INGOLD
Leading Process Analytics

5 Ways to Improve Bioprocessing with Digital Sensors

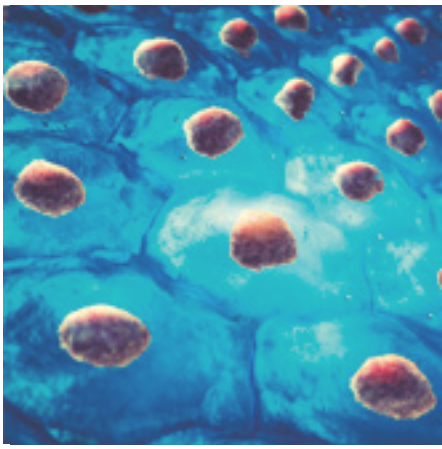
1 Enhance Bioreactor Performance
Integrating Digital pH & DO Sensors

2 Speed-up Process Development
from Benchtop to Production

3 Evaluate Respiratory Efficiency
With Continuous CO_2 Monitoring

4 Eliminate Possible Interference
By Shifting from Analog Sensors

5 Reduce Maintenance Efforts
Using Optical DO Measurement



METTLER TOLEDO

"Easy Digital Sensor Integration"

Highest Performance in Tissue Engineering Bioreactors

Tissue engineering demands the utmost accuracy and repeatability from sensor measurements. A manufacturer of cutting-edge bioreactors for tissue applications has selected METTLER TOLEDO Intelligent Sensor Management (ISM®) probes for their high reputation and problem-free integration.

Rapid growth in tissue engineering

The ex vivo engineering of living tissues is a young but rapidly developing field in the biotechnology industry. Tissue engineering utilizes human and animal cells, biomaterials, physical stimulation and biochemical factors to manipulate and grow tissue. Its applications are as wide ranging as replacement of animal testing during drug development to developing bio-artificial human organs to creating edible artificial meat. Worldwide, the tissue engineering market is estimated to have grown from US $ 15 billion in 2014 to US $ 32 billion this year.

Much of the development in tissue engineering requires advanced bioreactors that can standardize, control and automate the required growth and testing conditions in order to achieve high reproducibility.

At the forefront of bioreactor development

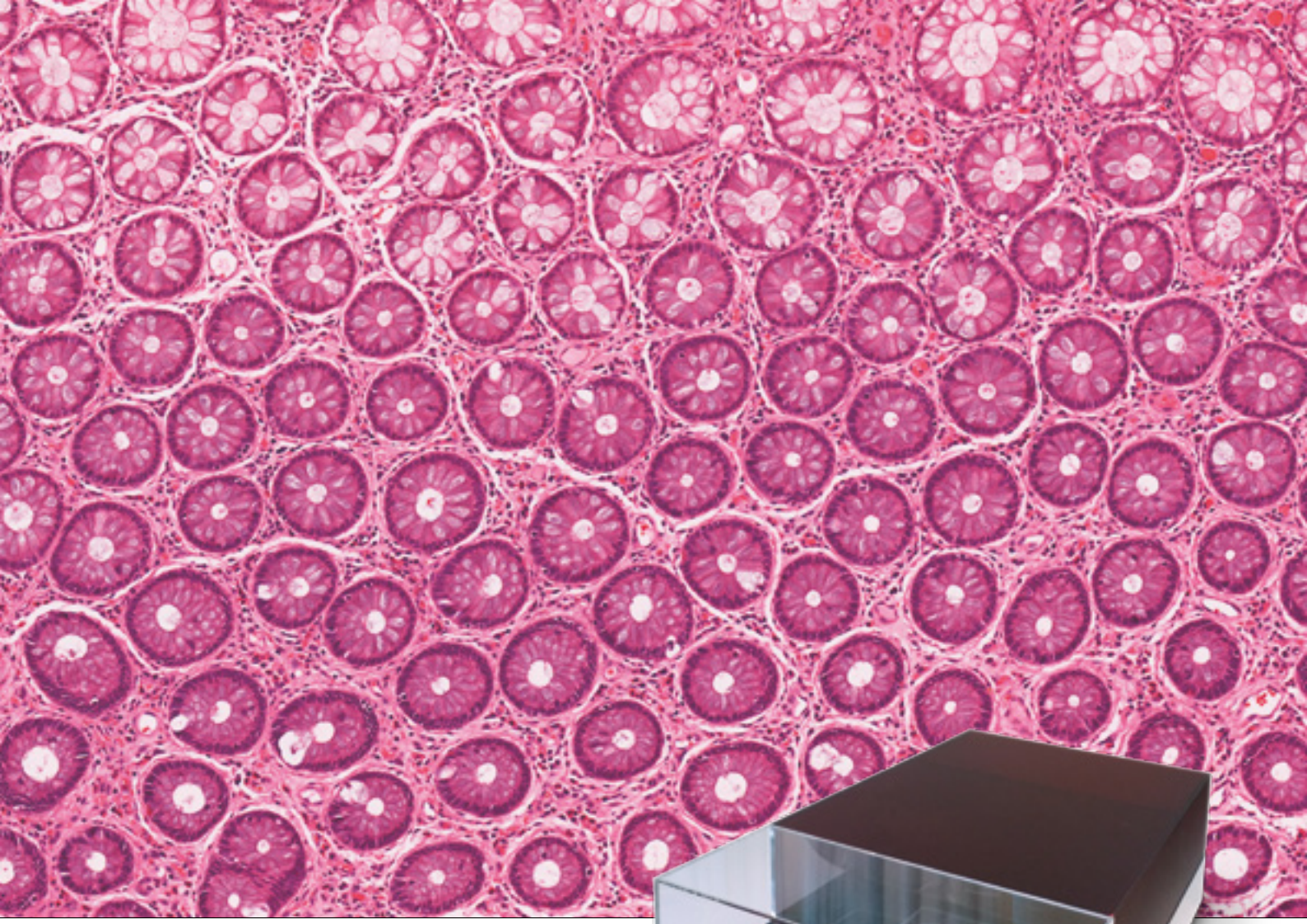
Ospin, in Berlin Germany, was founded in 2014 to provide technology solutions that increase throughput in tissue engineering research and which serve as development and production platforms for tissue applications.

