



INGOLD

Leading Process Analytics

InPro 5000 Enables Real-Time CO₂ Measurement in Cell-Culture Reactor

In the cultivation of mammalian cells, oxygen consumption is proportional to the rate of CO₂ production. By using an inline CO₂ measurement system from METTLER TOLEDO, the risk of overdosing of culture medium and an overproduction of substances toxic to the cells can be reduced to a minimum.

Aachen University, Germany

The FH Aachen with over 8000 students and more than 220 professors as well as about 200 lecturers and a further 450 staff members at its two locations, Aachen and Jülich, is among the largest universities of applied sciences in Germany. While the FH Aachen was founded in 1971, some predecessor institutions can look back on a partly centennial tradition.

CO₂-measurement in cell culture

In the biotechnology and pharmaceutical industry, the measurement of CO₂ has developed into a decisive parameter in mammalian cell culture processes. The physiological activity of the cells plays a key role in such processes. Measurement of the metabolic characteristics of mammalian cells enables the quantitative determina-

tion of substrate conversion and estimation of biomass concentration. The rate of oxygen consumption during aerobic cell growth shows a development directly proportional to the CO₂ production. The knowledge level on these two analysis parameters provides a suitable platform for the success-oriented control of the process.

FH Aachen research into cellular respiration

Characteristic of the FHAachen is the emphasis on practice-oriented education and research. This is reflected in the close cooperation with regional and international companies as well as prominent research establishments. Postgraduate Michelangelo Canzoneri, Department of Biotechnology, Cell Cultivation Technics, under Prof. Manfred Biselli, employed a CO₂



METTLER TOLEDO



measurement system from METTLER TOLEDO in his research assignment to investigate the respiration activity of mammalian cells. The final goal is to be able to produce recombinant pharmaceutical proteins as well as various antibodies under specially optimized conditions.

O₂ and CO₂ balance in fed-batch cultures

Knowledge about the respiration activity of mammalian cells is a crucial factor in the analysis of metabolism processes and the

optimization of process parameters relevant to metabolism. Fed-batch cultures provide the basis for this particular investigation, in which a constant feed of nutrient medium is effected without removing the spent medium.

In this way, the cells are continuously provided with a certain basic level of nutrients in order to ensure growth free of stress.



Fed-batch culture.

The growth phases of the cells are determined by nutrient (substrate) availability. As a rule the medium is made up of sugar, amino acids and fatty acids, as well as mineral nutrients and trace elements. Depending upon which of these constituents are being used by the cells will influence the balance between O₂ und CO₂.

The change in O₂ and CO₂ concentration in the fermenter provides two important facts for the operator:

1. Information on the status of metabolism of the cells, and
2. Information about which ingredient is most used by the cells.

The ratio and the concentration of the various constituents of the nutrient medium are decisive for O₂ consumption and the corresponding formation of CO₂.

Benefits of real-time measurement of O₂ and CO₂

The use of O₂ und CO₂ measurement provides important advantages for cell culture operators:

1. Development of an efficient feeding strategy
2. Optimization of the quality of protein and pharmaceutical products.
3. Real time measurement due to online measurement directly in the nutrient medium. Errors associated with pulling of grab samples for the laboratory (outgassing of sample) are thereby effectively avoided.

CO₂ measurement system from METTLER TOLEDO

For his investigations, M. Canzoneri used a CO₂ measurement system from METTLER TOLEDO consisting of a CO₂ sensor InPro 5000 and CO₂ Transmitter 5100e. The sensor is equipped with a semi-permeable, high-selectivity membrane and is both sterilizable and autoclavable in keeping with its hygienic design (EHEDG).

During measurement of the CO₂, the dissolved CO₂ diffuses through the sensor membrane to react with the electrolyte and form HCO₃⁻ and H⁺. From the pH value with simultaneous temperature measurement, the CO₂ concentration is then calculated by the transmitter and converted into CO₂ units. The change in pH value of the electrolyte correlates with the partial pressure of the CO₂:



Rapid response time of the InPro 5000

Handling of the measuring system is unproblematic and it can be installed in practically all types of existing reactors without any special effort. A major advantage of the CO₂-Sensors InPro 5000 is its fast response time, necessary in order to establish a viable feeding strategy for the cell culture. This makes it possible to prevent overdosing nutrient medium and to minimize the risk of overproduction of toxic by-products in the cell culture.

