

Contact: John Bonham-Carter
Tel +1 973 952 0002 jbonhamcarter@refinotech.com

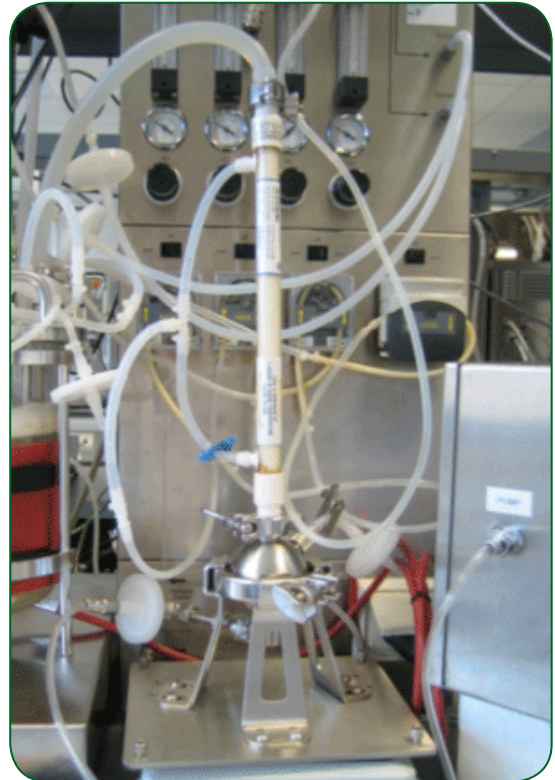
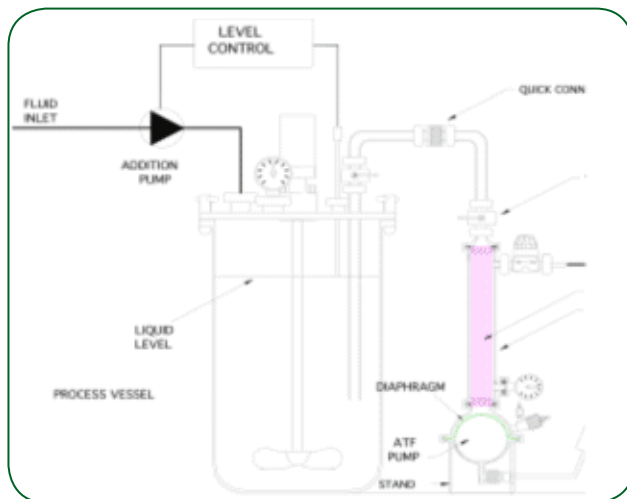
▶ Perfusion / Cell Retention Application

Set-up

There are three methods to attach an ATF™ System to a bioreactor, depending on the type of bioreactor you use.

Autoclavable glass reactors

The ATF is attached with special molded tubing via a diptube, and both are autoclaved together.



Single-use reactors

The ATF is autoclaved with tubing and a single-use aseptic connector, such as Pall Kleenpack or GE ReadyMate, and then a sterile connection is made to the reactor.

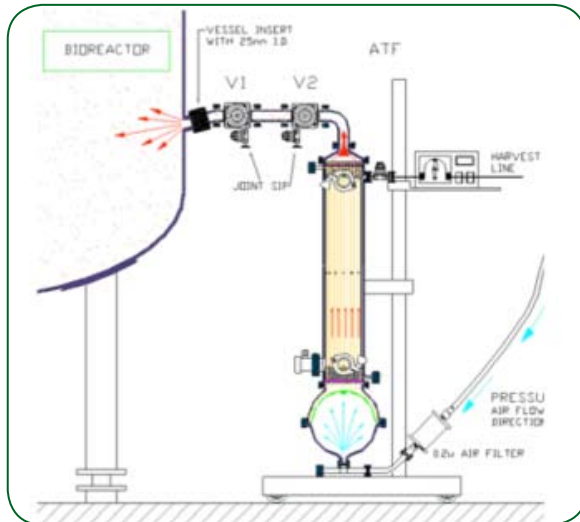


▷ Perfusion / Cell Retention Application

Set-up (cont.)

Steel reactors

The ATF is autoclaved and then connected to a reactor port via a steamed reesterilizable valve connection



Cell Culture Start-Up

The reactor is filled to its working volume with media and then inoculated with cells, e.g. 0.5×10^6 viable cells / ml. A batch culture is run for 2-3 days. The ATF System is then started and within 5-10 minutes the reactor and the ATF are in fast equilibrium. The ATF rate (cross-flow) is set to be at least 100 times faster than the filtrate (harvest) rate (e.g. for 140L / day harvest, the ATF will run at 14,000L / day = 10 L /min which is a typical flow for an ATF6 System). The filtrate pump is then started.

