Growth of mammalian cells at high oxygen concentrations

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Summary

Oxygen, although essential to the growth of mammalian cells in vitro and in vivo, has been widely reported to be toxic at concentrations at or above the oxygen concentration in culture medium equilibrated with air (approximately 200 μM). We were therefore surprised to note that a diploid human B-cell line (TK6) was able to proliferate normally while exposed to 380 μM-oxygen. This observation was extended to Vero (African Green monkey kidney) cells, and Sp2/0-derived murine transfectomas producing antibody. Using an experimental system with a high capacity for oxygen transfer, we determined the growth rates of the three cell lines at controlled oxygen concentrations ranging from 80 μM to 910 μM. Each of these cell types was able to grow normally at oxygen concentrations up to 360-380 μM. At oxygen concentrations above 380 μM, a significant increase in the apparent doubling times of the cells was observed. No adverse effect of oxygen on TK6 cell survival was seen for concentrations ranging from 60 μM to 410 μM. We conclude that exponential growth at nearly maximum growth rates was observed for Sp2/0-derived cells, TK6, and Vero cells at constant oxygen concentrations up to 400 μM (twice air saturation).

These findings have substantial implications for process control in the production of cells and cell-derived materials. The intrinsic oxygen transfer rate of any cell culture vessel can be increased severalfold by raising the oxygen gas concentration. In practice, this should permit significant increases in the operating cell density without the difficulty and expense of oxygen control feedback loops. Using this information it should be possible to eliminate oxygen transfer as a limiting factor in high-density cell culture technology.

Key words: oxygen, high cell density, lymphoblastoid cells, Vero cells, Sp2/0 cells.

Introduction

Oxygen is an essential requirement for the normal growth of mammalian cells, but concentrations in excess of 200 μM (160 mmHg partial pressure or 21 % oxygen in the gas phase) have been reported to inhibit the growth and metabolism of a number of cell types. Usually, cells are grown in culture equilibrated with air (21 % gas-phase oxygen). Under these conditions, the cells are exposed to dissolved oxygen concentrations of 200 μM or less. Cooper et al. (1959) found that, while embryo rabbit kidney cells grew well at 210 μM-oxygen, their growth rate was halved at just 230 μM, and concentrations of 240-285 μM were 'rapidly cytocidal'. Similarly, Balin et al. (1976) showed a marked inhibition in WI-38 human diploid fibroblast growth as the oxygen concentration was increased from 165 μM to 365 μM. Hyperatmospheric oxygen (465 μM) was reported by Balin et al. (1978) to reduce the rate of DNA synthesis and to delay mitosis in WI-38 cells. Taylor et al. (1974) found a tenfold increase in WI-38 plating efficiency at 10 μM as compared with 190 μM. The plating efficiency of Vero cells was reported to be unaffected in this range of oxygen concentrations.

The apparently narrow range of dissolved oxygen concentrations (between 40 μM and 140 μM) believed suitable for cell growth has had a pronounced effect on the design of large-scale mammalian cell production devices. At a density of 2×10^7cells ml^-1, typical human cells will...
consume 1–10 mmol oxygen l⁻¹h⁻¹ (Fleischaker & Sinskey, 1981). The low solubility of oxygen in aqueous medium (200 μM-oxygen at air equilibrium) relative to its rate of consumption causes its rate of supply to be the limiting factor for cell growth.

The oxygen transfer rate in a fermentor is described by:

\[ \text{OTR} = K_L a (C_{\text{gas}} - C_{\text{liq}}) \]

where \( \text{OTR} \) = oxygen transfer rate in μmol O₂ l⁻¹h⁻¹; \( K_L a \) = mass transfer coefficient in h⁻¹; \( C_{\text{gas}} \) = gas-phase O₂ (equilibrium) concentration in μM; and \( C_{\text{liq}} \) = liquid-phase O₂ concentration in μM. This equation implies that the OTR can be increased by either an increase in the \( K_L a \) or an increase in the driving force \( (C_{\text{gas}} - C_{\text{liq}}) \).

