INGOLD
Leading Process Analytics

5 Ways In-Line CO_2 Sensors Improve Bioprocessing

1 Managing CO_2 Accumulation
for Increased Productivity

2 Faster Process Development
from Benchtop to Production

3 Evaluating Respiratory Efficiency
for a Reproducible Process

4 Real-Time Responsiveness
Allows Better Process Control

5 Optimize Cell Growth
with Minimal Effort

METTLER TOLEDO

In-line Control of CO_2 Accumulation
for Improved Cell Culture Processes

High dissolved CO_2 concentration in bioreactors has a significant negative effect on cell culture: unwanted metabolic changes, growth inhibition and subsequent lower productivity. Continuous measurements directly in the bioreactor with an in-line sensor can lead to significant gains in both productivity and final-product quality.

Growth in culture yield

Over the past ten years, productivity in cell culture processes have increased up to 2 to 3 g/l at all scales from benchtop to production. However, these productivity gains have come with accompanying side issues, such as greater accumulation in bioreactors of unwanted byproducts such as lactate and dissolved CO_2.

Problems of high/low CO_2 levels

High dissolved CO_2 concentration in bioreactors alters the glycosylation pattern of recombinant proteins, especially with an expression model using mammalian cell lines. At very high levels (200–250 mmHg), CO_2 becomes cytotoxic for mammalian cells, and if not rapidly corrected can result in batch productivity loss.

Although less likely, due to natural cell production of CO_2 in the Krebs cycle, low CO_2 levels (below 5 %) cause mechanical stress on cells and also negatively impacts process productivity. Therefore, CO_2 should be kept within the required range throughout culturing.

The optimal operating range for CO_2 concentration for, as an example CHO cell culture, has commonly been reported as 5–30 %. The reason for the wide span is that the ideal range depends on the specific process, media and recombinant cell line being used.

Problems with blood gas analyzers and other off-line and at-line methods

Usually, off-line measurements with instruments such as blood gas analyzers (BGAs) are constrained by the low sampling frequency (usually one or two samples per day) and cannot be used for at-line or in-line monitoring. Further, sampling from the media carries potential issues that can alter sample characteristics. Temperature changes and time delay between samples being taken and measured can all affect CO_2 level. These issues are not just confined to BGAs: any off-line or at-line method can be subject to influences that alter the CO_2 concentration from its true level.

Intelligent Sensor Management – ISM®

ISM digital sensors ensure the accuracy and integrity of critical analytical measurements.

Predictive Diagnostics
Prevent sensor failure during a batch process, and support condition-based maintenance.

Plug and Measure Technology
Quicker start-up by pre-calibrating sensors before installation.

Electronic Documentation
Clear sensor usage records to simplify compliance.

Advantages of in-line measurement

Use of in-line CO_2 sensors provides accurate, real-time measurement without any of the problems of off-line or at-line analysis. Data from in-line sensors can be used to define CO_2 addition or removal regardless of bioreactor size, sparger design, type of gas used for stripping, and the cell line. It has been reported that tight CO_2 control can increase up to 30 % the productivity rate with mammalian and insect cells.

METTLER TOLEDO solution

The Severinghaus principle is the common CO_2 measurement method in off-line analyzers such as BGAs. Applying the same approach in an in-line sensor proved difficult, but METTLER TOLEDO's competencies in glass pH electrode design and construction solved the issues. The result is the only in-line CO_2 sensor for bioprocessing from the bench to manufacturing scale, available on the market.

InPro® 5000 i is hygienically designed and suitable to withstand sterilization cycles and cleaning-in-place operations. As pH is temperature dependent, a built-in temperature sensor is used to provide continuous, accurate temperature compensation.

The sensor's measurement precision (± 1 to 2 % of the reading), reliability and response time are not affected by pH variations or concentration of metabolites such as glucose, glutamine, glutamate, lactate, and ammonium.

An InPro 5000 i and METTLER TOLEDO transmitter form a system that not only measures CO_2, but allows its control via the transmitter's PID function.

