Single-Use Bioreactor

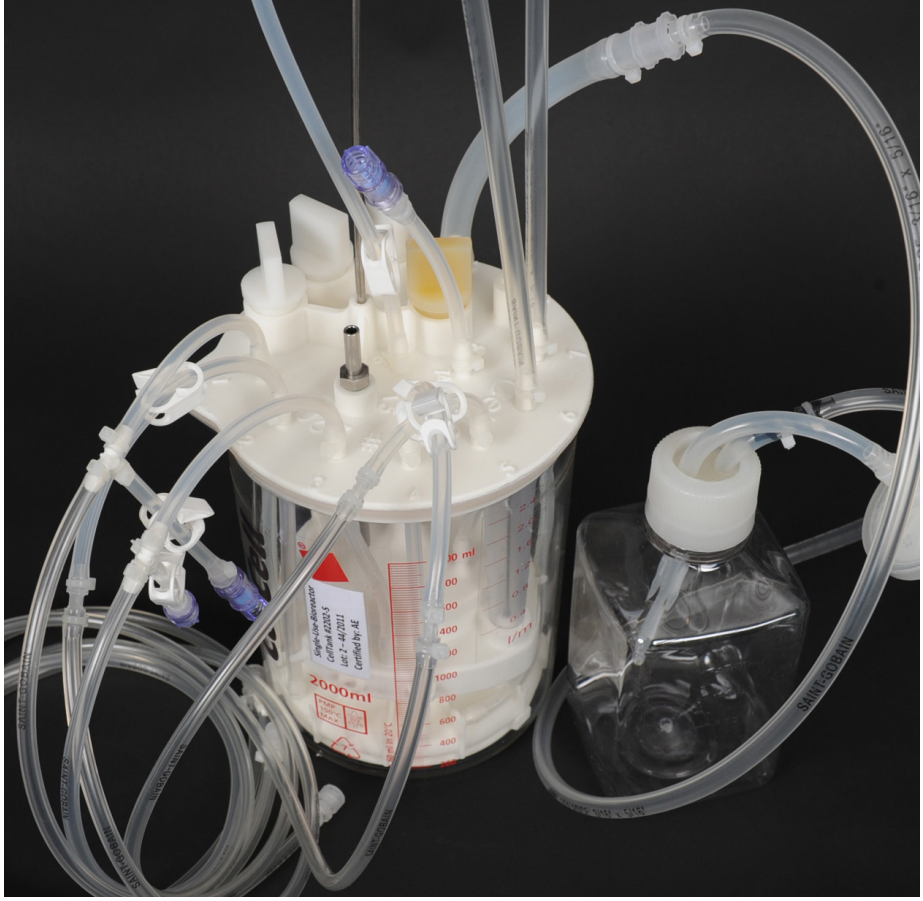
Scalable perfusion platform cultivating suspension and adherent cell lines

5 easy steps to single-use perfusion

1. Remove ready-to-use SUB delivered E- beam irradiated in dual foil bags
2. Connect CP-SUB with sterile media and harvest bag, sensors and mixed gas
3. Pump sterile media into the SUB and condition the temperature
4. Calibrate the pre-installed bio mass, pH and dO2 Single-Use-Sensor's
5. Inoculate suspension or adherent cell line and start cultivation for weeks of continuous product expression

ProlifeCell

2013



CellTank perfusion CP-SUB platform scalable 1:1000

Perfusion is the future method of cultivation and as the cells is harboroud inside the matrix exchange of fresh media to constant harvest is an easy task.

The CellTank replaces auto-claving for just 5 days batch operation and creates a single-use processing platform with extended cultivation time

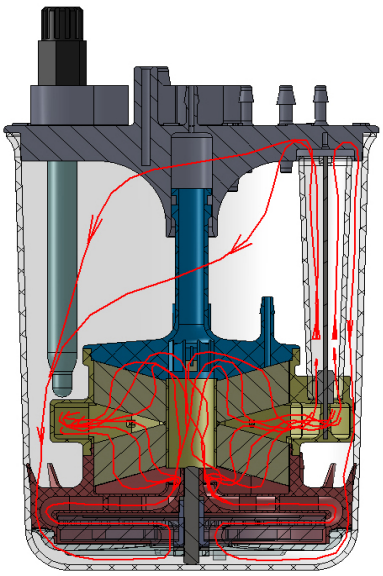
CellTank ranging 150 ml to CellReactor 1,500 ml matrix volume and future 15 litre in just one platform is radically and satisfy all cultivation needs from research to pilot scale production, etc.

