

Real-Time Off-Gas Analysis Improves Bioreactor Efficiency

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The desire to reduce development times for drug manufacture drives companies to look for reliable technologies to improve fermentation and cell culture production. Companies have invested millions of dollars in production plant and R&D facilities, and they want their expensive assets to be used as efficiently as possible. The shortfall of worldwide production capacity adds to their urgency: Even small yield increases at existing bioreactor facilities can forestall production shortages until new facilities come on-line.

Standard bioreactor design has changed little during the past 20 years, but the electronics and sensors available now are radically improved, and production efficiencies have increased



greatly. The trend toward better instrumentation and data collection continues. Extracting and analyzing more information from each bioreactor run is now a priority for process development teams. The many off-line measurement techniques currently available provide useful data, but what is needed for even more efficient control of bioreactors and the bioprocesses within them is on-line, real-time measurement information.

AN ALTERNATIVE TO MASS SPECTROMETRY

Mass spectrometers have traditionally been used to analyze the exhaust gas from a bioreactor to increase understanding of a bioprocess. These analyzers are, unfortunately, expensive tools, and they are often multiplexed to several bioreactors, with the result that detailed information about each individual unit may be lost. Furthermore, servicing a multiplexed analyzer means that a significant

number of a facility's bioreactors are idle at one time, costing money and causing scheduling uncertainty. Should the multiplexer or analyzer fail, all information on all the bioreactors is lost, possibly at the cost of an entire batch. The best solution would be a dedicated gas sensor on each bioreactor that can be reliably used for automatic measurement and control of the bioreactor's exhaust gas mixture, just as a dissolved oxygen probe is used inside the bioreactor.

DSM Biologics (Groningen, The Netherlands, www.dsm.com/en_US/html/biologics/home_biologics.htm) had laboratory and pilot bioreactors already installed and wanted to buy a flexible gas monitoring system. One of its manufacturing customers had developed a laboratory-scale process for the production of a recombinant subunit vaccine in *Escherichia coli*, for which off-gas analysis data were required not only to prove a successful technology transfer and scale up

PRODUCT FOCUS: PRODUCTS OF MICROBIAL FERMENTATION OR CELL CULTURE

PROCESS FOCUS: BIOREACTOR OPTIMIZATION, PRODUCTION, PROCESS DEVELOPMENT

WHO SHOULD READ: EARLY AND LATE-STAGE PROCESS DEVELOPMENT, PRODUCTION, AND BIOANALYSIS PERSONNEL

KEYWORDS: PROCESS CONTROL, AUTOMATION, OFF-GAS ANALYSIS

LEVEL: BIOTECH BASICS

(similar exit gas profiles), but also to support claims for process consistency and reproducibility (1). It chose the Tandem off-gas analyzer. The system was installed by DSM personnel without help from the vendor's engineers. Furthermore, DSM found that its personnel could easily transfer the analyzer among its many bioreactors.

Scale-up demands and process reproducibility are two of the parameters that best demonstrate the value of bioprocess off-gas analysis in process development. The use of gas sensors for metabolic analysis requirements and fed-batch control is also increasingly common. Although microbial fermenters still dominate the gas analysis market, changing market pressures are moving gas analysis into cell culture bioreactors also. For example, the worldwide shortage of cell culture production capacity is leading production and development managers to consider off-gas analysis to increase yields with existing bioreactors. However, many companies cannot afford to install expensive off-gas analysis systems, especially when such systems may require complex interfaces to work with existing instrumentation and software controls.

The Tandem analyzer provides continuous and accurate measurements of O₂ and CO₂ (± 0.01% partial pressure) and transmits these data by means of 4–20 mA or 0–10 V signals back to the control system. The Tandem scales up well with a bioprocess because of its relatively low per-unit cost, which allows individual installation on each bioreactor. The pitfalls of multiplexing a single expensive analyzer are thus avoided. Furthermore, the precision and accuracy of the Tandem competes well with other on-line analytical methods.

Many Applications: The process run at DSM required induction of recombinant protein expression at a predetermined optical density value. Using off-gas analysis with off-the-shelf data-logging software, DSM scientists could correlate that point accurately with a specific carbon dioxide concentration in the exit gas. In subsequent batches, a combination of the optical density data and the gas

Figure 1: Growth of *Escherichia coli* on a succession of substrates; CER = Carbon dioxide evolution rate; OUR = oxygen utilization rate (left); RQ = respiratory quotient (right)