Advanced sensor diagnostics

The InPro 5000 i features METTLER TOLEDO Intelligent Sensor Management (ISM®) digital technology. ISM offers a number of features that are not possible on analog probes. These include a robust digital signal between sensor and transmitter that remains stable over long cable runs and is unaffected by interference from surrounding equipment. Advanced predictive diagnostics calculate when sensor maintenance will need to be performed: preventing a failing sensor from being installed in a measurement point. ISM sensors retain their own calibration data, which allows them to be calibrated away from the process in a more convenient location.

Faster Process Development

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.

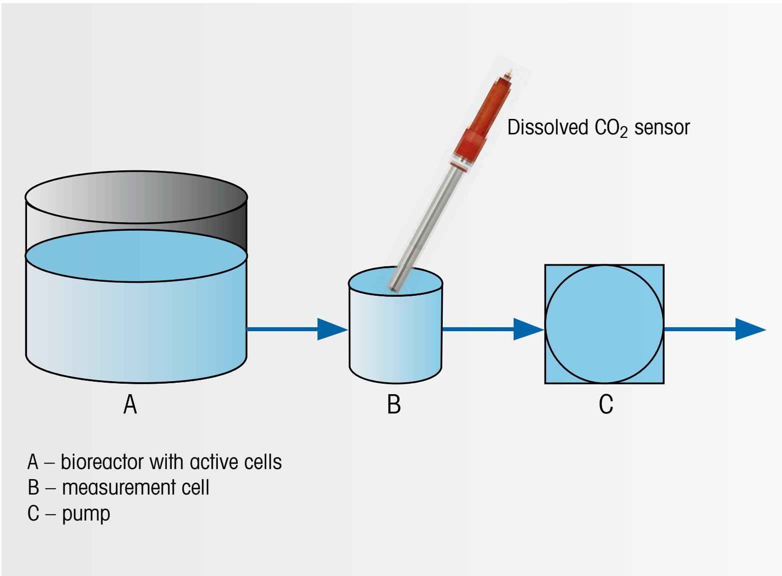
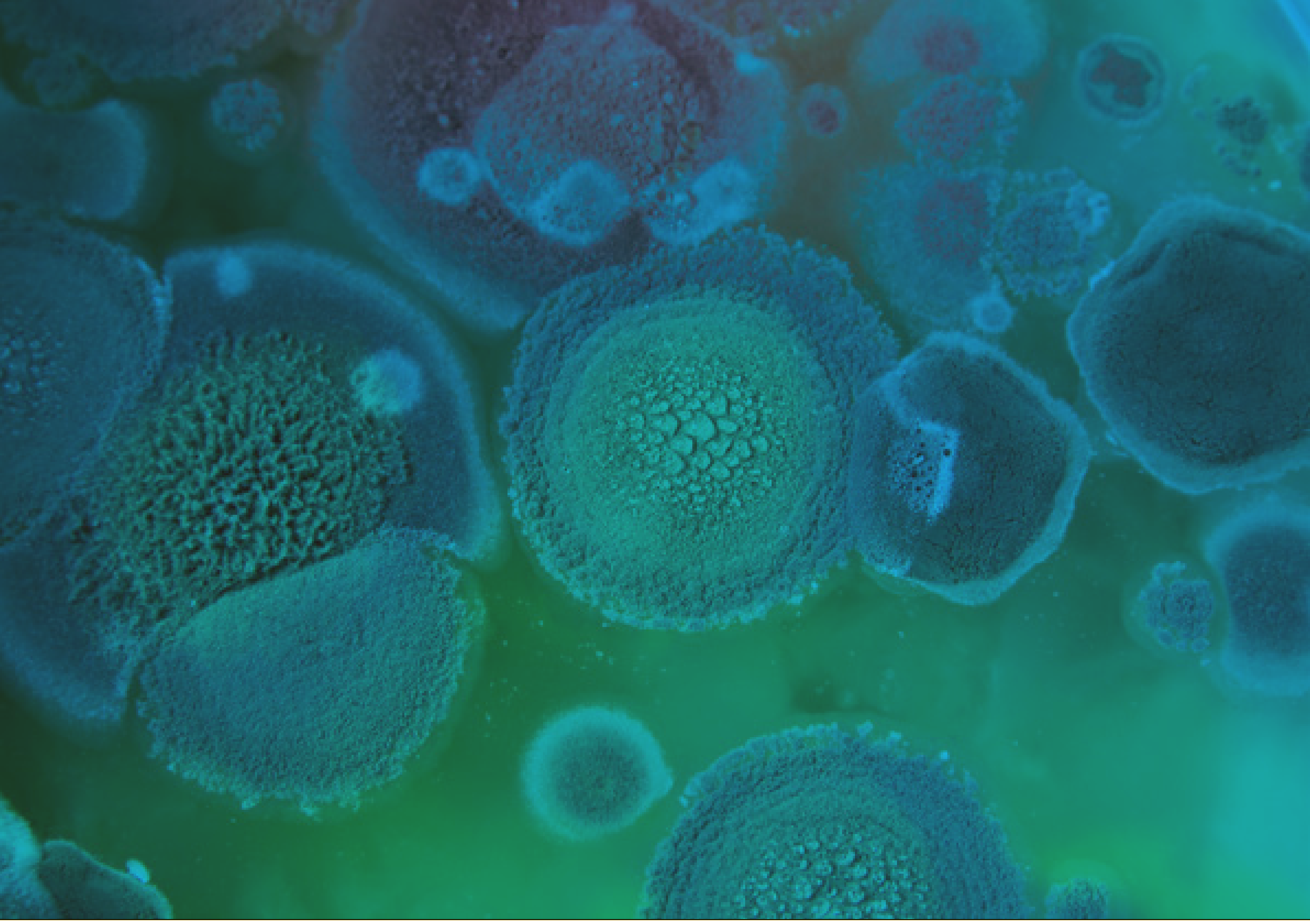