CellTank/CellReactor products are engineered:

- to increase volumetric productivity factor 10-50 on your existing Process-Control-System (PCS) in your existing facilities
- to eliminate investments in larger PCS and lab facilities with increasing demand of product
- with integrated classical signal Single-Use-Sensor's (SUS) pH, DO, level, and bio mass probe
- precision E-beam irradiated, disposable and ready to use right out of the bag
- Plug & Play operating on Magnetic-Stirrer-Table drive by servo motors controlled by your PCS

How does it work?

1. The reactor core design is a cylinder with stacked even number of circular envelopes. They are arranged parallel with radial inlet and axial outlet. As the envelope diameter and number is variable the incredible scalability is created. From a few cm^3 until >15,000 cm^3 is the span!
2. Internal re-circulation of media inside the SUB insure constant gradient free access to media, gasses for constant expression of product.
3. Media pump inlet is at the very bottom. The media passes the advanced impeller driven by external magnetic forces or a servo motor. Media exits the pump into the reactor core centre to the triangular volumes and flows further perpendicular through the matrix. Having passed the matrix media is collected in the hollow circumference collection volume in direct correspondence with the media flow instrument.



Illustation of re-circulation flow in CellTank-34

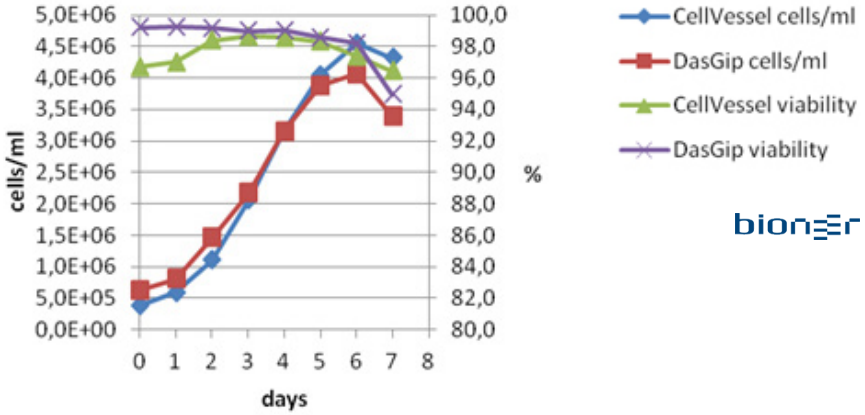
CellVessel single-use platform scalable 1:100

The CellVessel family makes single-use-bioreactor possible and economic instead of the conventional re-usable STR

The range from as small as 50 ml to 6,000 ml working volume in just one platform is radical and satisfy any need to cultivation and fermentation for research or small scale production, etc.

All CellVessel products are engineered:

- to operate 100 % identical to any traditional and autoclaved glass/steel bench-top STR
- to fit in between your cell lines and your existing Process-Control-System (PCS)
- to operate with a variety of turn tables or servo motor drives
- to use classical format and signal sensors with PG13.5 thread – RUS and SUS as you wish
- as precision E-beam sterilized, disposable and ready to use right out of the bag



CellVessel SUB vs classical STR

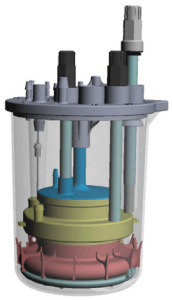
CellVessel STR is fully functional (cells survive, multiply, and produce antibodies) and it performs almost exactly as the DasGip STR (for this specific cell line).

Antibody produced amounts to 77 µg/ml for the CellVessel STR as compared to 76 µg/ml for the DasGip STR. Furthermore, the figure below clearly demonstrates, that cell viability during exponential growth are the same for the two reactors (well above 98 %) and that the maximal cell densities achieved are the same for the two reactors.

