

## Aeration and Gas Analysis in Cell Culture

1<sup>st</sup> January 2005

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### Dissolved CO<sub>2</sub> and Aeration Methods

*Thanks to Lonza Biologics for extracts for below*

In addition to CO<sub>2</sub> added to the culture as part of the pH control system, account must be taken of CO<sub>2</sub> generated by cell metabolism. In recent years it has been apparent that in some large-scale processes, CO<sub>2</sub> can accumulate to levels that inhibit cell growth and recombinant product formation (1,2-6). Inhibition may be caused by an effect of CO<sub>2</sub> on intracellular pH (even if culture pH is controlled) and may also be caused by an increase in osmolarity caused by the additional base required to maintain pH (2,7).

Some groups working with CHO cells in a high density perfused process, show that productivity of a recombinant product can be maximised when CO<sub>2</sub> was maintained in the range 30-76 mm Hg (6). Levels greater than 105mm Hg may result in inhibition of growth and productivity. CO<sub>2</sub> concentration may also influence glycosylation of recombinant proteins (7).

Aeration systems designed for efficient oxygen transfer will not necessarily be efficient at stripping out CO<sub>2</sub>. It has been reported that sparging with microbubbles of pure oxygen gave very efficient oxygen transfer but is inefficient for CO<sub>2</sub> removal because the bubbles dissolved before reaching the surface of the culture (6). Sparging with large bubbles (2-3mm diameter) improved CO<sub>2</sub> removal while retaining adequate oxygen transfer. Using sintered steel spargers often give good oxygen transfer but allow accumulation of CO<sub>2</sub> up to 200 mm Hg, depressing cell growth. By using a less efficient sparger (12mm x 1.5mm orifice ring sparger) CO<sub>2</sub> accumulation was reduced to a noninhibitory level (8).

However, damage may be caused to cells by interaction with bubbles, for example when they burst at the surface (damage from agitation at slow speed is not significant). While damage may not affect the performance of the cells to a great extent, the problem is readily overcome by incorporation of a protective polymer such as the non-ionic surfactant Pluronic F68 into the culture medium (2,9,10,11).

### Cell Culture Gas Mixing

Many small scale reactors are supplied with individual solenoid or pulsed gas control of CO<sub>2</sub> and/or oxygen to supplement the main gas flow which is air. Sometimes nitrogen is used at the early stages of a culture, particularly in air lift reactors. There are a number of processes which run a constant 95% air 5% CO<sub>2</sub> mix, which itself is supplemented by other gases.

Where solenoids are used, errors in calculation of gas mixes are regularly underestimated – a 1 second pulse at 1L/min (16.66cc3) may in fact range +/- 25% away from this ideal. This is caused by the inaccuracies of rotameters (+/-10-15%), the inaccuracies of measuring cumulative seconds in the controller (+/-5-10%) and by the deviation from a square wave of the opening and shutting of the valve itself (+/-10-15%). In early research, it may only be important that the cells are correctly controlled at the correct pH and DO levels. However, it is arguable that understanding different gas demands is crucial and such inaccuracies cannot be tolerated. There is now more pressure on the cell culture development scientist than before to improve productivity and gas inlet control is a simple first step.

In larger scale reactors, or when processes are being scaled up, it is imperative to have a good understanding, and control, over the mix of gases entering the reactor, hence massflow controllers for every gas are normally recommended, giving 1% or better accuracy.

## Cell Culture Gas Analysis

While analysis of exhaust gases is commonplace in microbial cultures, there is currently little carried out during cell culture processes. This is for several reasons, most notably the low CO<sub>2</sub> evolution from cells and the more complex gassing strategies used (see gas mixing above). However, gas analysis can still provide some useful information if detailed care is taken on both the measurement and control of the process. There are a few academic and industrial groups starting to analyse cell culture off-gas. Industry has been reluctant to share results, however, the following general observations and recommendations can be made.

Since metabolic understanding, based on derived variables such as CER, RQ etc., relies on the difference between inlet and outlet gas concentrations, the inlet gas stream must be analysed due to its mixed composition, as well as the off-gas. Some use good quality massflows to provide this information, otherwise, an analyser such as the Tandem can be used. For further accuracy, the time taken,  $t_{\text{transition}}$ , for any particular gas to pass through your reactor can be measured. This means that the gas mixture at time  $t$  entering the reactor is subtracted from the gas mixture analysed on exit at time  $t + t_{\text{transition}}$ , rather than at time  $t$  (as happens in microbial cultures). This should help eliminate spiking errors of CO<sub>2</sub> and O<sub>2</sub> introduced by the gas mixing control system. Older control systems may have trouble with this time delay calculation, so check with your manufacturer how to achieve it. However, it can be done through an on-line user-defined equation, and this method will also help you tweak any variations due to your equipment.

The low cell density and low productivity means the amount of CO<sub>2</sub> produced will also be low. Depending on the cells, it turns out that useful data from CO<sub>2</sub> analysis is generally limited to the last 1/2 to 1/3 of the process. OUR/CER values can be of the order of 5mmol/L/hr (12). Perfusion and continuous processes will clearly fare better. Academic work is also focussing on the O<sub>2</sub> data by itself, to get a better understanding of O<sub>2</sub> transfer and O<sub>2</sub> uptake rate.

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