

The development of PAT in biotech manufacturing

Quality by design and PAT approaches are increasingly being used for the biotech manufacturing of medicines. Complex manufacturing processes can not only be controlled using PAT principles, but optimized with respect to both product quality and economic value. This column describes how the fermentation process is often the first to benefit from this type of implementation.

Firms using biotechnology to manufacture medicines are embracing process analytical technology (PAT) at an ever-quickening pace as part of their quality by design (QBD) initiatives.

They are often faced with complex and fragile processes lasting many hours or days, and are looking towards PAT as a means of not only understanding the fundamentals of their processes, but also to offer more incisive process monitoring technologies, control and optimization strategies.

Commercial payback comes from both R&D — from being able to get more robust processes to market quicker — and from manufacturing — by being able to mitigate process risk, batch-on-batch, as well as through process optimization, which improves subprocess and final product yields.

This article will describe how aspects of PAT are applied to a fundamental process stream in biotechnology manufacturing — fermentation.

The biotech process challenge

Biological processes often have high variation levels, making the development of a robust and reliable process inherently difficult. Couple this with the high intrinsic value of the end product, and a biotech firm has to bear a degree of risk during its manufacturing process that is unseen in other parts of pharmaceutical manufacturing. Mitigating this manufacturing risk has been a biotech goal for many years, and current approaches correlate with the principles QBD and PAT:

- To identify critical quality attributes (CQAs) of the product and their acceptability limits.
- To identify the sources that lead to the variability in the CQAs.
- To identify the factors within the process that can be adjusted to control the variation in the CQAs.
- To determine a control strategy to ensure that the CQAs are held within predetermined limits during

the manufacture and lifetime of the product.

A typical fermentation-based biotech process flow is shown in Figure 1. From an economic and quality risk perspective, the critical parts of this process are the production of the active agent through the fermentation step, the recovery of the active agent in the purification step, and the stabilizing of the finished product prior to shipment during the freeze drying/lyophilization step.

QBD and PAT efforts are being put into this process, not only to aid understanding, but to control the critical steps and go on to optimize the subprocess yields from the fermentation, purification and lyophilization steps.

Brian Davies

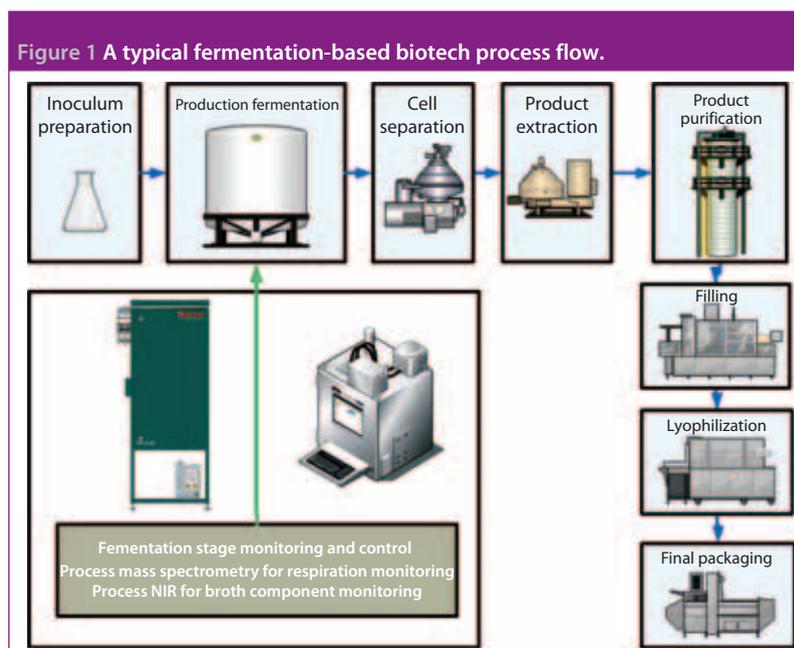
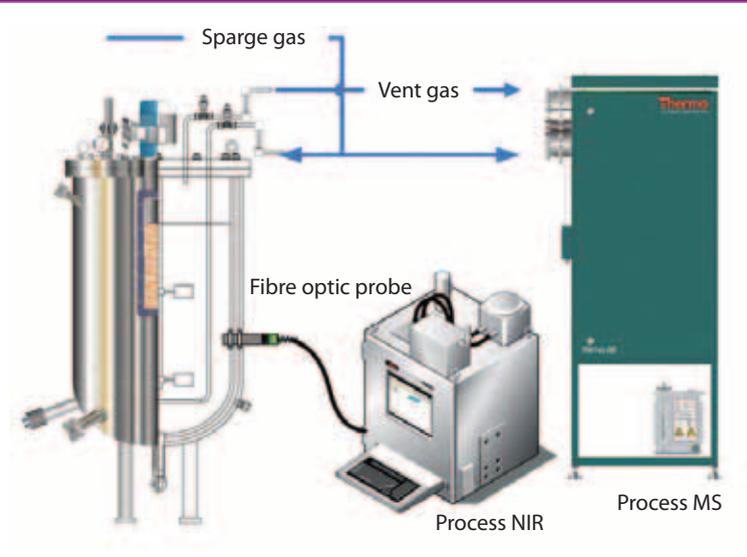


