KTH Biotechnology

Document type	Application note
Title	Study of perfusion process of CHO suspension cells with CellTank 2202 prototype, bench-top Single-Use-Bioreactors (SUB) with 150 cm^3 CellCore matrix at KTH
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2 Introduction

2.1 Perfusion study in CellTank bioreactor

The CellTank bioreactor (CerCell) has been developed to perfuse adherent or suspension high cell density mammalian cell cultures, i.e. cultivation systems with continuous renewal of the cultivation medium containing the nutrients. The CellTank is a matrix caged in a cassette, which is immersed in a larger tank or reservoir, see Figure 1. The cells are entrapped in the matrix and the reservoir contains circulating cultivation medium free from cells. The bioreactor has been designed to ensure a homogeneous distribution of the fluid component in the matrix. The medium is re-circulated through the matrix thanks to a rotating impeller pump. The liquid is pushed upwards from the reservoir bottom, entering at the matrix bottom, passing through the matrix and exiting at the top of the rotameter (see next Section), from where it is pouring back into the reservoir.

The CellTank bioreactor had been tested for adherent cells. The present study was a pioneer trial to test this bioreactor for suspension cells. The polymer matrix had been treated to favour cell adhesion of suspension cells despite the natural tendency of these cells to not anchor on support.

The objective of this work was to evaluate the operation of CellTank 2202 SUB prototype with a 150 cm^3 CellCore matrix in perfusion culture using a suspension CHO K1 cell line producing recombinant IgG. Two perfusion runs were performed in chronological order, BOL#1 and BOL#2. The purpose of the first run (BOL#1) was to reach a cell density as high as possible, to assess the cell density capacity of the CellTank 2202 SUB prototype. The purpose of the second run (BOL#2) was to study cell growth arrest at high cell density.

2.2 Perfusion setting

The main functions of the bioreactor and the perfusion process, represented in Figure 2, are the following:

Matrix, impeller and reservoir: The bioreactor is a non-woven polymer **matrix** in a cassette. The matrix acts as a depth filter entrapping the cells. It is immersed in a larger tank or **reservoir** in which a centrifugal **impeller** pump ensures a fast flow circulation through the matrix.

Rotameter: A conical part is sealed vertically on top of the matrix with the larger end upward some 3 cm above the liquid level and is functioning as a rotameter, i.e. a flow rate indicator. This is the only exit of the liquid flowing through the matrix and the harvest is

pumped from this part. Liquid coming out this conical part is pouring back to the liquid phase and has therefore a very effective exchange with the gas phase.

Magnetic stirrer: The bioreactor impeller was magnetically stirred.

Intermediate flask: The medium and supplementations of glucose and glutamine for one day were pumped in the intermediate flask. The liquid of the intermediate flask was pumped into the matrix. The pump was operating 24 h/day but intermittently in order to deliver accurate small flow rate. Usage of an intermediate pump allowed delivery of glucose and glutamine evenly distributed over a day instead of a bolus addition.

Medium Feed: Fresh cultivation medium for one day usage was pumped into the intermediate flask.

Glucose: Stock solution of glucose for one day usage was pumped into the intermediate flask.

Glutamine: Stock solution of glutamine for one day usage was pumped into the intermediate flask.

Alkali: Alkali solution was pumped into the bioreactor automatically under the control of the pH controller.

Harvest: The harvest was pumped out from the bioreactor, collected and weighted.

Gas IN to headspace: A continuous gas flow was blown into the headspace in order to steadily maintain a slight overpressure, ensuring the sterility of the system. A mix of air, N_2 and CO_2 was used. CO_2 was added continuously in order maintain $pCO_2 > 2$ kPa. N_2 was also mixed since otherwise the DO was too high.

Gas IN to sparger: Oxygen was pulsed automatically under control of the DO controller. Sparging was operated in the reservoir so that the sparged bubbles were never entering the matrix and therefore never in contact with the cells. A dip tube obturated at the end and punctured of holes was functioning as sparger.

Gas OUT: Gas outlet was expelled via a bubble flask, i.e. a sterile flask half filled with water in which the gas coming from the bioreactor is bubbling. The flask had a second opening ended by a sterile filter. This allowed a) condensing evaporation water contained in the outlet gas, which might otherwise block the gas filter and b) visual observation that the bioreactor overpressure was maintained.

Pressure indicator: The pressure in the matrix increased with increasing cell density. A tube was mounted vertically on top of- and connected to the matrix. The liquid level (or water column) was an indicator of the pressure in the matrix, i.e. similarly as a barometer.

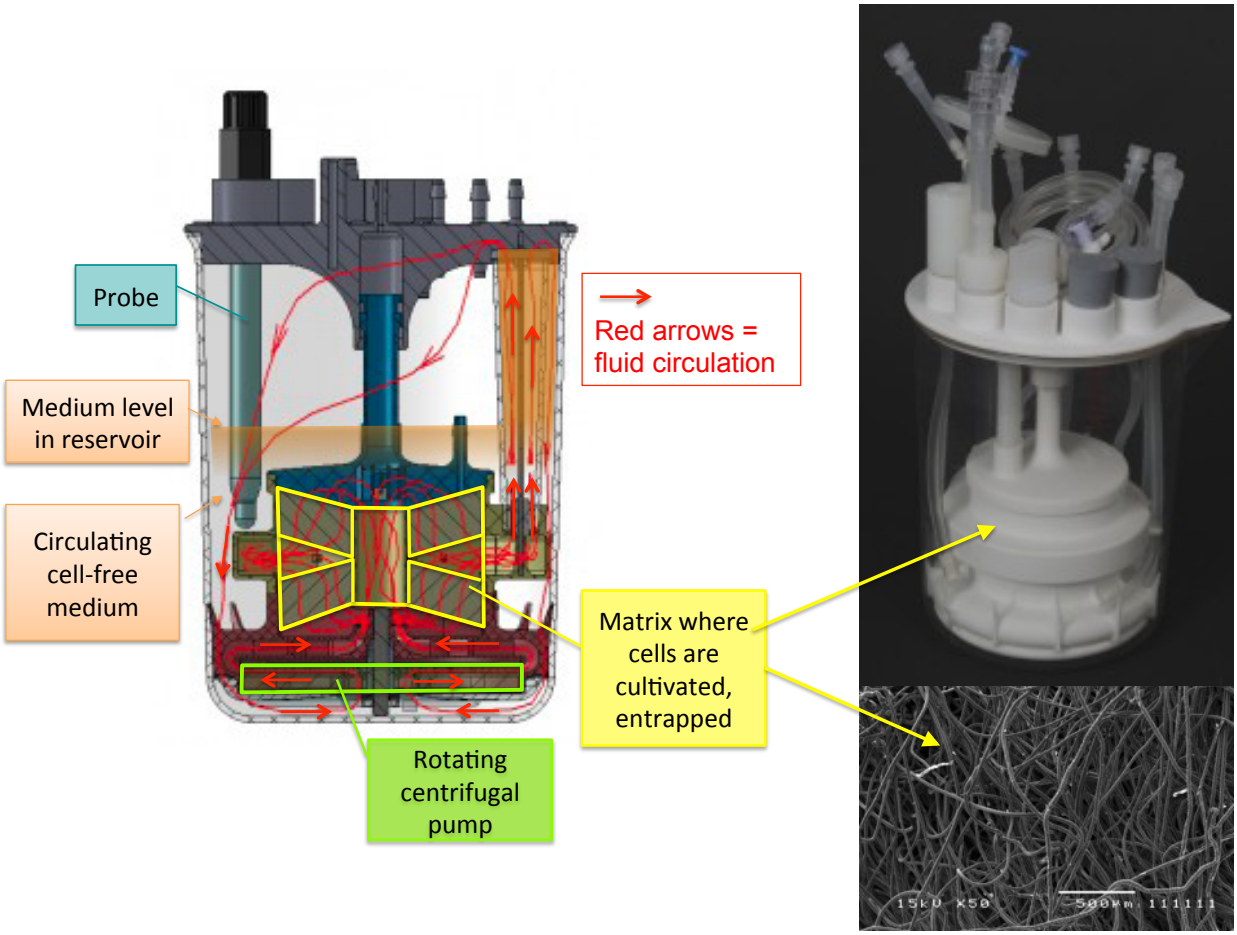

Figure 1: CellTank (modified from http://cercell.com/)

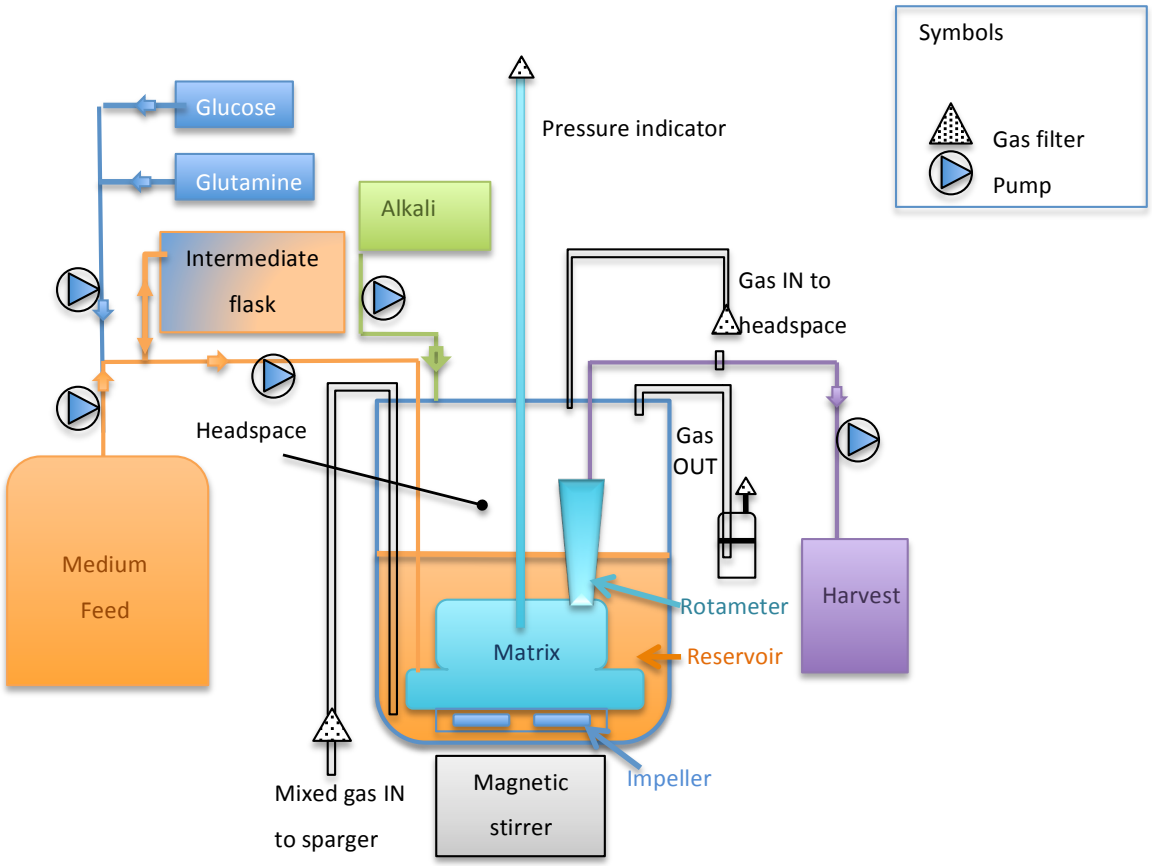

Figure 2: Main functions of the perfusion and bioreactor set-up

3 Materials

The following materials and sensors were used:

- Cell line: CHO DP-12 clone 1934 (ATCC).
- Base medium: IS CHO-CD XP with hydrolysate blend (Irvine Scientific, USA) supplemented with 3% IS CHO FEED-CD XP and 2 mM glutamine unless other specified in text. The medium was supplemented with a solution of antibiotics-antimycotic: streptomycin, Penicillin G and amphotericin (Sigma-Aldrich). This medium contains 1 g/L pluronic F-68. Liquid medium was reconstituted from powder form. The medium pH and osmolality were respectively adjusted to 7 (± 0.1) and to 300-330 mM and the medium was sterile filtrated through a 0.2 µm membrane (Sartorius).
- Additives: Supplementations of glucose (stock solution 2500 mM (45%) Sigma-Aldrich cat. nr. G6152) or glutamine (stock solution 200 mM Irvine Scientific cat. nr. 96700) were performed according to the cell need, targeting low glucose and glutamine residual concentration in the bioreactor.
- CellTank #2202 SUB prototype with 150 cm^3 CellCore matrix.
- Process control system: Belach Biophantom system.
- Gas: Air/O_2/CO_2/N_2
- Temperature controlled via 100 W, 24V heating blanket around the SUB.
- Internal re-circulation performed by the build-in rotating pump driven magnetically by a Belach stirrer table prototype (BOL#1) and a CerCell stirrer table (BOL#2).
- On-line sensors in CellTank:
    - Integrated EVO200 Fogale (PG13.5x120) biomass sensor mounted in the matrix for viable cell density in the bioreactor as well as cell diameter indicator;
    - FermProbe® pH Electrodes, Broadley-James (PG13.5x120) mounted in the reservoir;
    - Dissolved oxygen sensor METTLER TOLEDO (PG13.5x120) mounted in the reservoir;
    - Temperate sensor mounted in the reservoir: pt100;
    - Integrated re-circulation medium rotameter: 0-2.4 L/m;
    - Indicator of the pressure in the matrix: 0-350 mm water column (WC).
- Off-line sample measurements:
    - Concentrations of glucose, glutamine, lactate, glutamate, ammonium: Bioprofile FLEX (Nova Biomedical);
    - Cell count, viability and average diameter of cells leaking from matrix: Bioprofile FLEX (Nova Biomedical);
    - Cell viability: lactate dehydrogenase (LDH cytotoxicity assay);
    - pCO_2: Bioprofile FLEX (Nova Biomedical);
    - Product IgG: HPLC Protein A method.