Dissolved oxygen concentrations are frequently expressed as the oxygen concentration in the liquid in equilibrium with the partial pressure of oxygen in the gas phase (‘oxygen tension’, \( pO_2 \)). The gas \( pO_2 \) may be expressed as mmHg, where 760 mmHg corresponds to pure oxygen at 1 atmosphere, 150 mmHg is the partial pressure of oxygen in water vapor-saturated air, and 160 mmHg is the partial pressure of oxygen in dry air. Dissolved oxygen may also be expressed as the oxygen concentration in the liquid in equilibrium with % oxygen in the gas phase. Here, we define 100 % as one atmosphere of pure oxygen. Thus, air is 21 % oxygen.

We have estimated actual levels of dissolved oxygen in media by calculating the reduction in solubility in medium (due to salts) as compared to that in distilled water using the technique of Schumpe et al. (1978). The concentration of oxygen in medium in equilibrium with dry air at 37°C is approximately 200 μM. Saturation of the air with water vapor at 37°C reduces the equilibrium oxygen concentration to approximately 190 μM.

To overcome the limitation of the low solubility of oxygen in culture medium, microbial fermentations are typically aerated by direct sparging of air into the fermentor. However, sparging of mammalian cell cultures was reported by Kilburn & Webb (1968) and Telling & Radlett (1970) to be detrimental to cell growth rate, viability and maximum attainable cell density. Handa et al. (1987) recently related this effect to bubble size and frequency as well as cell type. Several alternative means have been developed to improve the oxygen transfer rate, including submerged silicone tubing (Tyo & Wang, 1980; Kuhlmann, 1987), microporous tubing (Lehmann et al. 1987), caged aeration (Whiteside et al., 1985), and fluorocarbon supports (Munder et al. 1971).

Most fermentor design in the past has centered on increasing \( K_L a \) (Spier & Griffiths, 1984; Aunins et al. 1986). The concentration of oxygen in the gas phase \( (C_{\text{gas}}) \), which is the driving force for oxygen transfer, has usually been kept low at the onset of growth, in an effort to avoid the putative deleterious effects of elevated oxygen concentrations. For example, Fazekas de St. Groth (1983) oxygenated hybridoma cultures using surface aeration with a 10 % oxygen gas mixture. This poses the problem of having reliable dissolved oxygen probes and feedback systems to monitor oxygen levels. Our observations suggest that some cell lines can tolerate oxygen at elevated concentrations (above 200 μM, air equilibrium); cultures could then be operated with an elevated oxygen concentration in the gas phase \( (C_{\text{gas}}) \). This would increase the driving force available during the later stages of the culture, overcome the limitations of the low solubility of oxygen in the medium, create a greater oxygen transfer rate, and ultimately result in an increased maximum cell density, without the necessity of utilizing costly oxygen feedback control loops.

During unrelated studies on the effects of oxygen on cell mutation rates, one of us (A.O.) observed that a human B-cell line (TK6) was able to grow normally at constant oxygen concentrations in the gas phase of 40 %, that is, approximately 380 μM dissolved oxygen. Furthermore, these cells were able to grow with no toxic effects (as determined by plating efficiency) at an oxygen concentration of 410 μM.

To see if this phenomenon was applicable to other cell lines, we investigated the effects of high oxygen concentrations on Vero (African Green monkey kidney) cells and murine cells transfected with chimeric antibody genes (Sp2/0 derived). Vero cells have widespread application in human virus vaccine production, and hybridomas and transfectomas are of central importance in the production of monoclonal antibodies.

Materials and methods

Cells

TK6 is a diploid human lymphoblastoid cell line derived from WI-L2 (Skopek et al. 1978). WI-L2 was originally isolated at the Wistar Institute (Philadelphia, PA) from the lymphocytes of a male donor. Vero cells are derived from African Green monkey kidney and are a stable, diploid, anchorage-dependent cell line obtained from the Institut Armand-Frappier (Laval, Quebec). Marline cells, obtained from Centocor, Inc. (Malvern, PA), are an Sp2/0-derived cell line, transfected with chimeric mouse–human antibody genes, which secretes IgG (Shaw et al. 1988).

