



**INGOLD**

Leading Process Analytics

## Double DO Measurement Controls Cell Culture Density

**Inlet and outlet dissolved oxygen (DO) measurement in packed-bed bioreactors (PBR) allow to control the growth rate of mammalian cell cultures with high precision and reliability.**

METTLER TOLEDO has evaluated and discussed with different customers a new way to control the cell density with a double measurement of dissolved oxygen, at the inlet and at the outlet of a packed-bed bioreactor (PBR).

### Raising demand for mammalian cell cultures

Mammalian cells are widely used to produce bio molecules like recombinant glycoprotein such as hormones, enzymes, cytokine and antibodies for human therapy. Due to their advantages, tremendous effort has been invested in developing animal cells as commercial vehicles. Bioreactors with a variety of cell culture systems are now available:

- Suspension cells, with or without cell retention devices

- Immobilized cells, with cells fixed on a support as packed beds

### Growing need for mammalian cell cultures

The demand for therapeutic proteins derived from mammalian cell cultures continues to grow with the approval of newer products. Some of these recently developed products need to be administered in higher doses and necessitate production of larger quantities than those that were required with older products. As a result, there is a growing need to increase the productivity of mammalian cell culture bioreactors.

### Perfusion cultures provide higher cell density

Most biopharmaceutical proteins are produced in stirred tank bioreactors in a



batch or fed-batch operation mode. It is relatively simple to scale-up the production in stirred bioreactors. However, it requires large volumes of the culture from 10 – 20 m<sup>3</sup> due to the relatively low cell densities attained in this process.

Typically the cell density in suspension cultures is between 10<sup>6</sup> and 10<sup>7</sup> cells/ml. In perfusion cultures, a nearly 10-fold higher cell density (10<sup>7</sup> – 10<sup>8</sup> cells/ml) can be attained in comparison to batch cultures in stirred bioreactors. In perfusion cultures, the medium is perfused at an appropriate rate and in a constant volume. Culture and cells are retained in the bioreactor by various means. In practice, a 10-fold higher yield of cells with the perfusion technique means that a 2 m<sup>3</sup> perfusion culture is as productive as a 20m<sup>3</sup> fed-batch culture! Besides this advantage it has to be mentioned that perfusion cultures are of high complexity and are not easy to be scaled-up.

### **Packed-bed bioreactors (PBR) show excellent volumetric productivity in comparison to batch cultures**

A packed-bed bioreactor typically consists of a packed-bed supporting cells on or within carriers and a reservoir that is used to re-circulate the oxygenated nutrient medium through the bed. It is a system that provides extremely high productivity within a compact size. Packed beds have been widely used for perfusion cultures of immobilized mammalian cells.

### **Oxygen availability as a limiting factor for maximum bed size**

Packed-beds cannot be scaled-up without limits. At the inlet of the bed the solubility of oxygen is quite low and limits the depth of the bed. Anywhere in the bed the oxygen concentration must not fall below a critical or it would jeopardize the survival of the cells and their ability to produce the desired protein.

### **Cell growth rate depends on oxygen consumption**

As the cells grow on the packed-bed, the cell density or growth rate can not be measured in the re-circulated nutrient medium. If the oxygen concentration is measured at the inlet (CO<sub>2</sub> – in) and the outlet (CO<sub>2</sub>-out) the cell density can be calculated from the oxygen consumption rate.

### **Double DO measurements an interesting application to control O<sub>2</sub> consumption**

A packed-bed bioreactor typically operates within a range of oxygen concentrations which could be less than 80% of oxygen saturation. CO<sub>2</sub>-out remains above 20% while the cells have a constant specific oxygen consumption rate of 2 x 10<sup>-13</sup> mol/cell/h. This application, which allows determining cell density with a double measurement of dissolved oxygen in the inlet and outlet of a PBR, has been evaluated at a large pharmaceutical company. The implementation of the PBR technology will open new possibilities in their production.



