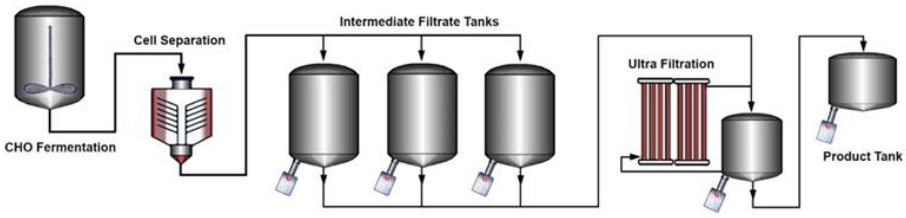
CHO fermentation and cell/protein separation

Protein

Benefits

- Inline measurement allows CHO cells to be cultured at large scales in bioreactors, facilitating mass production
- Advances in fermentation process control can lead to significant efficiency gains, allowing to produce 3 to 10 grams of recombinant protein per liter of culture.
- Inline monitoring reduces the need for manual sampling, which is time consuming and requires dedicated personnel.



Overview

Modern pharmaceutical production increasingly relies on fermentation processes, especially in the production of recombinant therapeutic proteins, such as antibodies. A key element of this process is the use of CHO fermentation and *immortalized cell lines*, particularly *Chinese Hamster Ovary (CHO)* cells, which are essential for the mass production of therapeutic proteins.

CHO cells are the most used mammalian cell line to produce therapeutic proteins. In fact, CHO cells account for approx. 70% of global monoclonal antibody production.

Refractive index measurement applications

CHO cells are genetically engineered to produce the desired proteins using *CRISPR technology*, a precise genome-editing tool. CRISPR allows for precise modifications to the CHO cells' DNA, ensuring high efficiency and yield in target protein production.

Fermentation process

Continuous fermentation method allows for ongoing production over extended periods, which can last for months. Continuous fermentation includes various techniques:

a. Chemostat cultures

Nutrients are continuously supplied to the culture, while products and some cells are removed. The cells can be separated and returned to the bioreactor. This allows for consistent production and easy adjustment of culture conditions.

b. Perfusion cultures

Cells are retained inside the bioreactor while nutrients are continuously fed, and waste products are removed. This maintains high cell viability and protein production over time.

c. Fed-batch processes

Nutrients are added at controlled intervals to maintain high cell densities and viability over a period of two to three weeks. This is the most widely used method in the industry due to its balance between production efficiency and scalability.

Downstream processing

Following fermentation, the next crucial step is the separation of cells and the target protein. This downstream process begins with centrifugation to remove cells from the culture medium, followed by filtration steps to further purify the protein. The final concentration of the protein is achieved through ultrafiltration.

Throughout these steps, process refractometers are employed to continuously monitor and ensure the consistency of protein concentration, up to the final filling stage. By ensuring that protein concentration remains within the desired range, the process can be optimized for maximum efficiency, reducing processing time and increasing throughput.

Inline monitoring reduces the need for manual sampling, which is not only time-consuming but also labor-intensive, requiring dedicated personnel. Manual sampling introduces a higher risk of contamination during downstream processing — a critical concern in biopharmaceutical production, where contamination can severely compromise the quality and safety of the final product. By employing inline monitoring, we can maintain a sterile environment and enhance overall product integrity

Maintaining a consistent protein concentration throughout downstream processing is also crucial for producing a uniform final product. Inline monitoring allows for continuous tracking of concentration levels, helping to prevent variations that could lead to batch inconsistencies. This consistency is vital for meeting regulatory standards and ensuring that the final product is safe and effective for therapeutic use.

Instrumentation and installation considerations

KxS process refractometer DCM-20 with Ingold process connection is designed with the highest hygienic industry standards to integrate in pilot and high-volume manufacturing bioreactors and filtration systems. KxS DCM-20 complies with food safety and safe food contact materials (FCMs) according to U.S. and EU regulations, including European Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food. We also comply with Commission Regulation (EC) No 2023/2006 on Good Manufacturing Practice (GMP), ensuring that our manufacturing processes are well-controlled so that specifications for FCMs remain in conformity with the legislation.

Measurement output options include both analog and digital communication protocols: dual analog 4-20mA and

Modbus TCP. The refractometer does not require any

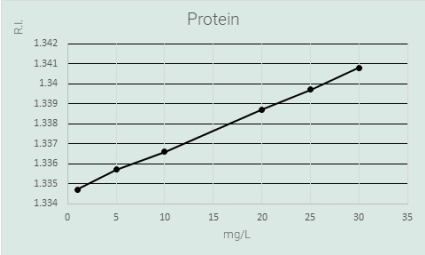
recalibration or regular maintenance. Furthermore, the calibration of each refractometer can be verified using NIST traceable standard refractive index liquids and easy verification procedure.

The refractometer is factory-calibrated for the full refractive index and temperature range, converting measured values directly to concentration units, e.g., for milligrams per liter.

Temperature variations are automatically compensated in the readings.

Chemical curve

Chemical curve for protein mg/L per R.I. at reference temperature of 20°C



When the reference method differs from Refractive Index related method, it's essential to use tailored calibrations for each individual stage.

For example, if the sensor result is compared to the protein peak in chromatography, the RI-value will represent the sum of all peak areas contributing to the density signal, not just the protein peak area. This means that if the ratio between the protein peak and other fermentation components varies across different tanks, separate calibration curves will be required to ensure accuracy.