HAMILTON

Biopharma PAT

Quality Attributes, Critical Process Parameters & Key Performance Indicators at the Bioreactor

May 2018

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Focus Spots

PAT Building Blocks

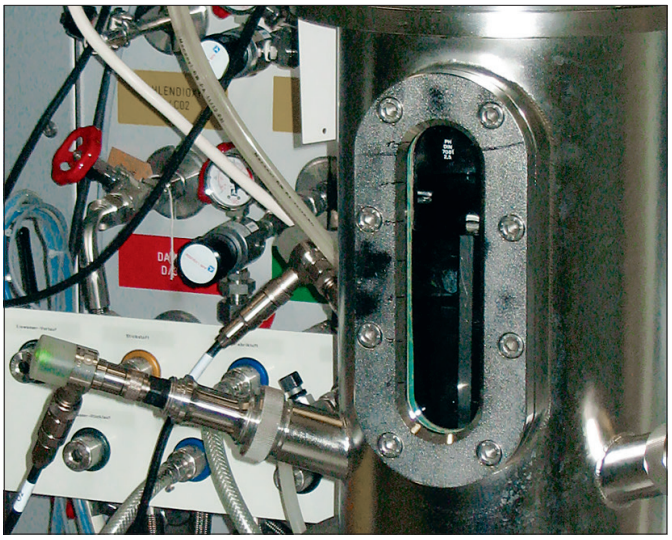
The Process Analytical Technology (PAT) Initiative originates from the 2004 guidance published by the U.S. Food & Drugs Administration (FDA)[1]. It is a part of the broader initiative «Pharmaceutical cGMP for the 21st century – A risk based approach»[2]. The focus of this initiative is to minimize risks to public health associated with pharmaceutical product manufacturing. PAT established a regulatory framework intended to facilitate the voluntary development and implementation of innovation in pharmaceutical development, manufacturing, and quality assurance. It focuses on enhancing the understanding and control of the manufacturing process to achieve Quality-by-Design (QbD): quality should be built into a product with an understanding of the product itself and the process by which it is developed and manufactured along with a knowledge of the risks involved in the manufacturing process and how best to mitigate those risks. PAT promotes a process which starts with the identification of each product's specific Critical Quality Attributes (CQAs), then proceeds with monitoring as often as possible the related Critical Process Parameters (CPPs) and of the Key Performance Indicators (KPIs), in order to automatically control them within pre-defined limits. The relationship between CQAs, CPP and KPI are described in their definitions from PAT literature: [3], [4]

- Critical Quality Attribute (CQA): a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.
- Critical Process Parameter (CPP): a process parameter whose variability has an impact on a critical quality attribute and, therefore, should be monitored or controlled to ensure the process obtains the desired quality.
- Key Performance Indicator (KPI): a metric for the status of each production step. KPIs are related to CQAs and therefore influenced, as well, by the CPPs. As the CPPs remain within the pre-defined limits, the KPIs should indicate that each production step proceeds accordingly resulting, in the end, in a product having its CQAs within the appropriate limits, too.

CQAs are still difficult to measure directly in production. Along the upstream and downstream portions of the manufacturing process it is most common to monitor the CPPs and KPIs related to the quality attributes. PAT recommends various tools for this purpose:

- Multivariate tools for process design, data acquisition and analysis
- Process analyzers (e.g. in-line sensors or automated at-line devices)
- Process control tools (e.g. statistical process control softwares)
- Continuous improvement and knowledge management tools

An appropriate combination of these tools may be applicable to a single-unit operation like a bioreactor, or to an entire manufacturing process portion like upstream or downstream. Process analyzers are a typical example of tools to measure process data. Their output is used for different scopes like univariate mechanistic modelling, process characterization or multivariate analysis such as the «golden-batch» prediction. This whitepaper focuses on process analyzers for the bioreactor. An overview of the most important performance indicators and process parameters will be provided, together with examples of the proper in-situ sensors and equipment used to monitor them. Figure 1 highlights an example of in-situ process sensors.



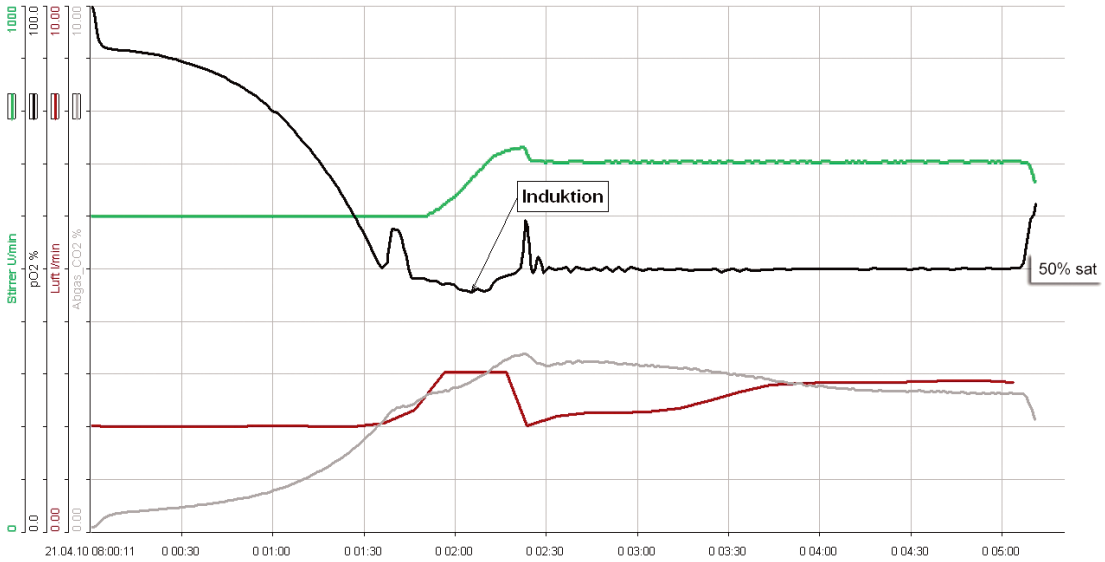


Figure 1: Example of a bioreactor with in-situ process sensors for a microbial fermentation.
The chart represents the real-time monitoring of CPPs such as the Dissolved Oxygen as % saturation (signal in black).