4 Equipment

4.1 CellTank 2202 prototype set-up

Figure 1 and 2 (see Introduction) introduced the general set-up including the main functions of the bioreactor and the perfusion process. Figure 3 represents the CellTank 2202. Notice that Figure 3 sketch is a version of the CellTank 2202 improved compared with the prototype used in the present study. Therefore the connections of Figure 3 might be slightly different than the ones listed in Table 1.

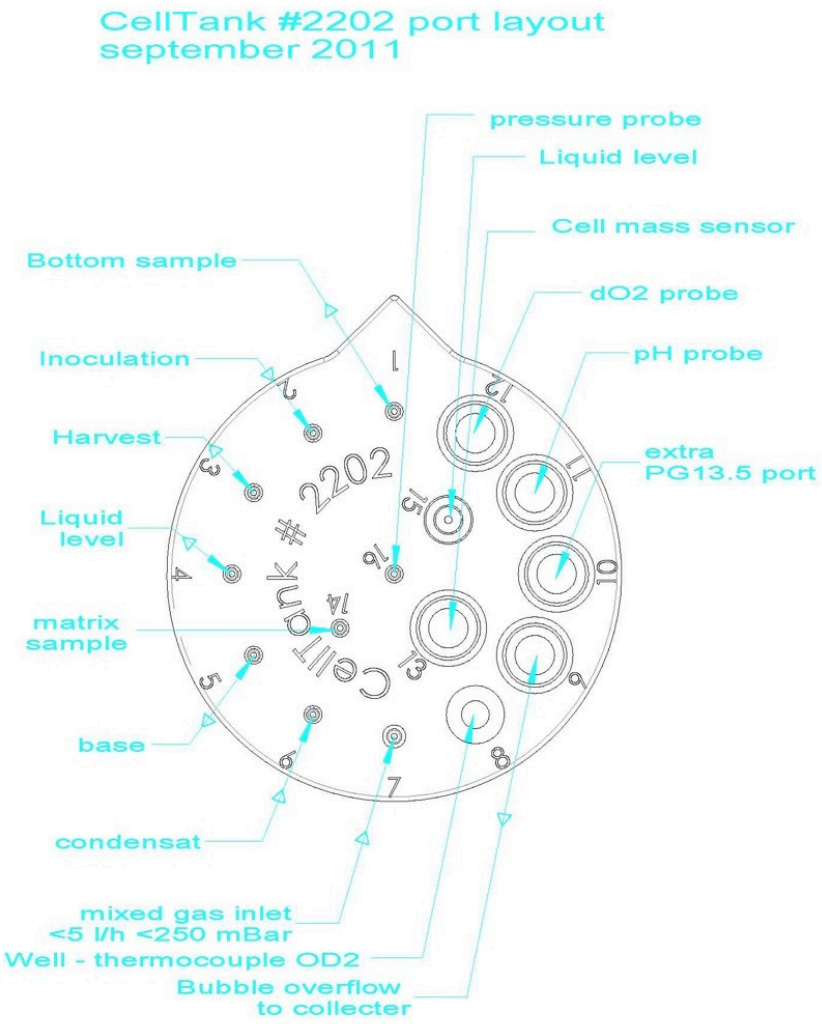

Figure 3: CellTank 2202 design (source: www.cercell.com, Home / Products / CellTank SUB / Guides system 22-0000 / Setup / CellTank connections)

Table 1: CellTank 2202 prototype set-up (in- and out ports)

[1]Port	BOL#1	BOL#2
1	Bottom sample port connected to a needleless connector for sampling	
2	Inoculation/Feed IN connected to tube + MPC connector[2]	
3	Harvest port connected to tube + MPC connector[2]	
4	Liquid level connected to tube + MPC connector[2]	
5	Matrix sample port connected to a needleless connector for sampling	
6	Base inlet connected to tube + MPC connector[2]	
7	Mixed gas inlet to sparger (sparger = obturated dip tube with 2 holes)	
8	Thermocouple temperature sensor	
9	Exhaust gas with sterile filter at the end of the tube	[3]Exhaust gas with sterile bubble flask at the end of the tube
10	Extra PG13.5 port	[4]Head space gas inlet with gas sterile filter
11	pH probe	
12	DO probe	
13	Cell mass sensor	
16	Pressure indicator (WC)	

[1]Same numbers as Figure 2

[2]Silicone tubes, mounted with a Luer connector at one end and an MPC connector at the other end (named 'tube + MPC connector' here), were connected at each port of the bioreactor ended by a Luer. This was performed in order both to obtain a longer tube facilitating sterile operations (e.g. replacement of a flask coupled to the bioreactor) and to use an MPC connector instead of a Luer connector (Luer connector can disconnect 'spontaneously' in case a tube is torqued).

[3,4]Modified for BOL#2 run based on the experience gained from BOL#1 run.

4.2 General Set-up

A scheme over the connections and settings is presented Figure 4. The cultivation medium was protected from light at all time, e.g. with back plastic film.

Pictures of the system used at KTH can be seen in Figures 5 and 6.

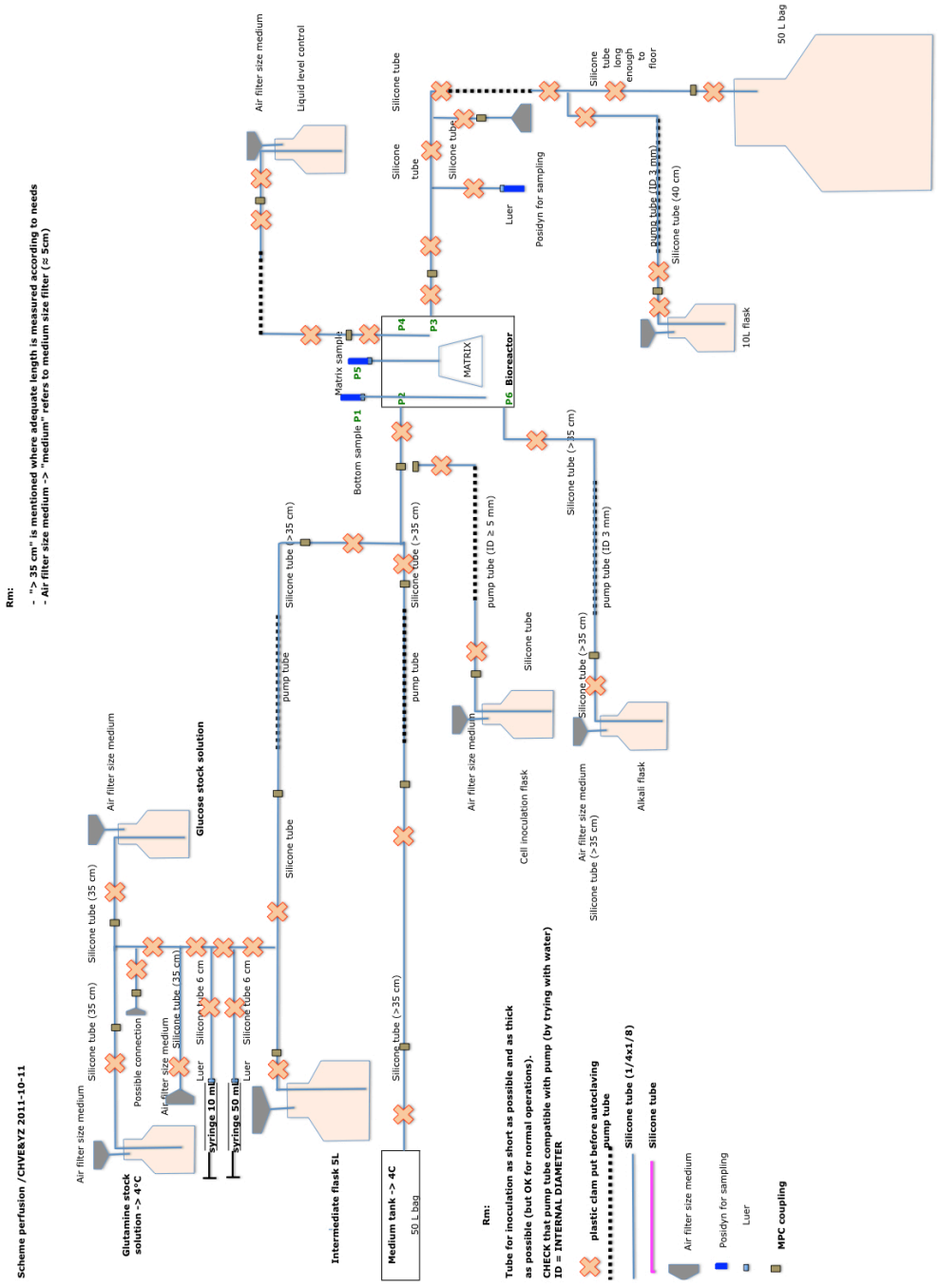

Figure 4: Scheme of the process set-up. All the tubes are silicone tubes except 'pump tubes'. 'Posidyn' are needleless connectors.

