Sparger and Surface Gas Transfer for Cell Culture Bioreactors

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Summary

The impact of bioreactor and sparger scale-up on gas transfer properties was investigated using four geometrically similar bioreactors ranging in size from 15-L to 2500-L total volume with the same sintered metal frit (microporous) sparger design. The mass transfer coefficients for both oxygen and carbon dioxide were measured at each scale at various flow rates. Scale did not directly affect gas transfer properties. Of the four bioreactors investigated, only the 200-L bioreactor had significantly different (higher) values for the oxygen and carbon dioxide sparger mass transfer coefficients.

The rate of carbon dioxide stripping from sparging in all vessels was much lower that expected based from film diffusion theories. The rate was limited by the saturation of the sparge gas by the carbon dioxide dissolved in the medium. The stripping rate was so low that sparging to provide oxygen would not remove enough carbon dioxide to balance cellular respiration.

The measured surface transfer of carbon dioxide and oxygen decreased directly with available surface area per unit volume. In agreement with observations, cultures that exhibit controlled carbon dioxide levels at small scale (15 L and less) may not have stable carbon dioxide levels at larger scale.

Introduction

To understand the effect of scale-up on dissolved gas concentrations in bioreactors, we investigated the impact of bioreactor and sparger scale-up on gas transfer properties with particular emphasis on carbon dioxide removal. Aunins (Aunins 1993) divided spargers into two categories, microporous and orifice. Microporous spargers are typically frits with holes less than 0.1 mm in diameter, whereas orifice spargers have drilled holes that are greater than 0.1 mm in diameter. Previous work has demonstrated that sintered metal frit spargers will provide high gas transfer coefficients by creating very small bubbles. The high oxygen transfer is several times what is achieved for orifice spargers, which create larger bubbles.(Aunins 1993), (Bovonsombut 1987), (Zhang 1992), (Zhang 1993), (Chisti 1993)

Authors have associated spargers generating very small bubbles with poor carbon dioxide removal(Aunins 1993). Gray (Gray 1996) attributed poor carbon dioxide removal to the shrinking of pure oxygen bubbles as oxygen is transferred to the media. Poor carbon dioxide removal can lead to difficulties in controlling pH (Aunins 1993), and accumulation of carbon dioxide can affect cell culture behavior, product titer and desired product quality(Zhangi 1999), (Kunkel 2000),(Jardon 2003),(Mostafa 2003). Mostafa and Gu (Mostafa 2003) found that replacing a frit sparger with an open pipe sparger, and adjusting the impeller position enhanced carbon dioxide removal in a large scale (1000-L) cell culture bioreactor, and resulted in increased titers.

To assess how scale affects the performance of these spargers, we studied four bioreactors ranging in size from 15-L to 2500-L total volume, with the same sintered metal frit sparger design. We measured mass transfer coefficients for oxygen and carbon dioxide for the sparger and liquid surface.

Material and Methods

Four Bioreactors were included in the study with nominal total volumes of 15-L, 200-L, 500-L and 2500-L. The physical dimensions are provided in Table 1. Marine impellers were used in all bioreactors. The bioreactors were filled to a liquid height to vessel

diameter of 1:1. Microporous (10 to 20 μm) frit spargers were installed in all vessels, and where feasible, spargers were scaled to provide the same sparger frit area per unit volume. Sparger dimensions are provided in Table 2. Spargers for the 15-L and 200-L bioreactors were provided by BioTech Services Inc. (Halesite, NY), and spargers for the 500-L and 2500-L bioreactors by the bioreactor manufacturer (Bioengineering, Wald, Switzerland).