Fig. 2: Experimental setup for dissolved CO_2 measurement outside the bioreactor.



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

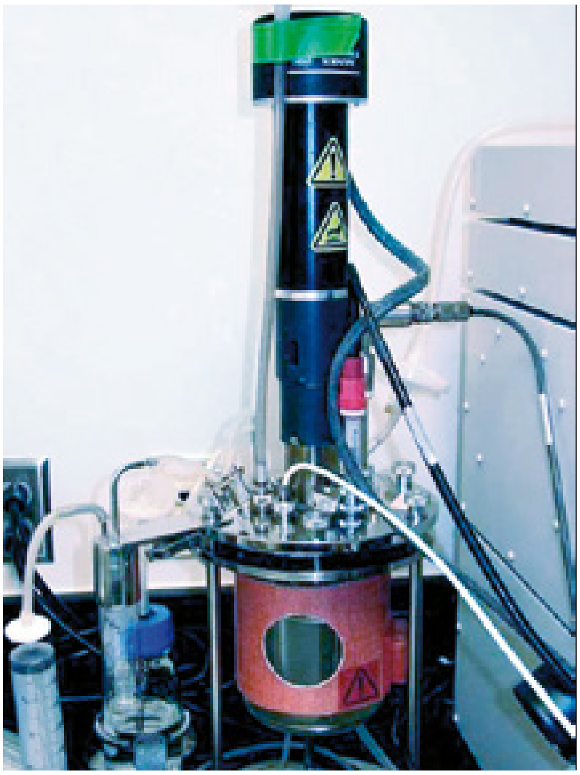
In respect of results, Frederik Aboka stated: "We are satisfied with the steady state measurement of dissolved CO_2 using the METTLER TOLEDO InPro 5000 sensor."