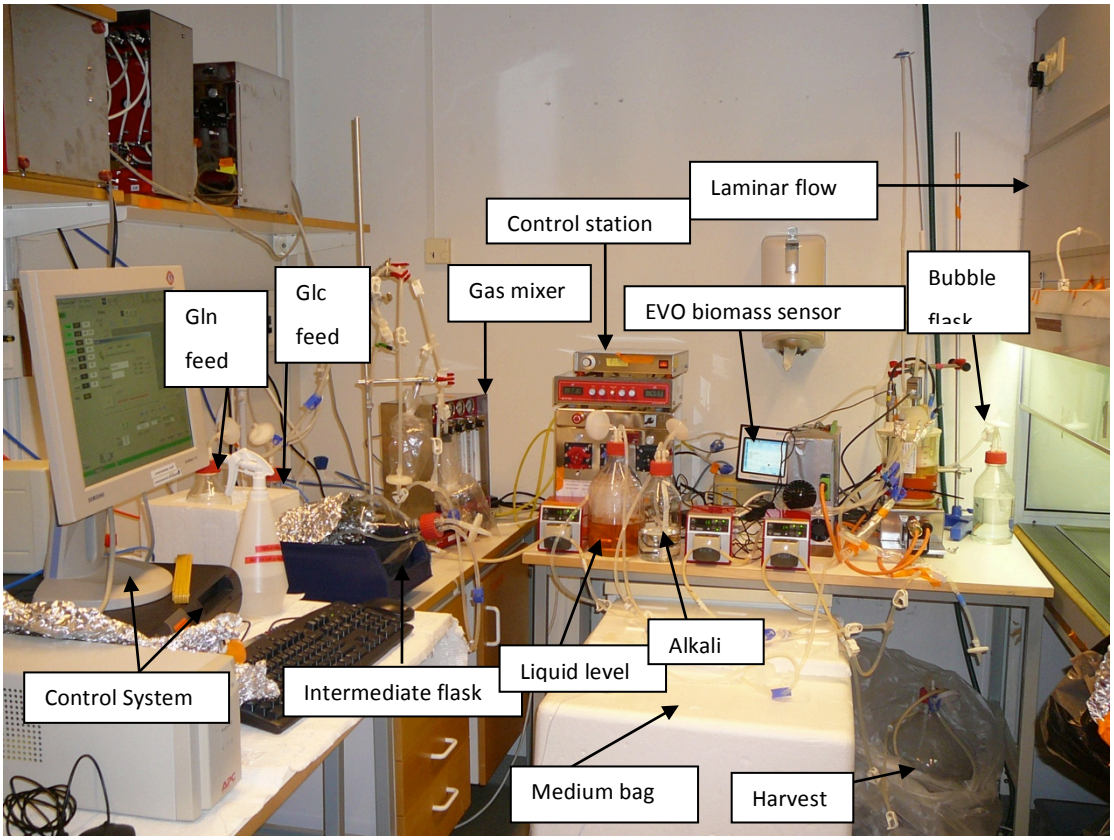

Figure 5: Bioreactor setup at KTH cell lab. Photo: Véronique Chotteau

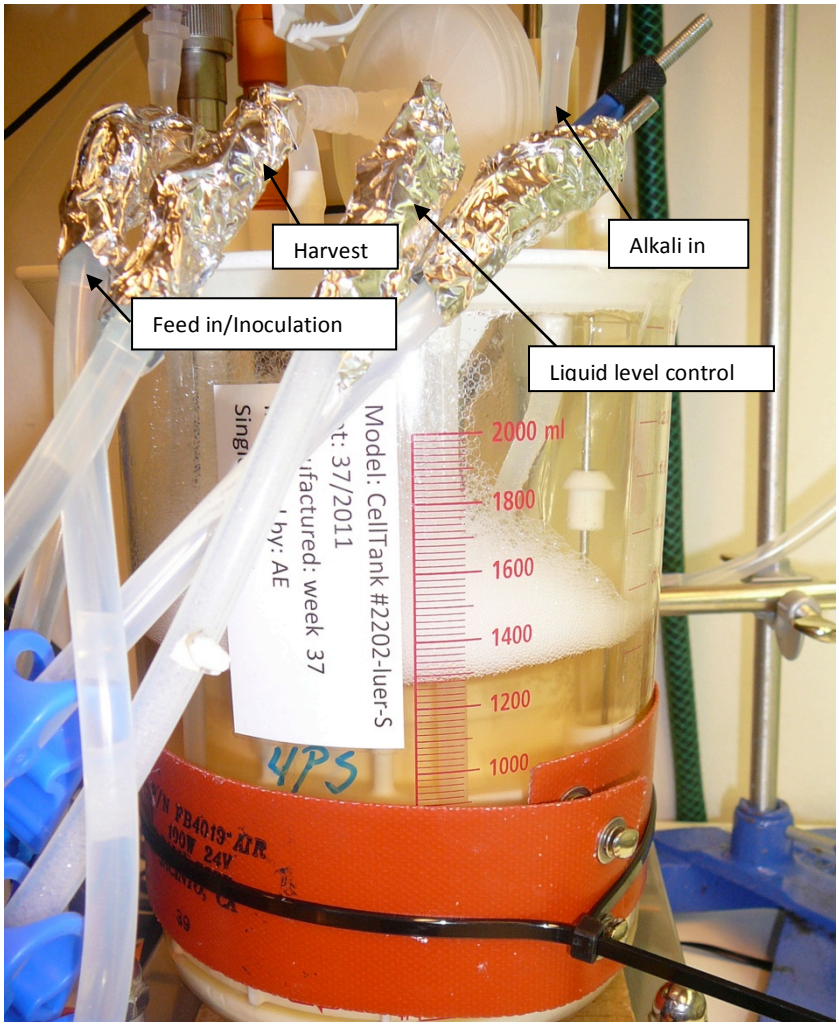

Figure 6: Bioreactor view at KTH cell lab. Photo: Véronique Chotteau

5 Method

The bioreactor was inoculated from shake flask culture. The cells for bioreactor inoculation were in exponential growth phase and not older than 3 days. After bioreactor inoculation, the cells were first grown in batch mode then the perfusion was initiated.

5.1 Cell expansion in shake flasks

Prior to seeding the CellTank, the cells were grown (for at least 4 passages) in shake flasks with IS CD XP medium supplemented with 3% of IS-CHO Feed-CD XP (both Irvine Scientific, USA).

Table 2: Shake flask culture volume

Shake flasks type (mL)	Culture volume (mL)
250	25 – 50
500	50 – 100

Table 3: Shake flask experimental conditions

Agitation rate (rpm)	100
CO2 (%)	5
Temperature (°C)	37.0
Seeding cell density (MVC/mL)	0.25 to 0.5

5.2 Controls and monitoring of the bioreactor

Table 4: Controls and monitoring

	Control upward	Control downward	Monitoring
pH	Alkali addition via controlled pump dropping in bioreactor	Controlled CO2 pulses addition in gas phase	On-line with BioPhantom, Belach
DO	Controlled O2 pulses addition via sparger (BOL#1 and BOL#2) or in headspace (BOL#2 last days)	Manually tuned continuous flow of N2 via sparger (BOL#1) or in headspace (BOL#2 last days)	On-line with BioPhantom, Belach
Temperatu -re	Controlled heating of belt	/	On-line with BioPhantom, Belach
Reservoir level	/	Continuous pumping of liquid above the maximum limit level	/
Bioreactor or matrix volume	/	/	Always filled at maximum volume
Perfusion rate	Manual tuning of pumps pumping the fresh medium in (feed) and pumping the harvest out	Manual tuning of pumps pumping the fresh medium in (feed) and pumping the harvest out	Off-line reading of harvest weight
Stirring speed	Manually tuned to achieve desired re-circulation flow	Manually tuned to achieve desired re-circulation flow	/
Re-circulation flow	Manually tuned by adjusting the stirring speed	Manually tuned by adjusting the stirring speed	Off-line reading of rotameter at harvest collection port
pCO_2	Manually tuned continuous addition of CO_2 in entering gas mix (via sparger in BOL#1 and into head space in BOL#2)	/	Off-line measurement
Cell density	/	/	On-line measurement by EVO 200
Pressure in matrix	/	/	Off-line reading of the pressure sensor

5.3 Set points and experimental conditions in the bioreactor

Table 5: Set points and experimental conditions

Parameters	BOL#1	BOL#2
DO (%)	45	40
pH	7.00	7.00
Temperature (ºC) A) → shifted to B) B) → shifted to C)	37.0	A) 37˚C B) 32˚C at 100 MVC/ml C) progressively shifted to 29˚C
Inoculation viable cell density (MVC/mL)	1.0	
Real culture working volume (mL):	150	
Reservoir volume (mL)	1280	
Perfusion rate (RV/day) (nL/cell/day)	1 or higher 0.05 or higher	
Re-circulation flow rate (L/min)	1.0[1]	1.6[1]
Alkali	0.5 M Na_2CO_3	
Maximum cell age at inoculation (passages)	12	

[1] The speed flow adjustment depends on the cell density and backpressure.

5.4 Perfusion settings and pumps

Table 6 presents the settings of the four peristaltic pumps (all Watson-Marlow 120U) used for the process.

Table 6: Perfusion and flow set points used in BOL#1 &BOL#2 perfusion cultivation

Perfusion rate	Started at 1 RV/day. Perfusion rate adjusted according to $\frac{cell\ density}{20}\ \left(\frac{RV}{day}\right)$ [eq. 1] where cell density (MVC/mL); and also increased to reduce lactate or NH_4^+ level if necessary	
Feed pump (P-1)	Continuous flow	1 mL/min
Harvest pump (P-2)	Continuous flow	1 mL/min
Liquid level control pump (P-3)	Continuous flow	1.1 mL/min
Glucose and glutamine feed pump (P-4)	operated when needed	
The pumps P-1, P-2 and P-3 were under the control of one timer, which made the pumps working intermittently and simultaneously; and allowed achieving very small flows accurately. For perfusion rate of 1 RV/day, the timer was ON 1 min of 10 min cycle (9 min OFF) so that the actual feeding rate was 0.1 mL/min with feed pump set at 1 mL/min. When the perfusion rate was increased the cycle time was kept, the ON time was increased and the OFF time was decreased. Above 10 RV/day, the timer was permanently ON and the pump speed was increased with the perfusion rate.		

5.5 Gas settings for the bioreactor operations

Table 7: Gases

Gas	Approx. flow (mL/min)	Mode	Inlet		Purpose
			BOL#1	BOL#2	
Air	20-100	Continuous / pulsed	Open tube sparger	Head space	Aeration and over-pressure
CO_2	0-10 (0-5% pCO_2)	Pulsed/continuous**	Open tube sparger	Head space	pH control or pCO_2 maintenance
O_2	0-50	Pulsed	Open tube sparger	Open tube sparger or head space*	Aeration
N_2	20-200	Continuous	Open tube sparger	Head space	Avoid too high DO

* From day 16 in BOL#2 experiment the O_2 inlet was switched from open tube to headspace.

** Continuous flow of CO_2 was applied to avoid too low pCO_2.

5.6 Analyses

5.6.1 Cell density, viability, pH, concentration of glucose, lactate, glutamine and ammonia

All these parameters were measured by Bioprofile FLEX (Nova Biomedical), which was also used to monitor the osmolality of the medium. Samples from matrix sample port (from a hole drilled in the matrix), harvest sample port (from the conical part sealed on top of the matrix) and bottom sample port (bottom of the reservoir) were daily taken and compared. The samples from different ports were kept daily for product (IgG) analysis.

A capacitance probe EVO 200 (Fogale-nanotech) was mounted to monitor the cell density in the bioreactor. One pF/cm read on the EVO system was equivalent to 1 MVC/mL.

5.6.2 IgG analysis by HPLC

The mAb concentration was measured by protein A HPLC.

5.7 Perfusion run

Action	Comment
Day -3	
The pH and DO probes were calibrated prior autoclaving: 1) Two points calibration for pH probe, zero and slope using buffers pH = 4 and pH = 7 2) Two points calibration of DO probe, 0 % (N_2) and 100 % air saturation.	The DO probe has to change from 5 % of its initial value to 95 % of its final value within 45 s (preferably 30 s) when going from 0 % to 100 % and 100 % to 0 %.
The pH, DO, biomass sensor probes were autoclaved in PBS, 35 min at 123˚C. The DO, pH and biomass sensor were mounted in the CellTank in the LAF (see Table 1). The CellTank was equipped according to Table 1 with needleless connectors for sampling, inlet/outlet connections, an extension of WC hose and a bubble flask (for BOL#2 only) in the LAF. The CellTank was moved to the working bench, the gas inlets/outlets were connected and sterile medium was pumped in. All the cables, the temperature sensor, the pumps, the pump timer, the medium bag, the harvest bag, the additive flask and the alkali flask were connected. The gas flows (through headspace and sparger) and bubble flask function were checked by gassing air into the gas mix inlet and observing bubble occurrence in the bubble flask. The pH control was started with CO_2 addition only. The temperature control and the agitation at 260 rpm (to reach 1.0 L/min re-circulation rate) were started. All the set points were the ones of the experiment. The sterility test was initiated.	A sterility test is recommended to verify that the bioreactor (when ready to be inoculated) is sterile. Typically three days at 37˚C temperature in presence of cultivation medium allow the detection of contamination if any. In the meanwhile, this time is used for DO probe calibration of 100 % air saturation (since this calibration needs to be re-done after autoclaving). The pH should always be controlled by CO_2 addition when the bioreactor is filled with medium (in particular when agitation is turned on) to avoid medium alkalisation. If the medium pH becomes higher than 8, it is recommended to replace the medium by fresh medium.

