t_e\) for the completion of mass transfer is fast, i.e., \(t_e < \tau\), \(q(t)\) is expressed as

\[
q(t) = \frac{1}{\tau} \int_{0}^{t_e} q(t, t') dt' = \frac{u\tau}{\alpha} (C_f - C(t))
\]

where \(u\) is a constant. Then, we have

\[
K = u\Omega
\]

in place of Eq. A12.

Nomenclature
\(C_0\), gas concentration in liquid at the beginning of air sparging [mol L\(^{-1}\)]
\(C_f\), gas concentration in saturated liquid [mol L\(^{-1}\)]
\(C_i\), concentration at the gas–liquid interface [mol L\(^{-1}\)]
\(C(t)\), gas concentration in liquid at sparging time \(t\) [mol L\(^{-1}\)]
\(\Delta CO_2\), ratio of mole of dissolved carbon dioxide at the beginning of air sparging to mole of total gas dissolved in medium [%]
\(\Delta CO_2\), ratio of mole of dissolved carbon dioxide at a steady state after a long sparging period to mole of total gas dissolved in medium [%]
\(\Delta DO\), ratio of mole of dissolved carbon dioxide at sparging time \(t\) to mole of total gas dissolved in medium [%]
\(\Delta DO\), dissolved oxygen concentration at the beginning of air sparging [mol L\(^{-1}\)]
\(\Delta DO\), dissolved oxygen concentration saturated in water [mol L\(^{-1}\)]
\(\Delta DO\), dissolved oxygen concentration at sparging time \(t\) [mol L\(^{-1}\)]
$K$, volumetric mass transfer coefficient [h$^{-1}$]  
$k_{da}$, volumetric oxygen transfer coefficient [h$^{-1}$]  
$k_{dCO_2}$, volumetric dissolved carbon dioxide transfer coefficient [h$^{-1}$]  
$k$, rate of mass transfer between bubble and liquid [m/s]  
$L$, liquid depth [mm]  
$N$, number of bubbles in bioreactor [-]  
$Q$, bubble volume sparged per unit time to bioreactor [L min$^{-1}$]  
$q(t, t')$, rate of mass transfer between bubble and liquid at time $t'$ [mol/s]  
$r$, radius of bubble [mm]  
$V$, liquid volume [L]  
v$_{b}$, volume of a bubble [mm$^3$]  
v$_{f}$, terminal velocity of bubble [mm/s]  
vvm, gas volume per liquid volume per min [L min$^{-1}$ L$^{-1}$]  
$\alpha$, coefficient due to agitation [-]  
$\Omega Q/V$, [L min$^{-1}$ L$^{-1}$]  
$\tau$, lifetime of bubble [s]  

References  