The company develops modular and scalable bioreactors that enable tissue production for a wide range of clinical and research applications. The bioreactors employ cloud computing and a web-based user interface to monitor and manage the tissue engineering and analyze the resulting data from the pH, DO and other sensors.

METTLER TOLEDO "the obvious solution"

Maintaining the desired growth environment in the bioreactors requires careful control of pH, dissolved oxygen (DO), and other parameters. This necessitates the use of highly reliable analytical sensors with excellent repeatability. For this, Ospin turned to METTLER TOLEDO.

Jan Saam, founder and CEO of Ospin, explains: "Our customers already use METTLER TOLEDO sensors in their biotech research and production, so



selecting them for our bioreactors was the obvious solution for pH and DO measurement. Also, the digital output from METTLER TOLEDO pH and electrochemical DO ISM probes made integration into our applications easy."

Error-free installation

ISM provides a number of other useful benefits that analog sensors cannot provide. These include Plug and Measure installation. Calibration and other sensor information is stored within the sensor. When connected to an ISM-ready transmitter, or controller – as is the case with Ospin bioreactors – the data is automatically uploaded and the controller configures itself appropriately without any operator intervention: saving time, and eliminating the possibility of human error.

Pre-batch diagnostics

Using METTLER TOLEDO's CFR-ready iSense™ software, Ospin's customers can run pre-batch diagnostics to identify any sensor maintenance required before a probe's installed in a bioreactor.

Future partnership

Ospin and METTLER TOLEDO hope to continue working together to produce high performance bioreactor platforms that will assist developments in the important field of tissue engineering.

Read more on ISM sensors:

► **www.mt.com/pro-digital-sensors**

Modeling with CO_2 Measurement Allows Faster Scale-up of Fermentation Processes

The InPro® 5000i sensor from METTLER TOLEDO enables precise and reliable in-line measurement of dissolved CO_2 throughout an entire cultivation period at variable gas transfer from liquid to gas phase.

Kluyver Center, Delft, Netherlands
The Kluyver Center for Genomics of Industrial Fermentation in Delft, Holland is a consortium of several different Universities and Research Centres. The center works closely together with Elscolab, a company which has represented METTLER TOLEDO Ingold in the Benelux countries in the field of process analytical measurement systems for many years.

Microbial genomics
The Kluyver Center applies microbial genomics to improve the performance of microorganisms in industrial fermentation processes. Fermentation is used in the production of renewable feedstocks for food products and ingredients, beverages, pharmaceutical compounds, nutraceuticals, through to fine and bulk chemicals.

Scope of possible application
In connection with research programs covering yeast fermentation, fungal fermentation, lactic acid fermentation, bio catalysis and genomic tools including bioinformatics, the center is always on the lookout for the latest developments in in-line measurement technology.

In this regard, Martin Hoogedoorn, Product Specialist at Elscolab Nederland B.V., introduced METTLER TOLEDO's in-line CO_2 sensor to Sjaak Lispet, head of instrumentation at the center.

Importance of CO_2 measurement
Sjaak Lispet stated that "dissolved CO_2 is, next to pH and dissolved oxygen, the most important measurement parameter for us". Frederik Aboka, a PhD student in Bioprocess Engineering explained the reason why it is so important to be able to measure dissolved CO_2 directly in-line: "Many aerobic microbial fermentations are stimulated or inhibited by dissolved carbon dioxide. It is therefore very important to measure carbon dioxide content in the fermentation broth".

Fig. 1: 4.0 Liter laboratory fermenter with side-mounted CO_2 sensor.