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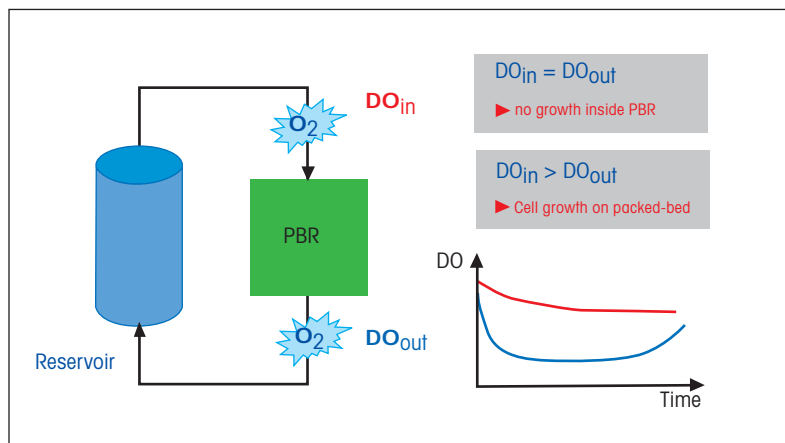
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### METTLER TOLEDO system recommendation

To get highly reliable results and to minimize the time for unnecessary maintenance, METTLER TOLEDO recommends the following measurement system for this application:

- 2 Oxygen sensors InPro 6800
- 2 M 700 transmitter equipped with DO modules.

METTLER TOLEDO Process Analytics is convinced that this way to control the cell density in packed-bed bioreactors will also be of interest for many other pharmaceutical companies.



► [www.mt.com/O2](http://www.mt.com/O2)

► [www.mt.com/transmitter](http://www.mt.com/transmitter)

## pH Control Improves Accuracy in Bacteria and Yeast Fermentation

**Former unstable bacteria and yeast fermentation process was brought under control with the implementation of the liquid filled pre-pressurized pH electrode InPro 3253 and allowed to get much higher accuracy.**

### Bharat Biotech International Ltd. Hyderabad, India.

Bharat Biotech International Limited (BBIL/Bharat Biotech), Hyderabad, India, is a multidimensional biotechnology company specializing in product-oriented research, development and manufacturing of vaccines and biotherapeutics. Bharat Biotech, established in the year 1996, is engaged in developing next-generation vaccines and bio-therapeutics through innovative and collaborative research. BBIL's state-of-the-art manufacturing plant is the largest of its kind in Asia-Pacific. This is first bio-pharmaceutical facility in the country to be audited and approved by Korean Food & Drugs Administration (KFDA), sprawls over a picturesque campus at Genome Valley,

Hyderabad. Built with an investment of over INR 1000 million, the facility's Manufacturing, Control Procedures and Protocols, conform to the stringent standards laid down by internationally recognized institutions such as USFDA, UKMCA and WHO. Bharat Biotech has set new benchmarks in Innovation and Quality that epitomizes the tremendous progress of Indian biotechnology in the global arena.

### A situation has to be improved

Mr. R. Venkatesan, a Senior plant Manager has initiated a fermentation process with bacteria and yeast cells in the pilot phase and in the production process. To control these processes he installed a pH measuring system of a competitor. The results however were not satisfying at all.



The performance of the pH electrode was poor and short-lived. There was deviation in coloration of the gel electrolyte and the electrode could not be used for more than 10 to 15 sterilization cycles. Because of these unsatisfying performances Mr. Venkatesan was ready to test and install a new measuring system which was recommended by METTLER TOLEDO. BBIL knew our company already from former business ventures.

### The process

Before the process could be started some preliminary work had to be done:

- Cleaning of the fermenter in a CIP-process
- Calibration of the pH electrode InPro 3253 with standard buffer

solutions (pH 9.21 and 4.01)

- Charging of the fermenter with the media and adjusting of the pH-value by adding NaOH or HCl automatically
- Automatic steam sterilization over 30 minutes at 121 °C
- Stabilisation of the temperature at 37 °C

With the addition of the inoculums (seeds) to the vessel the batch for the fermentation process was started. In the course of the process NaOH / HCl dosages were controlled by the transmitter pH 2050 e. The target was to maintain the process at 37 °C and pH 7.00.