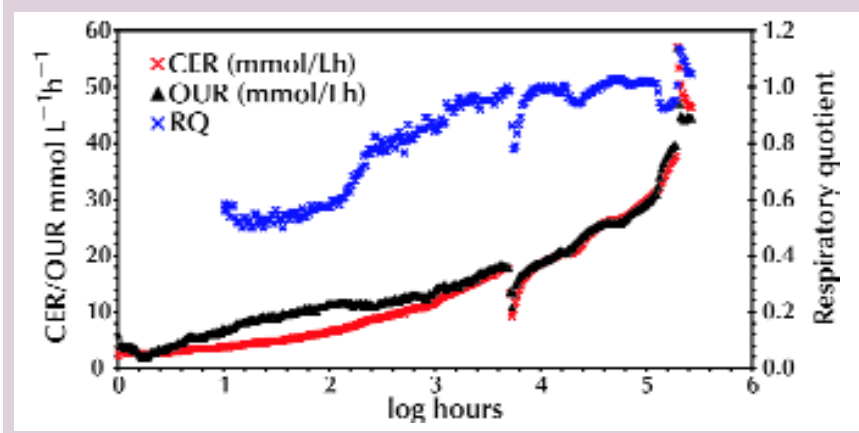
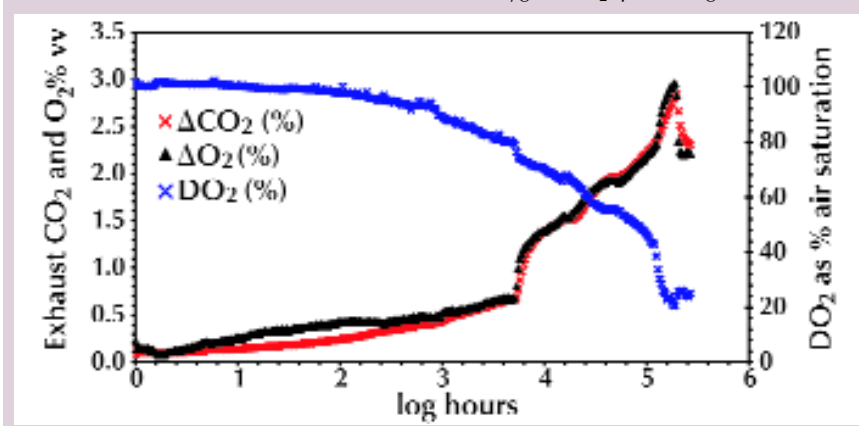


Figure 2: Growth of *Escherichia coli* on a succession of substrates; Raw CO₂ and O₂ profiles (difference of inlet minus outlet, left) and dissolved oxygen (DO₂) profile (right)



analysis data was used to pinpoint the moment to initiate recombinant protein expression. As a result, DSM was able to generate highly consistent and reproducible data for subsequent consistency batches. That success led to the introduction of the Tandem at DSM's GMP manufacturing plant in Canada. By attaching the Tandem to its existing system, the company increased the flexibility of its GMP plant. In addition, it now has a reliable system on which to base new control strategies.

Other companies are also taking advantage of the technology. David Anderson, research director at ChemGen Corporation (Gaithersburg, MD, www.chemgen.com), says "We use Tandems for analysis of laboratory fermentations. Using the instrument is a very easy and cost effective way to determine O₂ and CO₂ concentrations in the off gas." Paul Milner, of Microscience Ltd (Reading, UK, www.microscience.com), explains, "We have found the system to be both

reliable and consistent. The information on CO₂ evolution directly correlates with product formation, and so we now use it to monitor the physiological state of our cultures."

Researchers in development laboratories are becoming more clever in their analysis of gas data from bioreactors. Common derived variables such as the carbon dioxide evolution rate (CER) and respiratory quotient (RQ) are now frequently used for scale-up calculations and understanding metabolism for feeding control. Less used, but still important, are derived variables for growth rates and dissolved oxygen and carbon dioxide in the media. For example, engineers at production sites find that using gas analysis to produce an estimated dissolved oxygen (DO₂) level in large tanks helps them troubleshoot DO₂ probe readings.

Figure 3: Exhaust gas data for a fermentation producing a recombinant antibody fragment; The point of phosphate depletion is marked as sample 13

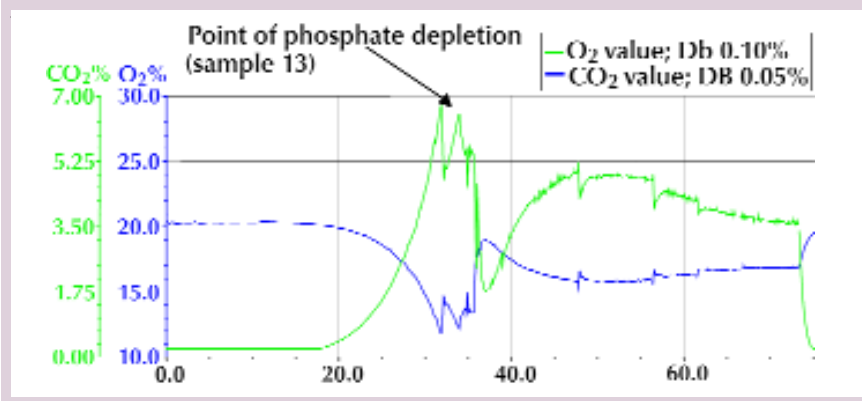
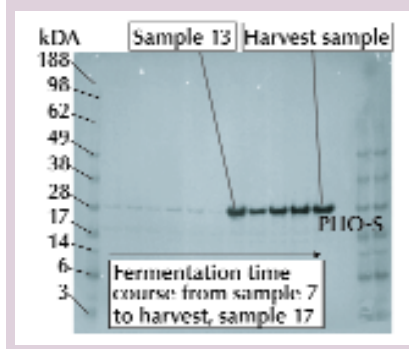