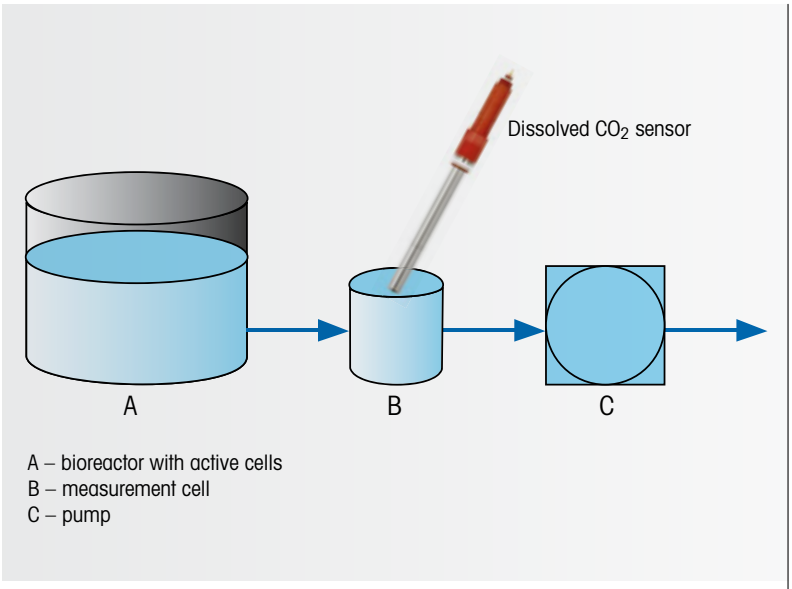
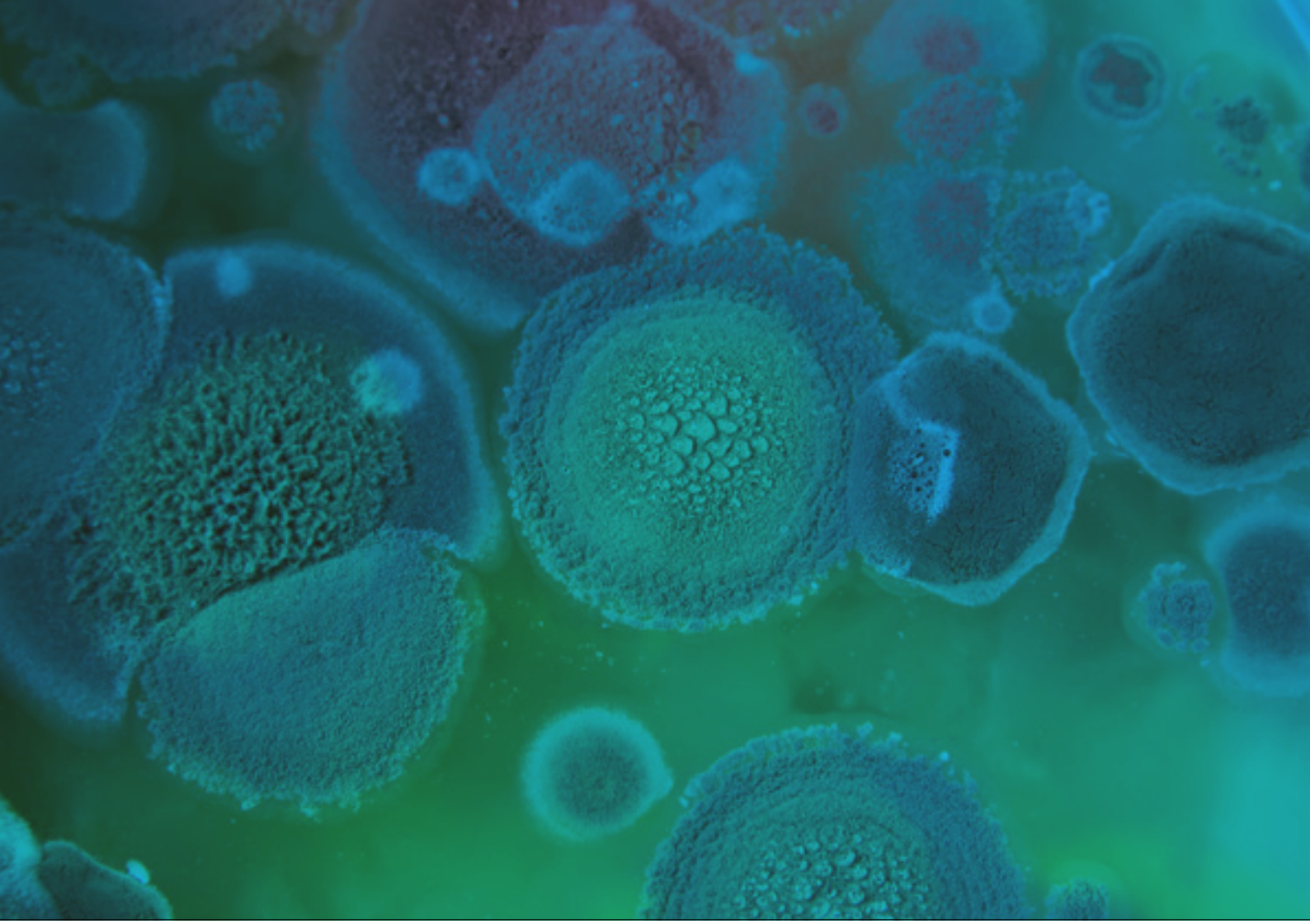


Fig. 2: Experimental setup for dissolved CO_2 measurement outside the bioreactor.



Faster scale-up

An in situ CO_2 sensor delivers information on the liquid phase of the bioreactor. It has to be considered that the liquid phase is generally never in a thermo-dynamic equilibrium with the gas phase during the process. A measurement in the liquid phase is directly related to actual condition values of the volumetric coefficient of transfer. This coefficient is an important design and scale-up parameter for bioreactors. In all processes where dissolved CO_2 plays a key role in the metabolism, in situ CO_2 measurement allows a faster scale-up during process development and a faster time to market for new products.