InPro 5000 provides following Customer benefits

- Process safety
- Reliability
- Rugged design
- Easy maintenance and service
- Proven repeatability

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 www.mt.com/CO2

Inline DO Measurement Accelerates Scaling-up During Production of Chiral Amines

Information about the kinetic parameters of the enzyme and data on inhibition and biocatalyst stability were obtained by monitoring and controlling the DO concentration during biotransformation. This real-time control of quality and quantity may be characterized as a PAT tool.

InPro 6800.



Ingenza, UK

Ingenza is a dynamic biotechnology and bioprocess company providing cost efficient and practical industrial scale methods for the manufacture of chiral chemicals. This includes the technology of chemo-enzymatic deracemisation of racemic mixtures.

Ingenza's deracemisation process

The oxidation half reaction of Ingenza's deracemisation process using dissolved oxygen monitoring proceeds as follows: Oxygen is sparged through the solution of DL-alanine to ensure saturation (~35-40mg/L oxygen concentration). On addition of the enzyme, the DO concentration drops very rapidly over about a minute to approx. 5mg/L. Sparging of oxygen gas is continued over the course of the reaction, and over approx. one hour the DO concentration slowly rises back to saturation level. The rate of reaction is limited by the rate at which oxygen is transferred into solution. The transfer rate is proportional to the difference between the actual oxygen concentration and the saturation limit (i.e. Δ in the graph).

Monitoring of oxygen concentration

Since oxidation of the D-enantiomer of the racemic mixture by D-amino acid oxidase consumes one mole of oxygen per mole of substrate turned over, it was reasoned that the reaction rate could be monitored continuously by measurement of the oxygen concentration.

METTLER TOLEDO solution

After a long search to find an instrument capable of data-logging and accurate, fast measurement of dissolved oxygen, Ingenza finally selected the following measuring system from METTLER TOLEDO:

- Oxygen sensor InPro 6800
- Multiparameter transmitter M700 equipped with a O₂ module

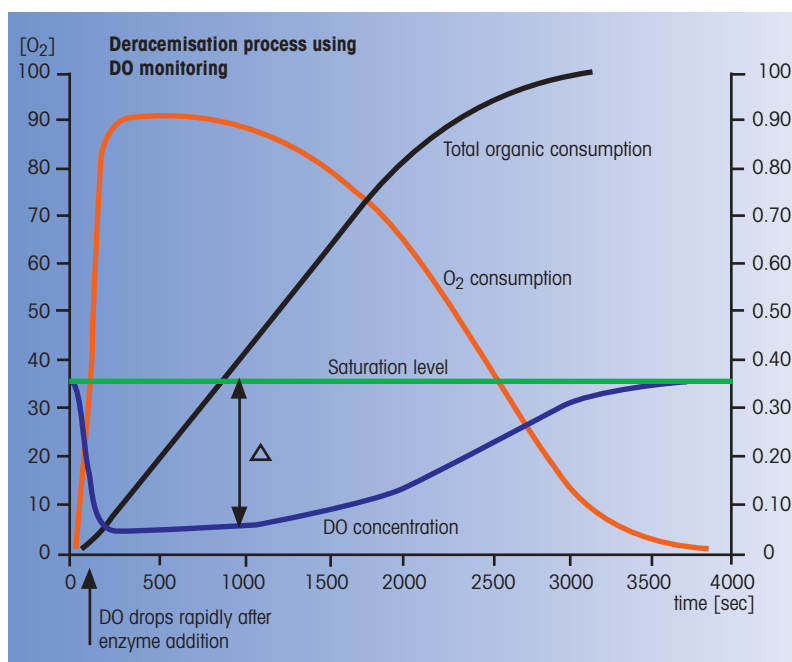
From pilot to full scale reaction

The technique is directly applicable to manufacturing scale process analytical technology. The chemistry will be scaled up to pilot level towards the end of 2006, and the same type of measurement instruments as used in the basic assessment program will be used to follow the reactions later run at full scale.