Media

TK6 cells were grown in suspension in RPMI 1640, containing 2 g l⁻¹ sodium bicarbonate, supplemented with 10 % horse serum. Vero cells were grown on DEAE–Sephadex microcarriers (Superbeads, Flow Laboratories, McLean, VA) in RPMI 1640 supplemented with 10 % horse serum. Sp2/0-derived cells were grown in suspension in DMEM, containing 3-7 g l⁻¹ sodium bicarbonate, supplemented with 2 mM-glutamine, 2 mM-pyruvate, 50 μM-B-mercaptoethanol, and 2-5 % each of horse serum and heat-treated fetal bovine serum. Media and sera were obtained from Gibco Labs (Grand Island, NY), and Hyclone (Logan, UT), respectively, and other media supplements were obtained from Sigma (St Louis, MO).

Gas mixtures

Premade gas mixtures were obtained commercially (Granite State Oxygen, Nashua, NH) or were made by combining individual gases (Middlesex Welding Supply, Everett, MA). Commercial gas mixtures contained 5 % carbon dioxide, the indicated proportion of oxygen, and the balance as nitrogen. Gas mixtures from individual gas tanks were prepared by
combining oxygen, air (21% oxygen), nitrogen and carbon dioxide, using a mass flowmeter (Matheson Gas Products, East Rutherford, NJ) to measure the flow rates. Dissolved oxygen probes (Ingold, Waltham, MA) were used to measure the final oxygen concentration in the medium equilibrated with each gas mixture.

Methods

TK6 cells were grown in 500 ml modified Ball canning jars with approximately 80 cm of silicone rubber tubing (Cole-Parmer, Chicago, IL), 3 inch inner diameter and 4 inch outer diameter, coiled inside against the walls with the outlet venting to the outside of the jar. Cells were counted and diluted daily to keep them in exponential growth (cell densities between $3 \times 10^5$ to $9 \times 10^6$ cells ml$^{-1}$). Dry gas mixtures at a constant flow rate of 500 ml min$^{-1}$ were passed through the silicone tubing and equilibrated with the medium. A Pasteur pipette and two sections of tubing permitted sterile sampling and diluting via siphoning. The $K_a$ for oxygen transfer in this system was $2.5$ h$^{-1}$. Cultures were grown at 37°C with constant stirring. Control cultures were grown without gas mixture being pumped through the tubing. Under these conditions, the oxygen levels in the control culture varied from 200 /iM immediately following dilution to 50 /iM prior to dilution on the next day. The pH of the medium varied daily from 6-9 to 7-4. No differences in pH were found between the control cultures growing without gas pumped through the tubing and the cultures grown at constant oxygen concentrations.

Toxicity measurements were made either by a cloning assay or by back extrapolation of growth curves after ceasing treatment using the technique of DeLuca et al. (1983). Both methods measure relative survival, and yielded the same survival estimates when used concurrently after exposure of the cells to high oxygen concentrations. In the cloning assay, relative plating efficiency at 2-4 cells/well was measured using 96-well microtiter plates.

Stock Vero cells were grown on polystyrene roller bottles (Corning), in RPMI 1640 supplemented with 10% horse serum. Freshly trypsinized cells were resuspended in fresh medium and counted using either a Coulter Counter or a hemocytometer. Viability was always in excess of 99% as judged by Trypan Blue exclusion.

Cultures were inoculated at a cell density of $2 \times 10^5$ cells ml$^{-1}$ with 4 g/l of microcarriers in a volume of approximately 150 ml. Cultures were grown in spinner flasks having a suspended magnetic stirrer and were sampled daily. The cell density was enumerated by counting stained nuclei (Sanford et al. 1951). The spinners were fed with fresh medium every 1-3 days, as needed to prevent medium acidification.