Use of the CO_2 sensor InPro 5000

Asked about the use of the CO_2 measurement system, Frederik Aboka explained: "In our lab, the sterilizable sensor InPro 5000 was employed in aerobic yeast fermentation in two ways. In the first instance, side-mounted in a fixed position in a 4.0L laboratory fermenter during the whole period of the cultivation. In the second instance, the sensor was installed as shown in the diagram (Fig. 2). In this configuration the sensor was placed in a small measurement cell of our own design. Culture broth is drawn off from the bioreactor and fed to the measurement cell by means of a pump.

Reliable and stable measurement

In respect of results, Frederik Aboka stated: "We are satisfied with the steady state measurement of dissolved CO_2 using the METTLER TOLEDO InPro 5000 sensor."

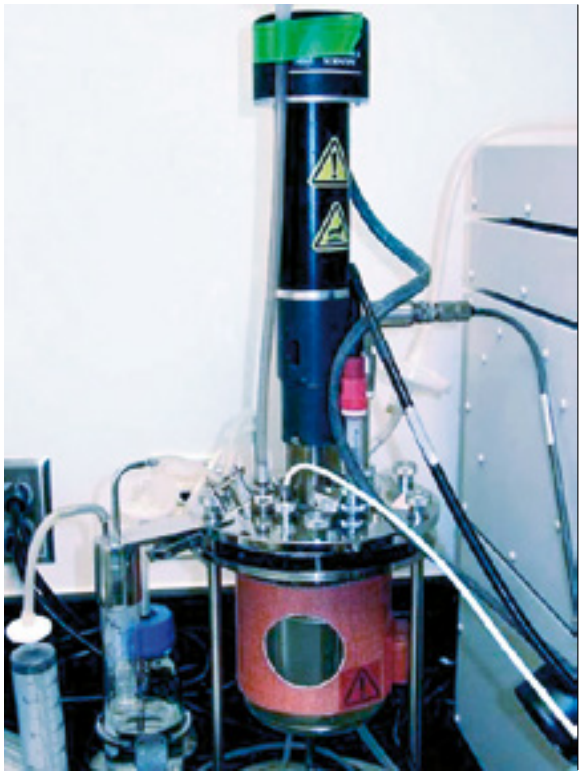
► **www.mt.com/InPro5000**

In Situ CO_2 Measurements
to Determine Growth Phase of Saccharomyces

In conjunction with pH and dissolved oxygen, in situ measurement of dissolved CO_2 is a critical parameter in evaluating the respiratory efficiency of microorganisms in cell culture. Virginia Bioinformatics Institute used the METTLER TOLEDO in situ CO_2 measurement system to study Saccharomyces cerevisiae cells (yeast) in batch culture in order to accurately and reproducibly determine the growth phase of the culture as a function of CO_2.

How CO_2 relates to optical density

The production of CO_2 by yeast in a batch culture can indicate the stage of growth for that culture. During the exponential phase, yeast cells grow by fermenting the available sugar and producing ethanol and CO_2 as byproducts. The amount of CO_2 generated during the exponential growth phase can be directly correlated to optical density values, which are generally used to determine the progression of the growth phase.



Experimental design

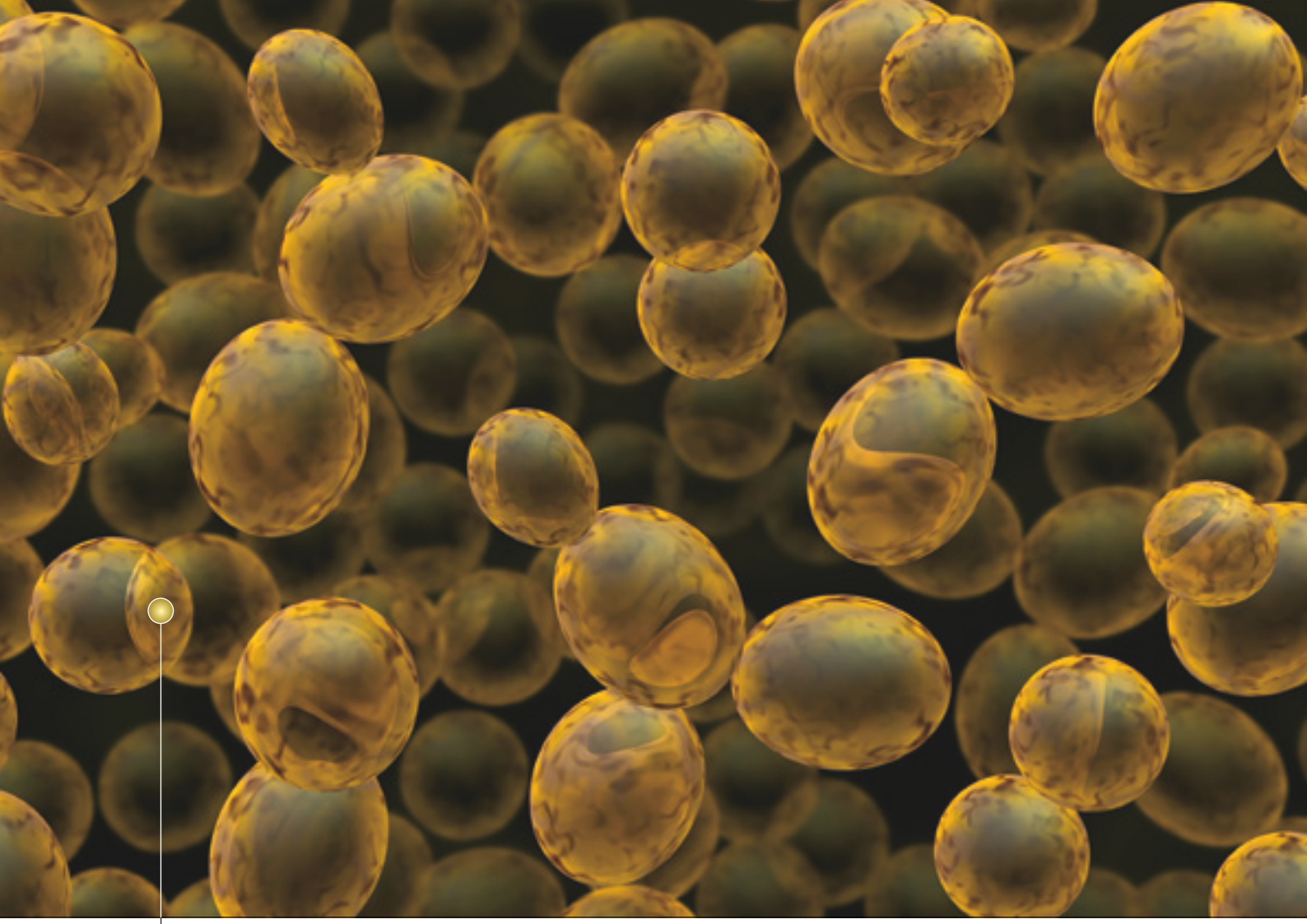
In a project developed by the Virginia Bioinformatics Institute, the response of Saccharomyces cerevisiae cells to oxidative stress induced by hydroperoxides, was studied. Yeast cells were grown in a fermenter with controlled conditions of pH and dissolved oxygen to ensure that the response obtained was due to oxidative stress and not to other environmental conditions. Monitoring CO_2 production allows for a more accurate determination of the growth phase of the culture as opposed to just using OD measurements. This is important because the oxidant must be added at a specific point of the growth curve. Hence the requirement of observing reproducible CO_2 curves during yeast growth.

