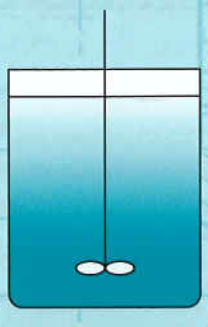
Technical Data Sheet

STS90

BioSep:
the advanced acoustic cell retention device

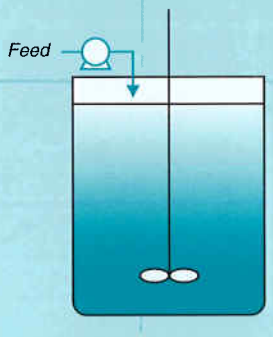


BATCH

Duration: 0.5-1 weeks

Cell density: 2 x 10^6 c/ml

Finish: no more nutrients



FED - BATCH

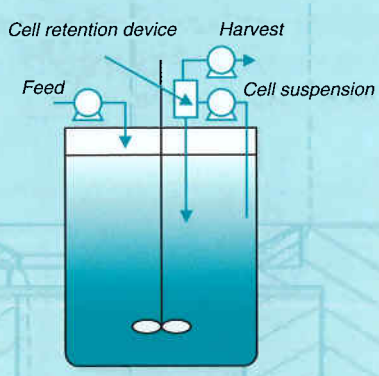
Addition of concentrated nutrients

=> higher product concentration.

Duration: 1-1.5 weeks

Cell density: 2.5 x 10^6 c/ml

Finish: Viability < 50%



PERFUSION

Addition of nutrients with cell retention.

Duration: 1-3 months

Cell density: 20 x 10^6 c/ml

AppliSens
SENSOR INNOVATION

With the progression of the genomics initiative, increasing numbers of proteins will need to be produced rapidly.

The growing demand for novel proteins has motivated the development of more efficient and reliable mammalian cell culture production technologies. This currently is resulting in a spreading use of simpler, more productive processes.

Perfusion is the technology to use, providing:

- ***high cell density***
- ***high (volumetric) productivity***
- ***cost-effective operation***