Control cultures were kept in a 37°C incubator with 5% CO2. Experimental cultures were grown at a constant dissolved oxygen level. Approximately 1.6 ml of silicone rubber tubing (Dow, Midland, MI), 0.028 inch inner diameter and 0.077 inch outer diameter, was formed into a coil and placed inside the spinner with the outlet venting to the headspace. The gas mixture with the desired proportion of oxygen and 5% CO2 was first humidified by passage through a column of water at 37°C, and then passed through the silicone tubing. Calculated oxygen concentrations for these experiments were corrected for the partial pressure of water vapor at 37°C (47 mmHg). The oxygen permeability of the tubing combined with surface aeration was determined to have a $K_a$ of about 10 h$^{-1}$ for this system. As was found for TK6 cultures, the pH was the same for control Vero cell cultures and cultures grown at constant oxygen.

Sp2/0-derived cells were grown in the same spinners described for Vero cells. Cells were inoculated from exponentially growing stock cultures at $3 \times 10^5$ cells ml$^{-1}$ and diluted to maintain the cell density between $3 \times 10^5$ and $12 \times 10^5$ cells ml$^{-1}$. Spinners were sampled and the cells counted daily. Control cultures were grown in a 37°C incubator with 5% CO2. Experimental culture oxygenation was as described for Vero cells.

Results

TK6 lymphoblasts

We examined the growth and viability of TK6 cells over a period of several days as a function of constant oxygen concentrations ranging from 60 /iM to 910 /iM. Control cultures were grown under the same conditions without regulation of the dissolved oxygen. The control vessels were sealed and gas exchange arose solely by surface aeration from headspace air. The oxygen concentration in the control cultures varied from 200 /iM immediately following dilution of the cells to 50 /iM prior to dilution the next day. Control cultures grew with a doubling time of 14-15 h. Figure 1A shows the growth curves of TK6 cells at various constant oxygen concentrations. Cell cultures were diluted daily and the relative cumulative cell number was plotted. TK6 cells grew between constant 100 /iM and 380 /iM oxygen with a doubling time of 15-16 h. At constant oxygen concentrations of 60 /iM or 410 /iM, cells grew with a doubling time of 18 h. At constant oxygen concentrations of 520 /iM and 650 /iM, the apparent doubling times were 20 and 33 h, respectively.

We report the apparent doubling time of these cultures because the information derived from the growth curves alone does not allow us to distinguish between cytostatic and cytotoxic effects of high oxygen concentrations. To discriminate between these two possible effects, we measured survival at different times during exposure, either by a cloning assay or by back extrapolation of growth curves after ceasing treatment. Table 1 shows the relative survival (viability) of TK6 cells at various times during growth at various constant oxygen concentrations. No adverse effects of oxygen on relative survival were detected for oxygen concentrations of 60 /iM, 100 /iM, 280 /iM, 380 /iM and 410 /iM. Thus, the inhibition of growth seen at 410 /iM (doubling time of 18 h) was not due to cell killing. However, at constant oxygen concentrations of 520 /iM and 650 /iM, survival of the cells decreased to 50% and 37%, respectively, after 2 days of exposure. This cytotoxic effect thus contributed to an apparent doubling time of 20 h and 33 h, respectively. At these oxygen concentrations, survival remained approximately constant with continuous exposure for 4 days and a partial recovery from the toxic effects of oxygen was observed after 4 days (data not shown). During continuous exposure to 910 /iM oxygen, a monotonic decrease in survival was detected. After 24 h, 70% of the cells were viable and after 48 h, about 40% of the cells were viable. These toxic effects were not reversible with time, as cell survival continued to decrease exponentially as a function of time of exposure.

The apparent doubling time of TK6 cells as a function of oxygen concentration is shown in Figure 2. These values are derived from the data of Figure 1A and show a plateau region between constant oxygen concentrations of...
60 and 280 μM. In this range of oxygen concentrations, cells can grow with an approximate doubling time of 14–18 h (0.9–1.2 times the control with no gas), without any effect on colony forming efficiency.