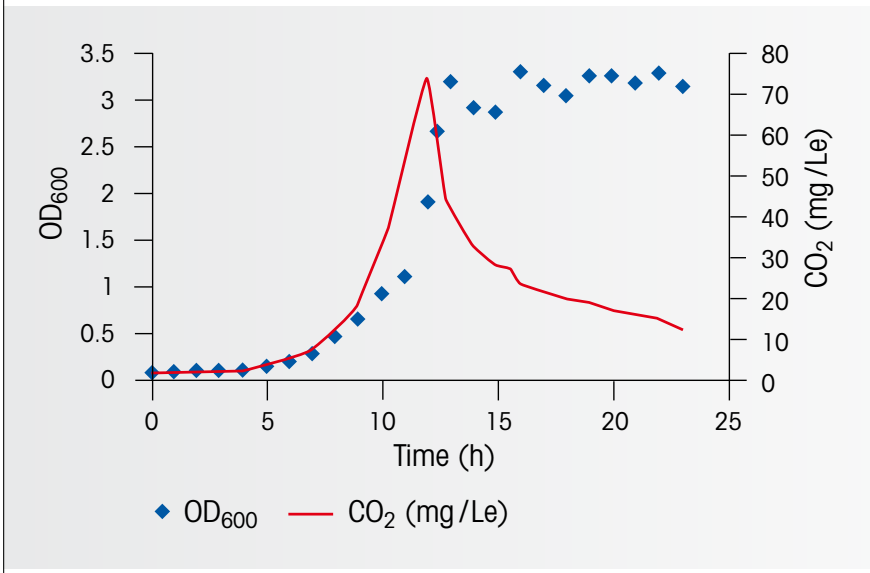
The first experiment was designed to test the reliability of the InPro® 5000 CO_2 sensor, comparing its measured values with those obtained via a GC-MS. A yeast culture was grown in a fermenter for 24 hours. Culture samples were collected from the fermenter for the determination of OD at 600 nm and headspace samples were collected for CO_2 analysis by gas chromatography – mass spectrometry (GC-MS). Samples of 100 µl drawn from the fermenter headspace were injected at a split ratio of 10:1 into a GC-MS system. Mass spectra were recorded at 10 scans per second over a range of 30 – 100 m/z.

The second experiment was designed to test the reproducibility of the InPro® 5000 CO_2 sensor. Two yeast cultures were grown in fermenters for 24 hours. Culture samples were collected for the determination of OD_{600}, and CO_2 measurements were recorded.

Easier handling and less contamination risk

Autumn Clapp, the Fermentation Microbiologist for the Virginia Bioinformatics Institute at Virginia Tech in Blacksburg, VA stated, "The results obtained from the METTLER TOLEDO InPro 5000 CO_2 sensor were much easier to obtain that those from GC-MS. Using the InPro 5000, there is less chance of contamination to the culture as the sensor may be sterilized within the fermenter. In order to take samples from the fermenter for GC-MS analysis, a syringe must be introduced into the system which may introduce contamination. The InPro 5000 CO_2 sensor and METTLER TOLEDO transmitter interface also lends to easy readouts with no wait time as compared to GC-MS, and there is also no need for extensive data processing that is sometimes involved with GC-MS results".