Figure 2 A biotech fermentor with a process MS and process FT-NIR attached. Each technique can be used to monitor different aspects of the process as it progresses.



Monitoring, control and optimization

Understanding and monitoring what is happening during the fermentation process is vital to ensure the healthy progression of the culture, as its health determines the conversion rate of nutrients to product, the formation of unwanted by-products, and in extreme cases, the onset of poisoning. Growth kinetics and substrate consumption are key indicators of the progress.

Process mass spectrometers (MS) and process FT-NIR spectrometers are widely used as the measurement technology components in these monitoring, control and optimization systems.

Figure 2 illustrates a biotech fermentor with a process MS and process FT-NIR attached. Each technique monitors different aspects of the process as it progresses.

Process mass spectroscopy

The MS allows for highly sensitive and precise measurements of the components of the gas streams into and out of the fermentor. Analysis of the respiratory gases being fed to and removed from the fermentor is an ideal means of characterizing the fermentation state. A key indicator of the fermentation progression is the respiratory quotient (RQ). Respiration is the process by which an organism oxidizes food to produce energy. The RQ is the ratio of the carbon dioxide evolution rate (CER) to the oxygen uptake rate (OUR).

Many fermentations are characterized by small changes in oxygen and carbon dioxide concentrations at critical phases of the fermentation. Therefore, the process MS (with ability to simultaneously measure multiple gas components, and use other components such as nitrogen and argon in the gas stream as reference gases) can very rapidly calculate this rate and feed it into a process control strategy to intervene, keeping the fermentation on track.

When equipped with a sample stream multiplexer, a single process MS can be attached to multiple fermentation vessels, offering a high degree of operating and economic efficiency.

Figure 3 shows the process MS generated trace of RQ versus fermentation time. Here, the RQ value falling below a predetermined level is used to trigger the addition of nutrients into the fermentor to keep the fermentation at an optimum state.

Figure 3 Fermentation control: nutrient addition triggered from process MS data.

