Integrating acoustic perfusion in mammalian cell culture

Scale-up and performance characterisation

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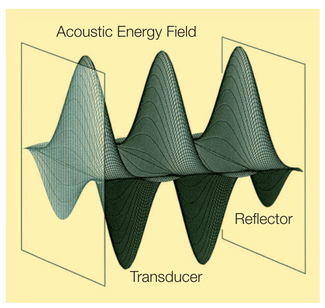
Principle of operation



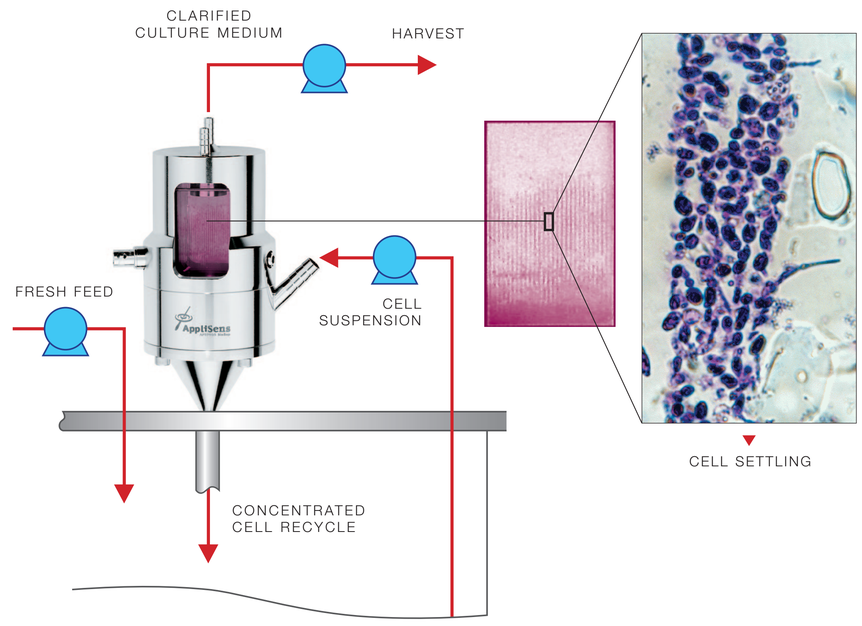
The BioSep is an acoustic cell separator, which is used for perfusion in mammalian cell culture. It is usually mounted on top of the reactor and it retains the cells with a virtual, acoustic field. In the BioSep cells are filtered from the medium and returned to the bioreactor by a recirculation flow. The BioSep has a perfusion capacity of 1 to 250 L/day, divided over three different models (referred to as a 10L, 50L and 250L BioSep). In this study all models were tested for performance and capacity. Yeast was used as a model particle at different cell densities comparable to cell densities as achieved in a mammalian cell culture. To achieve the highest separation efficiency possible power and timer settings were varied. Separation efficiency was high over a large range of cell densities and flow rates, more than 99%. Power and timer settings of the controller of the BioSep became more important at the higher cell densities and flow rates. More power was needed at the higher flow rates/cell densities, ranging from 3-8 W for the smallest device. With timer setting the acoustic field can be shut down periodically to enhance sedimentation. Run time of the controller was decreased (from 10 min to 1 min) at higher flow rates/cell density allowing more stops, of 3 sec. Similar behavior with regards to the operation parameters of three different models allows for consistent scale-up of the perfused cell culture.

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 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 BioSep system can also be set up to allow for semicontinuous operation or alternative strategies of cell recirculation. Acoustic perfusion is generally applicable for suspended mammalian and animal cell culture, but can also be adopted 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 created within the BioSep constitutes “virtual”, thus superior-, non-contact, non-fouling, non-moving filtration means allowing for up to thousands of hours of continuous operation. As a result, greatly increased steady state cell density, productivity, and product quality is obtained.

Figure 1 | Typical configuration of acoustic cell retention system.



In industry the acoustic perfusion has been applied in a wide variety of applications. Results have been published amongst which are high-density perfusion cultures of insect cells (Baculovirus expression systems). Reported are cell densities of over 30 million cells/mL with a cell viability greater than 90%.

Introduction

The BioSep has a capacity from 1 to 250 L/day and is available in three different models, the 10L Biosep (figure 2a), the 50L BioSep (figure 2b), and the 250L 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 10L system the nominal capacity should be 10L/day. In this study the three models of the BioSep are characterised for separation efficiency[1,2] at a large range of flow rates and cell densities. Special attention is paid to optimisation of operational parameters and to the scale-up of the system.

Materials and methods

Suspensions of dried yeast cells, in a physiological salt solution (9 g/L NaCl), were made at different concentrations in the range from 0.2 g/l to 20 g/l. Yeast cells were used as model particles in stead of mammalian cells since they are easy to obtain and handle. The suspension was kept at 37°C, which is the usual operating temperature for mammalian cell cultures. 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.

The separation efficiency was calculated by dividing the amount of cells in the harvest stream by the amount of cells in the suspension

$$\textit{Separation efficiency} = \left(1 - \frac{C_h}{C_s}\right) * 100\%$$

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 count 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 also counted to determine the amount of cells per gram.

To optimise the separation efficiency the power output and settings of the integrated BioSep timer were varied. With the timer, the field can be switched on for 10-600 s and off for 1-60 sec. Using the Interface port of the Biosep, operation of the harvest pump was synchronised with the ultrasonic field being engaged. As a result, the harvest pump stopped when the ultrasonic field was switched OFF, enhancing sedimentation during field-off periods.

