Integrating Acoustic Perfusion in Mammalian Cell Culture


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Abstract

Acoustic Perfusion Process

An acoustic perfusion process using the BioSep typically involves continuous addition of fresh medium to the bioreactor, while cells are filtered from the harvest streams by the resonator chamber and returned to the bioreactor. The BioSep can be directly mounted onto the bioreactor head plate. One standard mode of operation employs, for example, a harvest pump at the exit port of the resonator chamber, and a recirculation pump for the small, apart from the acoustic field. The BioSep system can also be set up to allow for semicontinuous operation or an alternative strategies of cell recirculation.

Results

The separation efficiency was more than 99.5%, for a large range of flow rates and cell densities. At a cell density of 10 g/L, >99% separation efficiency was still reached at a harvest flow rate of 15 L/day for the 15 L system. However, at higher flow rates, the agglomerates became too large for the liquid to pass through. Cells were dragged along into the harvest resulting in a decreased separation efficiency. This was at 20 g/L and 15 L/day for the 15 L BioSep.

The separation efficiency was more than 99% for the three models of the BioSep, the 10L BioSep (99.8% at 10 g/L), the 50L BioSep (98.9% at 10 g/L), and the 250L BioSep (99.5% at 10 g/L) at different flow rates and cell densities. The separation efficiency on the 10L BioSep was found to be significantly lower than on the other two BioSep systems. The separation efficiency at 10 g/L was 98.9% at 2 L/day and 98.5% at 10 L/day. At high cell concentrations, the separation efficiency decreased at high flow rates (20 g/L). However, the systems are limited at high cell density where the agglomerates settling out of the acoustic field followed by immediate recirculation to the bioreactor.

Conclusion

The separation efficiency of the models was high for a large range of flow rates and cell densities. The separation efficiency was higher than 99% in suspensions of yeast cells up to 10 g/L, at flow rates beyond the nominal capacity of the BioSep. At 20 g/L, the nominal capacities were the maximum flow rates where 95% separation efficiency could be reached. Power output and run time became more important at high flow rate and cell density.

Scale-up and performance characteristics

The BioSep has a capacity from 1 to 250 L/day and is available in three different models, the 15 L BioSep (figure 2a), the 50 L BioSep (figure 2b), and the 250 L BioSep (figure 2c). The nominal capacity of the BioSep is given by the maximum harvest flow rate at which an acceptable separation efficiency can still be reached. For the 15 L system, the nominal capacity should be 10 L/day. In this study the three models of the BioSep are characterised for separation efficiency and scale-up properties. Special attention is paid to optimisation of operational parameters and to the scale-up of the system.

Method and materials

Suspensions of yeast cells, in a physiological salt solution (9 g/L, NaCl), were made at different concentrations in the range from 2-20 g/L. Yeast cells were used as model particles in stead of mammalian cells since they are easy to obtain and handle. The harvest was kept at 27°C, which is the usual operating temperature for mammalian cell culture. To test the separation efficiency the medium was circulated through the BioSep using a circulation pump and a harvest pump (figure 1). In this case the harvest stream was also returned to the reactor to maintain the same cell concentration in the reactor during the experiment.

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Sep. eff = \frac{[C]_{\text{harvest}}}{[C]_{\text{suspension}}} \times 100%

with Ch = cell concentration (g/L) in harvest and Cs = cell concentration (g/L) in suspension. The cell concentration was determined by measuring the optical density (OD) of samples from the harvest and the reactor. The optical density was compared to a cell count. A calibration curve between the optical density and cell number determined the linearity between the optical density and amount of cells. After the cells were weighed and diluted the amount of cells per ml was measured.

To optimise the separation efficiency the output power and settings of the integrated BioSep timer were varied. With the timer, the field can be switched on for 10 to 600 s and off for 1-600 s. Using the integrated timer, the BioSep operation was synchronized with the ultrasonic field being engaged. As a result, the harvest pump stopped when the ultrasonic field was switched OFF, enhancing sedimentation during flow-off periods.
**Acoustic Perfusion Process**

An acoustic perfusion process using the BioSep typically involves a continuous addition of fresh medium to the bioreactor, while cells are filtered from the harvest stream by the resonator chamber and returned to the bioreactor. The BioSep can be directly mounted onto the bioreactor head plate. One standard mode of operation employs, for example, a harvest pump at the exit port of the resonator chamber, and a recirculation pump for the return of separated cells that settled from the acoustic energy field within the resonator chamber. Alternatively, the BioSys system can also be set up to allow for semicontinuous operation or an alternative strategy of cell recirculation.