Conclusions and implications

By simple mathematical manipulation of the DO concentration during the course of the biotransformation the conversion of D-amino acid against time may be determined. This information may then be used to determine kinetic parameters of the enzyme as well as giving data on inhibition and biocatalyst stability.

► www.mt.com/O2



What are the Trends in Process Analytics for Pharmaceutical Applications?

Kurt Hiltbrunner, an analytical chemist, has been in the employ of METTLER TOLEDO for many years as a market specialist. In this function he has gained a comprehensive overview of the market and gathered invaluable experience. In the following interview he highlights some important trends in the pharma industry.



Kurt Hiltbrunner.

In which process stages do process analytics currently play an important role?

I paid visits to several different companies manufacturing active substances, intermediates or end products such as tablets, ointments or injection solutions. Primarily, our process analytical sensors and systems are of interest to producers of active substances. Automation and inline process analytics have however not everywhere reached that standardization level possible today. This means that QA does not check the process itself but only the actual product during final inspection. At end product facilities, process analytical measurement systems are used in water preparation, such as in the production of ultrapure water or water for injection.

You mention different types of manufacturers. Has this any consequence for the pharmaceuticals industry in the future?

Well, "Contract Manufacturing" of active substances and end products at special plants is very much on the rise. Even large pharmaceutical companies are outsourcing some of their production. The greatest

increase in pharmaceuticals manufacture is seen in India and China. Although currently it mainly concerns chemical processes, the importance of biotechnology, even at these same plants, is strongly on the increase. The first biotech generics will soon be on the market and it won't take long until contract manufacturers get into this technology too. Only then will inline analytics start to come into wider use.

What are the most important demands on products in the area of process analytics?

Batch processes are common both in chemical synthesis and in fermentation operations. Here, great emphasis is placed on reproducibility, and consequently on process analytics for process reliability. Retained batches lead to an enormous rise in production costs.

Safety against contamination is also frequently mentioned. In fermentation processes, sensors and housings have to be guarded against contamination.

Since mammalian cell cultures grow very slowly and a batch loss due to a defective sensor after a few weeks cultivation can be fatal and extremely expensive, sensor quality plays a central role. A sensor must be able to last several batches and withstand repeated sterilization without loss of performance. Sensors equipped with the new ISM technology contribute firmly to the security of the process.

PAT (Process Analytical Technology) and QbD (Quality by Design) have in the past few years become the buzz words. In how far has the pharma industry already implemented these concepts?

Principally, PAT and QbD are important issues in large pharmaceutical companies. Even if PAT is not widely applied in the short term, manufacturers using PAT will be at an advantage, since validations will be quicker, and this in turn reflected in product quality. Therefore a change in the pattern of thinking is anticipated. Quality based purely on documents will be replaced by scientific and risk-based quality management.

The introduction of PAT should already start during the lengthy and expensive product development phase, because when a process has been validated, the manufacturer would wish to keep by the same configuration as long as possible. INGOLD sensors are ideal in such cases as they can be used equally well at all levels, from gram scale through to actual production level. The uniform sensor characteristic facilitates scale-up to larger batch sizes.

**INGOLD sensors
are considered as
ideal PAT tools**



At congresses and in magazines there are often reports on spectroscopic PAT tools. Can pH, oxygen, CO₂ and turbidity sensors also be regarded as PAT tools?

Electrochemical and optical sensors and systems from METTLER TOLEDO can clearly be regarded as “classic” PAT tools if they are not just used for measurement and recording purposes alone. If measured values are employed to influence a process one can then speak of PAT, under the condition that understanding of the process is thereby continuously improved. pH, oxygen, CO₂ and turbidity sensors are in this respect well-proven PAT tools.