### The customer's expectations and satisfaction

Accurate pH control and -adjustment plays an important role in achieving required growth of bacteria and yeast cells. After 24 hours of growing and multiplying of seeds the fermentation process was

stopped and the harvested material could be extracted after final centrifugation. Thus it is of utmost importance to optimize the fermentation process. The results were very satisfying and has improved the following:

- Accuracy of measurements
- Life time of the electrode
- More sterilization cycles (55 to 60) per electrode
- Reliability of measuring results
- Stability in cultivation mode
- Response time of the electrode
- Temperature control due to built-in sensor
- Maintenance and handling

### The measuring system

METTLER TOLEDO installed four measuring loops consisting of the following elements:

- pH electrode InPro 3253 / 120 / Pt 100
- pH transmitter pH 2050 e

### Conclusions

The right selection of a measuring system can help in improving not only the quality of the whole process but also saves time and money even if the initial costs may be slightly higher. The four fold number of sterilization cycles made the decision easy.



InPro 3253.

### InPro 3253 – for biotechnological applications

- pH sensitive glass, minimizes zero point shift following sterilization
- Highest precision at low to medium operating pressures, due to self-cleaning action at the diaphragm
- Designed for use at process pressures up to 4 bar overpressure
- Fully autoclavable or in-situ sterilizable
- Greatest degree of reliability of measurement values guaranteed by the patented silver-ion trap and built-in temperature sensor
- Measurement of ORP, enhanced diagnostics and shielding against earth potentials are optional
- Conformance with all relevant hygiene and safety regulations.

 [www.mt.com/pro-pH](http://www.mt.com/pro-pH)



# Manage your ISM Sensors the Easy Way



**iSense ISM Asset Suite for the Process Analytics represents a big step into a more controllable future of your sensors life – starting at the first implementation into your process and ending with the decision to eliminate the sensor.**

## Raising demand on control data

With the volume of calibrations performed each year, calibration information has to be collected, managed and analyzed efficiently and consistently to satisfy regulatory requirements and in order to utilize company's time and resources to their maximum.

## ISM technology

The digital "Intelligent Sensor Management®" concept makes it much easier to operate process analytical systems from initial installation to maintenance right through to sensor exchange. It ideally supports the vertical integration of diagnostics information into the process control environment. Predictive sensor wear or adaptive calibration timer information can be integrated into a PLC via fieldbus technology.

## iSense – the key to maximizing the benefits of the ISM technology

iSense allows verification and calibration of digital ISM pH electrodes and digital DO sensors by its capability to measure dissolved oxygen in laboratory conditions.

## Easy connection to your PC

Simply connect your ISM sensor via a USB port to your computer and follow the instructions of your iSense software. No transmitter is required as an interface.

## Comply your documentation requirements

The documentation of the entire sensor history is a result of the iSense Asset Suite. This is ideal for organizations looking for a complete and reliable stand alone calibration solution making it easy to comply with the demanding documentation requirements.

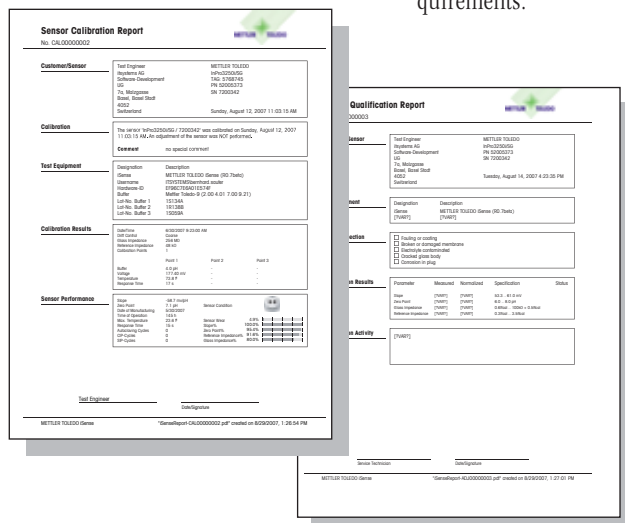
## Intuitive calibration support

Enhance your productivity with the advanced software application for your sensor calibration management. An analysis has shown that you can reduce drastically duration and frequency of calibration resulting in a saving of about 25 % with a ROI as low as 3 months!