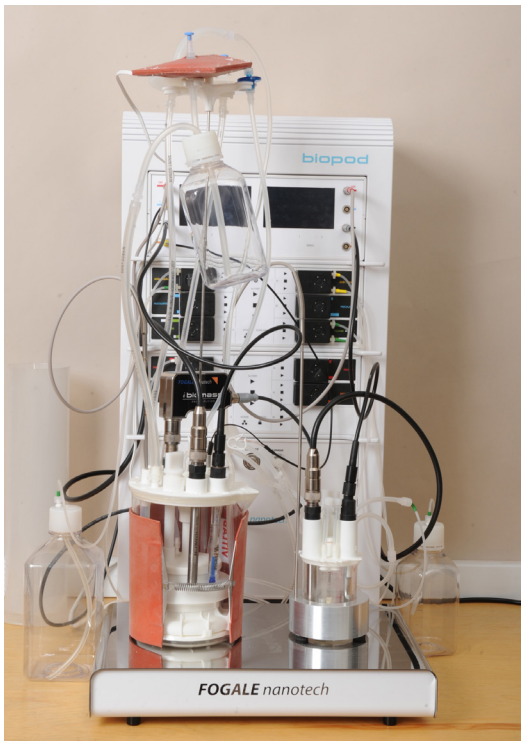
If anything, cell density achieved for the CellVessel STR (4.6x10E+06 cells/ml) are somewhat higher than for the DasGip counterpart (4.1x10E+06 cells/ml).
The work was performed Q1/2012 by senior scientist Holger K. Riemann at www.bioneer.dk

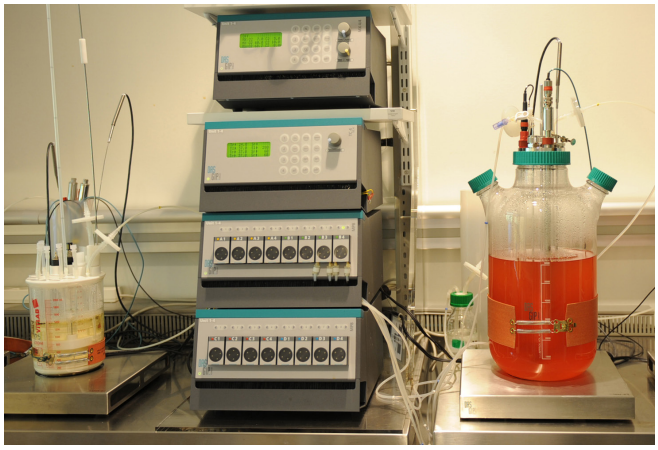
SUB and STR easily adaptable to all controllers

Applikon



Belach

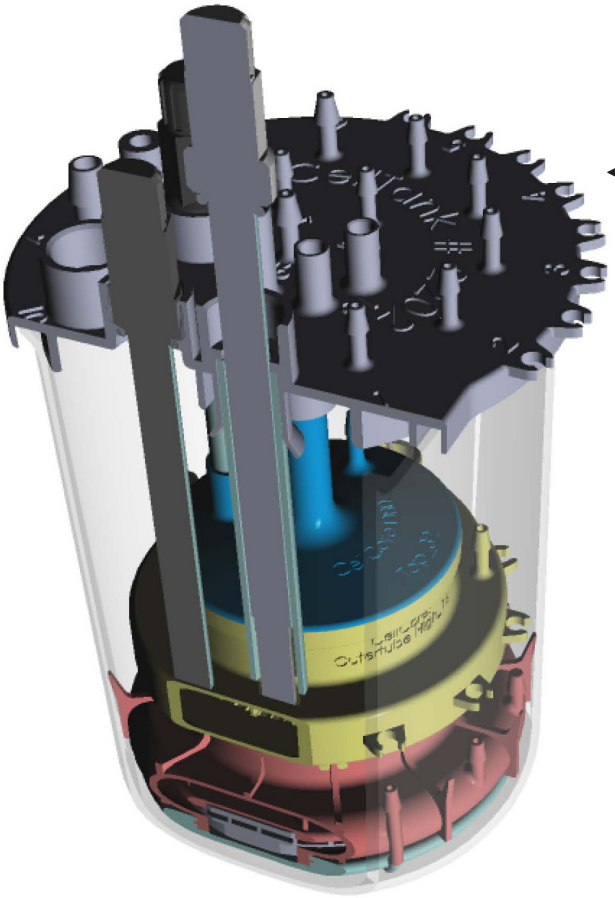




◀

Work at Bioneer in Denmark on CellTank-34 in perfusion on DasGip MP8 with identical suspension CHO cell lines compared against autoclaved 8 litre glass STR. Both STR and CP-SUB hold 1.5x10E10 cells. The SUB operates for weeks or month in perfusion setup. The traditional STR with 6 litre ww was cultivated for 6 days before termination as to extensive lactate levels.

