Optimal production results thanks to continuous monitoring of the cell density

Cell cultures and microorganisms play an essential role in the development processes in modern pharmaceutics and biotechnology. Since they react very sensitively to external influences, the conditions during the reproduction process must be precisely managed and monitored. This includes the temperature, pH value or dissolved oxygen, for example. The cell density numbers among the most important process characteristics and can be determined on the basis of the optical density of a medium, for example.

Biotechnological processes are used whenever complex active ingredients for pharmaceutical production cannot be produced through chemical synthesis. Here, monoclonal antibodies or other recombinant proteins, for example, are produced using genetically modified organisms. Bacteria, yeast or mammalian cells, for example, are used for this depending on the later use and the post translational modification or protein folding required, among other things. The complexity of the active ingredient is often directly related to the effort and vulnerability of the process here. Even the smallest changes here can have a significant impact on the properties and thus the effectiveness of the substance obtained. Biopharmaceutical processes therefore number among the most challenging processes in the production of active ingredients.

When we consider all of the necessary production steps, the upstream process in particular gains special importance. Here, the cultivation of the cells and their subsequent reproduction is done in bioreactors by means of batch, fed batch or perfusion processes, among others. In order to produce the greatest possible volume of the desired substance in an appropriate quality, the conditions in the bioreactor must be optimally adjusted. The most important parameters here include the temperature, pH value, stirring rate and the concentration of culture medium and dissolved oxygen, for example.

Determination of the cell growth on the basis of the optical density (OD)

Determination of the optical density, which can be determined by means of absorption measurement, for example, is an indicator of the successful reproduction of the cell cultures. Here, a beam of light is conducted into the medium and the light loss is determined by means of a detector. The reduction in the light emitted here correlates directly to the actual cell density. This numbers among the most important process characteristics because the volume of the target protein is often determined by the number of cells. It is needed in order to determine the time for a feed, changing the carbon source, induction or harvesting. Dilution rates can also be calculated using the optical density.

In order to achieve absorptions which are optimal for most cells and exclude colour influences, sensors with a near-infrared (NIR) wavelength are generally used for determination of the cell density. Here, all particles which scatter or absorb light are detected. In addition to living cells, this also includes dead cells and cell debris. However, the type of sensor described has proven itself to be particularly effective at providing reliable values during the growth phase thanks to quick and easy to implement in-line measurements. Early detection of process variations which can become noticeable through reduced cell growth are another of the strengths of OD measurement.

Continuous monitoring of the cell density in the laboratory and later in the production process

In order to be able to benefit from the strengths of OD measurement, however, a correlation to other measurement values such as total number of cells and

cell dry weight must already be established in the development process. Dielectric spectroscopy is given as another example. With this method, the permittivity, also known as dielectric conductivity or dielectric function, is determined. Since living cells have the capacity to store electrical charges, changes to the permittivity allow conclusions to be drawn concerning defective or dead cells. Accordingly, dielectric spectroscopy, in contrast to OD measurement, provides information about the number of living cells.

In many development laboratories and to some extent in downstream production processes, the optical density is generally only determined “offline” in order to draw conclusions with regard to process progress or cell growth. This means that an employee has to manually take a sample at defined times in order to analyse it. In the times between this or if there are no employees on site (e.g. overnight), valuable data is lost because no process monitoring is done here. Through removal from the process and analysis outside the bioreactor, possible contamination of the sample also cannot be ruled out.

Permanent and unbroken monitoring and control of all critical and quality-related parameters are key elements in bioprocess development. Only through its correct adjustment can an environment in which the cells used can optimally thrive be created. In order to improve the product quality and process performance, sensors and measuring instruments which allow for continuous in-situ monitoring and recording of all of the necessary data and which can also be used in the later production process are increasingly used. As a result, the reproducibility is significantly improved and the process yields are increased. If the same sensors and measuring instruments are used, then laborious correlation between the measurement technology used in the laboratory and that installed in the process is avoided.

Requirements for the measuring probe used to determine cell density

Since biopharmaceutical processes are very challenging processes which react very sensitively to external influences, high quality in the components, e.g. bioreactor, agitators and measuring probe, is essential. In addition to the selection of the materials used, generally stainless steels, the relevant surfaces which, in the case of stainless steel, have a very low roughness depth and are generally electropolished are also key factors here.

Contamination of the medium is effectively prevented through a design for the measuring probe which is as gap-free as possible, and thus has little dead space. All recesses and undercuts which are required by the design, particularly those which are located in areas which come into contact with the medium, should also be easy to clean. By now, it is standard for the measuring instruments used to be CIP/SIP-compatible and autoclavable.

Thanks to continuous detection and recording of the absorption values, the measuring probe offers up-to-date monitoring of the cell growth. Sample preparation such as dilution of the sample is also not necessary. Errors in the provision of the data can thus be avoided. The measuring probe does not need recalibration during the process and provides a drift-free signal. The data collected is easy to interpret and therefore does not require any special processing. However, no correlations to measurement values which were collected using conventional methods such as dry cell weight or cell count can be established. Thanks to the design specified above, the probe can be easily installed and qualified together with the bioreactor.