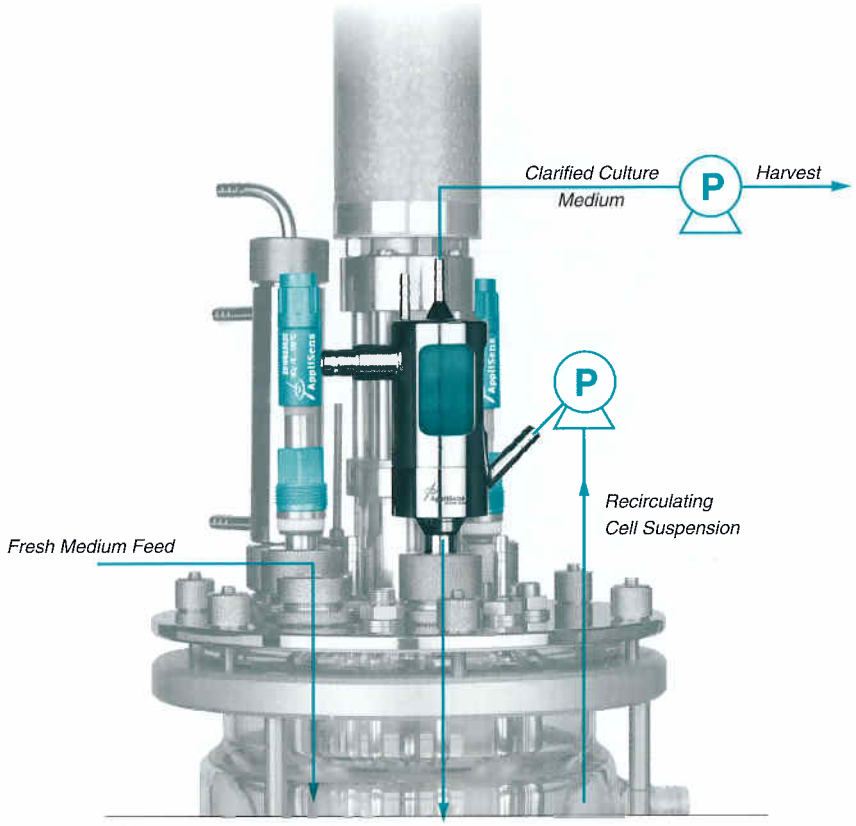


Fig. 1: *perfusion set-up with BioSep.*

In stirred perfusion cultures, high cell densities (over 10^7 cells ml^{-1}) can be achieved by separating cells from the outflow stream, and retaining them in the reactor (fig. 1) while fresh medium is added.

The volumetric production in perfusion cultures can be almost two orders of magnitude higher than in a batch. In some cases the product concentration is increasing up to 5-fold:
➔ the required bioreactor volume can be reduced dramatically (100-fold)!

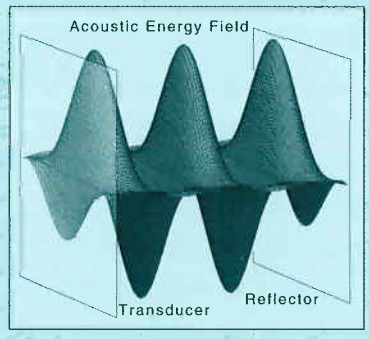
Because perfusion cultures can last for months, it's obvious that there are economic benefits amongst which are reduced labor requirements for bioreactor inoculation and turnaround.

With the innovative technology of the ultrasonic separation, production costs in pharmaceutical industry can be dramatically reduced.

Purely based on sound, an invisible energy mesh is created: **the BioSep, a filter that never will foul.**

Result of the invisible and harmless energy mesh

Cells appear as evenly spaced visible vertical lines in the viewing window of the BioSep chamber. They are held by ultrasonic forces against the upward flow of the culture medium. The acoustic forces form a barrier to the cells, eliminating the need for mesh or membrane filters.



The BioSep from AppliSens is the first reliable and economical solution for the realization of mammalian cell perfusion processes.
The acoustic separation technology of the BioSep can be applied on research, pilot and production scale.

Perfusion processes using the BioSep acoustic separator typically involve continuous addition of fresh medium to the bioreactor, while cells are filtered from the harvest stream by the BioSep chamber and returned to the bioreactor. The BioSep chamber can directly be mounted onto the bioreactor head plate.

