Case study: dead-end filtration of post-centrifugation bioreactor broth

A subunit vaccine substance has been expressed in a microorganism, which has been cultivated in a bioreactor in batch mode. In order to obtain the active substance, cells and inclusion bodies need to be burst open by shear force. This is achieved by high-pressure homogenization, where the harvested liquid from the bioreactor is exposed to high shear force in order to burst open the cells and access the substances within. This results in a liquid that contains four protein components, which we will refer to as A, B, C and D, where A is the active vaccine substance, and the remaining are impurities that need to be removed by means of chromatography. However, the liquid also contains fragments of the cells that were burst open by homogenization. This cell debris needs to be removed before the liquid can be purified by chromatography. This is achieved by centrifugation, which reduces the amount of solid cell debris in the liquid by some amount. The remaining cell debris removal is performed by dead-end filtration.

High-pressure homogenization was performed in two different configurations (combinations of pressure and number of passes), and the particle size distribution was then measured via dynamic light scattering. The results of these measurements are summarized in Tables 1 and 2.

Table 1: results from high-pressure homogenization using Configuration 1.

|  |  |  |
| --- | --- | --- |
|  | Size | % of total mass |
| Particle 1 | 27.5 nm | 46.6% |
| Particle 2 | 101.1 nm | 27.6% |
| Particle 3 | 315.4 nm | 25.8% |

Table 2: results from high-pressure homogenization using Configuration 2.

|  |  |  |
| --- | --- | --- |
|  | Size | % of total mass |
| Particle 1 | 144.0 nm | 51.9% |
| Particle 2 | 1174 nm | 48.1% |

After centrifugation, the total amount of cell debris was reduced by 30% in Configuration 1, and by 60% in Configuration 2.

Assume that each particle is spherical and has a density 10% higher than that of water. We can obtain the molecular weight of each particle by first computing the volume of the particle, converting it to mass using the density, and dividing by Avogadro’s number.

Now, a dead-end filtration unit needs to be designed to remove the remaining cell debris. Assume that the size distribution remains the same after centrifugation, i.e. that the total concentration of cell debris of each size is reduced equally. For a 100 l bioreactor with a cell density of

* Density of the cell broth
* Cake density
* **Cut-off: smooth or harsh**
* **Flowrates/pressures**
* **Area/resistance of the membrane**
* **Size distribution data (ask UCL)**