PAT for Biopharma

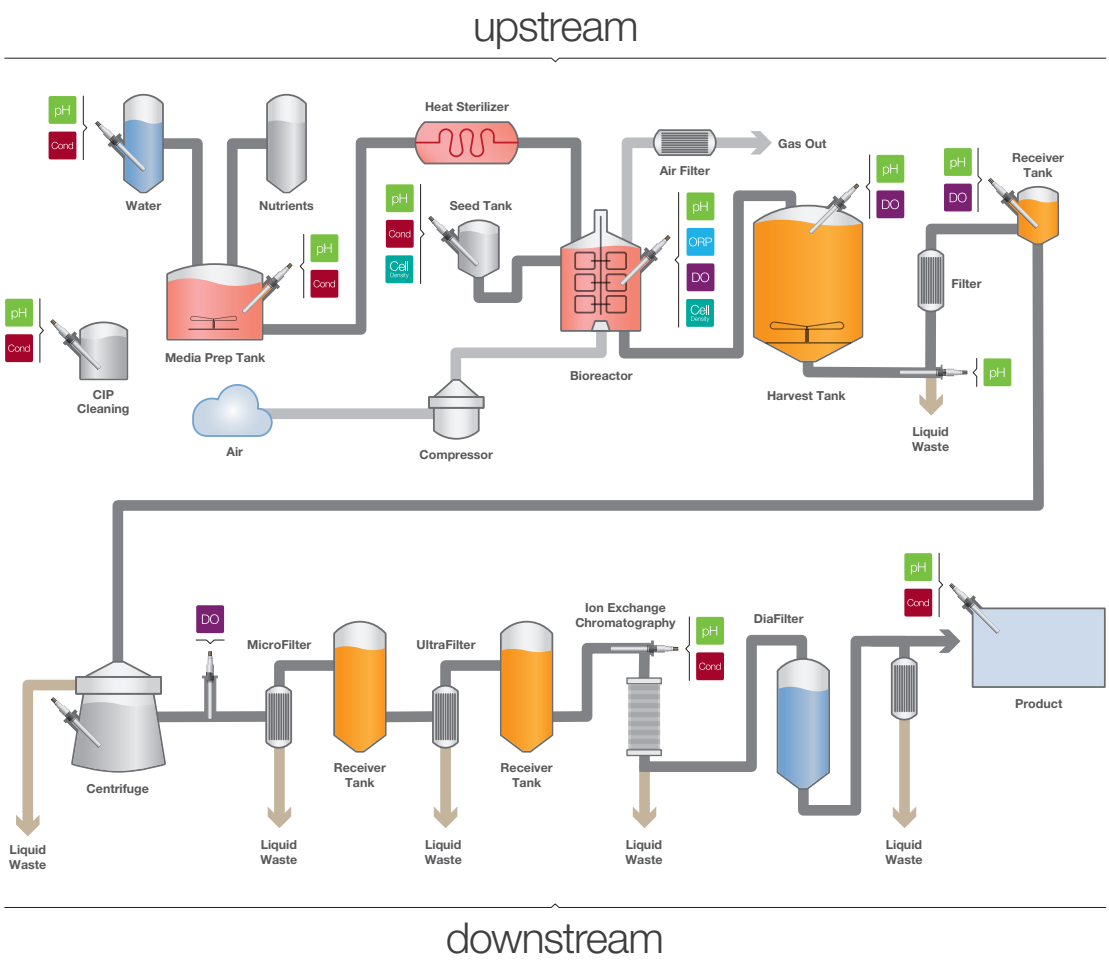


Figure 2: Biopharmaceutical manufacturing process. This map shows the commonly monitored real-time Critical Control Parameters pH in green, ORP in blue, DO in violet, Conductivity in red and the Key Performance Indicator Cell Density in teal.

Manufacturing of biopharmaceutical products is a complex process[5]. The complexity is due to the heterogeneity typical for the bioprocesses. In the upstream process the heterogeneity arises from the cells who are living subjects. According to minimal variations of the environment, they can produce higher or lower yield of product with a quality within the pre-defined limits. Or, in other words, with a molecular conformation bioactive enough to deliver the expected healing effect on patients. The upstream heterogeneity transfers to the subsequent product purification steps in downstream processes, too.

Existing scientific literature[6] already describes how the application of PAT to these complex processes could enable significant improvement in upstream through the use of performance indicators (e.g. viable cell density). In downstream the application of PAT results in higher quality and purity of the final product (e.g. protein, vaccines, etc.).

It is universally acknowledged that to properly apply PAT, it is essential to move from the manual sampling and laboratory measurement procedures to automated control[7]. As even minimal variations of process parameters have a major influence on the final product, controlling them in real-time minimizes the risk of lower yield and purity.

Real-time monitoring is possible due to sensors which can withstand the Cleaning-In-Place (CIP) and Sterilization-In-Place (SIP) procedures required to minimize the risk of contamination. This is already common for the fundamental CPPs: e.g. pH, dissolved oxygen (DO) and conductivity (Figure 2). Further advances in CIP and SIP compliant sensors have been developed to directly measure KPIs such as Total Cell Density (TCD) and Viable Cell Density (VCD). These will be described in the following paragraphs.