Action	Comment
Day 0	
Calibration of pH and DO probes post-autoclaving: 1) One point pH calibration: a sample of the bioreactor medium was taken and pH was measured externally (BioProfile Flex). A single point calibration adjustment was performed on the pH control unit according to the off-line result if the difference was larger than 0.03 pH units. 2) One point (100 % air saturation) DO calibration was performed on the DO control unit when the signals of DO, pH and temperature had been stable for at least 45 minutes and the pH one-point calibration had been performed.	
After the temperature, pH and re-circulation rate at 1 L/min had been stable for at least 45 min in the bioreactor, the cells were inoculated using the inoculation/feed in line: a flask containing the cell inoculate was connected sterilly in the LAF and the cells were pumped into the bioreactor at a final cell density of 1 MVC/mL in 150 mL (bioreactor working volume). Sampling from bottom and matrix sampling ports were taken to monitor the cells anchorage by the matrix.	

Action	Comment
Day 1 to end	
The perfusion was started when the viable cell density shown on Biomass sensor was > 2 MVC/mL, typically on day 1. Parameters measured on-line: viable cell density (EVO), pH, temperature, DO, gas flows, stirring speed, back pressure. The stirrer speed was switched to 300 rpm resulting in 1.6 L/min re-circulation flow rate in BOL#2 run. The pump timer allowed an accurate low flow rate using intermittent mode. The harvest weight was recorded to monitor the perfusion rate and the pump settings were adjusted if necessary. The perfusion rate was adjusted according to the cell density (see Table 6) and the lactate and NH_4^+ concentrations. Parameters measured off-line: counting and viability of non anchored cells, pH, concentrations of glucose, lactate, glutamine, glutamate, ammonium, osmolality, IgG, pCO_2. Glucose and glutamine were added if needed to compensate for cellular consumption (calculated the last 24 hours) and for difference between target and measured concentrations. Supplementation of glucose and glutamine were added into the culture via an intermediate flask in BOL#2 run (rm.: it was added directly into the bioreactor in BOL#1 run since the intermediate flask had not been mounted). The fresh medium with glucose/glutamine addition for one-day usage was mixed in an intermediate flask and the medium was pumped (on a period of 24 hours) from this intermediate flask to obtain glucose/glutamine addition evenly distributed in time.	Refer to 4.2-4.5 1) The re-circulation flow rate dropped of ≈ 10-20 % each day due to increasing biomass → it was re-adjusted to 1.6 L/mL daily by increasing the stirring speed of ≈ 10-15 rpm. 2) Even with pre-extended WC hose, it was observed that the WC hose filter got easily clogged by the medium. The filter needed to be changed when wet to ensure the sterility and functionality (i.e. no filter clogging) of the whole system. 3) Online pH value was adjusted according to the offline pH value if the difference between them was > 0.03 pH units.

6 Results and discussion

Two runs were performed, BOL#1 and BOL#2, in chronological order. The purpose of the first run (BOL#1) was to reach a cell density as high as possible, to assess the cell density capacity of the CellTank 2202 SUB prototype. The purpose of the second run (BOL#2) was to study cell growth arrest at high cell density.

6.1 Cell Growth

After inoculation of BOL#1 run, 75 % of the cells were trapped in the matrix after 20 min and 95 % of the cells were trapped in the matrix after 2 hours. It took a little bit more than 4 hours for the EVO 200 sensor signal to be stable with the cells harbored inside the matrix. During the first two weeks, the growth was slow due to some troubleshooting and adjustments, so this period should not be taken into account. From day 14, the system was satisfying and the cells grew exponentially, reaching a maximal cell density of 200 MVC/mL after 25 days of cultivation, see Figures 7 and 9. Unfortunately, on day 27 a technical problem occurred with the stirrer causing BOL#1 run termination. The number of cells leaking from the matrix was very low: the cell density of non-anchored cells in the matrix was maximum 0.3 MVC/mL measured in the matrix and 0.8 MVC/mL measured in the harvest line, see Figure 8. The viability (measured by LDH and calibrated with LDH content in a known number of viable cells) was ≥ 95 % during the whole run, see Figure 8. At a viable cell density of 200 MVC/mL, the viability was 98 %. The perfusion rate (Figure 7) was adjusted according to eq. 1 (Table 6) based on the cell density, but was also increased if necessary based on lactate and NH_4^+ levels. At 200 MVC/mL cell density, the perfusion rate was 10 RV/day. It was then increased to 15 RV/d on day 26 in an attempt to increase this cell density. The perfusion rate was increased above the value given by eq. 1 at days 5 and 21 when ammonium reached 4.8 mM and 6 mM respectively.

The cell growth in BOL#2 run was exponential from day 1, see Figure 9 and 10. Since the aim of BOL#2 run, was to study the cell growth arrest at high cell density, the temperature was lowered to 32˚C when the cell density reached 100 MVC/mL at day 10. The cell growth was notably reduced as can be observed in Figure 9 however not stopped, so further temperature reduction was performed on day 11 to 31˚C and to 30˚C on day 14. Day 16, the temperature was even decreased to 29˚C resulting in total growth arrest. The temperature reduction resulted in a viable cell density around 130 MVC/mL maintained for 14 days, from day 10 to day 24. The number of cells leaking from the matrix was very low here as well: the cell density of non-anchored cells in the matrix was maximum 0.2 MVC/mL measured in the matrix and 1.2 MVC/mL measured in the harvest line (Figure 10). The viability (measured by LDH) was ≥ 96 % during the whole run (Figure 10). Despite this very high value, one could observe a slight viability decrease after the temperature was decreased ≤ 30˚C. The perfusion rate was adjusted according eq. 1 (Table 6) except when the lactate or ammonium concentrations were too high (performed two times, once when ammonium level reached 5 mM at day 5 and then when lactate and ammonium levels reached 50 and 8 mM

respectively at day 8). Furthermore despite a reduced metabolism, consecutive to growth arrest, the lactate accumulated, see Figure 16; hence the perfusion rate was progressively increased up to 10 RV/d. Reducing the perfusion rate to 8 RV/d at day 21 resulted in a new increase of the lactate level. The cell specific perfusion rate was lower in BOL#2 run than in BOL#1 run as can be seen in Figure 12.

The signals measured by the EVO 200 detector for BOL#1 run are given in Figures 13 and 14. Fc is an indicator of average cell size. Between days 16 and 21, Fc was stable, it was then decreasing indicating a larger cell diameter. After day 24, it decreased again, coinciding with a smaller diameter. Alpha is an indicator of the size homogeneity. During the run, alpha was increasing, indicating an increasing inhomogeneity of the cell sizes (or different radii if the cells were not spherical anymore). Due to the very high viable cell density (200 MVC/mL), the limit for DeltaEps signal measured by the EVO 200 detector was reached – it seems to be 1000 pF/cm. From day 19 the conductivity was dropping to almost half value at the end, which might be due to the fact that some ions have been consumed while not fully replaced by the perfusion medium. There was a very nice correlation between the cell density (calculated from 2 frequencies) and DeltaEps (all frequencies).

To assess the cell density reached in BOL#1, the CellTank was disassembled and the cells were tentatively removed from the matrix by thorough PBS rinsing. From this operation ≈ 131 x MVC/mL were recovered, corresponding to 66 % of the measured cell density by EVO detector. The same yield was observed by other groups (personal communication) hence the value of 200 MVC/mL cell density given by EVO 200 sensor in BOL#1 is deemed to be reliable.

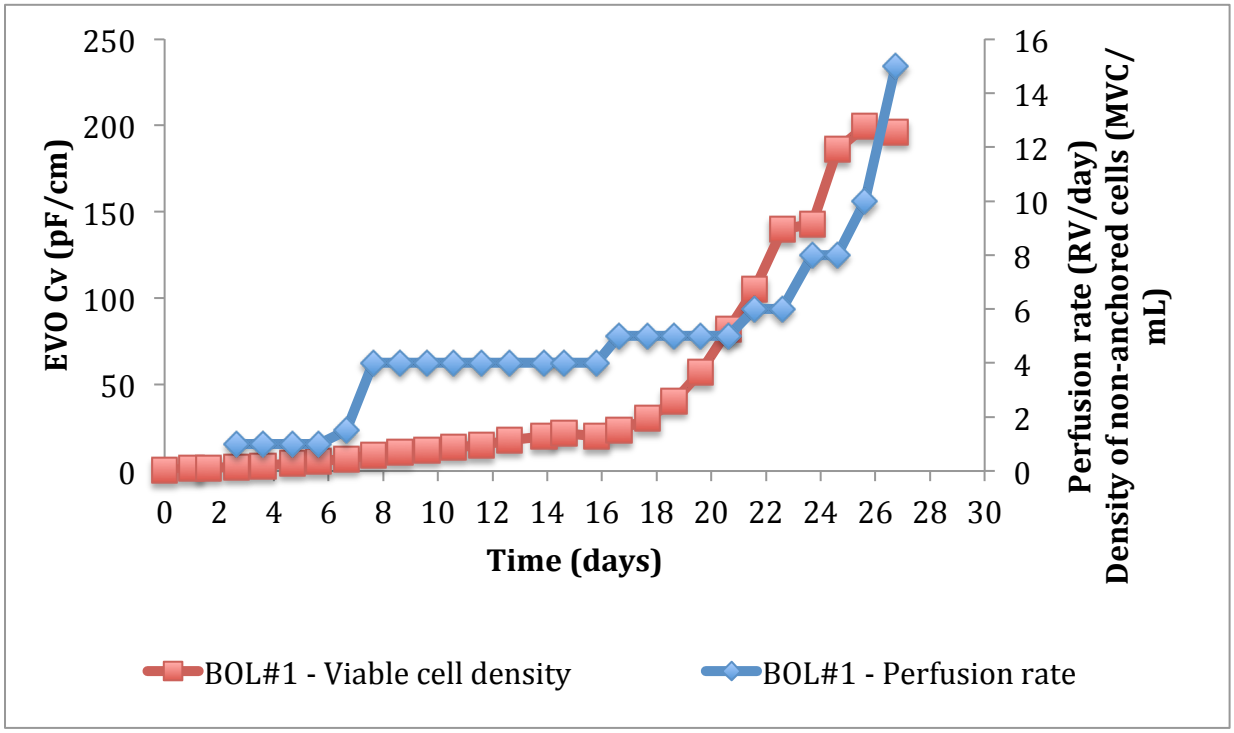

Figure 7: Viable cell density measured by EVO 200 detector and perfusion rate in the 27-day perfusion run BOL#1. One pF/cm read on the EVO 200 system is equivalent to 1 MVC/mL.