Table 1: Bioreactor Dimensions

Total Volume	Fill Volume	Vessel Diameter	Impeller Diameter
15-L	8 L	20 cm	10 cm
200-L	160 L	61 cm	40 cm
500-L	420 L	80 cm	50 cm
2,500-L	2,000 L	150 cm	90 cm

Table 2: Sparger Dimensions

Bioreactor	(Number x) Length, Diameter
15 L	(1x) 5 cm, d = 1.0 cm
200 L	(4x) 25 cm, d = 1.0 cm
500 L	(2x) 14cm + (2x) 23 cm, d = 1.4 cm
2,500 L	(2x) 14cm + (2x) 23 cm, d=3.8 cm

Dissolved oxygen and pH were monitored continuously with probes installed through 25 mm Ingold ports. pH probes (model GT-DJ-PG13.5-DTCH, ISI) were calibrated prior to insertion into the vessel. Each vessel was sterilized by steam-in-place, in accordance with established procedures. The pH readings were periodically confirmed with at-line measurements of samples from the bioreactor. Dissolved oxygen probes (model 7803-31535, Mettler Ingold) were calibrated to 100% after the media was equilibrated with air (typically overnight). All vessels were filled with purchased DMEM (HyClone) media with 10% Fetal Calf Serum (HyClone) to a liquid height equal to the diameter of the vessel. The agitation rate for each bioreactor was selected to provide a power per unit volume of approximately 2.5 mW/m^3, i.e. 90, 30, 27 and 17 rpm for the 15-L, 200-L, 500-L and 2,500-L bioreactors respectively. For all experiments, gas was sent to the

head space of the bioreactor at a flowrate of approximately 0.05 liters per minute per filled reactor volume (0.05 vvm).

Determination of sparger oxygen mass transfer coefficient $k_{L,Ox}a_B$

Oxygen transfer in cell culture media has been extensively studied at small scale for various sparger designs (Aunin 1993), (Sambanis 1993), (Zhang 1992), (Zhang 1993), (Emery 1995), (Fenge 1993). Oxygen transfer can be described by the mass transfer model for a sparingly soluble gas into a liquid, where the driving force for oxygen transfer is the oxygen concentration that would be in equilibrium with the sparger gas, $[O_2]^*$, minus the dissolved oxygen concentration, $[O_2]$. The oxygen transfer rate, $d[O_2]/dt$ is the oxygen mass transfer coefficient for the bulk ($k_{L,Ox}a_B$) times the driving force:

Equation 1

$$d[O_2]/dt = k_{L,Ox}a([O_2]- [O_2]^*)$$

A similar equation can be written for oxygen surface transfer, using the oxygen mass transfer coefficient for the surface ($k_{L,Ox}a_S$) and the driving force from the headspace gas $([O_2]^*-[O_2])_S$. For larger bioreactors, the surface oxygen transfer is much smaller than the sparger oxygen transfer, and can be neglected during sparging.

To determine the sparger oxygen mass transfer coefficient ($k_{L,Ox}a_B$) for each bioreactor, nitrogen was sparged to reduce the dissolved oxygen to less than 10%, based on 100% is in equilibrium with air. Air or oxygen was then sparged until the dissolved oxygen was $k_{L,Ox}a_B$ over 90%. During the sparging of nitrogen, air or oxygen, the $k_{L,Ox}a_B$ was determined by a linear fit of $\ln / [O_2]^*- [O_2] /$ vs time.

Determination of sparger carbon dioxide mass transfer coefficient $k_{L,CO}a_B$

In many media commonly used for cell culture, the pH is maintained by a carbon dioxide – bicarbonate buffer. Several reactions are required to fully describe the behavior of this buffer system, but in the pH range 6.5 to 8.0, the reactions can be summarized in one combined reaction: (Ho, 1986)

Equation 2

$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^-$$

The carbonic acid (H_2CO_3) and carbonate ions (CO_3^{-2}) concentrations are not significant compared to bicarbonate and carbon dioxide concentrations in the pH range from 6.5 to 8.0(Stumm, 1981), (Gray 1996). The equilibrium constant for the above reaction in cell culture media (Gray 1996) is about $8x10^{-7}$. Using a mass balance for the species, H^+, CO_2 and HCO_3^- and eliminating insignificant terms, we can obtain the carbon dioxide concentration $[CO_2]$ during stripping at any time relative to an initial value $[CO_2]_0$ as a function of pH:

Equation 3

$$[CO_2]=10^{pH0-pH}[CO_2]_0$$

The rate of carbon dioxide stripping, $d[CO_2]/dt$ is describe by a differential mass balance similar to that for oxygen transfer, except it is based on the carbon dioxide mass transfer coefficient for the bulk ($k_{L,CO}a_B$) and carbon dioxide is not present in the sparger gas ($[CO_2]^*=0$):

Equation 4

$$d[CO_2]/dt=-k_{L,CO}a_B[CO_2]$$

Combining the equilibrium relationship with a mass balance for carbon dioxide, we can show that without the addition of other acids or bases, the bicarbonate level will remain constant, i. e. $[HCO_3^-] =[HCO_3^-]_0$, and the mass transfer coefficients will be directly proportional to the rate of change of the pH, independent of the total inorganic carbon concentration.

Equation 5

$$k_{L,CO}a_B=2.303\ dpH/dt$$

A similar relation can be written for surface CO_2 removal using the carbon dioxide mass transfer coefficient for the surface, $k_{L,CO}a_S$.

To determine the sparger carbon dioxide mass transfer coefficient ($k_{L,CO}a_B$) for each bioreactor, the pH was reduced to between 7.00 and 7.20 by sparging with CO_2. These sparges were not used to determine the $k_{L,CO}a_B$, since the changes in pH with sparging CO2 may not represent the equilibrium of CO_2 related species in water. The pH was allowed to stabilize for approximately 15 minutes before measuring the stripping rate, d(pH)/dt. Nitrogen, air or oxygen was then sparged until a pH change of at least 0.04 units was observed. The stripping rate, d(pH)/dt, was determined by a linear fit to pH vs time and the mass transfer coefficient was then calculated directly from $k_{L,CO}a_B = 2.303$ d(pH)/dt.

Determination of surface oxygen mass transfer coefficient $k_{L,Ox}a_S$ *and surface carbon dioxide mass transfer coefficient* $k_{L,CO}a_s$

To determine the surface mass transfer coefficients for each reactor, nitrogen was first sparged to reduce the dissolved oxygen to less than 10% and the pH was reduced to between 7.00 and 7.20 by sparging with CO_2. Air was sent to the headspace at 0.05 vvm, and the $[O_2]$ and pH was monitored for several hours, typically overnight. After an initial transitional period associated with the dissipation of the foam on the surface, the $k_{L,Ox}a_B$ was determined by the slope of the least squares fit of $\ln / [O_2]^* - [O_2] /$ vs time, and stripping rate, d(pH)/dt, was determined by a linear fit to pH vs time. The surface carbon dioxide mass transfer coefficient was calculated directly from $k_{L,CO}a_S = 2.303$ d(pH)/dt.

Results and Discussion

No statistically significant differences were found between determinations made by sparging with air, nitrogen or oxygen. Results for all three gases were therefore combined in the analysis. As can be seen in Table 3, there is no trend with bioreactor scale in the oxygen or carbon dioxide sparger mass transfer coefficients ($k_{L,Ox}a_B$ and $k_{L,CO}a_B$) under typical operating conditions. The mass transfer coefficients of the 15-L, 500-L, and 2,500-L bioreactors are not significantly different from each other.

Table 3: Gas transfer coefficients at 0.005 vvm with scale. Ranges are 95% confidence limits.

Bioreactor	$k_{L,Ox}a_B$ (1/hr)	$k_{L,CO}a_B$ (1/hr)
15-L	3.4±1.2	0.46±0.09
200-L	9.3±1.0	0.65±0.25
500-L	3.0±1.0	0.28±0.05
2,500-L	3.7±1.1	0.42±0.08

The observed ratio of the carbon dioxide to the oxygen mass transfer coefficient ($k_{L,CO}a_B$ / $k_{L,Ox}a_B$), about 0.1, is much lower than would be expected based on film diffusion based theories. The ratio of the mass transfer coefficients should be between 0.8 and 0.9. (Aunins 1993), (Ho 1986), (Ho 1988) Gray proposed that for small oxygen bubbles, the shrinking of bubbles as they traveled to the surface would reduce the stripping of CO_2.(Gray 1996) However, we observed no significant differences in the CO_2 stripping rate for nitrogen, air or oxygen, so this mechanism does not explain these results.