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

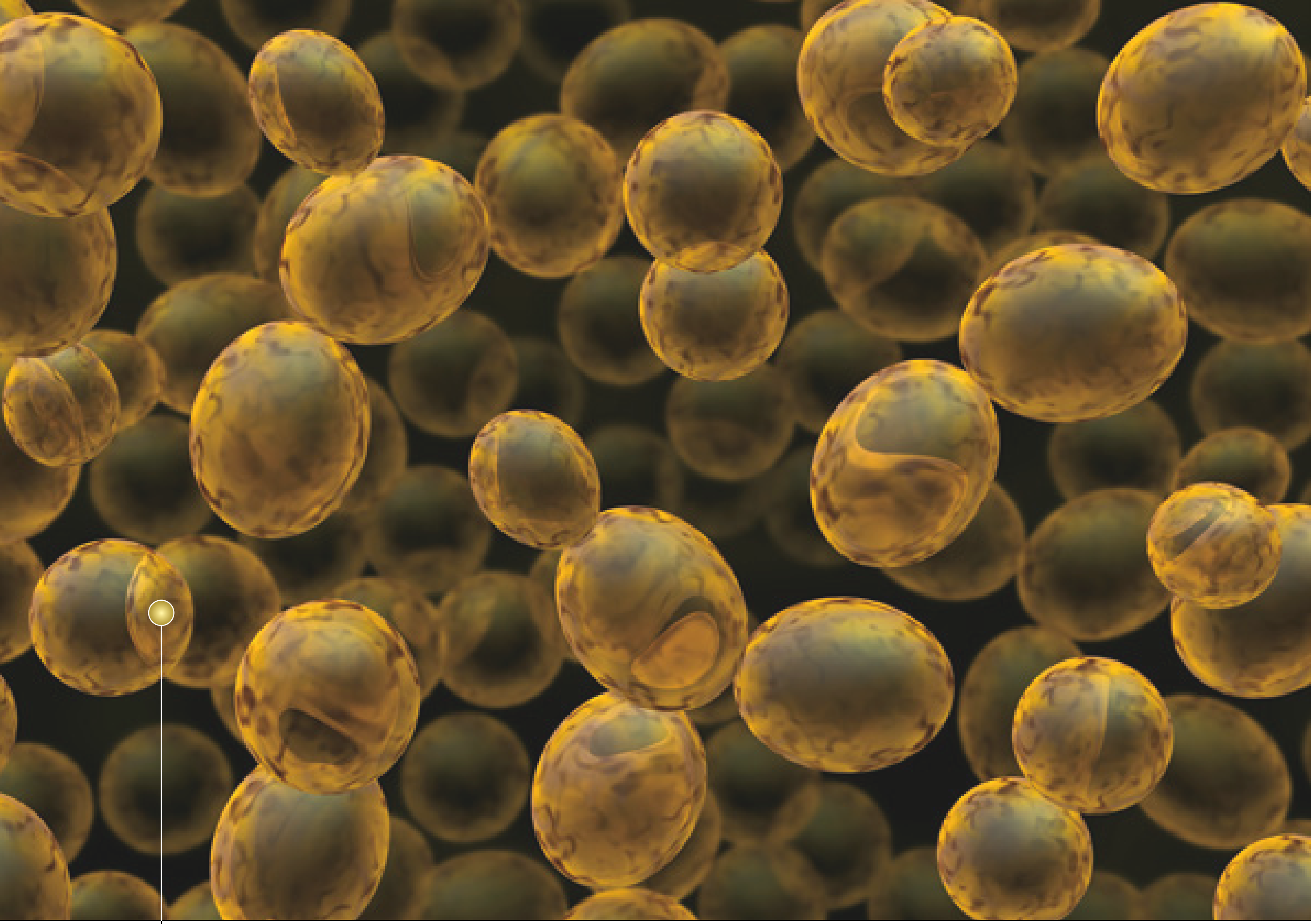
In a project developed by the Virginia Bioinformatics Institute, the response of Saccharomyces cerevisiae cells to oxidative stress induced by hydroperoxides, was studied. Yeast cells were grown in a fermenter with controlled conditions of pH and dissolved oxygen to ensure that the response obtained 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.