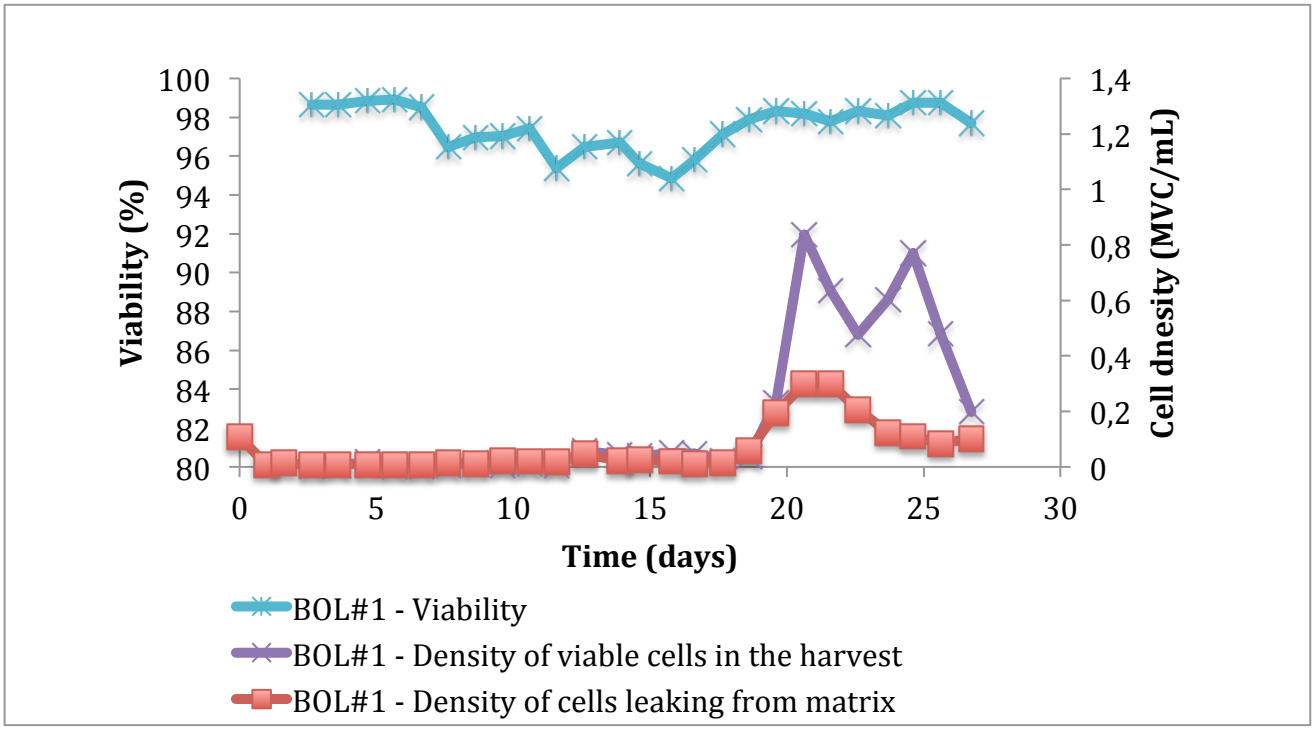

Figure 8: Viability measured by LDH analysis of the supernatant, density of viable cells leaking from the matrix (i.e. non-anchored) and viable cell density of the cells leaking found in the harvest line in BOL#1 run

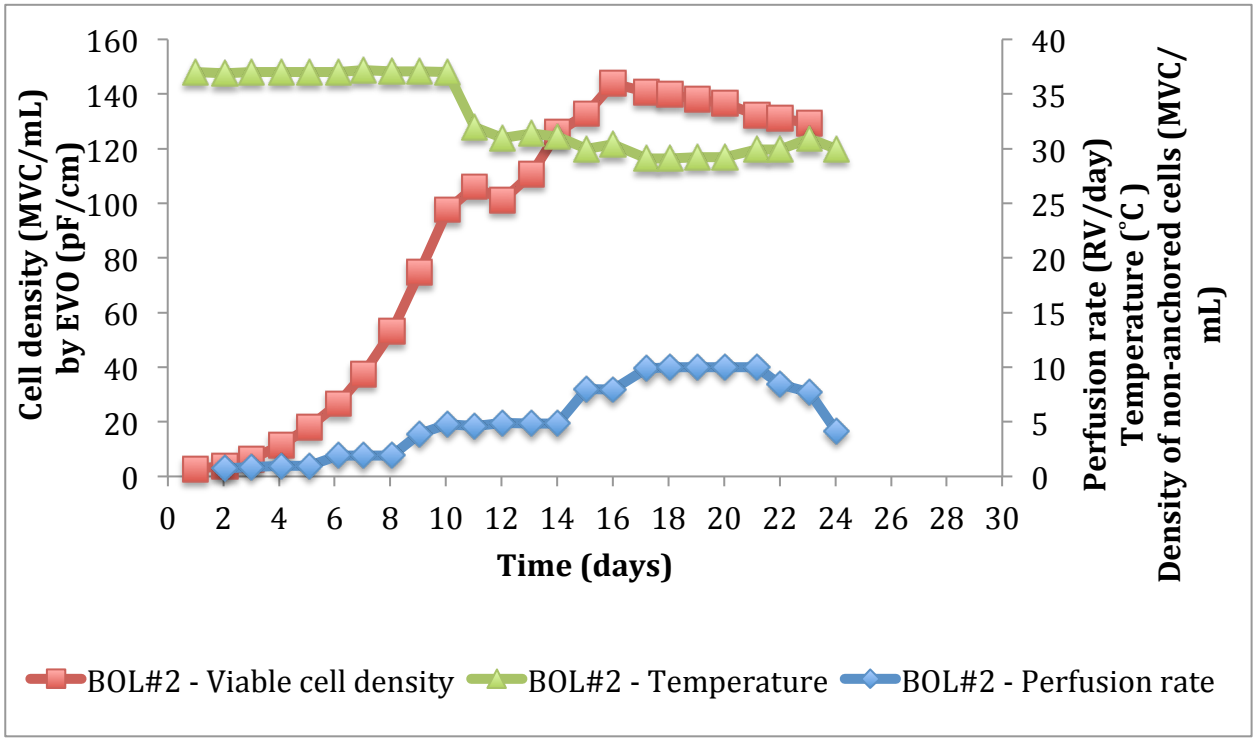

Figure 9: Viable cell density measured by EVO 200 detector, perfusion rate and temperature during the 24-day perfusion run BOL#2. One pF/cm read on the EVO 200 system is equivalent to 1 MVC/mL.

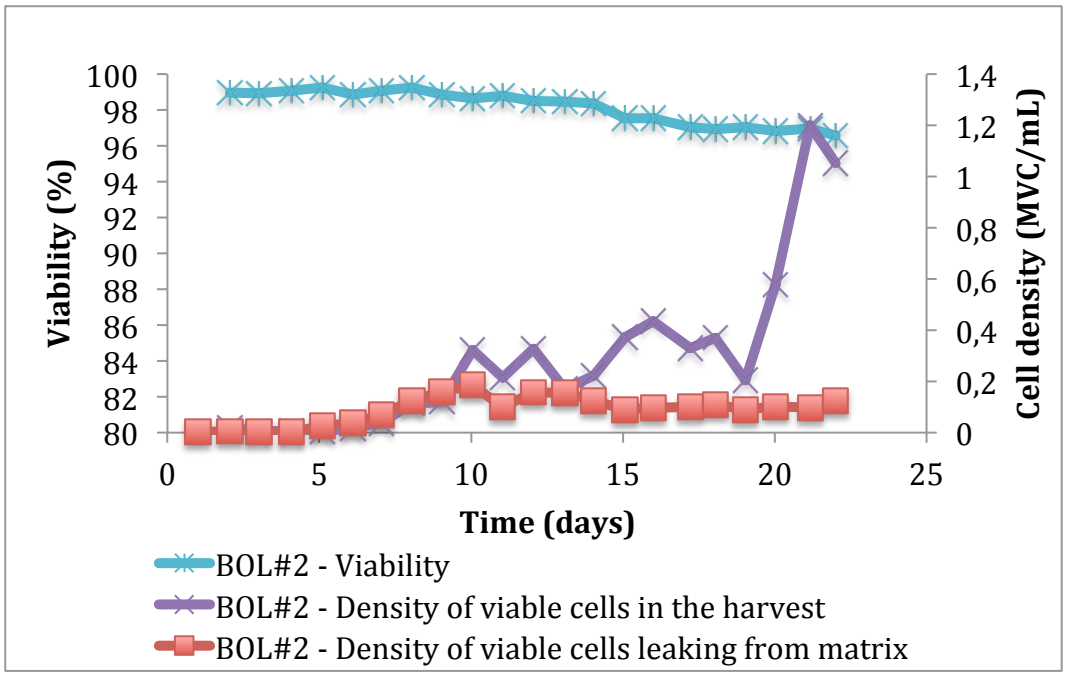

Figure 10: Viability measured by LDH analysis of the supernatant, density of viable cells leaking from the matrix (i.e. non-anchored) and viable cell density of the cells leaking found in the harvest line in BOL#2 run, both measured by Bioprofile FLEX