Culture & Fermentation Process Types

Bioreactors are vessels used for cultivating mammalian cells (such as CHO), microbial cells (such as E. Coli) and yeast or small plant cells (such as moss). These cells work like small factories to produce the desired compounds. Through a fermentation process they transform nutrients like glucose and amino acids into high-value biopharmaceutical products as vaccines, monoclonal antibodies (mAbs), and other therapeutic proteins. Such variability in the processes makes the application of PAT at the bioreactor unique according to the culture used and the fermentation process type required[8].

Culture – Mammalian

MAbs and therapeutic proteins are produced mainly by means of mammalian cells, especially if the needed therapeutic agent must be compliant with human biology.

The main cell lines are: CHO, BHK, and NSO-GS. Such cells typically exhibit a growth rate of doubling their numbers every 24 hours. This is relatively slow, therefore monitoring & control strategies should benefit from longer working times. Nonetheless, mammalian cells have a less robust outer membrane compared to microbial cells, thus they are more fragile against changing process conditions: they have to be constantly controlled. Huge cost comes from batch loss due to equipment not working properly to maintain the desired process conditions[9]. Equipment failures – particularly at the commercial scale – are extremely costly, resulting in lost batches, a repeat of the bioprocess studies to satisfy regulators, and other such problems[10]. Due to these reasons, the PAT push for real-time monitoring and automated control means significant improvements for bioprocesses using mammalian cells.

Culture – Microbial

Several recombinant proteins and vaccines are produced with microbes such as bacteria[11] and yeasts[12], applications of these cells are widespread due to their robustness and ease of cultivation.

Compared to mammalian cells, microbials typically have shorter fermentation times as well as higher chemical and physical protein stability. Bacterial cultures can double within 20 to 30 minutes, which is why CPPs such as pH, DO, cell density and feed rates need to be measured as frequently as possible. Once again real-time control becomes necessary to achieve true QbD.

Fermentation Process Type – Batch

Batch fermentation processes are often considered the first processes adopted by the biopharmaceutical industry. Microorganisms are added to culture media in the bioreactor, which has been pre-filled with nutrients like glucose, glutamine, other amino acids and minerals. The media remains the same during the entire process and is not supplemented, refilled or exchanged at any time.

After an initial lag phase, the number of microorganisms increase sharply during the growth phase. Then, after a stationary phase of suspended population, the culture population drops off in a death phase. The cause for the population drop-off can be tied to the depletion of nutrient media and the accumulation of toxic substances.

The PAT strategies are limited just to the Critical Process Parameters which can be modified in real-time and therefore can be controlled: e.g. pH, DO and temperature.

Fermentation Process Type – Fed-Batch

Fed-batch has been the dominant bioprocessing method for decades[8]. The fed-batch process differs from the traditional batch process by adding nutrients in stages to maximize cell growth. The bioreactor is filled with a base amount of media to support initial cell growth. Feed media is added when needed to replace nutrients depleted by the increasing cell population. The cells and their product(s) remain in the bioreactor until the end of the run. With this setup it is possible to automatically regulate the addition of feed media according to nutrient levels or viable cell density.

Fermentation Process Type – Perfusion

The term «continuous bioprocessing» generally refers to perfusion technologies. The bioreactor runs at a fixed volume and fixed cell concentration for 30–90 days or longer depending on cell line. During this time the feed media is constantly refreshed and the secondary toxic metabolites eliminated while cells are simultaneously harvested for

further processing. Perfusion technology is one of the newest methods for cell culture processes. Despite the benefits of perfusions, regulatory issues are still a hurdle for its implementation: problems with the «batch» definition in a continuous process make release procedures more complex. Therefore, even more than for the other processes' types, perfusion is highly dependent on QbD and PAT in order to work properly and be accepted by regulatory authorities.

Monitoring Methods

According to the PAT guidance[1] and the scientific literature[8], the monitoring of the critical process parameters and quality attributes can be performed following different methods, like those represented in Figure 3. Although the in-line (or in-situ) and on-line are the methods of choice for real-time monitoring, at-line and off-line are still options for those CPPs, KPIs and even CQAs which cannot be accurately measured in the bioreactor. The following paragraphs detail such differences.

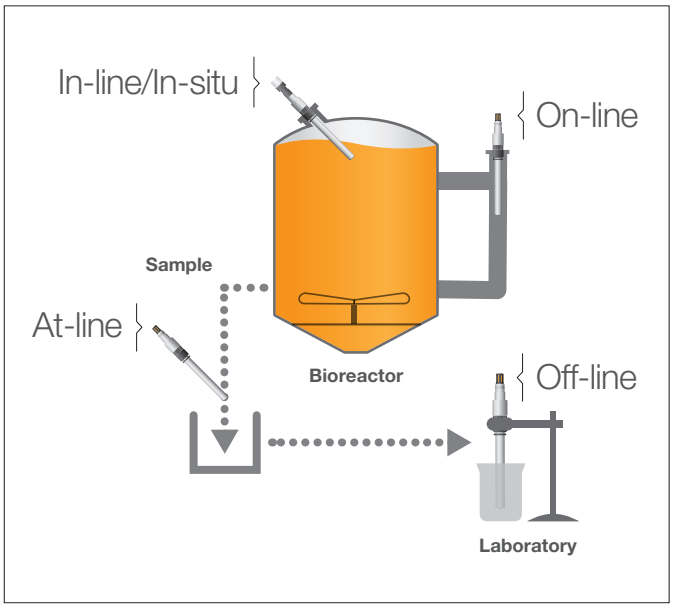


Figure 3: Different methods of process monitoring according to the PAT guidance (2004) & later scientific literature

Off-line

The sample is taken out of the bioreactor in sterile conditions and analyzed in the lab after physical pretreatments (e.g. filtration and dilution). The preparation and handling require clear Standard Operating Procedures (SOPs) as well as skilled personnel. If problems occur during these stages, the accuracy of the results will decrease. Together with the complexity involved in manual handling, the major disadvantage of off-line measurement is the time delay, which results in lower measurement frequency. Due to these issues off-line measurements should not be considered true PAT unless there are no other measurement possibilities (e.g. HPLC for product titer or mass spectroscopy for product quality). In these examples, automated controls are not a possibility.