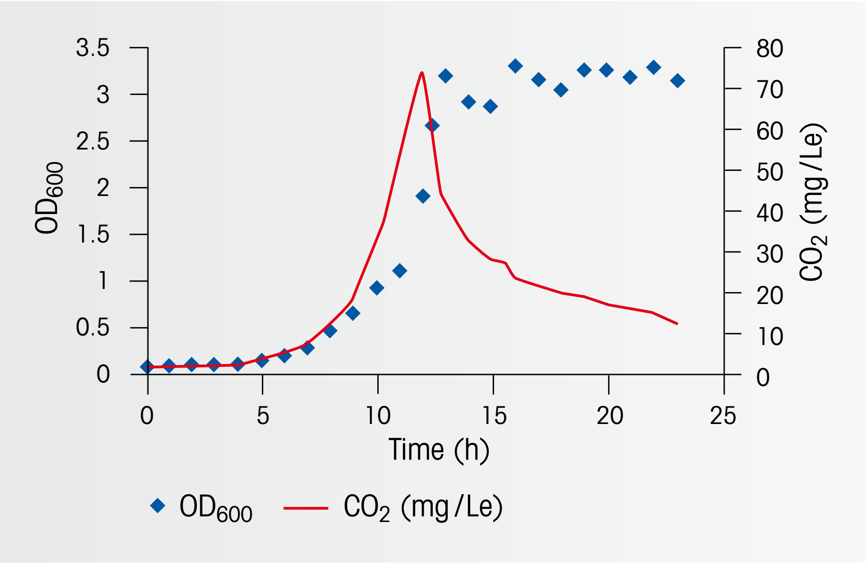
The first experiment was designed to test the reliability of the InPro® 5000 CO_2 sensor, comparing its measured values with those obtained via a GC-MS. A yeast culture was grown in a fermenter for 24 hours. Culture samples were collected from the fermenter for the determination of OD at 600 nm and headspace 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".





Superimposition 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

Digital CO_2 Sensors on Analog Biocontrollers
Convenient, Reliable, In Situ Measurement

More and more biopharmaceutical companies want to benefit from digital analytical sensors, while retaining their analog biocontrollers. A smart solution from METTLER TOLEDO has greatly impressed a major US pharmaceutical company for its high accuracy and low maintenance.

Reducing the need for blood gas analyzers

The use of blood gas analyzers to measure dissolved CO_2 during mammalian cell culture processes is common practice. However, it is time-consuming and for large R&D/Process Development facilities with many biocontrollers, can become a bottleneck.

A convenient, real-time measurement alternative is provided by the METTLER TOLEDO InPro® 5000, an in situ sensor for accurate measurement and control of dissolved CO_2 in biopharmaceutical applications. A major biopharma facility in California had been using the InPro 5000 successfully for many years, but the company was interested in switching to the digital Intelligent Sensor Management (ISM®) version of the sensor, the InPro 5000i; however, they wanted to continue using their installed analog biocontrollers.

Simple upgrade from analog to digital sensors

METTLER TOLEDO's local agent in the region, Optimal Biotech Group (OBG), arranged a meeting with the biopharma company to demonstrate how the upgrade would be possible and how it would provide better measurement accuracy, reliability, stability and ease of sensor operation.

At the meeting, OBG showed that the key to integrating the digital InPro 5000i into analog biocontrollers lies with the M100 SM. This is a small, single-channel transmitter that connects directly to the top of the InPro 5000i and can also be used with ISM pH and dissolved oxygen probes. The M100 SM converts the digital signal from the attached sensor to analog, while still allowing user access to the probe's valuable ISM features:

Predictive diagnostics
Determine whether a sensor can be safely used for the next fermentation/cell culture run

Plug and Measure
For fast, trouble-free sensor setup

Sensor deactivation
Prevents a failed sensor being used on a biocontroller

Electronic signatures
Compliance with 21 CFR Part 11 and GMP Annex 11 using iSense™ software

Compact sensor mount transmitter

The M100 Sensor Mount is a single-channel transmitter. It allows the connection on analog or digital biocontrollers of ISM sensors for measuring pH, DO (amperometric and optical) and CO_2. The M100 SM has a Bluetooth 4.0 interface which is compatible with the mobile version of iSense software for ISM. This means that sensor diagnostics can be accessed at any time from a smartphone.

Two independent interfaces are implemented: two configurable 4/20 mA analog outputs and one digital MODBUS RTU. LEDs clearly indicate sensor status, alarms and warnings.