Integrated Single-Use Sensors



◀ **3D cut through the CellTank** illustrating at left the 120 mm pH SUS and at right the re-usable Visiferm dO2 probe fitted to the non-invasive well with the SUS optical membrane in the front. Visible 120 mm SUS bio mass sensor behind pH sensor.

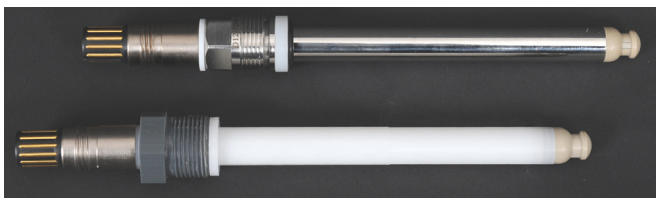
Photo of CellTank-34 below show:

- non-invasive well with inserted dO2 probe
- non-invasive well for temperatur, empty
- single-use ph sensor installed
- single-use bio mass sensor installed
- single-use mass flow rotameter installed

▼

Hamilton Single-Use-Sensor's (SUS) for dO^2 and pH offer the following advantages:

- Integrated SUS eliminate contamination risk
- Save hours of prep time and labor, as no autoclaving or cleaning is needed
- Enable SUS setup right on the bench – no biosafety cabinet / hood needed for operation
- Optical membrane in non-invasive well for re-usable VisiFerm classical DO probe
- Extend DO probe life, as it is never autoclaved
- Classical pH probe for extended lifetime needed for months of perfusion cultivation
- Classical pH and dO2 sensor signal fit any PCS



▲

CellTank´s integration of Fogale's single-use capacitive sensing technology allows precise on-line monitoring of the cell mass, viable cell density as well as cell physiological state. Users can also track cell cycle changes, model apoptosis, and predict protein titer all in real time, and this from inside the scaffolding matrix.

Study of perfusion process of CHO cells in CellTank bench-top Single-Use-Bioreactors with 150 cm³ matrix

Introduction

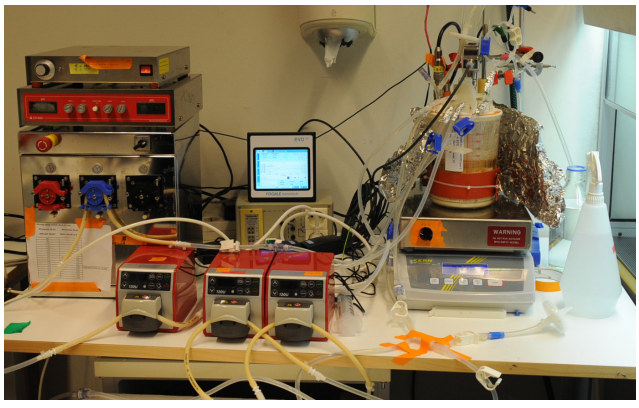
Major advantages of perfusion are high cell numbers and high total production in a relatively small size bioreactor. Moreover, perfusion is optimal when the product of interest is unstable or if the cell line product yield is low. On the other hand, disadvantages are for example technical challenges originating from non-robust cell separation devices as well as sterility concerns from the more complex set-up needed.

Recently, ProlifeCell (Denmark) has developed a perfusion integrated Single-Use-Bioreactor (SUB) named CellTank that operate on magnetic-stirrer-tables. A revolutionary matrix for cell densities beyond 100 million cells/ml is integrated in the CellTank and the suspension cells stay harboured inside the matrix.