Off-line laboratory measurements are commonly used to monitor and validate the accuracy of the in-line/ on-line process analyzers. However, factors such as temperature changes and de-gasing can negatively influence the accuracy of these reference measurements.

At-line

In at-line measurement, the sample is removed and analyzed in close proximity to the production process, either manually or by using automated sampling devices. Similar to off-line measurement, sterile conditions must be maintained for accurate results. At-line measurement is most common for parameters which cannot be measured accurately in-situ or on-line.

Advantages of at-line measurement include shortened time delay (relative to off-line), and the possibility for automated control; however the final results might be too slow to effectively monitor cultures with fast growth rates such as microbial cultures, according to PAT principles.

On-line

In on-line measurement, the sample is diverted from the manufacturing process with a by-pass stream and may be returned to the bioreactor. The sample is automatically measured in the by-pass by process sensors. The advantages of this method lie in the simple sterilization and the straightforward access to the sample in stationary conditions. The implementation of such a solution requires a specifically designed or modified bioreactor. The added complexity of set-up makes this method less common than in-line monitoring, yet it is one of the two methods with which constant monitoring and, therefore, control are possible in real-time.

In-line or In-situ

In in-line or in-situ, the measurement occurs directly in the bioreactor with a process sensor. The generated measurements are sent in real-time to PLC/ SCADA systems for automated control. Process parameters such as pH, ORP (redox potential), dissolved oxygen, dissolved CO_2 (DCO_2), temperature, and conductivity are all common in-situ measurements.

In-line and on-line sensors are the optimal choice for application of PAT principles. They are required to accurately measure without manual intervention over the entire process run, which can last several weeks or even months. Therefore, the operation and maintenance of the sensor should not be underestimated to guarantee reliable, accurate measurement. Preventative measures such as calibration and cleaning should be implemented at specified intervals to avoid drift or loss of signal. The sensors need to be compatible with repeated CIP and SIP cleanings. Extended time at temperatures of 120 to 130 °C should not affect the sensor‘s performance.

Critical Process Parameters

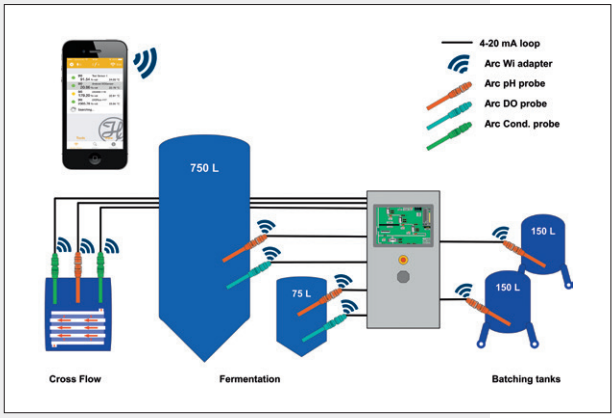
Physical and chemical critical process parameters are commonly monitored using in-line/ on-line process sensors or at-line process analyzers. This chapter provides an overview of each parameter. Application examples are also presented in focus spots 1 and 2.

pH

The most classical example of PAT applied to bioprocesses is the maintaining of culture pH at a pre-defined set-point based on an in-situ electrochemical sensor signal. The signal is used to automatically regulate the addition of a base or acid (or the controls of CO_2 for mammalian cells). The working range varies according to the applications. For example, mammalian cells vary between 6.8 - 7.4 pH, while others, like insect cells, are optimized around 6.3 pH. Tight control of this parameter is crucial. Drifting pH measurement often negatively influences the product's yield in large scale manufacturing operations[7]. Keeping the pH in the correct working range has an impact both on cell viability as well as on the product's quality. An example of the latter is how pH directly affects the therapeutic affect of mAbs: too low pH level negatively influences the

FOCUS 1 Intelligent Arc Sensors for pH and DO in-situ Measurement

Efficient, reliable, compact design, and precise process control – these are the factors that GEA Diessel GmbH requires for monitoring the fermentation plant. They were found in Hamilton Arc sensors[A]. The measurement of the pH and dissolved oxygen takes place in the pre-fermenter as well as in the main fermenter, in this process.



- The VisiFerm DO Arc optical dissolved oxygen sensor was chosen for its compact design and low maintenance costs. The optical sensing element is unaffected by pressure fluctuations and does not require polarization time at startup.
- pH is measured with the EasyFerm Plus Arc sensor. Its pressurized reference and low drift after repeated sterilization cycles make it ideal for fermentation processes.
- All Arc sensors include an integrated micro-transmitter which transfers the measurement, CIP and SIP data, and sensor quality, as well as data regarding the operating life, via a wireless connection to the Hamilton ArcAir App for sensor management.

protein's glycosylation pattern resulting in a loss of their bioactivity[20]. Glycosylation is one of the most important CQAs for monoclonal antibody production.