Filtrate Flow Rate

In the simplest state and under manual control, for example, the filtrate rate starts at 0.5 vessel volumes per day and is increased by 0.5vvd every 3-4 days until either 2 or 3 vessel volumes per day is reached (dependent on whether the cell line can be more productive at 3vvd than 2vvd). The rate then stays constant while a 5% or 10% bleed may be introduced if desired.

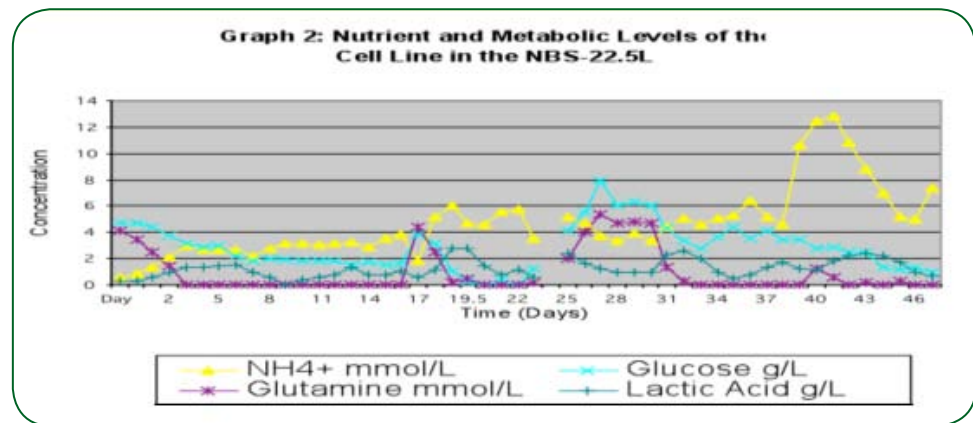
However, it is better to control the filtrate rate to be more closely aligned to the viable cell concentration and the metabolic demands of the cells – a cell density or capacitive probe is ideal to link via the bioreactor control system which then controls the filtrate pump. Once a day a sample can be taken to ensure that the sensor is correct. If a probe is not available, the off-line measurement can be used to make a once a day adjustment to the filtrate flow rate manually. It is important to measure the glucose, amino acids, waste metabolites and any other known critical parameter, and make appropriate filtrate rate adjustments, to ensure the cells are constantly kept in the same healthy environment.

A guideline for optimized media would be that each vessel volume of media per day could support from 30 to 60 x 10^6 viable cells /ml in a steady state production, depending on the cell line used.

▷ Perfusion / Cell Retention Application

Set-up (cont.)

For example, it may be that the glucose concentration seems sufficient, but that the ammonia or another by-product concentration is rising and requires removal via a higher filtrate rate. This would lead to wasting media. Therefore media composition and its development is key to ensuring a complete yet low cost solution, and the cell metabolism must be looked at in both inputs and outputs to ensure the optimum media composition is designed.



In this graph you can see that the glucose level is kept at about 2g/L until day 18, when it collapses to near zero. At the same time, the other metabolites monitored also move quickly away from the optimum. Several days later the glucose is then brought back up higher, and the other concentrations move back towards optimum levels, while the ammonia level moves gently up all the time.

From analysis of only this graph, you can guess that the viable cell concentration in the reactor grows well until about day 18, and then falls, possibly significantly. With the recovery of glucose, the viable cell concentration can rise again and probably achieves a good level based upon the lactate and ammonia we see produced. This is in fact what happens.

Choice of Filtrate Pump and Control Loop

The peristaltic pump must make a tight enough seal to ensure the vacuum on the filtrate side is maintained (which is the transmembrane pressure), e.g. a pump with at least 4 roller heads. This is because the ATF exerts a backflush on each cycle that pulls a small amount of liquid from the filtrate back across the filter into the reactor. The ATF System does not control the pump flow rate – it is controlled manually by the user or automatically by the bioreactor control system.

There are two common types of pump used, intelligent and simple.

In manual control – without any on-line sensor – a calibrated peristaltic pump, for example including an integrated microprocessor, is used to control the filtrate flow and adjusted daily, or twice daily, to ensure the correct flows are being reached. Generally, only 10-20% accuracy is possible with this system due to variations and stress in the tubing (which must be regularly checked) and, at small scale, the fact the pump will most likely be on a proportional timer which is subject to inertial errors (e.g. on for 1 second, off for 9 seconds will not give an accurate flow of 10%).

▷ Perfusion / Cell Retention Application

Set-up (cont.)