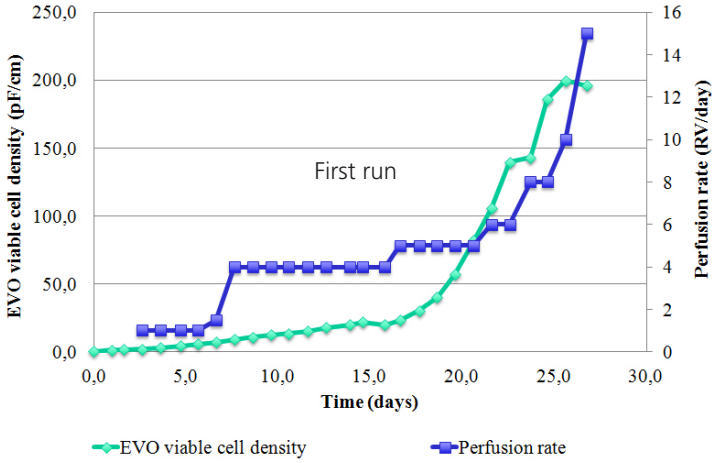
The EVO 200 iBiomass System (FOGALE nanotech) uses the dielectric properties of living cells and is capable of measuring on-line the live cell density independently of cell size variations.

In the present work, the CellTank-34 bench-top CP-SUB with 150 cm3 polyester fibre matrix was investigated. Perfusion cultivations were performed using a recombinant CHO cell line producing a monoclonal antibody as a model system.

The first trial was a capacity test; the system was pushed to its extremity in regards of maxium cell number and a cell density of ~2x10E+08 cells/ml was achieved. In the second trial the aim was to achieve 1x10E+08 cells and evaluate stability in the system.



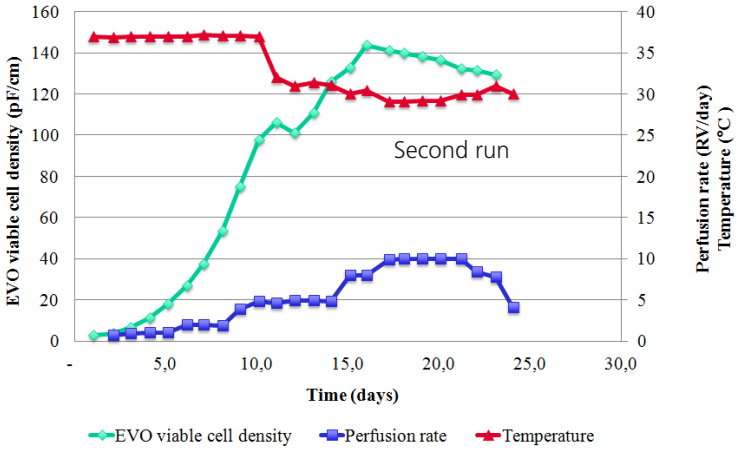
Experiemental setup at KTH on Belach system.



One pF/cm read on the EVO bio mass sensor system is equivalent with 1x10E+06 viable cell/ml

First run (medium and setting adjustments days 0 to 14) followed by exponential growth

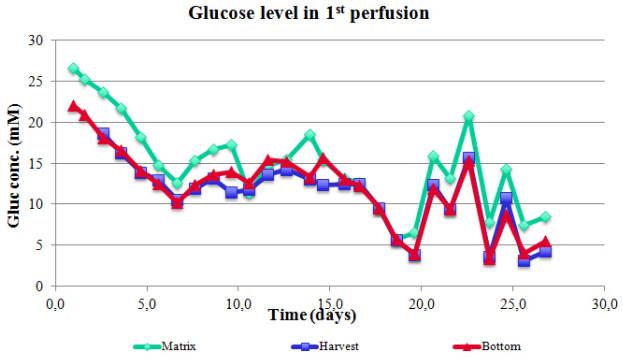
Cell density up to 2x10E+08 viable cells/ml after 25 days of cultivation at a perfusion rate 10RV/day