What particular trends can be seen regarding process analytics?

In starter cultures and bioreactors, inline systems for pH and dissolved oxygen are standard. They serve to optimize the mi-

lieu for microbial growth as well as the aeration rate. “Remote calibration“ using ISM technology is particularly interesting for large bioreactors.

Process analytics is frequently used in cellcultures and fermentations.

Biomass concentration is now being increasingly monitored by inline turbidity measurement which is not possible with the conventional laboratory method of optical density measurement in grab samples. The inline method allows immediate corrective action.

In the fed-batch cultivation of mammalian cells, yield can be substantially improved by incorporating CO₂ measurement. Separation, liquid chromatography

and also crystallization are typical unit operations in downstream processing. Turbidity measurement is applied in separation processes, and pH measurement in liquid chromatography. In the preparation of nutrient solutions and chromatography buffers it is necessary to use ultra-pure water, the production quality of which is guaranteed through continuous monitoring by highly sensitive conductivity and TOC measuring systems. This ensures conformance with the most stringent of regulations.

Mr. Hiltbrunner, we thank you very much for this insight into so many different aspects.



Reliable Process Connections with INGOLD Sanitary Sockets™

The INGOLD Socket, long the industry standard port connection, has been re-engineered. This INGOLD Sanitary Socket now offers significant improvements in hygienic operations, operator safety, and cleanability.

INGOLD sockets – industry standard since the 1960s

Mettler-Toledo's Ingold sockets have been an industry standard instrument port since the 1960s. (Fig. 1) Since its inception, the INGOLD socket has been successfully installed in a variety of pharmaceutical industrial applications.

Functionality

Sensors are connected to the socket either directly or through the use of housings. The non-tapered straight thread with a wide pitch provides consistent and repeatable depth of insertion and also minimizes risks of cross-threading. The threads are non-wetted with sealing against the wall of the INGOLD socket. Sensor and housing connections are accomplished by simple hand tightening of cap nut.

Installation Options

The INGOLD socket is available in 316L stainless steel, but also in Hastelloy C-22, Titanium, PVDF, and PP. It can be installed in a variety of locations including vessels, reactors and piping systems. The available length varies from 40 – 60mm (1.57" – 2.36"). Because most liquid-based, electro-chemical process analytics require an installation with a 15° slope the standard optional INGOLD sockets are available with an integral 15° slope.

The INGOLD Sanitary Socket™

METTLER TOLEDO improved upon the industry-proven INGOLD Socket with its INGOLD Sanitary Socket™; initially known as the Safety Socket. It provides nu-

merous advantages to hygienic applications within the pharmaceutical and biotechnology industries. The advantages of the INGOLD Sanitary Socket™ stem from the two main design enhancements:

1. An added safety feature to prevent accidental release of housings under unexpected system pressure
2. A conical, internal bevel at the point of insertion

Safety Features

The INGOLD Safety Socket™ provides increased protection in the event of any premature attempt to remove the housing when the reactor or pipe is still under pressure or filled with medium. The socket is designed to prevent possible injury, damage or loss of medium (Fig.2).



Function

When the ring nut (2) is turned in order to release the housing from the socket (1), the housing retracts slightly due to the effect of the spring snap ring (3). This movement causes the O-ring (4) of the housing to dislocate from the 25 mm diameter area, and it no longer seals. The ring nut (2) is still lightly engaged on the thread G 11/4". The medium under pressure flows past the O-ring (4) and leaks out through the thread (5). This is a sign that the process has not been shut down. By retightening the ring nut (2), escape of medium and pressure can be stopped.