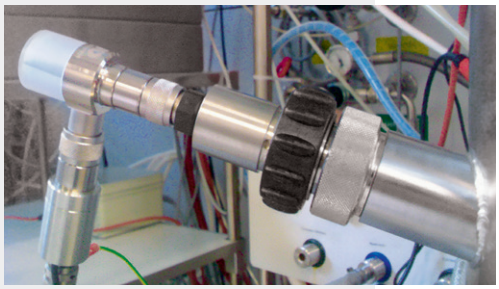
The most common process pH sensors are electrochemical combination glass electrodes which are designed to withstand multiple autoclavations, CIP and SIP cycles. Alternative pH measurement solutions, such as optical sensors, exist as well; however they have limited measurement range, can only be sterilized by gamma radiation, and exhibit substantial drift to guarantee accurate measurement.

Dissolved Oxygen

Dissolved oxygen (DO or pO2) is another critical process parameter. Air or oxygen-enriched air is supplied to the bioreactor to support cell demand. Oxygen is used for cellular respiration and cellular growth. While important, DO can be controlled over a broader range than pH without too significantly impacting cell growth rates or product quality. Typical DO operating ranges for aerobic cultures lie between 30 to 40% air saturation. DO levels below this range will affect cell viability, whereas excessive DO levels can oxidize the end-product.

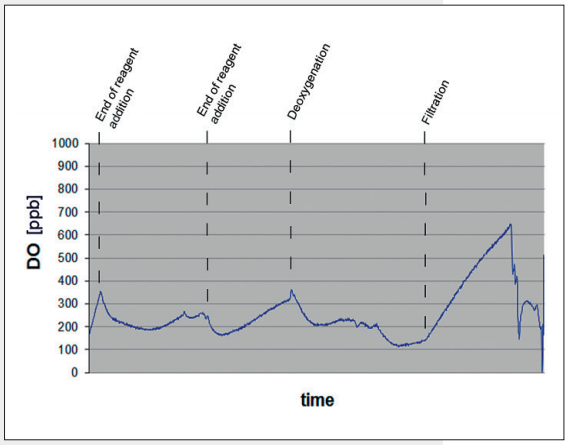
FOCUS 2 Dissolved Oxygen User's Experiences

- **Maintaining precision over multiple CIP/SIP cycles**
  Dissolved oxygen concentration is directly related to cell growth and high protein yield. Roche Pharmaceuticals uses the optical VisiFerm DO sensor due to its robustness, enabling them to survive in their applications[B]. Each sensor is sterilized over 25 minutes at 121°C followed by a deionized water rinse. This procedure is repeated several times a week. Roche reports no degradation of the sensor and precise measurement over time.



- **Arc technology prevents downtime and allows reliable fermentation control**
  UK based Albumedix focused their production on Saccharomyces to produce recombinant proteins[C]. An average fed-batch production run last approximately 5 days. During this time dissolved oxygen is reduced from 98% saturation to a designated control point using, as well, the optical VisiFerm DO sensor. The sensor outputs a 4-20 mA signal directly from the integrated microtransmitter into the biocontroller. Sensor status and actual measurement values can be easily checked through use of the Hamilton ArcAir app at any time.

■ **VisiPro DO Ex for low dissolved oxygen content in ATEX zones**
The manufacturer of pharmaceutical agents (APIs) Medichem relies on an optical dissolved oxygen sensor[D]. The high dissolved oxygen sensitivity of sulfur compounds requires a sensor that measures reliably at ppb levels. In this application, the dissolved oxygen sensors were located in potentially explosive atmosphere thus ATEX certification was required. The optical VisiPro DO Ex Sensor provides quick response time and simplified maintenance compared to polarographic sensors. Its integrated micro-transmitter outputs 4-20mA with HART protocol directly into the control system for simple integration.



Two types of bioprocess DO sensors are commonly used: polarographic and optical. Polarographic sensors utilize an electrochemical cell (sometimes referred to as a Clark cell). This design was the first to market, and has limitations of high maintenance costs, extended startup time, and measurement errors due to fouling by CO_2 and other gases. Optical DO sensors based on the newer quenched luminescent technology have begun to supplant polarographic technology and are now considered state-of-the-art for in-situ measurement.

Dissolved Carbon Dioxide

Dissolved CO_2 (DCO_2 or pCO_2) is a parameter which is monitored due to its influence on pH values in mammalian cells and fatty acid synthesis. A higher dissolved carbon dioxide level can inhibit cell growth and reduce production of secondary metabolites. Carbon dioxide is especially critical in cell culture (mammalian) processes and must be kept within 5 - 10% air saturation. Process DCO_2 sensors based on electrochemical principals have been available for many years. They are affected by high maintenance costs to ensure an acceptable measurement accuracy.

Exhaust O_2 & CO_2 – Off-gas

In some bioprocesses, like those using yeast for antibiotics production, culture viability is controlled by monitoring indirect indicators like Oxygen Uptake Rate (OUR) and Carbon Dioxide Evolution rate (CER). The ratio between O_2 and CO_2 entering the bioreactor and the exhaust/off-gas measured after air filter is used as a proxy of culture viability. Exhaust/off-gas can be measured with at-line mass spectrometers or with on-line process sensors based on galvanic measurement and infrared (IR) absorption. These measurements are mainly implemented for microbial culture, where the measurement is considered complicated and often not enough reliable.

Nutrients & Metabolites

Proper monitoring of nutrient (or substrates) concentration, as well as measurement of secondary metabolites, is important, especially for fed-batch and perfusion processes, as the feeding strategies can be controlled during the process. Glucose or glycerol are the main C-source (carbon source), while glutamine is the main N-source (nitrogen source), together with other amino acids in these bioprocesses. During the fermentation they are consumed, and secondary metabolites such as lactate, acetate and ammonium are produced. Suboptimal feeding strategies can produce excessive secondary metabolites which hinder cell viability and product yield. For example, the accumulation of lactate in mammalian cultures has long been recognized as an inhibitory factor for cell growth and recombinant protein production[13]. Therefore, control of nutrient feeding is of paramount importance (along with pH, DO and temperature), for process optimization.