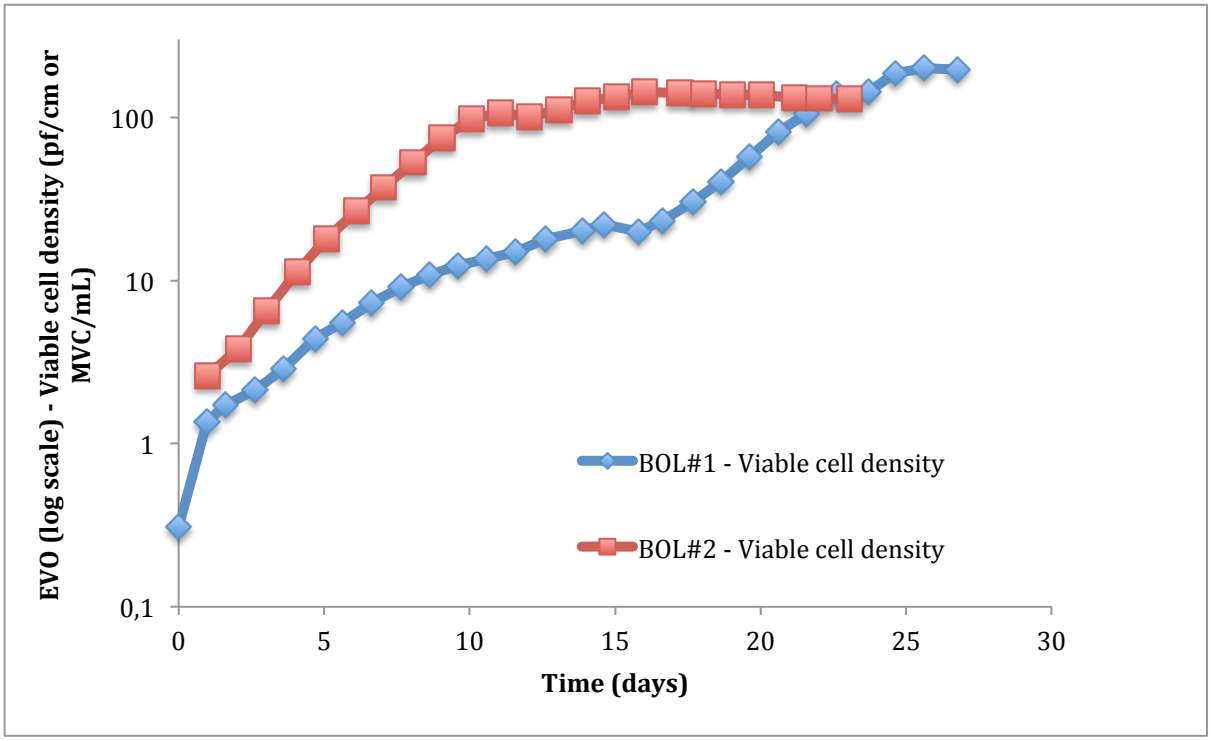

Figure 11: EVO Viable cell density (log scale) in BOL#1 and BOL#2 runs

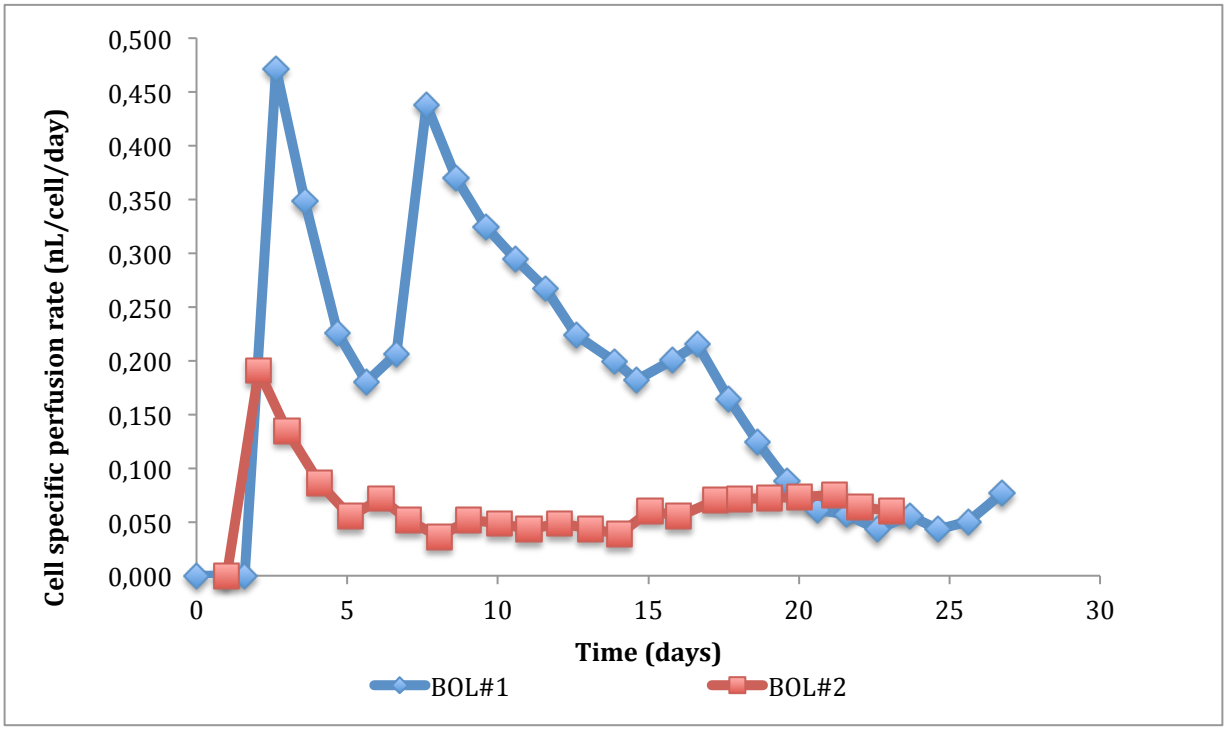

Figure 12: Cell specific perfusion rate in BOL#1 and BOL#2 runs

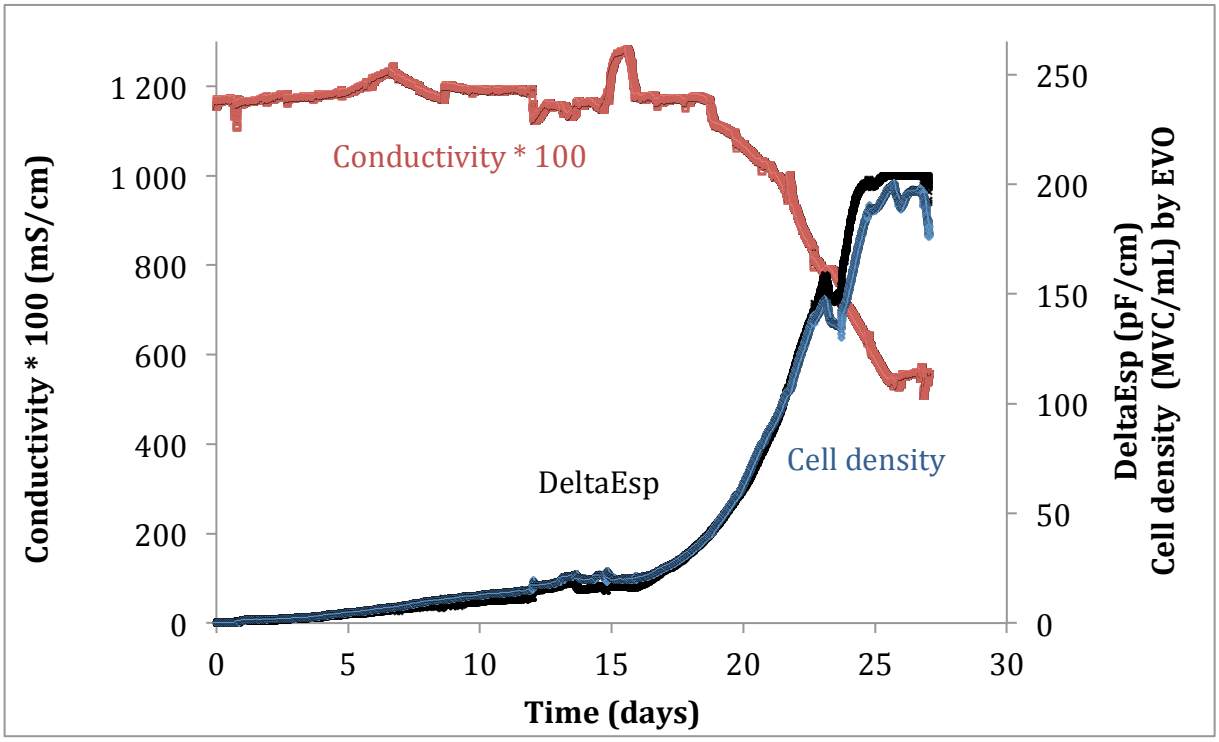

Figure 13: EVO 200 signals during BOL#1 run: biomass calculated by EVO 200 software, DeltaEps and conductivity (multiplied by 100)

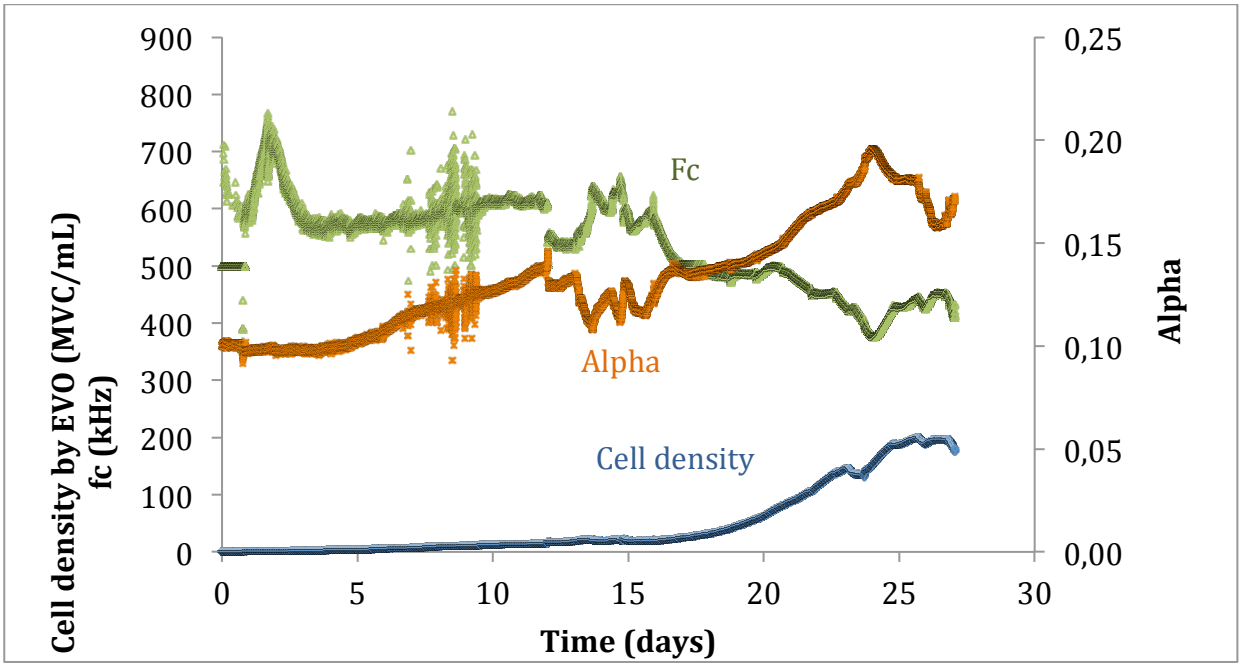

Figure 14: EVO 200 signals during BOL#1 run: biomass calculated by EVO 200 software, Fc and Alpha

6.1.1 **Cell Metabolism**

The concentrations of glucose, lactate, glutamine and ammonium measured during BOL#1 and BOL#2 are shown in Figures 15 to 19. The accumulation of lactate was high, reaching around 60 mM and 90 mM in BOL#1 and BOL#2 runs respectively. The ammonium concentration was transitory very high, 8 mM, in BOL#2 run. In BOL#1 run, it accumulated more from day 21, reaching 6 mM. The temperature reduction caused a decrease in ammonium production and an increase in lactate production when the temperature ≤ 29˚C. The concentrations of glucose, lactate, glutamine and ammonium were similar in the matrix, the harvest and in the bottom of the reservoir as can be observed in Figure 19, illustrating that the fluid is highly homogeneous in the whole system including the matrix and the reservoir.