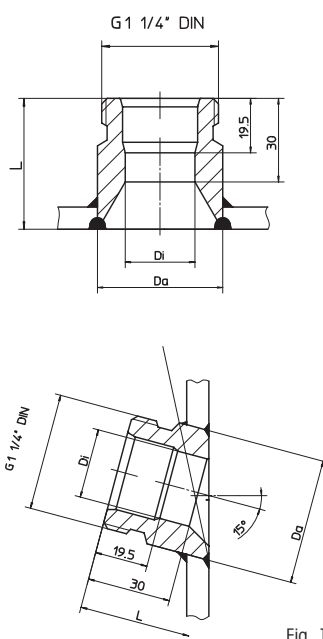


Fig. 1

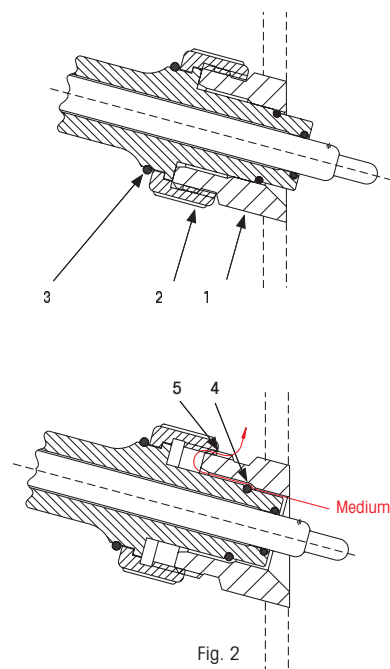


Fig. 2



Bioreactor.

Mandatory Requirement

The safety function is effective only if the appropriately designed housing (InFit 761/NC) or 25 mm dissolved oxygen sensor (InPro 6800) is used (Fig.3). All previous types of housings or 25 mm dissolved oxygen sensors can still be used, but without the benefit of the safety feature. Installation of CIP housings in standard INGOLD Sockets is also possible, but also without the safety feature.

It is important to note many imitation INGOLD sockets exists in today's market. These sockets do not all share the stringent quality demand, nor have the safety functionality of the INGOLD Sanitary Socket™. We recommend to ensure that the INGOLD Sanitary Socket™ is specified by name to guarantee safety functionality.

Hygienic Design

Standard INGOLD Sockets have been successfully employed in demanding biotechnology applications for decades. In thousands of validated systems worldwide one can find the INGOLD sockets successfully installed. It is a recommended vessel connection per ASME Bioprocessing Equipment guidelines as noted in the “Design for Sterility and Cleanability” Section. However, Mettler-Toledo Ingold had recognized improvements in hygienic design were possible and engineered the INGOLD Sanitary Socket™ to fulfill the potential of superior performance.

Through the engineered beveled interior and relocated optimal point of sealing the INGOLD Sanitary Socket™ provides more efficient hygienic performance. Housing or sensor O-rings are optimally located to prevent media hold-up and contamination. Sealing on the interior edge of the INGOLD Sanitary Socket™ bevel allows SIP or CIP processes to more thoroughly clean and sterilize the entire wetted surface of the instrument housing or sensor and its process connection. The result is superior hygienic and documented performance during production and through cleaning/sterilization procedures.



www.mt.com/housing



Fig. 3
EHEDG has certified the INGOLD hygienic sanitary socket together with the housing InFit 761/NC.

Original INGOLD Accessories

Keep your Measuring Systems Running

METTLER TOLEDO not only provides complete measuring systems to control parameters such as pH/ORP, dissolved and gaseous oxygen, CO₂, conductivity and turbidity, it also offers you a comprehensive and well-balanced package of accessories.

pH and ORP Accessories

METTLER TOLEDO offers a wide selection of pH buffers, electrolytes, cleaning and storage solutions to facilitate operation and maintenance of its high-accuracy pH measurement systems.



Oxygen Accessories and Maintenance

To maintain the membrane integrity of oxygen sensors, kits of multiple membrane types, including membrane body, electrolytes and O-rings are offered.

Continued Support

Many customers still rely on our previous generations of products. We are committed to continue to provide maintenance materials, service and technical support for all of these products.



For more information, we invite you to visit:

 www.mt.com/pro-service

Mettler-Toledo AG

Process Analytics
Im Hackacker 15
CH-8902 Urdorf, Switzerland

Your METTLER TOLEDO contact:

www.mt.com/pro

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