In-line and on-line sensors are typically based on molecular spectroscopy technologies like Near-Infrared (NIR) and Raman. They are secondary measurement technologies, meaning measurement with off-line reference methods are required to calibrate them through use of statistical multi variate data analysis (MVDA). The measurement accuracy of these methods is strictly related to the specific bioprocess environment and to the quality of the off-line measurements used for calibration. There have been no published studies which show the use of the same global MVDA calibrations to predict different cell processes with an acceptable accuracy[7]. For all such reasons, they are considered too labor-intensive and too expensive for a successful implementation in production environments.

The complexity of these measurements elucidate why at-line and/or off-line methods are still the most common option for nutrient and metabolite monitoring, despite not being optimal for PAT compliance. This equipment may utilize different technologies: HPLC, glucose oxidase, or other biochemical analysis to perform the measurement. These analyzers can be automated or semi-automated. They often require separate devices for sterile sampling and measurement cycles often require a relatively long measurement time (minutes)[14]. Yet, for the reason explained, they remain the only option available for measuring the culture's substrate and secondary metabolites.

Temperature, Pressure & ORP

Temperature is a fundamental and well-controlled parameter in bioprocesses. Bioprocesses are typically monitored and controlled tightly between 0 and 60°C, including during sterilization cycles. Several devices and measuring principles are common to measure temperature in bioreactors such as thermistors, resistance and bimetallic thermometers[8].

Other physical and chemical CPPs, such as pressure and ORP can be controlled to

optimize the cultures fermentation processes, as well. Pressure is an important control parameter because it affects not only the bioprocess but also safety. In general, it influences the saturation concentration of the gases dissolved in the liquid phase, like DO and DCO_2. Most common measuring options are represented by piezoelectric-based and filled diaphragm transducers.

Monitoring the oxidation reduction potential (ORP) provides information regarding the concentration of oxidizing or reducing molecules. ORP can provide important feedback for understanding the process: e.g. optimizing the yield from mammalian cells. Similar to pH sensors, the most common in-situ monitoring option is a combination reference/ measurement electrode.

Critical Quality Attributes & Key Performance Indicators

Monitoring CPPs makes it possible to maintain the related Critical Quality Attributes and Key Performance Indicators within the pre-defined limits. Collected process data are used in case of need for root cause analysis or for process characterization studies based on experimental design (e.g. for scale-up and scale-down). PAT will be best fulfilled when CQAs and KPIs can be directly measured as often as possible, as explained in the following paragraphs.

Product Quality & Product Titer

The main goal of bioreactor operation is to produce as much product as possible with the quality needed to make it functional for its therapeutic purpose. Quality and yield are important as the product often requires further purification in downstream processes that may cause additional modifications or losses.

As previously mentioned, the most important biopharmaceutical products are: monoclonal antibodies, recombinant proteins, or other types of therapeutic proteins (like vaccines). Therefore, bioprocess CQAs are often considered attributes specific to the protein's quality such as the glycosylation patterns or molecular-size distribution[4]. The most commonly used KPI at the bioreactor is the total protein titer and eventually specific titer for the protein type (e.g. IgG).

The most promising results for in-situ measurement of product titer and quality have been obtained with spectroscopic technologies, which have the same limitations described for nutrient and metabolite measurements. Likewise, HPLC, mass spectrometers, NMR, fluorescence or super-resolution microscopy, capillary electrophoresis or biochemical

analyzers installed at-line or off-line are often seen as more reliable solutions to quantify the mentioned CQAs and KPIs at the bioreactor[14]. Again, sterile sampling technologies and procedures make these quality attributes and process indicators limited with respect to PAT control guidelines.

Total and Viable Cell Density

Other Key Performance Indicators successfully used for in-situ control at the bioareactor are Total Cell Density (TCD) and Viable Cell Density (VCD). TCD indicates the total amount of cells in the bioreactor, while VCD is an indicator of the viable cells (alive and still productive). VCD is directly correlated with final product yield and is thus of high importance.

Different off-line measurement technologies have been established over the years[8]. For example, total cell density, as well as viable cell density, can be measured via off-line automated cell counter systems. A major disadvantage is that these methods are based on time-consuming sampling procedures which reduce the possibility that the cell growth is monitored in a process-safe way compatible with PAT principles. For this reason several efforts have been put forth over the years to find technologies suited for accurate and reproducible real-time measurements.

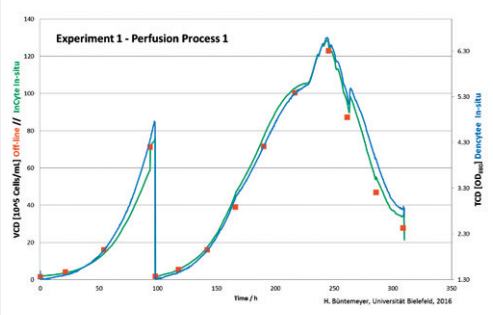
Some efforts for real-time cell density are based on the use of molecular spectroscopy, others on soft sensing techniques (e.g. algorithms based on the evolution of OUR and CER), both requiring MVDA to generate application specific calibrations which are labor-intensive to maintain.