► www.mt.com/M100SM

Streamlined processes

Lab managers from the facility could clearly see how the InPro 5000i/ M100 SM solution would streamline their processes while also reducing their reliance on blood gas analyzers. Four systems were installed on the facility's Finesse biocontrollers running DeltaV control. Tests showed high measurement accuracy from the InPro 5000i, even after repeated sterilization.

The managers also appreciated the sensor's low maintenance requirement, which can be conducted in just a few minutes.

Some months later, the facility lab managers report high satisfaction with the METTLER TOLEDO equipment and a strong desire to convert all remaining biocontrollers to the same solution.

ISM®

In situ CO_2 sensor

The InPro 5000i's measuring technology is based on the Severinghaus principle of potentiometric CO_2 measurement, which has been widely accepted for almost 50 years. A high surface finish on the stainless steel shaft prevents contamination, and the sensor is fully sterilizable either in situ or in an autoclave. The design of the membrane reduces service time to a few minutes. InPro 5000i's interior body, a high performance pH electrode, can be replaced on site easily and quickly.

► **www.mt.com/InPro5000**

Low Maintenance

Optimal Growth, Higher Yield

In-situ CO_2 Measurement for Fermentation Control

Carbon dioxide is a critical parameter influencing growth conditions in a number of bioprocesses. The new InPro 5000 i CO_2 sensor combines precise and reliable in-line measurement of dissolved CO_2, with the performance benefits of Intelligent Sensor Management.

Same sensor family from lab to production

In striving to achieve the goals of PAT and QbD, in-line monitoring and subsequent control of CO_2, pH and temperature during fermentation processes can be of great value. Furthermore, analytical measurements are of enormous benefit in yield optimization and product quality assurance.

Real-time, in-situ monitoring of the dissolved CO_2 levels in the fermentation medium allows timely actions for maintaining ideal process conditions. CO_2 measurement with the same family of sensors permits faster scale-up during process development, and quicker time to market for new products.

CO_2 sensor specifically for biopharma

The new InPro 5000 i is a fully sterilizable-in-place and autoclavable in-line CO_2 sensor. It features a class N5 surface and EHEDG certification for compliance with hygienic requirements. The sensor's membrane provides an excellent barrier against volatile organic acids, ensuring errorless measurement.

Easy installation and maintenance

For measurement accuracy where it matters most, the InPro 5000 i CO_2 sensor is designed for direct mounting into fermenters. Its modular design makes maintenance and spare part replacement fast and convenient. The ideal housing for the sensor is the retractable InTrac® 797 as its integrated flushing chamber allows easy sensor calibration and cleaning, even during a running fermentation.

Intelligent sensors offer a host of benefits

ISM®

The InPro 5000 i is the latest member of METTLER TOLEDO's Intelligent Sensor Management (ISM®) family of sensors and transmitters. Benefits of ISM include:

Predictive diagnostics

Determine whether a sensor can be safely used for the next fermentation run.