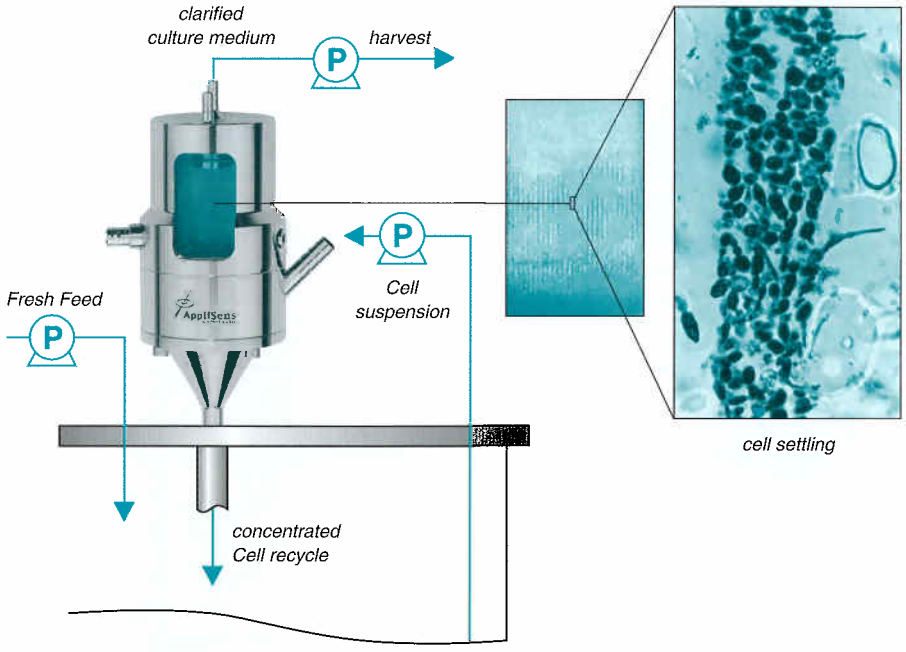


Fig. 2: *Typical configuration of the acoustic cell retention system.*

Several modes of operation are available making acoustic perfusion generally applicable for suspended mammalian and animal cell culture, but also for anchorage dependent cell lines, or for the perfused culture of plant cells (see literature reference list).

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 a “virtual”, thus superior non-contact, non-fouling, non-moving filtration means. The technology allows for up to thousands of hours of continuous operation. As a result, greatly increased steady state cell density, productivity, and product quality is obtained.

BioSep acoustic filters are not designed to ultra-purify the harvest stream from any cells. In contrast, a small escape rate allows for controlled cell bleeding and positively contributes to the viability of the culture (see publications).
Typical separation efficiency of the BioSep ranges from 90-99%.

The BioSep chamber is mounted above the bioreactor head plate. The cell suspension is pumped into the chamber by the recirculation pump. The flow is then split into the harvest flow and the return flow. The flow rate through the BioSep is controlled by the harvest pump. The ultrasonic forces in the BioSep aggregate and hold the suspended cells stationary against the harvest flow, thereby clarifying the harvest stream. The planar aggregates appear as parallel lines when seen from the side through the viewing window. Aggregated cells that settle from the resonator are rapidly recycled to the bioreactor in the return stream where they are dispersed by the impeller.

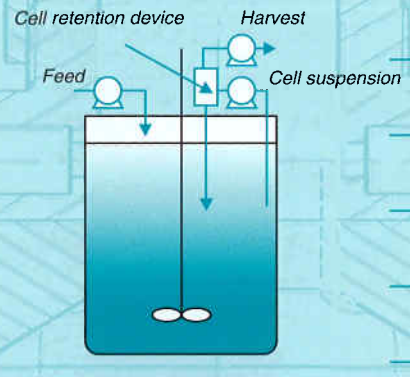
Conventional cell retention devices include filters, settlers and centrifuges. Regardless of their design, the filter surfaces are susceptible to fouling. Settling chambers and centrifuges solely rely on the difference in density between cells and medium.

Settling chambers	***Centrifugation***	**The BioSep**
require, a large settling area and long settling times due to the small difference in density. This leads to prolonged exposure of the cells to an uncontrolled environment.	*the sedimentation process is enhanced by centrifugal forces many times the force of gravity. The separation efficiency of a centrifuge is a function of a multitude of operating parameters. Mechanical systems such as centrifuges are susceptible to failure and cells are exposed to high shear forces.*	**simple and compact non-mechanical device in which only harmless sound waves are exploited to separate the cells from the suspending medium.**

BioSep 10 L

BioSep 50 L

BioSep 200 L



PERFUSION

Compared to technologies such as filters, centrifuges and settlers, the BioSep offers an economic separation technique in perfusion cultures:

- **surprisingly simple**
- **highly reliable**

The BioSep chamber assembly is entirely solid state and is unaffected by fouling, rendering it reliable for thousands of hours of continuous operation.
*The **BioSep 10L** is designed to operate at a perfusion harvest rate between 1 and 10L/day.*
*The **BioSep 50L** operation range is between 5 and 50L/day*
*The **BioSep 200L** is designed for both pilot- and production scale. The operating range is between 20L and 200L/day.*