The most reliable measurements are obtained using near-infrared light to measure culture turbidity in microbial fermentations, or by using capacitance sensors to measure cell viability (especially for mammalian cell cultures). Turbidity and capacitance are currently the most common technologies used to measure TCD and VCD in real-time. These sensors can withstand autoclavations, CIP and SIP cycles to meet hygienic standards. Examples of the application of such technologies are provided in focus spot 3 and 4, as well as those presented in the next chapter.

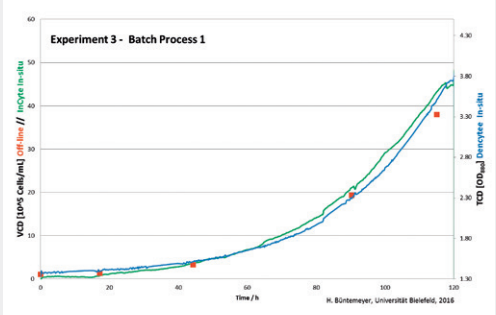
FOCUS 3 In-Situ Cell Density for Batch and Perfusion mAb Production

The monitoring of KPIs such as Total Cell Density and Viable Cell Density are currently available as in-situ measurements with process sensors. Studying these indicators allows the real-time control of the nutrient feed rate based on the growth rate of cell cultures. The Cell Culture Research Team of the University of Bielefeld investigated the accuracy of Incyte and Dencytee (Hamilton VCD and TCD sensors), for both perfusion and batch production of MAb using CHO cells[E]. The test demonstrated the following benefits:

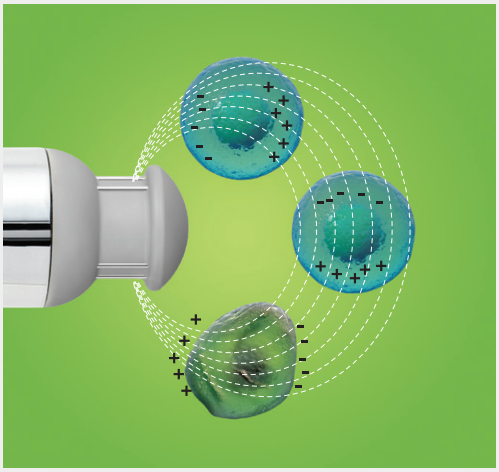
- Accurate measurement of the cell growth enabling real-time control
- Better insight about cell health based on the parallel measurement of TCD and VCD
- Reliable and stable measurements for long-lasting continuous fermentations



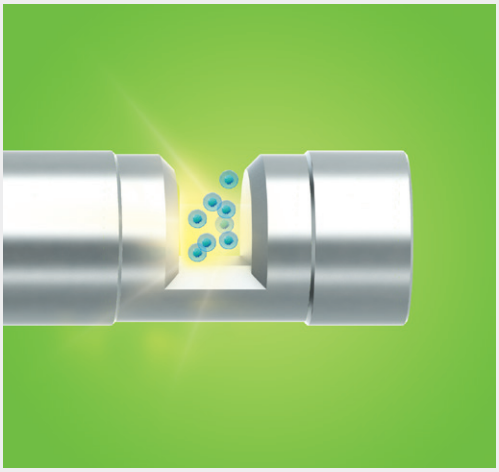
Performance comparison of cell density measurement for the perfusion set-up.



Performance comparison of cell density measurement for the batch set-up.



The Incyte is based on capacitance principles. In an alternating electrical field, viable cells behave like small capacitors. The charge from these small capacitors is measured by the sensor and reported as permittivity (capacitance per area).



The Dencytee is based on turbidity measurement of a suspension at NIR wavelengths. All particles and molecules that scatter the NIR light will be detected and can be correlated to the total cell density.

Recent Applications of In-situ VCD & TCD

The possibility to accurately and reliably measure TCD and VCD in-situ makes them the most important Key Performance Indicators at the bioreactor. This is consistent with the increasing number of biopharma related publications, such as the ones presented in the following overview.

4TH BioProScale Symposium 2016

At the «Bioprocess intensification through Process Analytical Technology (PAT) and Quality by Design» Symposium in Berlin, the Max Planck Institute of Magdeburg presented a poster[15] about Online-monitoring tools in batch and (semi-)perfusion cultivations for vaccine production. They reported that the data obtained from capacitance and turbidity sensors can be useful to monitor cultivations and to manually and automatically control perfusion cultures.

ZHAW University of Applied Sciences

A recent study conducted by the Zurich University for Applied Sciences found that capacitance measurements for VCD were the most suitable for integration into PAT systems[16]. The viable cell density was measured with in-situ sensors which do not require any sample dilution and therefore save time compared to at-line and off-line analyzers. Such capacitance sensors provide measurements robust enough to be used for automated control loops.

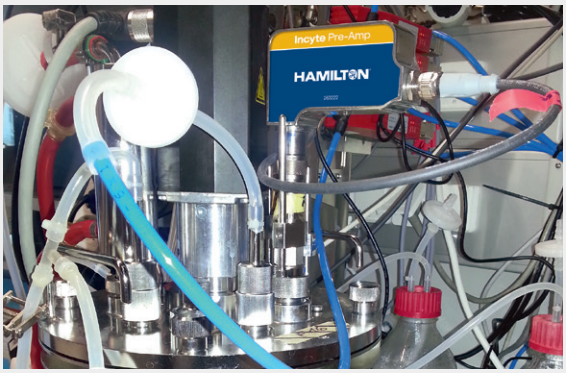
ESACT 2017

Multiple posters on in-situ TCD and VCD were presented at the 2017 European Society for Animal Cell Technology (ESACT) in Lausanne, Switzerland.