Work supported by the European Commission’s TMR Programme, Contract No. ERBFMRXCT97-0156, EuroUltraSonoSep.

Figure 2a | 10L BioSep (1-10L/day capacity)

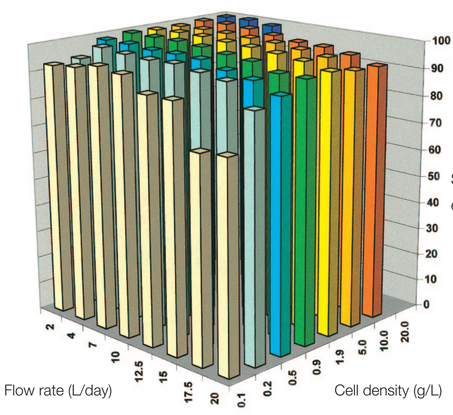


Figure 3a | Separation efficiency of the 10L BioSep at different flow rates and cell densitie

Figure 2b | 50L BioSep (5-50L/day capacity)

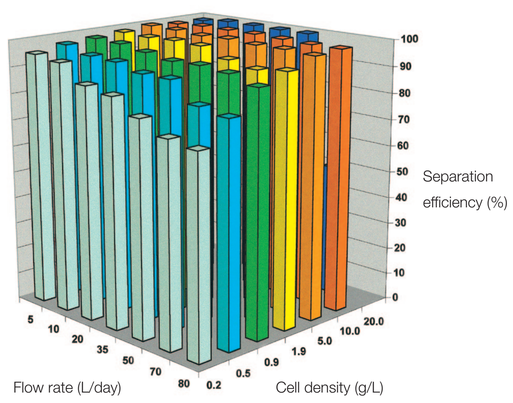


Figure 3b | Separation efficiency of the 50L BioSep at different flow rates and cell densities

Figure 2c | 250L BioSep (25-250L/day capacity)

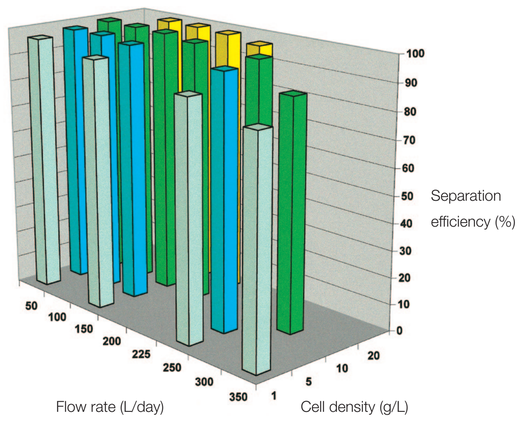


Figure 3c | Separation efficiency of the 250L BioSep at different flow rates and cell densities

Results

1 g/L of yeast suspension contained 2*107 cells/ml. Therefore 1 g/L yeast (d=5µm) is comparable by volume to 1*106 mammalian cells/ml (d=14µm).

The separation efficiency was more than 99%, for a large range of flow rates and cell densities, (figure 3a/b/c). At a cell density of 10 g/L, 95% separation efficiency was still reached at a harvest flow of 18 L/day for the 10L system. However the systems are limited at high cell density where 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 10L/day for the 10L BioSep.

With the 10L BioSep operated at low cell densities and flow rates ranging at 2-4 L/day, the power output needed to achieve the optimal separation efficiency was found at a relatively low level, 3W. When more power was supplied to the resonator chamber the ultrasonic field became stronger. However, any increase in power input resulted into increased dissipation of acoustic energy causing temperature gradients within the resonator volume and, thus, convective streaming of medium, which was observed to disturb trapping of cells within the acoustic field. Thus, at low flow settings, with increased power input decreased separation efficiencies were recorded. However, at 20 g/L and 10 L/day the power output had to be increased to 8 W to achieve the optimal separation efficiency. The same tendency was observed for the 50L (4-10 W) and 250L (40-80 W).

Both, harvest pump and ultrasonic field were periodically switched off, with the integrated timer, to help the cells sediment faster. When the field was switched off the cells were no longer captured by the field and the agglomerated cells sediment back into the recirculation flow and were carried back into the reactor. Such periodic stops did not appear to be of significant influence for achieving a high separation efficiency at low cell density and flow rate. However proper timing of on/off periods became more important at higher flow rates and cell densities: The run time had to be decreased from 600s at low flow rate to approximately 60s at high flow rate (nominal capacity), to give the cells more time to sediment. At high cell concentrations more cells came into the resonator chamber and the field had to be switched off more often to achieve the highest separation efficiency.

Conclusion

The separation efficiency of the BioSeps was high for a large range of flow rates and cell densities. The separation efficiency was higher than 95% in suspensions of yeast cells up to 10 g/l, at flow rates beyond the nominal capacity of the BioSeps. At 20 g/L the nominal capacities were the maximum flow rates where 90-95% separation efficiency could be reached. Power output and run time became more important at high flow rate and cell density. The separation efficiency of the 3 scales investigated had similar behavior with regards to operational parameters (relative to the size) which means that the range of BioSep models allow for consistent scale up of perfused cell culture.

References

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- Picture taken by Lisa Gherardini

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