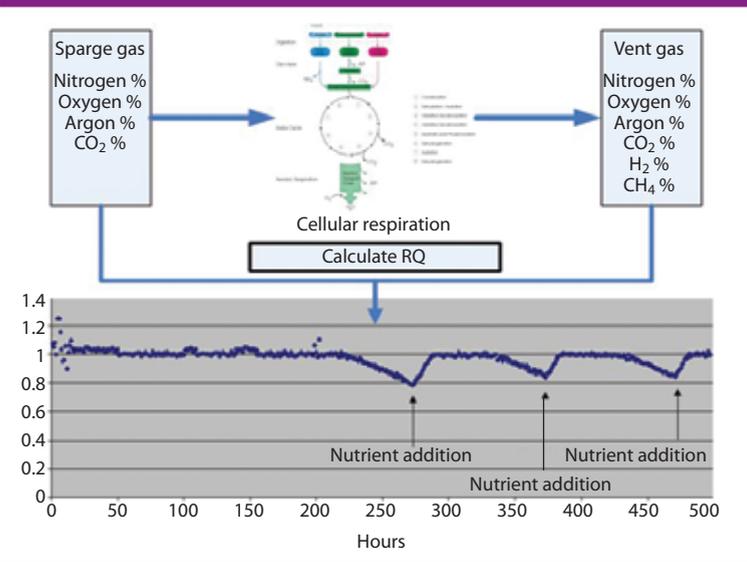
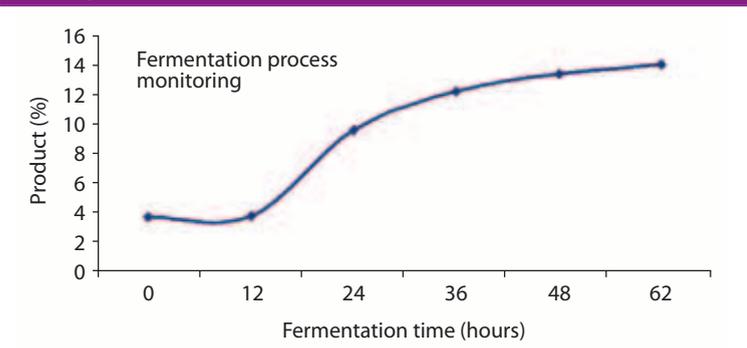


Figure 4 Monitoring fermentation product concentration using a process FT-NIR spectrometer.



Process FT-NIR

Making direct measurements of fermentation components within the fermentor itself allows additional information to be obtained on the progress and state of the fermentation.

Here, FT-NIR using a fibre optic probe immersed in the bulk of the fermentor liquor is used. The components measured by the FT-NIR vary by fermentation type, but can include:

- product concentration
- nutrient concentration
- biomass.

Making measurements directly in a fermentation liquor can be inherently difficult because of the changing chemical and physical properties of the medium during the process. For example, as biomass is produced during a process, the liquor may go from being a relatively clear liquid to a turbid

liquid with a high degree of suspended solids; this can confound the measurements of the critical components.

Where transmission spectroscopy would work well at the beginning of this process, it is likely that reflectance spectroscopy would be the best technique to use as the medium turbidity increases.

The latest generation of multipurpose fibre optic probes with both transmission and reflectance optics can be linked to a spectrometer with multiplexed measurement channels. This allows the system to be controlled through adaptive software work flows and can easily track these physical changes automatically. This enables the system to work at the optimum sampling regime for the most accurate and precise component concentration; ideal for automated process measurement and linking to a process control system.

Figure 4 shows the data from using an FT-NIR to determine the concentration of a product during the fermentation process cycle.

Rich data analysis

A fermentation stage with process MS and FT-NIR capturing large quantities of chemical component data coupled with classical process parameters such as temperature, pH, stir speed and gas rates being logged elsewhere, is ideal for investigating with multivariate data analysis tools. Currently, work is being conducted on this rich data pool and process fingerprints, multiplicative statistical process control, 'biological time' and model predictive control approaches are developing for use in control and optimization strategies.

Summary

Although the processes involved in biotechnology manufacturing differ significantly from those of small molecule medicines, the underlying principles of QBD and PAT are being applied enthusiastically.

The nature of biotechnology subprocesses allows control strategies to be extended into optimization strategies, and create sound economic value for firms.

We can expect to see more of this pragmatic application of PAT in this part of the pharma industry, as measurement and control technologies develop, and are applied to other sections of the process, especially those downstream of the fermentation step that carry a high degree of residual risk.

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