- A too high nutrient feed can lead to an accumulation of metabolites and therefore a sub-optimal cell culture development. Boehringer Ingelheim California presented a poster[17] about using a capacitance sensor for real-time VCD data and control of the process. An automatic feed-strategy was developed using VCD as an alternative to the previous bolus feeding strategy.
- The University of CNRS-Lorraine[18] monitors CHO cells for the production of monoclonal anti-bodies using in-situ cell density sensors to comply with PAT principles. The method allows for real-time measurement and records critical conditions immediately.
- Zoetis Spain[19] has presented applications illustrating the possibility of using both capacitance and turbidity sensors in cell density measurements in different scale-up phases with both conventional and single-use bioreactors.

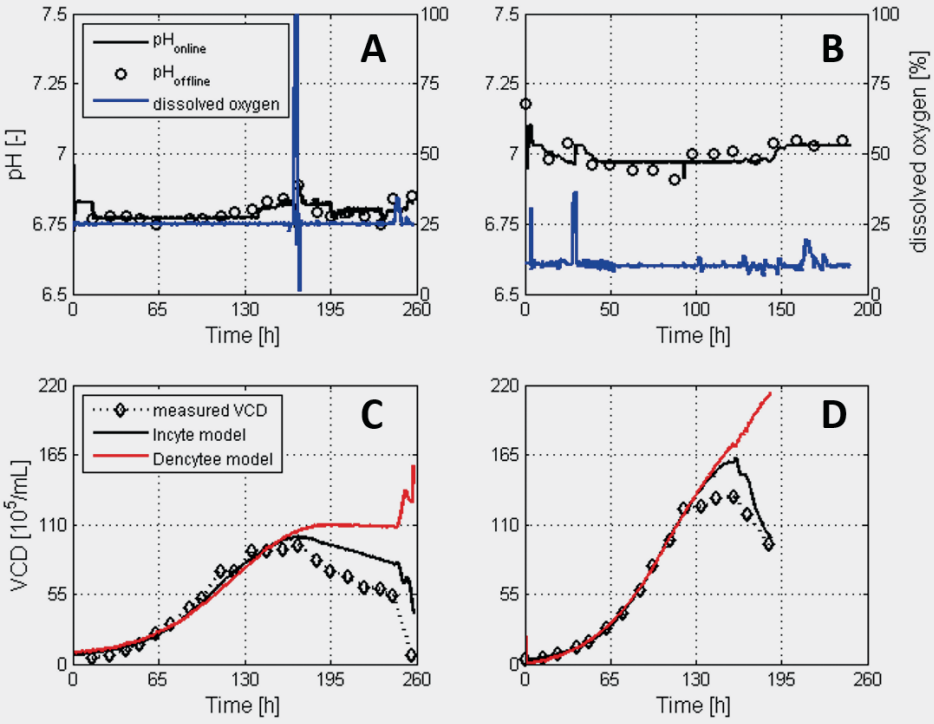
FOCUS 4 Strict Control of Mammalian Cultures through in-situ Process Monitoring

Mammalian cell cultures are used for the production of different recombinant proteins for clinical applications such as vaccines, anti-bodies or drugs. Vienna University of Technology[F] (TU Wien) determined that strict control of parameters such as pH, dissolved oxygen and cell density directly impacted product quality and yield.



- Even small fluctuations of each parameter within the process affect cell growth, which is why real-time measurement provides the best solution.
- In-situ measurements not only provide better process data but also better correlation with cell viability and growth.

The process is monitored and controlled using the Dencytee optical sensor (TCD) and the Incyte capacitance sensor (VCD). Both provide valuable insight in regard to the exponential growth phase, which lasts over 130 hours. The sensors are easily operated and do not need to be calibrated during the process.



Conclusions

With the implementation of PAT in bioprocesses, Critical Quality Attributes, Critical Process Parameters and Key Performance Indicators must be monitored. The most valuable measurements are performed in-situ to allow for real-time control strategies. The data derived from these continuous measurements are also valuable for use in root cause analysis or for process characterization such as scale-up and scale-down studies.

Table 1 summarizes the most important CPPs, CQAs and KPIs at the bioreactor, with an overview of the available measuring methods along with their typical accuracy and limitations.

The crucial CPPs allowing for real-time control strategies are pH, DO, and temperature. Other parameter's measurement such as DCO_2, nutrients and metabolites are accurate and repeatable just through at-line or off-line analyzers, making them complicate for real-time control strategies. In regards to CQAs and KPIs, those related to the product quality and product titer are important, nonetheless they are likewise complicate to measure in-line with acceptable accuracy. In conclusion, the real-time monitoring of the mentioned crucial CPPs, together with the in-line measurement of TCD and VCD represents currently the best PAT option to control product yield and quality at the bioreactor.

Table 1: Summary of the CPPs, CQAs and KPIs at the bioreactor

		PAT Method of Choice	Alternative Methods	
		In-line / In-situ Sensor	On-line Analyzer	At-line & Off-line Analyzer
Critical Process Parameter	pH	♦	♦	●
	DO	♦	♦	●
	DCO_2	●	●	●
	Temperature	♦	♦	■
	Exhaust O_2 / CO_2	■	♦	♦
	Nutrients e.g. Glucose, Glutamine	●	●	♦
	Metabolites e.g. Lactate, Ammonium	●	●	♦
Critical Quality Attribute	Product Quality e.g. Protein Glycosylation	▲	▲	♦
Key Performance Indicator	Product Titer	●	●	♦
	Total Cell Density	♦	♦	♦
	Viable Cell Density	♦	♦	♦

The availability of monitoring methods according to the scientific literature is represented with the indication of the corresponding measurement accuracy and robustness:

♦ *Accuracy, robustness and repeatability good enough to be commonly implemented for process control*
● *Accuracy, robustness and repeatability not commonly accepted for process control*
▲ *No true option available*
■ *Not required*

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