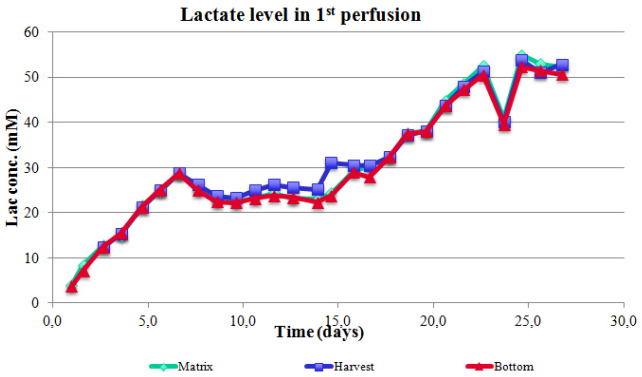


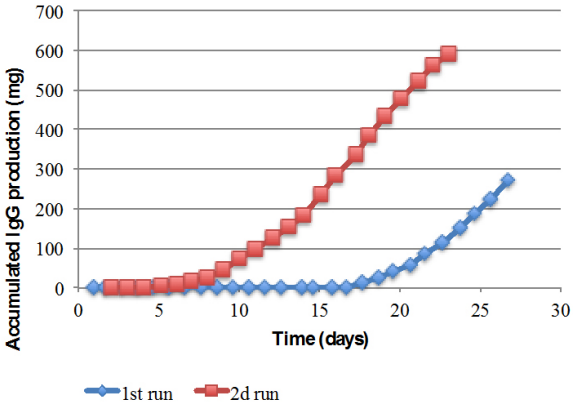
Cell density kept ≈1.3x10E+08 viable cells/ml at perfusion rate of 8-10 RV/day for over 10 days

Temperature lowered from 37°C to 32°C on day 10, to 31°C on day 11 and to 30°C on day 14, resulting in only partial growth arrest.

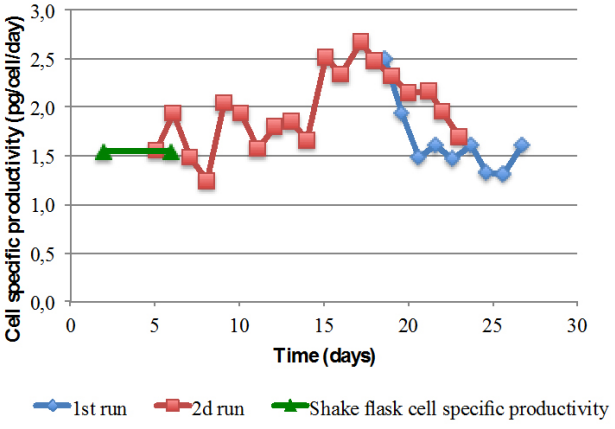
Temperature decreased to 29°C on day 16, resulting in complete cell growth arrest. The cell density was then maintained at a stable level for the following 9 days until test end.







Product accumulated nicely with time and increasing cell density (first after day 14 for 1st run)



Cell specific productivity in perfusion mode comparable to shake flask productivity except at 30˚C where productivity was ≈40 % higher.

Samples taken from matrix sample port, harvest sample port and bottom (reservoir) sample port.

- There are no obvious difference among different sample locations
- Glucose and glutamine concentrations slightly higher in matrix samples
- Lactate and NH4+ concentrations slightly higher in harvest samples
- In agreement with the fact that the fresh medium goes into the CellTank core directly

Conclusion of KTH

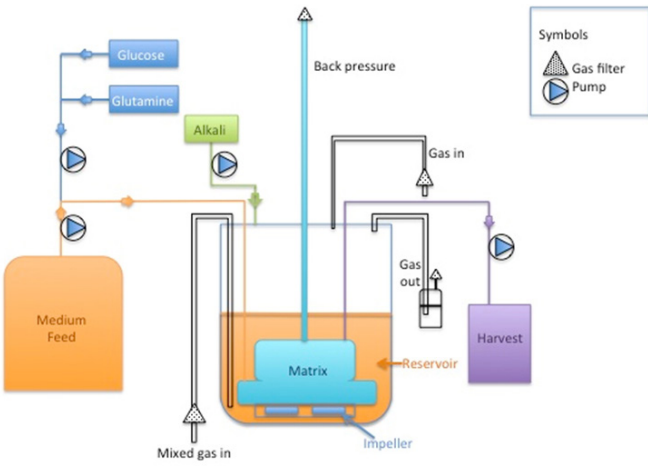
- Very high density of 2x10E+08 viable cells/ml achieved at perfusion rate 10RV/day (1RV=150ml).
- Very high cell density of 1.3x10E+08 viable cells/ml maintained for 11 days at perfusion rate 8-10RV/day with temperature lowered gradually from 37°C to 29°C.
- The use of a Single-Use-Bioreactor equipped with a real-time cell mass sensor offers a solution alleviating technical and sterility challenges occurring in perfusion processes. Operation using CellTank SUB is much easier and more handy, compared with traditional perfusion technologies, with robust integrated perfusion device. The CellTank is very lightweight, easy to transfer into laminar flow cabinet if sterile operation is required.
- IgG accumulated nicely with time and increasing cell density with a cell specific productivity comparable or higher to batch culture. No retention of IgG was found in the matrix.