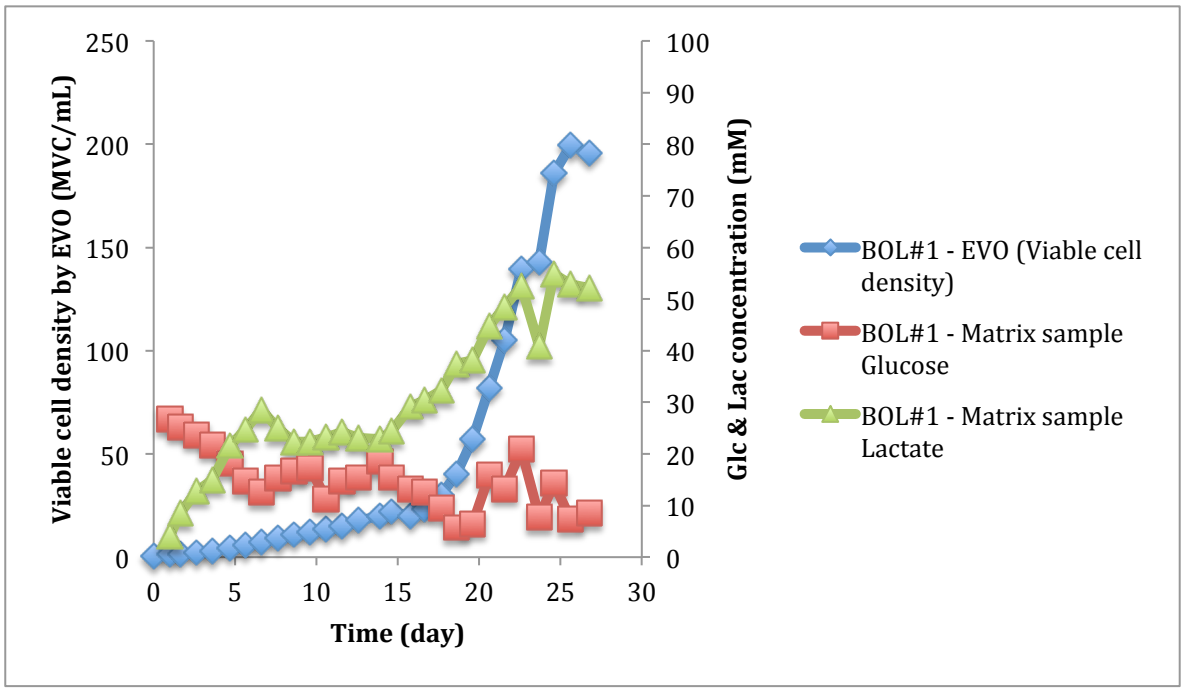

Figure 15: Kinetics of glucose and lactate of in BOL#1 run

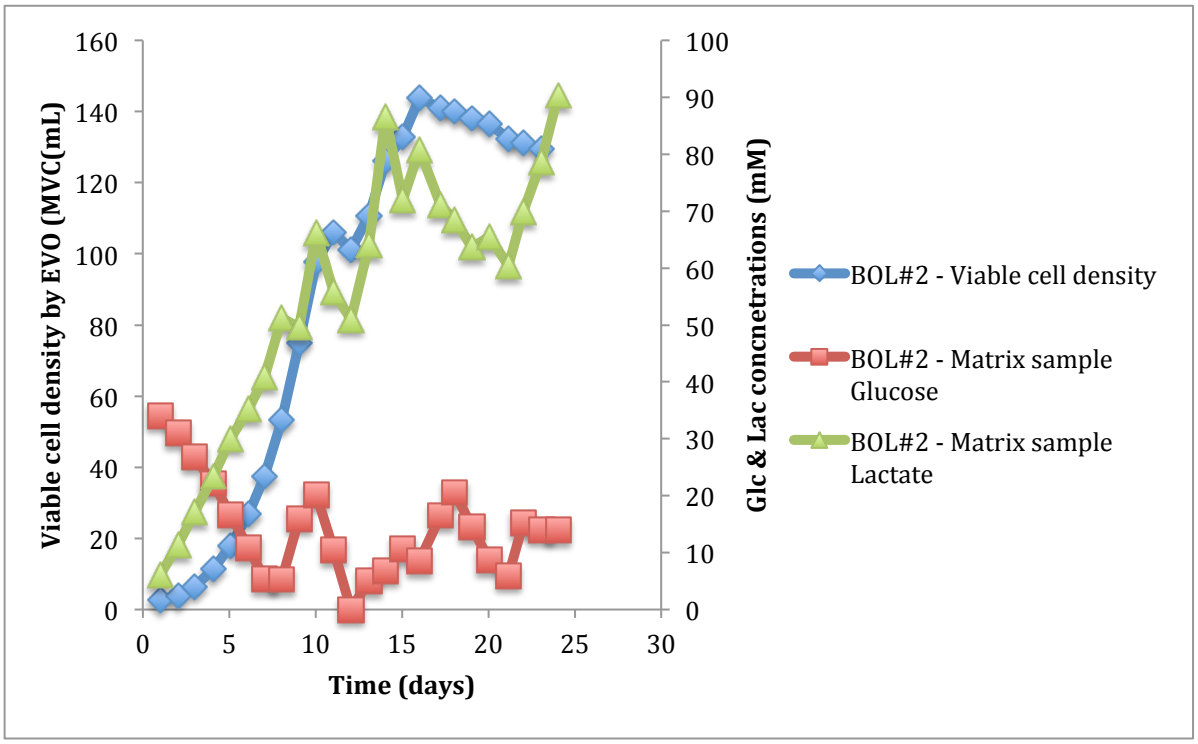

Figure16: Kinetics of glucose and lactate in BOL#2 run

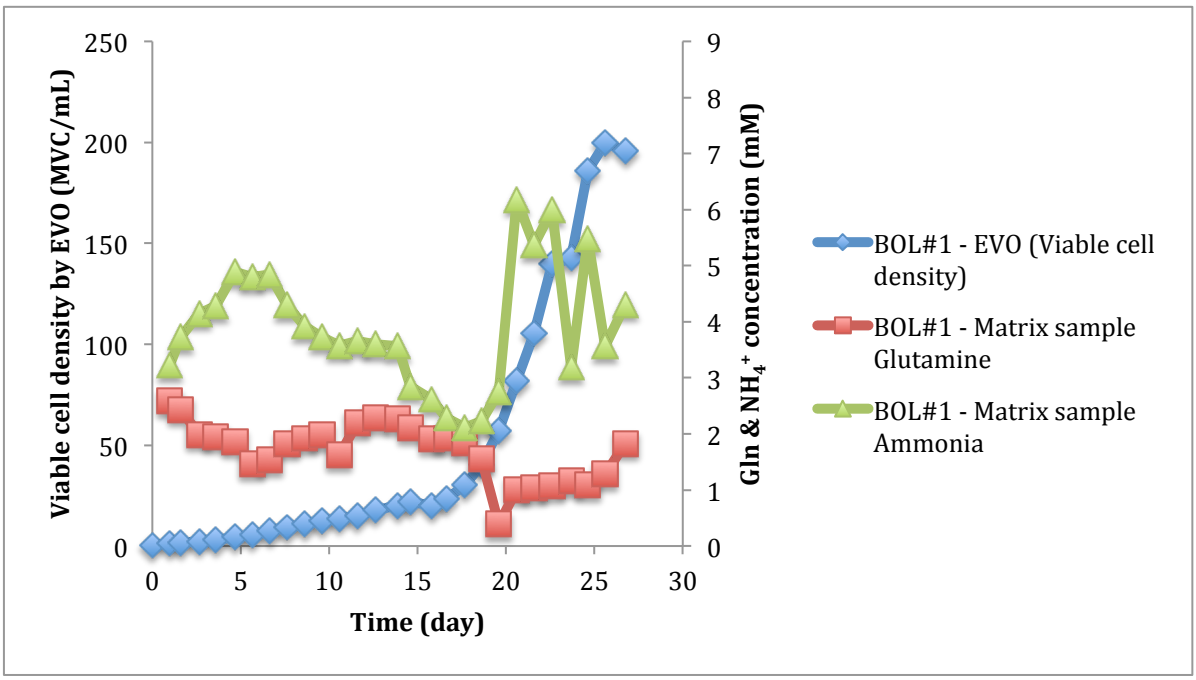

Figure 17: Kinetics of glutamine and NH_4^+ in BOL#1 run

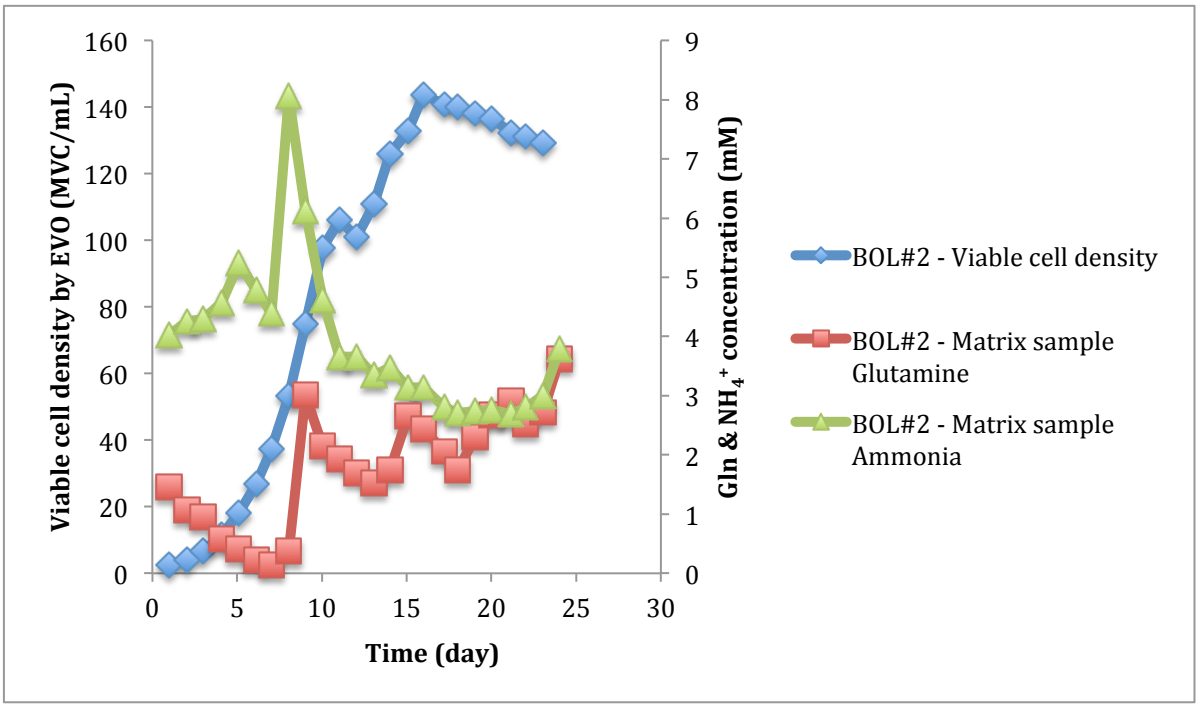

Figure 18: Kinetics of glutamine and NH_4^+ in BOL#2 run

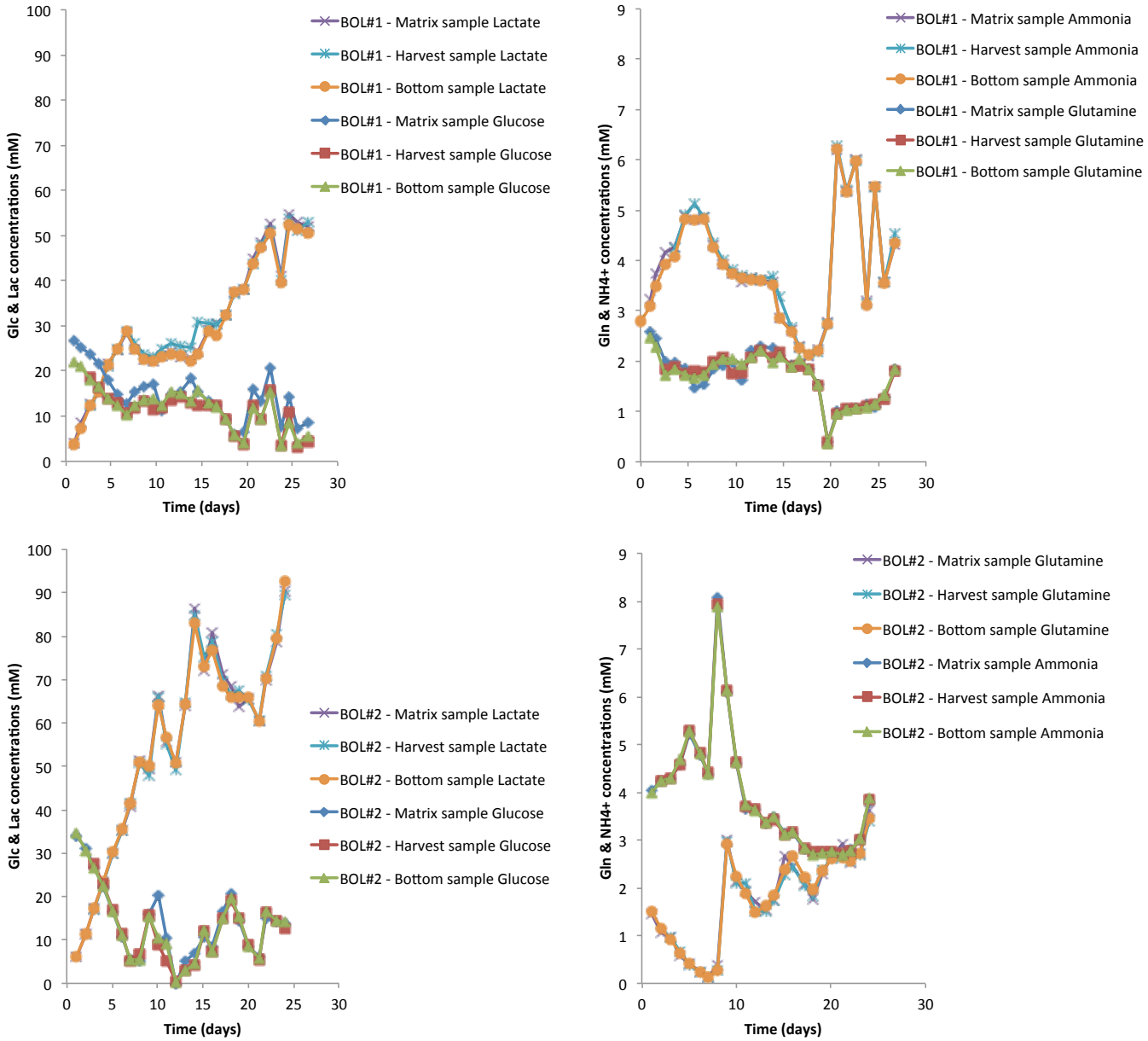

Figure 19: Concentrations of glucose, lactate, glutamine and ammonium in the matrix, the harvest and in the bottom of the reservoir ('bottom').

6.1.2 IgG production in BOL#1 and BOL#2

The IgG concentrations, accumulated IgG productions and cell specific productivities are given in Figures 20-22. The IgG concentrations in the bioreactor and the harvest line were similar so a yield of about 100 % was obtained indicating no retention of the IgG in the bioreactor. The harvested IgG amount accumulated until the end of the runs and 600 mg were finally produced after 24 days in the 150 mL bioreactor despite the low cell specific productivity of this cell line. The cell specific productivity was in average 1.7 pg/cell/day in BOL#1 and BOL#2 until day 14. This value was comparable or slightly higher than 1.5 pg/cell/day cell specific productivity measured in batch shake flask. When the temperature was decreased to 30˚C the cell specific productivity increased to an average of 2.5 pg/cell/day, i.e. 47 % higher than at 37˚C. The cell specific productivity decreased at the end of BOL#2 run, potentially due to non-optimal temperature (29˚C).