Acoustic perfusion is generally applicable for suspended mammalian and animal cell culture, but can also be adapted for anchorage-dependent cell lines, or the perfused culture of plant cells.

The BioSep separation principle is purely based on gentle, acoustically induced loose aggregation followed by sedimentation. In contrast to other cell separation techniques, the acoustic energy mesh acts as a virtual acoustic standing field is established within the cell suspension in which the cells are filtered from the harvest stream by the resonator chamber and returned to the bioreactor. The BioSys system can also be set up to allow for semicontinuous operation or an alternative strategy of cell recirculation. However, as found with this investigation presented operation at 1.4 MHz was more effective than at 2.1 MHz. This pumping system has been designed for batch culture, but can also be adapted for semicontinuous operation.

**Results**

**Characterisation**

The separation efficiencies observed with experiments at 1.4 MHz were comparable to those obtained at 2.1 MHz (figure 3). At low flow rates and cell densities, relatively low power output of 1.5 W to 2.45 W was used. This requirement increased with higher flow rates and cell densities up to 275 W at 10 L/day and 200 L. With low cell densities, experiments at 1.4 MHz revealed lower separation efficiencies than at 2.1 MHz (figure 3), although still above 95%. However, at 20 g/L, which is a typical biocomponent concentration observed with high cell density cultivation, the maximum flow capacity achieved was clearly larger for the 1.4 MHz (figure 3). At 10 L/day and 12.5 L/day, the separation efficiency of the 1.4 MHz system was recorded at 83% when it was only 47% for the 2.1 MHz version. This improvement of performance is believed to be related to the increase of separation efficiencies from 0.035 mm at 1.4 MHz to 0.05 mm at 1.4 MHz, reducing the hydrodynamic flow barrier posed by the aggregated particles within the displacement antinode (= pressure node) regions of the field.

**Perfusion run with CHO cells**

The culture was inoculated on day 1 and perfusion was started on the third day. The cell density increased considerably over the first two weeks and the viability was more than 95% (figure 4). At day 10 the viable cell density had increased to 35.1 mg/L. The flow rate was increased to approximately 5 L/day. The separation efficiency was above 95%. When the flow rate was increased to 7 L/day the separation efficiency dropped to 87%, and became too low to maintain such high cell concentration within the bioreactor. This limitation was probably due to the relatively low HF output power of only 30 mW available at this time (by the modified controller unit). The viability in the harvest stream was approximately the same as in the reactor. Therefore there was no significant selective retenion of dead or living cells. The harvest stream during this run was very high, above 90% also at high cell density. It was therefore concluded that the 1.4 MHz frequency has no negative effect on the culture.

**Conclusion**

Although the separation efficiency of the 1.4 MHz system was compatible to the separation efficiency of the 2.1 MHz in a large range, it was even higher at the higher cell densities and flow rates. This increases the capacity of the system since the separation efficiency becomes the limiting factor at high cell density. The power output needed to achieve the same separation efficiency was significantly lower.

The performance in a mammalian cell culture was also very good. The viability of the culture was observed to be above the 90% for more than 2 weeks at a maximum viable cell density of 25.5 mg/L.

**References**


**Figures**

- **Fig. 1:** Typical configuration of acoustic cell retention system.
- **Fig. 2:** Separation efficiency of the 1.40 MHz BioSep.
- **Fig. 3:** Comparison between the separation efficiencies from the 1.40 MHz (closed line) and the 2.10 MHz (interrupted line) experiments (* = 98% separation efficiency, ** = 95% separation efficiency).
- **Fig. 4:** CHO cell perfusion run with 1.40 MHz BioSep. Viable cell density (x), viability (y), and flow rate (z).