## Manage your ISM sensor over the entire life cycle

A centric view of the sensor data given by SQL data base allows the detection of all pieces of equipment ever been used in operation and delivers various data export possibilities. This guarantees protection of your sensor assets.

[www.mt.com/ISM](http://www.mt.com/ISM)



**iSense**  
ISM Asset Suite

## Conductivity Measurement for High Process Security

**In-line conductivity measurement technology plays an essential role in the efficient operation of Cleaning-In-Place systems, ensuring the highest possible levels of cleanliness as well as optimal control over the cleaning solutions.**

### **Cleaning-in-Place (CIP) equipment, a part of the production system**

CIP systems are an integral part of production systems in the pharmaceutical industry. Cleaning is carried out in compliance with clearly defined official regulations. At the end of each cleaning cycle, the cleaning solutions involved are returned to storage tanks for later reuse. Controlled recovery of the acid and alkaline solutions enables to determine their degree of reusability.

### **Control of acids and alkalines via conductivity measurement**

The level of concentration of the recovered acids and alkalines is established through conductivity measurement. In order to ensure the correct concentration in the individual tanks, the recovered solutions are automatically re-concentrated to the required strength.

### **Cleaning procedure**

Control of various CIP process stages such as start of dosing of alkaline or acid, or rinsing with water, is carried out efficiently and cost-effectively via in-line measurement of conductivity. Conductivity measurement allows to establish the degree of contamination of the cleaning solutions in the return pipe and thereby also allows to determine whether the solutions can be recycled or should be rejected.

The CIP cleaning procedure is a multi step process. In a first step, the different wash solutions are prepared in storage tanks with the assistance of conductivity measurement. Final rinse with ultrapure water takes place at the end of the procedure. Conductivity measurement value of the ultrapure water determines the end of the CIP cycle.

### **Conductivity controls CIP process**

Conductivity measurements allow to distinguish between the separate cleaning agents, the product, and the rinse water. Contamination can be successfully detected and the concentration of each wash solution can be established very accurately. The cleaning program is simple to control via the limit switch contacts of the transmitter, and easy to integrate into a Process Control System (PCS). The conductivity sensors used to monitor the dosing apparatus are built directly into the solution tanks, so that if a concentration falls below a set value, the appropriate preprogrammed transmitter limit contact is activated and the acid or alkaline feed pump switches on accordingly.

### **InPro 7108-VAR-VP, widely used in the pharmaceutical industry**

Establishing the contamination level of the CIP cleaning solutions via conductivity measurement greatly contributes to quality control before and after the cleaning cycle. The InPro 7108-VAR-VP is optimally suited for applications with high

requirements on hygiene and designed to suppress the growth of unwanted bacteria. Process safety and minimization of production losses and laboratory costs clearly take first priority. The precision of the in-line conductivity measurement for quality control of the different solutions before and after the cleaning process, and assessment of the degree of reusability of the cleaning solutions, combine to underline the advantages of inline conductivity measurement in CIP plant in the pharmaceutical industry.

#### **InPro 7108: User benefits**

- No dead pockets
- Designed for hygiene requirements
- Guards against bacterial growth
- No contamination of wetted parts
- Minimized production losses
- Short process cycle time

► [www.mt.com/cond](http://www.mt.com/cond)



InPro 7108.

## Control of Turbidity Optimizes Crystallization Process

**On-line turbidity measurements provide early indication of quality deviation in the precipitation reaction of the crystallization process. It saves time and costs in downstream processing.**

### Background

An amine ( $R-NH_2$ ) produced by synthesis in the pharmaceutical industry exists in the form of a hydrochloride salt ( $[R-NH_3]^+ Cl^-$ ) and is precipitated from a clear solution through the addition of caustic soda (NaOH). Samples of the precipitated amine revealed strong and unacceptable fluctuations in product purity.

### Clear turbidity signal of crystallization is required

Precipitation conditions, solvent composition, concentration and dosage rate of the caustic soda all have an influence on the quality of the intermediate product.