Figure 4: A Western blot using an anti-PHO-S antibody to reveal the point of PHO-S induction, which indicates phosphate depletion



CASE STUDY: SPEEDING PROCESS DEVELOPMENT

GlaxoSmithKline (GSK, Ulverston, UK, www.gsk.com) ran a fed-batch *E. coli* fermentation using several different substrate feeds and a variety of control protocols. Figures 1 and 2, used here by kind permission of Andrew Collis in GSK operations, show data from the same batch.

The RQ should be approximately 1 for aerobic growth on carbohydrates, an artificially low initial value was observed, a consequence of the initially high solubility of CO₂ in the medium. Later variations were associated with changing the carbon and nitrogen substrates. The first switch in substrates is clearly visible. Small variations in the RQ may reflect metabolism of the combined carbon and nitrogen sources but could also be due to pH fluctuations and consequent changes in CO₂ solubility.

By using a Tandem analyzer, GSK gained fine detail (which would have been unavailable using mass spectrometry) on the real-time activity of the organisms in fermentation. The operations staff redefined their feeding profiles using that information to optimize their process. And feedback from the balances allowed for better pump control.

CASE STUDY: PREDICTING METABOLIC EVENTS

E. coli is an attractive host for recombinant protein production because it can be grown to high cell densities, is amenable to genetic manipulation, and can secrete and fold recombinant protein (2–5). A wide

range of promoters is available for the induction of recombinant proteins. One of them is the *E. coli* alkaline phosphatase (*phoA*) promoter, with which transcription of the recombinant protein starts when phosphate is depleted (6).

In microbial fermentations, it is essential to measure both on- and off-line parameters to monitor growth and evaluate the physiological state of cultured organisms. Analysis of fermentation off-gas composition offers a convenient and sensitive way of following physiological events in close to real time. In particular, derivations of O₂ and CO₂ concentrations, such as RQ and specific oxygen utilization rates (OURs), provide a way to evaluate physiological phenomena that cannot be readily examined by biochemical analysis of off-line samples.

Celltech Group (Slough, UK, www.celltechgroup.com) used the Tandem gas analyzer to determine the point of phosphate depletion. A sharp fall in carbon dioxide and a rise in oxygen in the exhaust gas corresponded to a metabolic event (Figure 3). It is also possible to calculate the CER and the OUR on-line using these data.

A Western blot targeting a protein induced under phosphate-depleted conditions, PHO-S, confirmed that the metabolic event depicted in Figure 3 was, in fact, phosphate depletion (Figure 4). Fermentation samples were taken every 15 minutes before, during, and after the PHO-S induction period. The samples were centrifuged to pellets. Those pelleted samples were resuspended in an osmotic shock buffer to a normalized optical density (OD) photospectrometry reading (at 600-nm

absorbance) of 7. This cancelled the effect that cell density differences would have on the PHO-S quantity. The resuspensions were extracted overnight and then centrifuged to pellets and a clarified extract.

The clarified extracts were treated with Novex NuPAGE nonreducing loading buffers and run on a 4–12% NuPAGE bis-tris gel (Invitrogen, <http://invitrogen.com>). The marker used was See-blue plus prestained marker obtained from Novex. The gel was run and blotted onto PVDF membrane following the Novex NuPAGE standard protocols. The anti-PHO-S antibody was a gift from Humphreys et al (7). The blot was revealed using a metal-enhanced DAB substrate kit from Pierce (www.piercenet.com).

MULTIPLE APPLICATIONS

Magellan Instruments (London, UK, www.magellaninstruments.com) has acquired the rights to the Tandem gas analyzer from Adaptive Biosystems and now manufactures it. The company intends to expand production based on the product's success. The Tandem is now in use worldwide on bioreactors ranging from less than 1 L to greater than 100 m³. The gas analysis market in cell culture is still tiny, but as cell densities increase, the Tandem will begin to offer the same benefits to cell culture as those already available for microbial fermentation. Even companies with mass spectrometers have bought the Tandem to use during maintenance or unexpected breakdowns. In those situations, it can also be used as a portable calibration and verification unit for bioreactors with an unusual off-gas reading.



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