Superimposion of graphs to show relationship between cell growth and CO_2 production.

Benefits of the METTLER TOLEDO in-situ CO_2 system

The METTLER TOLEDO in-situ CO_2 system provides an accurate measure of CO_2 production that is comparable to a GC-MS, but the METTLER TOLEDO unit has several obvious advantages. The clearest advantage is that the InPro 5000 sensor is present inside the fermenter and provides real-time measurement data that can be collected instantly, whereas an off-line system creates a lag time in the generation of results. The in-situ CO_2 system also is engineered with the highest possible hygienic design, which virtually eliminates contamination, whereas off-line systems require user sampling that may compromise the fermentation.

Autumn Clapp, Virginia Bioinformatics Institute at Virginia Tech, USA

▶ **www.mt.com/InPro5000**

Eliminate Analog Measurement Issues
with Digital Sensors

Digital sensor technology does not suffer from sensor measurement and calibration issues caused by insufficient electrical isolation on analog equipment. METTLER TOLEDO digital pH and DO solutions have restored measurement confidence for one of Big Pharma's companies.

Global pharmaceutical leader

One of the world's leading pharmaceutical companies, based in the USA, operates a large biopharmaceutical facility in New York State. Several critical functions are located at this site, including the manufacture of biologic medicines for use in clinical trials and for commercial use. As with all biopharmaceutical manufacturers, the revenue value of each production batch is worth millions of dollars; therefore, anything that can risk yield or drug quality must be avoided.

Poor electrical isolation threatens productivity

pH and dissolved oxygen (DO) monitoring and control on bioreactors is central to optimizing productivity, so high performance of measurement systems for these parameters is essential. Facility process scientists and maintenance engineers were experiencing an issue with this equipment that was causing a loss in confidence in the data they were receiving.

The personnel noticed that pH and DO levels displayed on transmitters changed whenever they approached the instruments. And due to the close proximity necessary during sensor calibration, the effect at these times was particularly pronounced: data from sensors would rapidly fluctuate, resulting in a lack of assurance in calibration accuracy. Investigations revealed the cause of the problem to be poor electrical isolation on the transmitters.

Further, the sensor-transmitter cables would often fail after only a few months of operation. As the replacement cost per cable was USD 600, facility managers were keen to find a better solution.

Digital technology provides the answer

METTLER TOLEDO met with a team of decision makers at the plant to discuss our Intelligent Sensor Management (ISM®) digital technology, as we were certain we could restore total confidence for process scientists and

ISM sensor offering
The parameters covered by ISM sensors is wide and includes pH, dissolved oxygen, gas phase oxygen, conductivity and TOC.

maintenance engineers in these important measurements.

The combination of our multi-parameter M400 transmitter and AK9 sensor-transmitter cables provides the electrical stability and low cost of ownership that are essential in any manufacturing environment. A demonstration of the solution clearly showed that measurements from sensors and calibration data on the transmitter and iSense remained stable regardless of personnel proximity.

An even more attractive aspect of our ISM technology for facility personnel is the ability to maintain and calibrate ISM pH and DO sensors in a remote lab via our iSense™ PC software. This allows convenient sensor calibration and verification as well as documentation of sensor operations away from the production environment.

Perhaps the most desired deliverable that any customer wants is superior customer support. The availability of local, competent support from our New York agent made the METTLER TOLEDO solution even more attractive.

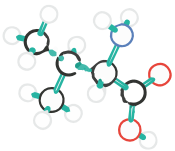
Problems eliminated, confidence restored

Certain that our systems would solve their pH and DO measurement issues, the decision was taken to convert over 80 installation points at the facility to METTLER TOLEDO ISM technology. Now, as expected, problems with noisy signals, questionable calibrations, and failing cables have been eliminated. The high performance and reliability of the equipment has since prompted other facilities operated by the company to also transfer to ISM solutions.

Find out more about ISM:
▶ **www.mt.com/pro-pharma-whitepaper**

30 % Drop in Cost of Consumables
by Going Optical

Amperometric dissolved oxygen (DO) sensors are abundant in pharmaceutical processes, but their requirement for regular maintenance results in high running costs. Optical DO sensors are an advanced, low maintenance alternative.



High maintenance expense

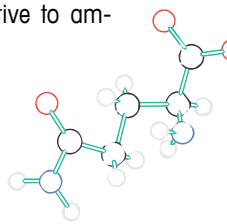
Maintaining amperometric DO sensors can be time-demanding and regular maintenance is prone to errors. Such probes require regular refilling of electrolyte and frequent membrane replacement. A Chinese producer of amino acids calculated that each of their amperometric DO sensors cost around 8,700 Yuan (USD 1,300) per year in consumables, in addition to the time for technicians to carry out the maintenance.

Sensor drift affects measurement confidence

A further issue with aged amperometric probes that company production staff noticed, is low signal stability over long fermentation runs, which puts into question the accuracy of sensor readings.

Threonine production requires vigilant DO control

For one of the company's products, threonine, control of oxygenation during fermentation is particularly critical as cell growth and metabolism is very sensitive to less than optimum DO levels during fermentation at any stage of the process. Although high DO levels do not negatively affect fermentation, they represent unnecessary running of air compressors. Production managers at the facility wanted a low maintenance, low drift alternative to amperometric sensors.