**Vero cells**

Growth of Vero cells in batch culture was examined for oxygen concentrations between 180 μM and 715 μM. Control cultures were grown without regulation of oxygen levels. Oxygenation was provided by surface aeration from the headspace, which was open to the 5% CO2 incubator. The doubling time of control cultures ranged from 31 to 43 h using RPMI 1640 medium. Vero cells grow at faster growth rates in other media such as Leibovitz L-15 or Dulbecco's modified essential medium (data not shown). Figure 1B shows the growth curves for Vero cells at various constant oxygen concentrations, as well as two control cultures without oxygen regulation. Relative cell number was plotted as a function of time. The doubling time at 180 μM was 45 h. Apparent doubling times at 360 μM and 540 μM were 48 and 68 h, respectively. No significant growth was observed at 715 μM. The cells from the cultures grown without oxygen regulation (controls), as well as the cultures grown at constant oxygen concentrations of 180 μM and 360 μM appeared cytologically normal and were morphologically indistinguishable (data not shown). In the cultures grown at constant oxygen concentrations of 540 μM and 715 μM there were fewer microcarrier-attached cells and many cell fragments in the medium. The sample supernatants were clear for the controls and the

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**Fig. 1.** Growth curves at various oxygen concentrations. A. TK6 cells. (●) 80 μM (60 mmHg); (○) 100 μM (80 mmHg); (▲) 280 μM (215 mmHg); (△) 410 μM (310 mmHg); (■) 520 μM (425 mmHg); (□) 650 μM (510 mmHg); (●) 910 μM (720 mmHg). B. Vero cells. (●) No oxygen control; (○) 180 μM (143 mmHg); (▲) 360 μM (285 mmHg); (△) 540 μM (428 mmHg); (■) 715 μM (570 mmHg). C. Sp2/0-derived cells. (●) No oxygen control; (○) 90 μM (71 mmHg); (▲) 180 μM (143 mmHg); (△) 360 μM (285 mmHg); (■) 540 μM (428 mmHg).

**Fig. 2.** Apparent doubling time as a function of constant oxygen concentration. (●) TK6 cells; (■) Vero cells; (▲) Sp2/0-derived cells. 1 atmosphere = 101 325 Pa.
Sp2/0-derived cells

tion of dissolved oxygen. Doubling times were estimated in replicate experiments, n.d., not determined.
of growth curves after ceasing treatment. The absolute plating efficiency of TK6 human lymphoblasts permitted us to distinguish between cytostatic and cytotoxic effects of oxygen. Therefore, the cell counts may overestimate the number of potential colony forming cells.

Figure 2 shows the apparent doubling time as a function of dissolved oxygen. Doubling times were estimated for days 1–3 in culture. Both the maximum cell density achieved and the growth rate during the exponential phase were reduced at oxygen concentrations of 360 and 540 μM. Thus, the Vero cells appear to be somewhat more sensitive to higher oxygen concentrations than the TK6 cells, but the relationship between dissolved oxygen and minimum doubling times is similar. Thus it appears that oxygen concentrations as high as 360 μM can be used for fermentor growth of Vero cells.

Sp2/0-derived cells

Growth of Sp2/0-derived cells was examined at constant oxygen concentrations ranging from 90 μM to 540 μM. Again, oxygenation of unregulated control cultures was provided by surface aeration from the headspace, which was open to the 5 % CO2 incubator. The doubling time of control cultures was 14–15 h. Figure 1C presents the growth curves of the hybridomas at various concentrations of dissolved oxygen, shown as relative cumulative cell number. Doubling times were approximately constant over a 5- to 7-day period for all cultures and were calculated from growth data between days 1 and 5. Doubling times for cultures at constant 90 μM-oxygen were 14–15 h, identical to the control cultures. Doubling times at 180 μM, 360 μM and 540 μM-oxygen were 15:8-16:2, 19:5 and 31 h, respectively. The doubling times as a function of the dissolved oxygen concentration are shown in Figure 2. It can be seen that growth at 360 μM-oxygen was slightly slower in relation to that in the control cultures. Doubling time as a function of dissolved oxygen concentration essentially parallels the results seen for TK6 and Vero cells.

