On-line measurement of cell density leads to better quality and yield

Hamilton offers new ways for bioprocesses with Incyte and Dencytee sensors

Biological processes have become a significant industry in constant growth. Development in the field of vaccines, antibodies and pharmaceuticals is very popular. The physiology of the cells used is very sensitive and requires quick adjustments to maintain accurate control of the culture media. Several parameters must be controlled; pH, dissolved oxygen, carbon dioxide and cell density. These needs are fulfilled by modern on-line measurement sensors. This information can be used to create an optimized environment for the culture, which leads to higher quality and better yields. Inattention to these measurements can lead to negative consequences.

Fast detection of parameter changes enables short term adjustments to the culture conditions

On-line measurements for determining pH or dissolved oxygen, have long been used in biological processes. Other parameters that provide important insights to the cell physiology have traditionally been measured with individual, time-consuming and less precise off-line methods. An important advancement towards on-line measurement is achieved with the new Incyte and Dencytee sensors from Hamilton. These sensors provide a means for direct real-time measurement of viable cell density and the total cell density. Continual data, never available before is now available constantly. Continual information concern-

ing critical process deviations that would have been missed before are detected instantly due to the new sensors. Monitoring total cell density, which measures both viable and dead cells along with all other particles, is a reliable method for determining the cell growth in the early stages of a culture. The most relevant information is obtained during the lag and growth phases before significant cell death occurs. Viable cell density measures only the living cells, which gives useful insight into the total progress of the culture up to and including pinpointing the beginning of the death phase. Additionally, viable cell density can provide information on changes in cell physiology. By means of theses on-line measurements it is now possible to recognize process deviations and quickly perform the necessary adjustments to enhance control of the process. On-line measurement provides faster insights for process changes and reduces risk from grab sampling.

Combined measurement of different parameters provides optimal results

Simultaneous on-line measurement of pH enables continuous pH adjustment to reduce this stress factor and allow an optimized environment chemistry for the production of the culture. Dissolved oxygen also plays an important role in bioprocesses. Too little oxygen can result in apoptosis (sudden cell death) or anaerobic digestion, which in turn drastically reduces the culture's viability and yield. On-line measurement of dissolved oxygen ensures optimal oxygen content so high-quality products can be obtained.

Incyte and Dencytee open new paths

The Incyte viable cell density sensor is targeted to mammalian cell cultures, yeast and high-density bacterial fermentations. It measures only viable cells and is not influenced by changes in the media, microcarriers, dead cells and debris. The functional principle is based on capacitance measurements. In an alternating electrical field, viable cells behave like small capacitors. The charge of these small capacitors is measured by the sensor and reported as permittivity. Improvement in yield and the reduced costs are advantages of this technology, as is the immediate detection of cell physiology changes. Precise monitoring in favor of creating an optimal cultivation environment and the early determination of process changes provide the foundation for a successful use of the Incyte sensor.

The Dencytee sensor is an optical-based technology that measures the total cell density by means of the cell suspension's turbidity. The measurement is made at near infra-red wavelengths and is therefore insensitive to changes in media color. All particles and molecules that scatter

light at 880 nm will be detected – viable as well as dead cells. This technology is also particularly effective when cells expand quickly. Fast and simple measurement of the cell growth, reliable values during the growth phase as well as the early detection of process deviations are counted among the sensors' strengths.

Nothing remains undetected: Practical test was mastered with flying colors

Hamilton proved the quality and reliability of each parameter's measurement and the measurement of different factors in combination, with the pilot project. The accuracy of the measured results was evaluated via off-line measurements. An optimal cultivation of CHO-cells (Chinese Hamster Ovary) was established in a bioreactor for the production of the monoclonal antibodies as part of a project. To achieve a high reproducibility, a tight control of all relevant parameters including pH, dissolved oxygen and the viable as well as the total cell density was set. Even slight process deviations have significant effects on the cell growth concerning these cultures. Determining the pH and dissolved oxygen are only linked indirectly with the cell status and cell growth. The capacitive Incyte sensors, combined with the pH and dissolved oxygen sensor, allow a continuous measurement of all relevant data of bioprocesses. Hence, the biological processes are significantly more transparent and can furthermore be optimized prospectively. The velocity and accuracy of the measurements were also increased, which leads to more efficient and standardized procedures. Hamilton's sensors are able to perform on-line measurements reliably, which serves the optimal process monitoring and control. During the pilot project, eventual measurement deviations were eliminated altogether.