See appendix next page for more info

The work was performed Q4/2011 at School of Biotechnology, Cell Technology Group (CETEG), Royal Institute of Technology (KTH), Stockholm, Sweden by MSc Ye Zhang, PhD Veronique Chotteau and financed by the Swedish Governmental Agency for Innovation Systems (VINNOVA).

Appendix for KTH

CHO DP-12 clone 1934 (ATCC) producing monoclonal antibody was used for this study. A prototype CellTank-34 Single-Use-Bioreactor with 150 cm3 PET matrix was equipped with Fogale bio mass sensor. The cultivation system was controlled by Belach PCS, Phantom software and CerCell MST. Watson-Marlow 120U pumps were used intermittently for fresh medium feeding, harvest and supplementary glucose or glutamine additions. The set-points of DO, pH and temperature were 40%, 7 and 37°C respectively. The internal re-circulation flow targeted to 1,6L/min. The actual impeller speed (range 280 – 500 rpm) was adjusted daily to obtain the target re-circulation flow due to the increasing back pressure as a function of bio mass. During the 1st experiment, the air, O2, CO2 and N2 was mixed before adding into the reactor through the open tube sparger. A general setup for the system is given here. N2 was continuously added to avoid too high DO in the culture. During the 2nd experiment, only O2 was blown through the open tube sparger, while the addition of the other gases were performed to the headspace.



The cells was cultivated in animal-component free IS CHO CD XP medium (Irvine Scientific, USA) with hydrolysate, supplemented with 3% of IS-CHO Feed-CD XP (Irvine Scientific, USA) and 2 mM glutamine. Supplementations of glucose or glutamine were performed according to the cell need. The pH was controlled by adding 0,5 M Na2CO3 or pulsing CO2 into the mixed gas inlet (Exp.1) or headspace (Exp.2). The viable cell density was measured by EVO 200 biomass sensor (Fogale nanotech) mounted with measuring electrodes inside the matrix. pH, pCO2, concentrations of glucose, lactate,glutamine, glutamate, ammonia and osmolality were measured by Bioprofile FLEX (Nova Biomedical). The mAb concentrations were analyzed by protein A HPLC.

Appendix for Bioneer

Experimental design

Suspension cultures of identical cells grown in:

- CerCell product CellVessel p/n 21-2000 single-use magnetically agitated spinner vessel, 300-1.000 ml wv
- DasGip p/n BSO500TPSS magnetically agitated glass spinner vessel with stainless steel head plate and pitched blade impeller, 300-900 ml wv
- Batch-mode for both reactors (i.e. no perfusion and no sugar shots)
- Same CD-medium used for both cultures
- Same seeding density for both reactors: Approx. 5x10E+05 cells/ml
- Both reactors are sparged from below through tubing
- The two reactors are equipped with identical autoclaved PG13,5 x 120 mm sensors (oxygen, pH)
- Both bioreactors are guided by the same DasGip MP8 controllers and M10 magnetic/inductive drive
- Controller settings identical for both reactors:
  - Oxygen tension: 30% of ambient air
  - pH 7,0
  - 37°C
- MP8 controller settings for the two reactors:
  - Stirring: 80 rpm for the DasGip STR versus 60 rpm for the CellVessel STR
  - Aeration: 3L/hr for the DasGip STR versus 1L/hr for the CellVessel STR

More at www.prolifecell.com