Optical technology provides the answer

At a meeting with the company, METTLER TOLEDO demonstrated our optical DO sensor, InPro® 6860i. Optical measurement technology does not involve electrolyte or membranes, and unlike amperometric sensors optical probes do not require polarization. The only maintenance needed on the InPro 6860i is replacement of the sensor's oxygen-sensing element (OptoCap™) approximately once per year.

Anti-Bubble OptoCap

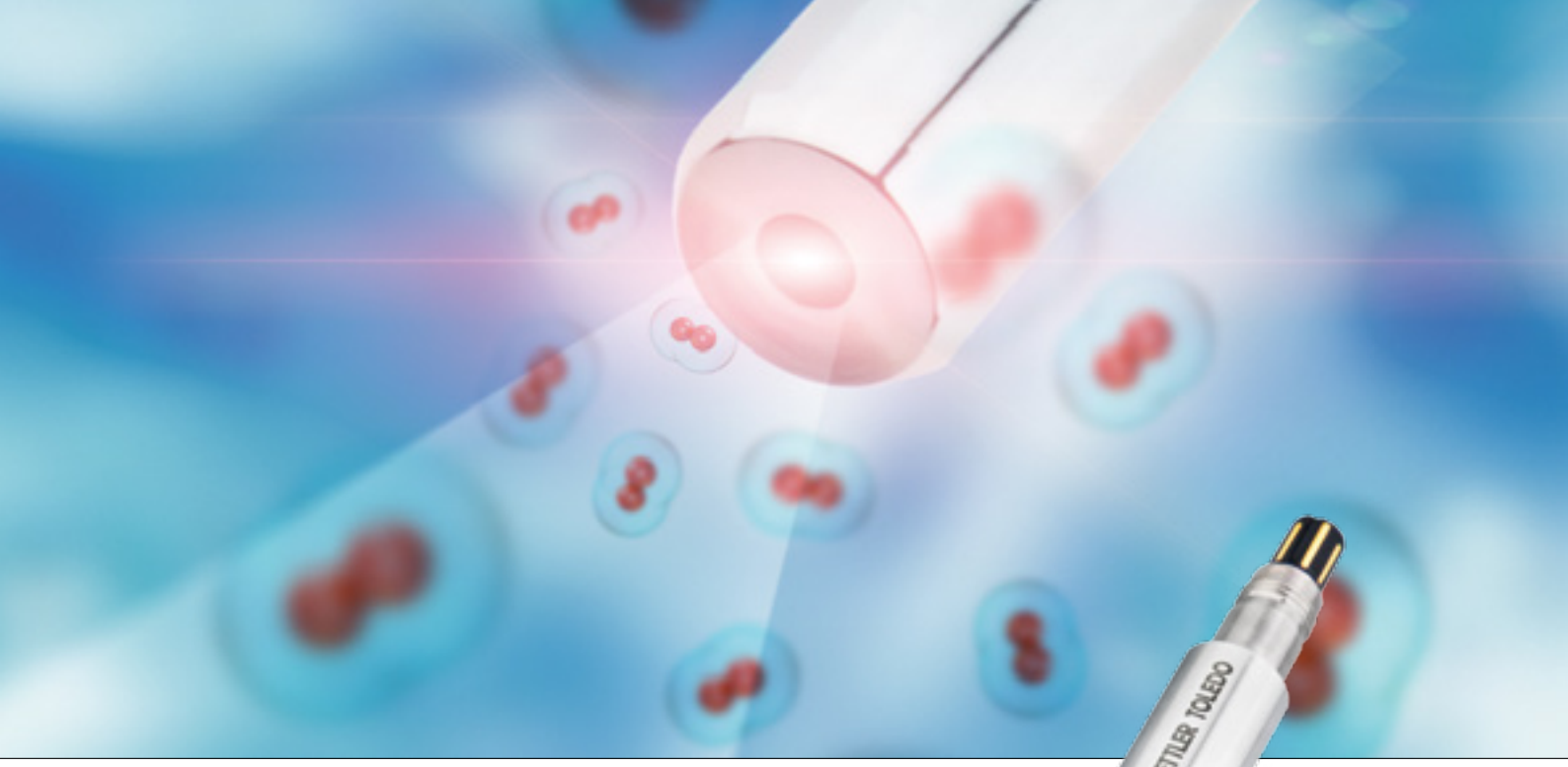
Optical DO sensors that are installed vertically in bioreactors can output a noisy signal due to bubble accumulation at the sensor tip.

The new anti-bubble OptoCap from METTLER TOLEDO prevents bubble formation:

- Angled tip and hydrophilic surface
- Stable and reliable DO measurements
- Repeatable and consistent results

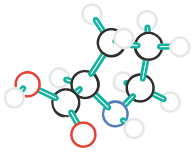
Watch the video:

▶ **www.mt.com/InPro6860i**



In respect to drift, the unique Automatic Stability Control feature ensures measurement confidence is always high. Electronic documentation of sensor history and advanced predictive diagnostics from Intelligent Sensor Management (ISM®) technology brings other benefits that the company representatives appreciated.

Convinced that the InPro 6860i would meet their requirements, 14 sensors along with M800 multi-channel transmitters were installed on seed tanks and production bioreactors.