The use of an in-line turbidity measurement system has provided important information contributing to optimization of this reaction step. Fig. 1 graphically illustrates the gradient of the signals of the turbidity sensor in relation to the dosing rate(s) of caustic soda. A linear increase in the dosing rates was accompanied by an increase in the level of turbidity, fol-

lowed by signal decay before the concentration of solid particles slowly evened out to a stable value.

The decay of the turbidity signal points to conglomeration of the finer crystals, possibly also includes entrapped impurities. A more rapid, optimized dosing of caustic soda leads to spontaneous precipitation of the whole product which, furthermore, also displays a higher degree of purity.

### The goal: time saving and avoidance of faulty charges

Detailed understanding of the optimum precipitation conditions therefore results in being able to achieve a product with the required degree of purity. The use of an in-line turbidity sensor in the process provides information on how these conditions are to be maintained and therefore gives early indication of any quality deviation in the precipitation reaction. This successfully prevents time-consuming and costly downstream processing of faulty charges.

### Reliable measurement with METTLER TOLEDO solution

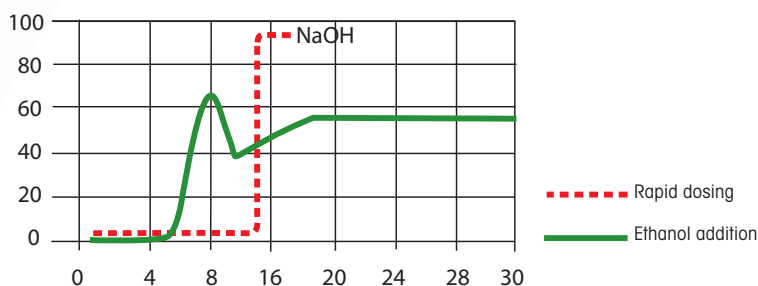
The turbidity sensor InPro 8200 has been proven to be the ideal solution for this type of application. The use of scratchproof sapphire windows allows long-term operation even in the presence of abrasive crystals. Signal transmission between sensor and transmitter employing optical fiber cable technology in conjunction with the principle of backscattered light results in a sensor with a compact design of which installation is equally possible in small research reactors and in large production-scale vessels.

### Features of the InPro 8200

Ideal for medium to low concentration and where high resolution is required.

- Two optical fibres
- Smooth sensor design prevents fouling and reduces maintenance
- Wide measuring range
- High accuracy
- Sapphire window for fiber protection

Fig.1: Gradients of the turbidity signal.



InPro 8200.

[www.mt.com/turb](http://www.mt.com/turb)

# New Developments in Process Analytics

**METTLER TOLEDO delivers powerful solutions to optimize your processes and reduce maintenance costs. Recently, we introduced new intelligent technologies that allow you to improve handling and optimize maintenance thus addressing your most pressing needs.**

## **ISM – the next generation of intelligent process analytics!**

With the groundbreaking ISM technology METTLER TOLEDO provides another milestone in process analytics measurement!

Dissolved oxygen sensors and pH electrodes with integrated preamplifier are using a new technology with “Plug and Measure” and intelligent diagnostics functionalities. The Intelligent Sensor Management (ISM) technology simplifies all maintenance operations of the sensor. Process interruptions are shorter or even avoided, leading to enhanced productivity.

## **iSense – the key to maximize the benefits of the ISM technology**

iSense ISM Asset Suite allows efficient and easy verification and calibration of METTLER TOLEDO digital ISM pH and DO sensors in an instant with an intuitive software application that includes advanced analysis and documentation functionalities to support your sensor management.

## **Digital transmitter line M300**

The digital M300 transmitter represents an easy-to-use version of the M300 transmitter line. Its unique “Plug and Measure” features enable a fast start-up and robust measurements for digital pH/ORP and dissolved oxygen sensors. Its versatility and reliability make this instrument the ideal choice for a wide range of applications.

If you want to take advantage of these advanced products ask your local METTLER TOLEDO representative or visit [www.mt.com/ISM](http://www.mt.com/ISM).

# ISM



**iSense**  
ISM Asset Suite



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