An explanation that fits our results well is that the sparge gas is saturating with CO2 prior to reaching the headspace. When this occurs, the total amount of stripping can only be the gas volume sparged times the equilibrated gas phase concentration. Using Henry's law, the ideal gas law, and the volume of sparger gas per bioreactor volume per minute (vvm), we can derive an apparent maximum gas transfer coefficient, $(k_{L,CO}a)_{SAT}$.

Equation

$$(k_{L,CO}a)_{SAT} = (H/RT)(60)(vvm)$$

For CO_2 in media at 37 ^{o}C, the Henry's Law constant, H, is ~40 atm-L/mol (Onda 1970), (Yagi 1977) and $(k_{L,CO}a)$SAT will be ~94 vvm or 0.47 at 0.005 vvm. The saturation value is consistent with the measured values in table 3. This is so low, sparging with pure oxygen will not remove as much carbon dioxide as is formed by respiration. For typical cell culture conditions, i.e. 10% dissolved CO_2 ([CO_2]~2.5 mM) and sparging

with pure O_2 ($[O_2]^*$- $[O_2]$)~0.9 mM), the amount of carbon dioxide stripped will be less than half of the carbon dioxide produced by respiration.

To avoid accumulation, CO_2 must be removed by another mechanism such as surface stripping. The surface gas mass transfer coefficients were proportional to the available surface area to volume ratio with scale (Table 4). The ratio $k_{L,Ox}a_s / k_{L,CO}a_s$ for surface stripping agreed better with film diffusion theory, consistent with the headspace gas, having 10 times the flowrate, does not saturate with carbon dioxide.

Table 4: Surface Gas transfer coefficients with scale.

Bioreactor	$k_{L,Ox}a_s$ (1/hr)	$k_{L,CO}a_s$ (1/hr)	Ratio
15 L	0.25	0.15	0.6
200 L	0.10	0.08	0.8
500 L	0.06	0.04	0.7
2500 L	0.05	0.03	0.6

These results also are consistent the problem of carbon dioxide accumulation with scale-up. For the smallest bioreactor, the $k_{L,CO}a_B$ was about 0.15/hr. For typical cell culture conditions (10% dissolved CO_2), the rate of carbon dioxide removal through the surface would be 0.38 mmoles/L-hr, or equivalent to the carbon dioxide produced by cellular respiration when the OUR is about 2.5% per minute. For the largest reactor, the $k_{L,CO}a_B$ is about 0.03/hr and could only remove the carbon dioxide produced by cellular respiration when the OUR is about 0.5% per minute. A process with cellular OUR in the range of 2% to 5% per minute would not have carbon dioxide accumulate in the small reactor, but would have carbon dioxide accumulate in the largest reactor.

Conclusions

The oxygen gas transfer coefficient of the sparger, $k_{L,Ox}a_B$, was not dependent on scale. Of the four bioreactors investigated, only the 200 L had statistically significantly

different (higher) values for the oxygen transfer coefficient at the operating condition of 0.005 vvm. As the gas flow rate was increased, the oxygen mass transfer coefficients increased until reaching a maximum. Because of the non-linearity of the relationship between oxygen mass transfer coefficient and flow rate, lowering the flow rate may lead to less gas sparged, and less foam formed in the bioreactor.

The carbon dioxide transfer coefficient, $k_{L,CO}a_B$ was nearly the same for the four bioreactors and was not dependent on sparge gas. The measured values were much lower than what would be expected based on film diffusion theories. The values were consistent with the saturation of sparge gas with carbon dioxide. Because of the low values this sparger design may not remove enough carbon dioxide to keep the levels balanced. As expected, the surface stripping of carbon dioxide decreased directly with the available surface area per unit volume. With this sparger design, cultures that exhibit balanced carbon dioxide levels at small scale (15-L and less) may not have stable CO_2 levels at larger scale.

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