Significant saving

After a year of operation the company calculated that costs for consumables for the InPro 6860i was 30 % less than for the previous amperometric sensors. Taken with the consequential drop in technician time spent on maintenance, and improved control of the aeration process, the company has realized a substantial saving.

Find out more at:
▶ **www.mt.com/InPro6860i**

InPro 6860i Optical DO Sensor

Easy Handling

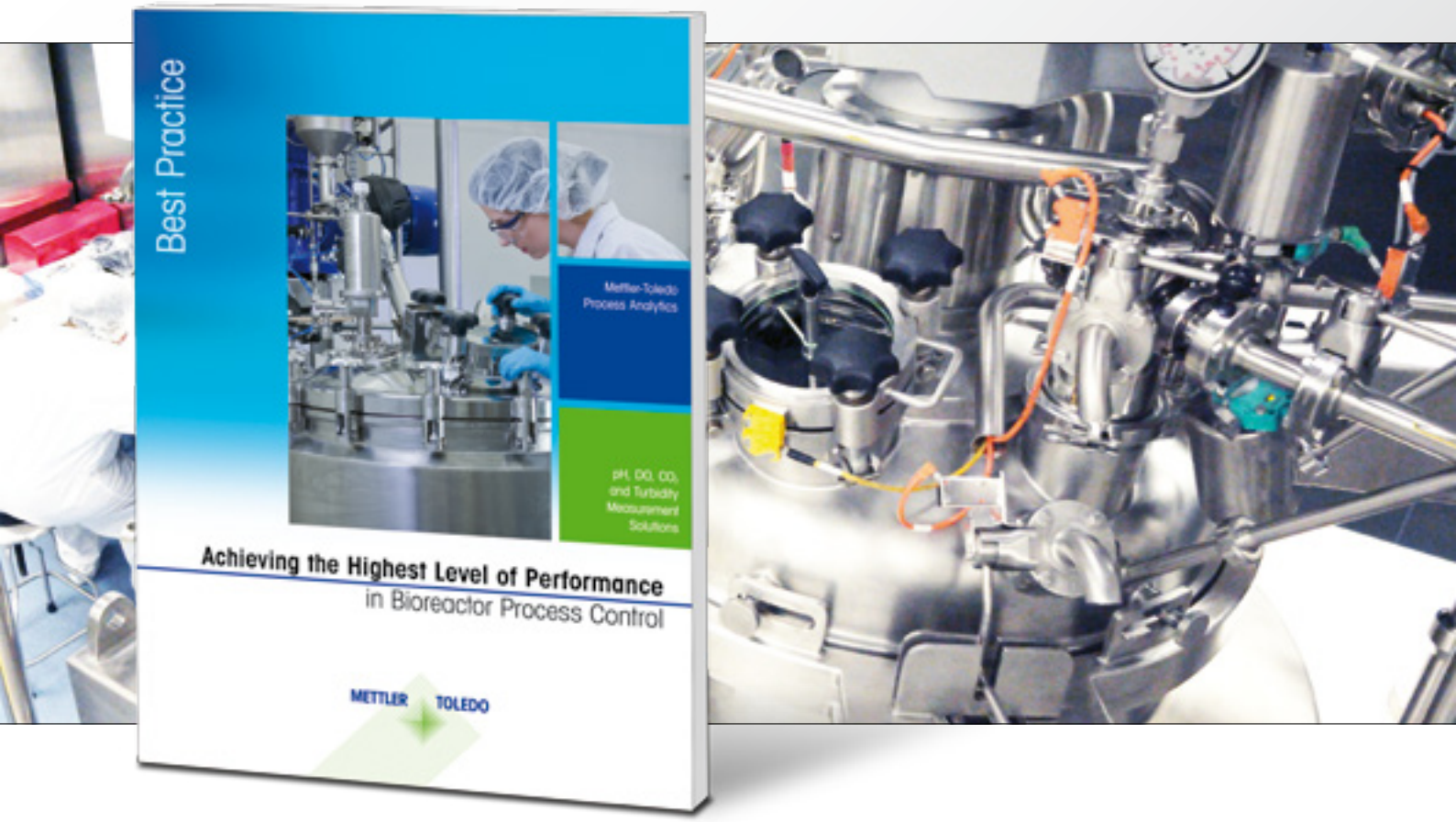
- Plug and Measure for fast startup
- Pre-calibration in the lab with METTLER TOLEDO iSense software
- Compatible with digital and analog installations

Exceptional performance

- Improved optical technology ensures high accuracy
- Automatic Stability Control for superior measurement stability

Uncompromised reliability

- Predictive maintenance
- Real-time status information through ISM diagnostics
- Pre-batch diagnostics with iSense™ software



Bioreactor Productivity Best Practice Guide

A Guide to Achieving the Highest Level of Performance in Bioreactor Process Control

How do analytical parameters impact fermentation and cell culture? What are the different analytical measurement requirements during bioreactor control? Our complimentary guide outlines several case studies with solutions to critical process challenges.

This guide summarizes how to achieve:

- Reliable, repeatable fermentation processes and better process understanding
- Batch-to-batch consistency and increased yield
- Easy traceability and error-free documentation
- Simpler compliance with regulations

Download your free copy of the METTLER TOLEDO Bioreactor Productivity Best Practice Guide:

▶ **www.mt.com/pro-biopharma-guide**

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