Discussion

Oxygen transfer is frequently the limiting factor for scale-up of animal cell culture processes. To assess the oxygen tolerance of three different cell lines, we examined their growth rates under a variety of controlled oxygen concentrations: Doubling times were used as the principal indicators of the overall health of the cultures. We observed that these cell lines could grow at or near their maximum rates at concentrations of 300–400 μM-oxygen, well in excess of air saturation (200 μM). Moreover, for all these lines, continued exponential growth was seen at concentrations up to 540 μM-oxygen.

The influence of dissolved oxygen on the plating efficiency of TK6 human lymphoblasts permitted us to distinguish the reversible effects of oxygen on cell growth rates from its irreversible effects on cell survival. Exposure of the cells to constant oxygen levels up to 410 μM-oxygen did not affect cell survival. Oxygen levels of 520, 650 and 910 μM did result in a loss of cell viability. Interestingly, it appeared that cultures may regain faster doubling times after 4 days at 520 μM or 650 μM-oxygen. This point deserves further study.

The particular cell lines chosen for this investigation, TK6 human lymphoblasts, Vero monkey cells and Sp2/0-derived murine cells, were not chosen for their oxygen tolerance and are not known to be unusually tolerant of oxygen. Vero cells were chosen because of their widespread use in the commercial production of viral vaccines. Sp2/0 cells are frequently used as fusion partners for hybridoma production and also as recipients for transfection of antibody genes, and TK6 cells are used in genetic toxicology. We were therefore surprised to note that three different cell lines are capable of exponential growth at near-maximum rates in the presence of oxygen at twice the level of atmospheric saturation and four times the level of 100 μM-oxygen generally used as a set point for mammalian cell fermentor oxygen regulation.

It is possible that widespread misconceptions concerning oxygen intolerance arose from the fact that many early investigators used normal cells, rather than established cell lines, in their experiments. Established cell lines also differ from normal cells in their metabolism; for

Table 1. Percentage relative survival of TK6 cells during growth at various constant oxygen concentrations

<table>
<thead>
<tr>
<th>Oxygen concentration (μM)</th>
<th>Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>60</td>
<td>n.d.</td>
<td>86</td>
<td>94</td>
<td>107</td>
<td>n.d.</td>
</tr>
<tr>
<td>100</td>
<td>n = 1</td>
<td>89</td>
<td>101</td>
<td>n = 1</td>
<td></td>
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<tr>
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<tr>
<td>380</td>
<td>n = 1</td>
<td>96</td>
<td>98</td>
<td>n = 1</td>
<td></td>
</tr>
<tr>
<td>410</td>
<td>n = 1</td>
<td>92</td>
<td>102</td>
<td>n = 2</td>
<td></td>
</tr>
<tr>
<td>520</td>
<td>n.d.</td>
<td>50</td>
<td>n.d.</td>
<td>40</td>
<td>n = 1</td>
</tr>
<tr>
<td>650</td>
<td>n.d.</td>
<td>37</td>
<td>n.d.</td>
<td>36</td>
<td>n = 1</td>
</tr>
<tr>
<td>910</td>
<td>n = 10</td>
<td>36</td>
<td>n = 10</td>
<td>20</td>
<td>n = 3</td>
</tr>
</tbody>
</table>

Upper right corner: method used to measure relative survival; PE, plating efficiency relative to control culture; GB, back extrapolation of growth curves after ceasing treatment. The absolute plating efficiency of control cultures was between 60 and 80 %. Lower right corner: n, number of replicate experiments. n.d., not determined.
example, they exhibit enhanced glycolytic rates (Lanks & Li, 1988). Cells reported to be sensitive to atmospheric oxygen levels were either embryonic in origin (Cooper et al. 1959) or were mortal diploid fibroblast strains (Balin et al. 1976, 1978). However, those who investigated HeLa cells (Rueckert & Mueller, 1960; Joenje et al. 1975) reported cell growth up to 300 µM-oxygen. Now that several established cell lines are licensed for the production of vaccines and other products, a reconsideration of oxygen tolerance for each cell line would be appropriate.