Sensor de-activation

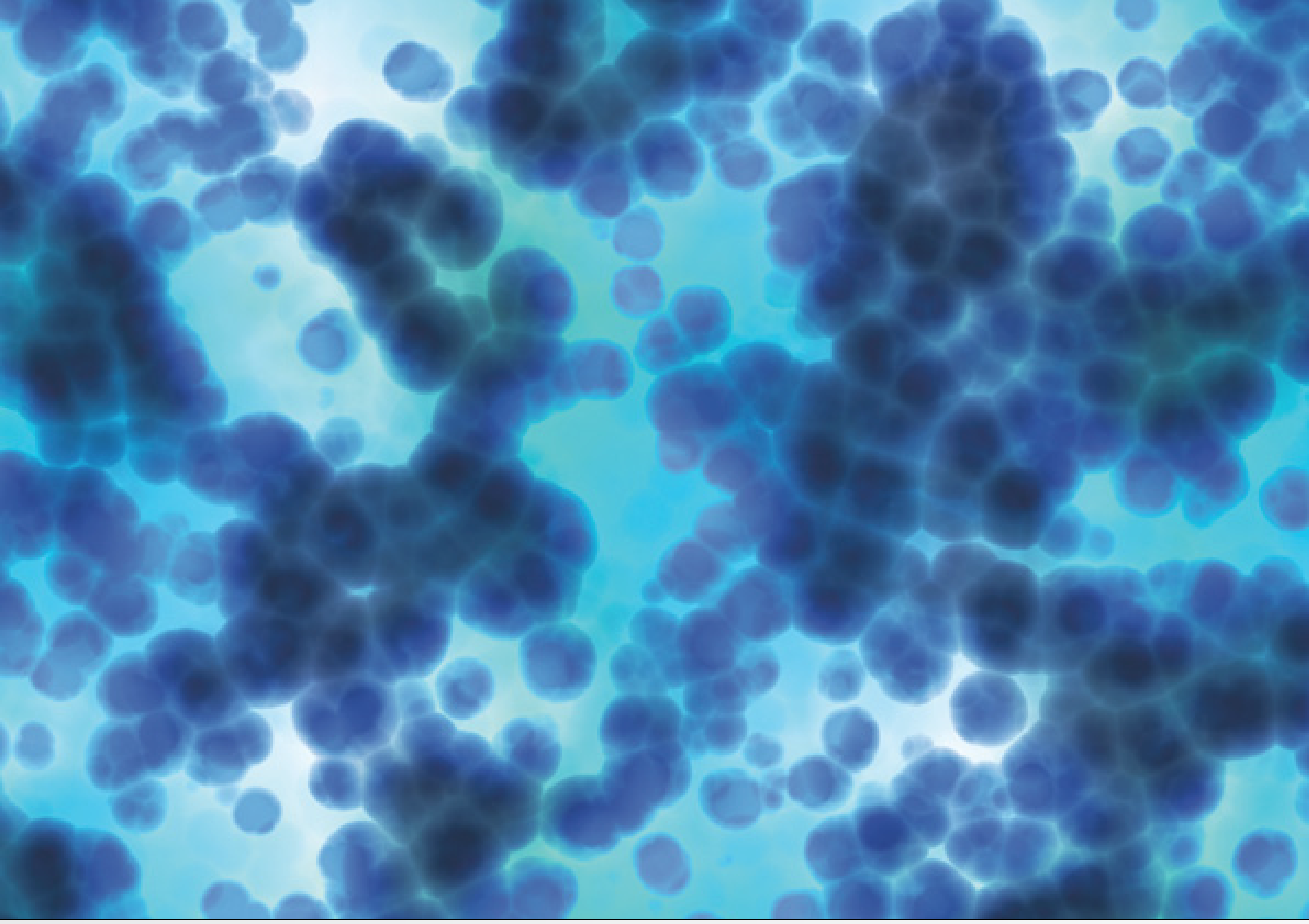
Prevents a failed sensor being used at the measurement point.

eDocumentation

Provides full traceability of sensor calibration and maintenance.

Digital sensor to transmitter signal

Measurement data is unaffected by cable length, interference or moisture in the environment.



How In-line Measurement of Dissolved CO_2 Became Possible

Technology Limitations Solved

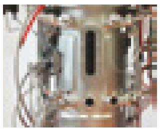
Severinghaus measurement reduced to compact size and made more robust to withstand sterilization

Off-line Blood Gas Analyzer

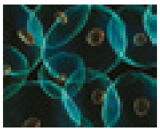
In-line CO_2 Sensor

Why Measure CO_2 in Bioprocessing?