Using a simple pump will require it is integrated to the reactor control system which then controls the flow rate (similar to fed-batch operation). This allows for more accurate control, via analogue output and/or feedback from a weigh scale, plus has the advantage of being able to datalog the flow. Additionally, the flow rate can be under closed loop control with an on-line measurement feedback, such as glucose or cell concentration. However, this requires a little knowledge to program the control system correctly.

Feed Rate

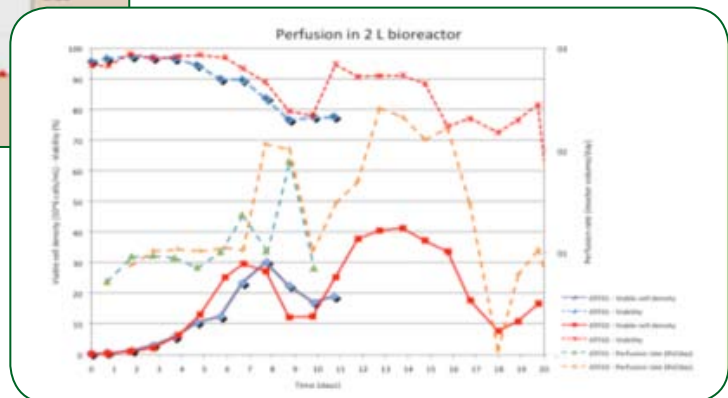
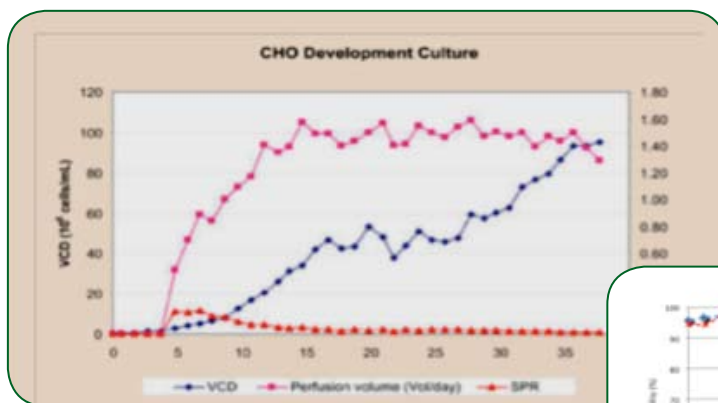
The feed into the reactor is of a complete medium (as described above). The rate should be controlled via a level probe or weigh scale, as the filtrate pump removes material, the level control detects a drop in the reactor and turns on the media feed pump.

If a level sensor is not available (e.g. with a Wave reactor), then we advise the reactor is placed on a weigh scale or load cell and a constant weight is maintained.

General Process Operation

The bioreactor control system measures and controls all cell related parameters, including all media flows and peristaltic pumps. The ATF controller only controls the ATF rate (which is the cross-flow rate). During the cell cultivation process, the ATF often requires no intervention. The increase in filtrate/perfusion rate during the run will be very small in comparison to the ATF rate.

Results and Discussion



▶ Perfusion / Cell Retention Application

Set-up (cont.)

(note the discussion is purely the opinion of Refine Technology with limited input from organizations involved).

The two graphs shown here are produced with kind permission of CMC Biologics (left) and KTH, Royal Stockholm University (right). The left graph (CMC) shows that perfusion starts on day 4 at 0.5vvd, whereas on the right (KTH) perfusion starts on day 1 at approximately 0.75vvd. CMC continues to increase the rate more or less following growth rate until 1.5vvd is reached after 2 weeks and with about 35m viable cells. KTH keeps the perfusion rate constant from the beginning and shifts it up as the viability drops on day 7 when the viable cell count is about 30m – a point only reached by CMC a week later. Unfortunately the reactor control system at KTH was not able to handle the oxygen demand coupled with the extra foam and so the process becomes unstable, even though 40m viable cells are produced after two weeks. CMC however controls the viable cell concentration well as it rises to nearly 100m after a month while keeping the perfusion rate rather low at only 1.5vvd - this is useful as a process suited to support Tox or phase 1 manufacture.

It is clear that for a University with limited equipment there are problems to control such high cell densities, however it is also useful to note that on the very first run with the ATF with this limited equipment, with unoptimized media and a weak cell line, such high cell concentrations are achievable!

In order to develop a reliable process with cell concentrations of 70-80m or more, good oxygen control and two spargers is recommended. Attention must be paid to minimize the generation of foam and its control in the bioreactor.

Cleaning

The ATF System is broken down into parts and placed in a washing machine. No CIP is required.

Sterilization

The ATF System is always autoclaved and is never sterilized in place (SIP). Even at manufacturing scale, the ATF System is autoclaved.

With thanks to Veronique Chotteau, KTH, and Jacob Jensen, CMC Biologics.