Unlike many previous authors, who adjusted only the oxygen content of the gas phase, we have taken special care to control the dissolved oxygen content of the medium in a well-mixed fermentor, as opposed to controlling only the gas phase. It was pointed out by McLimans et al. (1968) that cells grown in a stationary monolayer culture (such as in a Petri dish) will establish a diffusional concentration gradient of oxygen, resulting in an oxygen concentration of nearly zero in the immediate vicinity of the cells. Even well-mixed suspension systems will exhibit significant differences between the oxygen content of the headspace gas and that dissolved in the medium, dependent on both the KLa and the oxygen uptake rate. Through the use of a substantial quantity of submerged silicone tubing for oxygen transfer (to obtain a high KLa) and regular dilution of the experimental cultures (to reduce the cell concentration and therefore oxygen demand), we have minimized the gas–liquid concentration gradient necessary to maintain the desired oxygen concentration in the medium.

Our observations lead us to suggest that a simple method of increasing oxygen transfer rate and fulfilling the oxygen demand of the cells is to increase the oxygen concentration in the gas phase. During cell growth, as the cell density increases the oxygen concentration in the liquid decreases. However, this decrease in oxygen concentration causes the rate of oxygen transfer to increase. Therefore, as the cell density increases the oxygen demand of the cells is continuously satisfied until the cellular oxygen uptake rate exceeds the rate at which oxygen can be supplied to the culture. However, through the use of high oxygen concentrations in the gas phase, the oxygen transfer rate can be several times higher than that obtained using air alone. This allows cells to grow to a higher density before the rate of oxygen transfer becomes the limiting factor for cell growth.

A demonstration of the utility of our findings is shown by considering two similar cell cultures in the following example. We will assume that one fermentor is gassed with air, and that the other is gassed with 42% oxygen gas. Neither vessel has an external oxygen controller. Immediately after inoculation, the medium in both vessels will be close to equilibrium with the gas phase. The cells will grow exponentially in both cultures, and the dissolved oxygen concentrations in both will fall until the concentrations reach some final rate-limiting value (assumed here for purposes of illustration to be 60 µM). The oxygen uptake rate of Vero cells was determined to be 0.24 × 10^{-6} µmol oxygen cell^{-1} h^{-1} (data not shown). At a KLa of 10 h^{-1} (obtainable using silicone tubing) and a final liquid oxygen concentration of 60 µM, the oxygen transfer rate attainable using air is 1400 µmol h^{-1}. Therefore, gassing the culture vessel with air can permit Vero cells to grow exponentially up to a density of 5-8 × 10^5 cells ml^{-1}. On the other hand, if the other culture vessel is gassed with an oxygen-enriched gas mixture consisting of 42% oxygen, the oxygen transfer rate will be approximately 3400 µmol h^{-1} (again assuming a final liquid oxygen concentration of 60 µM). This is sufficient to support exponential growth up to a cell density of about 1-42 × 10^6 cells ml^{-1}, representing a 2-4-fold increase over that obtainable through the use of air alone. Of course, such a culture would also require medium perfusion to attain such a high cell density.

In summary, the previously unrecognized ability of human lymphoblasts, Vero cells and murine transfectoma cells to grow exponentially in the presence of high concentrations of oxygen enables the operation of a culture vessel with either oxygen-enriched gas mixtures or with air under pressure. This design provides a simple way of increasing the maximum attainable cell density and/or maximum culture size, without the expense and complication of oxygen control feedback loops. The use of oxygen-enriched gas for aeration provides an attractive alternative technique for the scale-up of high-density mammalian cell cultures.

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