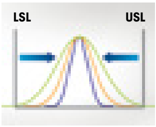
Improved process understanding



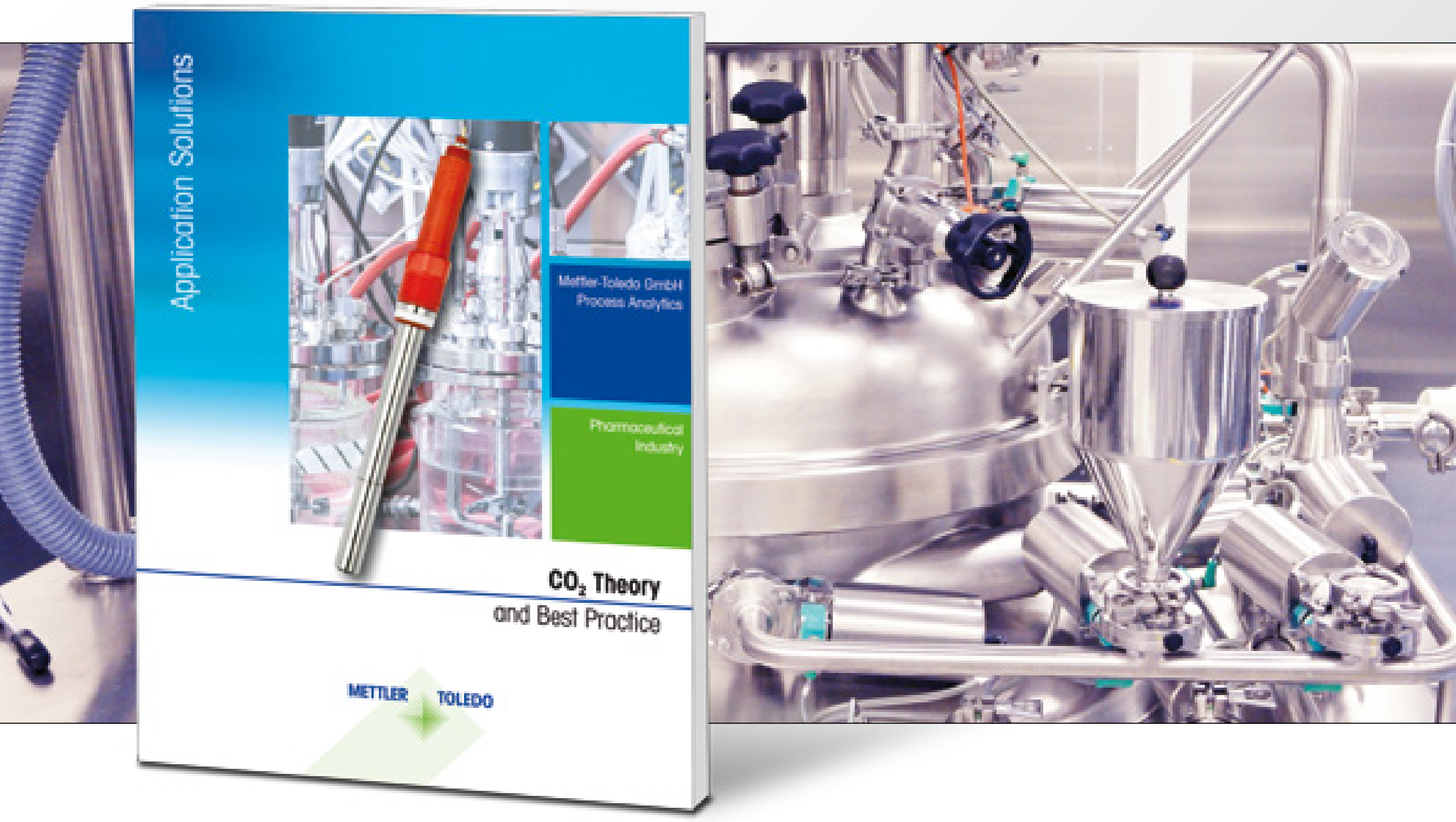
Increased cellular productivity



Reduced variability



► **www.mt.com/InPro5000**



Dissolved CO_2 Measurement Theory Guide

Learn the background and theory of dissolved CO_2 measurement

In this 60-page booklet you will learn about the physical background of dissolved CO_2 measurement, including the basic units of measurement for CO_2, influence of temperature, and the influence of vapor pressure.

The guide also explores dissolved CO_2 measurement technology. After providing an overview of the Severinghaus measurement principle of dissolved CO_2 measurement, it provides details on the technology of the InPro 5000i dissolved CO_2 sensor, which uses that principle to provide con tinuous dissolved CO_2 measurement.

Download the CO_2 Theory Guide today to learn more about how accurate dissolved CO_2 measurement can help in the understanding, control, and productivity optimization of biological processes.

▶ **www.mt.com/pro-pharma-co2**

Request a quote on InPro 5000i

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