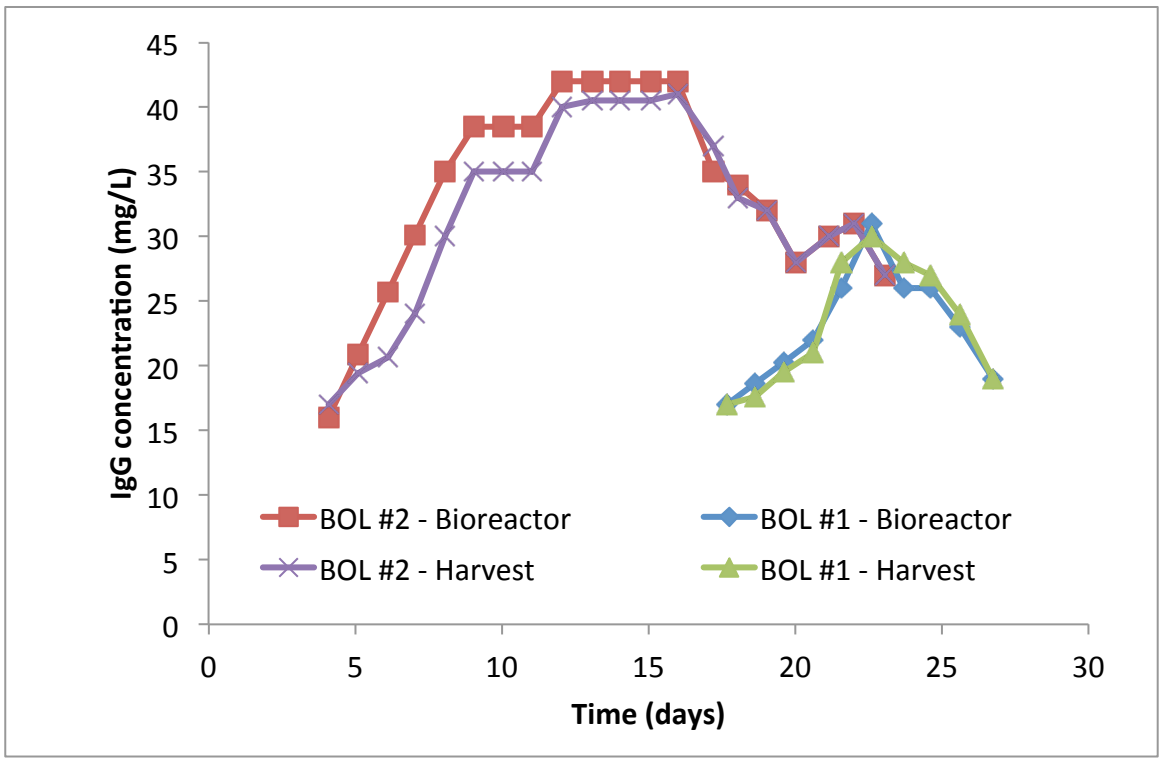

Figure 20: Kinetics of recombinant IgG production in BOL#1 and BOL#2 cultivation runs

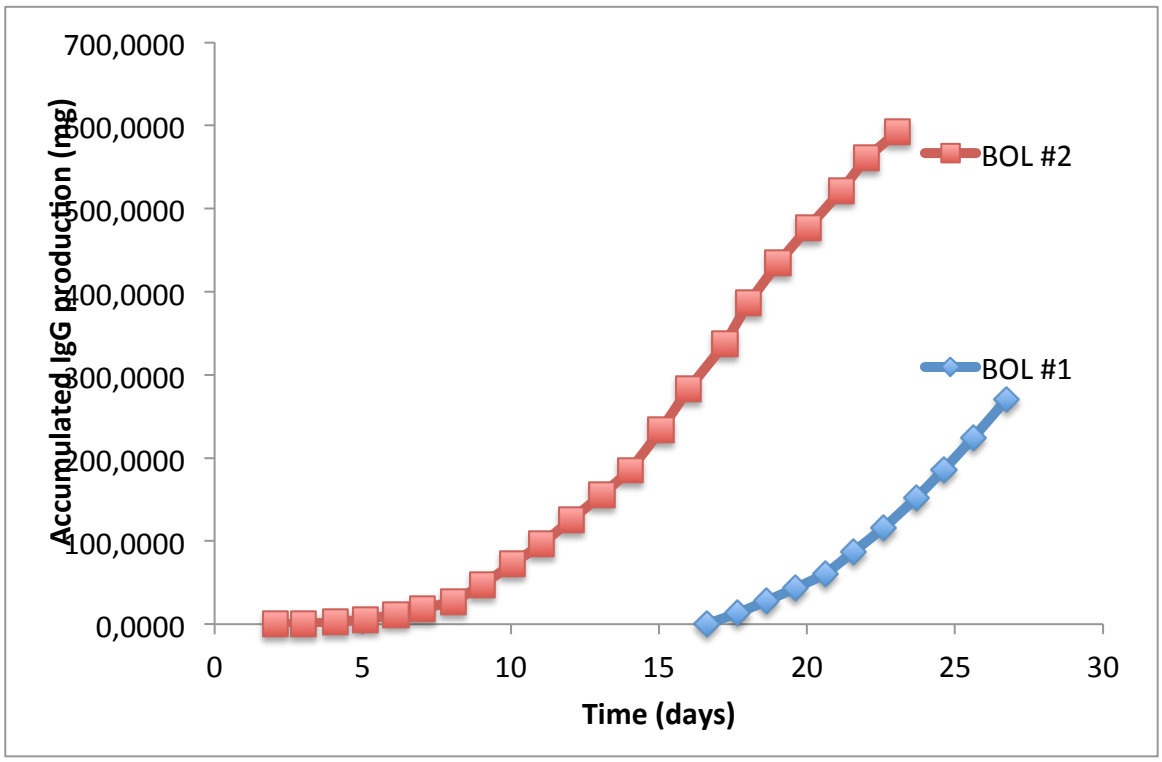

Figure 21: Accumulation of recombinant IgG in BOL#1 and BOL#2 cultivation runs

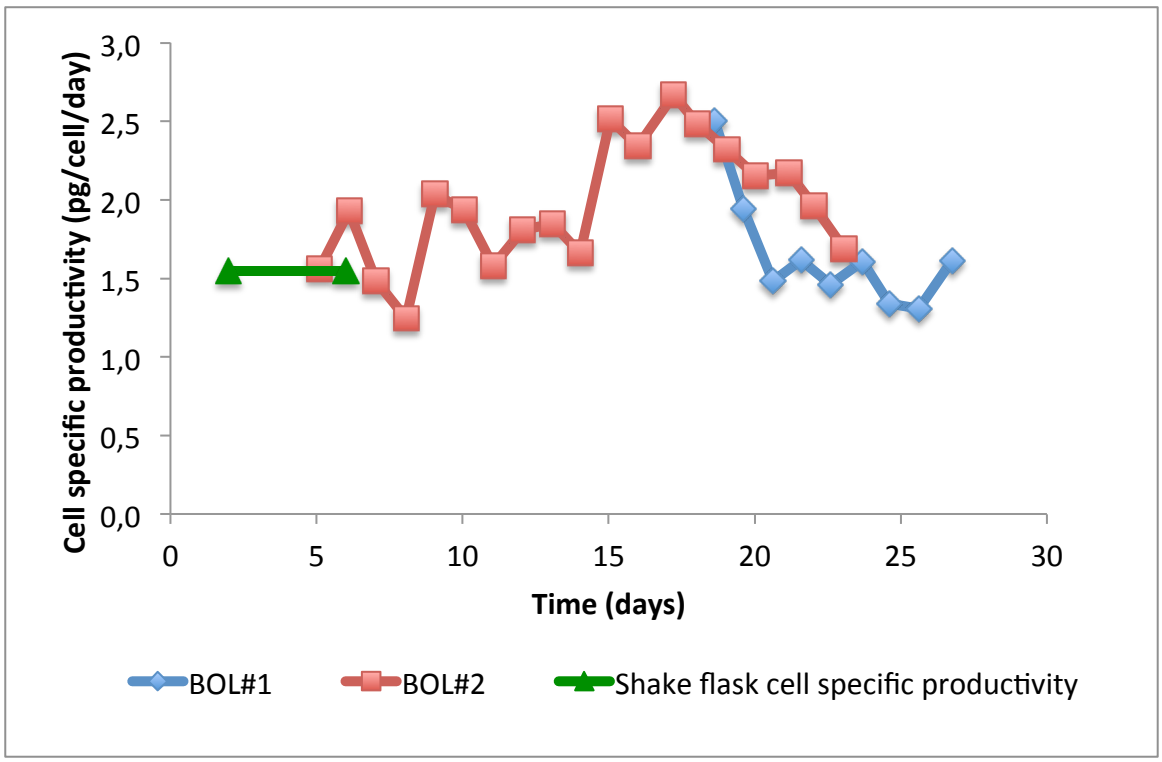

Figure 22: Cell specific productivity in BOL#1 and BOL#2 cultivation runs, compared with cell specific productivity in shake flasks in the same medium

6.1.3 Partial pressure of carbon dioxide

The pCO_2 dropped the first days of the cultures. To compensate for the CO_2 removal from headspace swiping, CO_2 was continuously added, resulting in a concentration ≥ 2 kPa, favourable for the cell growth.

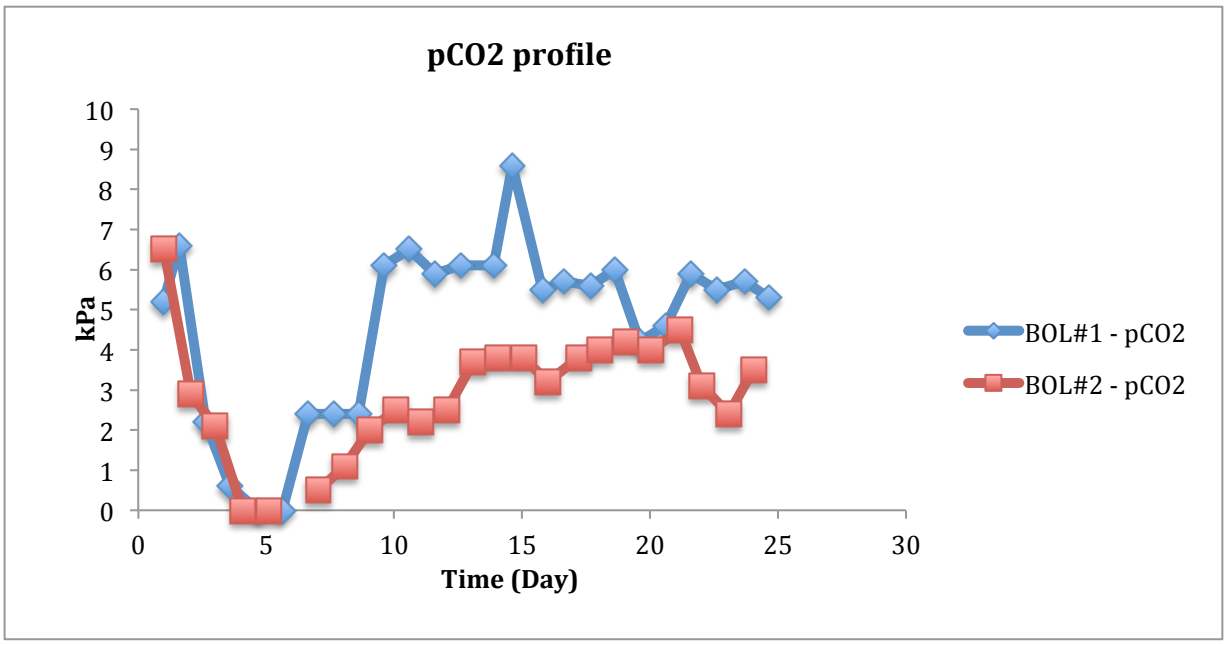

Figure 23: pCO_2 profile in BOL#1 and BOL#2 runs

7 Conclusions

A perfusion system based on the CellTank bioreactor was successfully developed. Two perfusion runs were performed during 24 and 27 days. An exponential growth was observed in both runs. A very high cell density of 200 MVC/mL was obtained during BOL#1 run. In BOL#2 run, cell growth arrest was obtained by reducing the temperature when the cell density reached 100 MVC/mL. A viable cell density around 130 MVC/mL was then maintained for 14 days with a low temperature, ≤ 32˚C.

The perfusion device was robust and integrated in the CellTank system, alleviating the operations. Operation using CellTank SUB was easy and highly simplified compared with traditional perfusion technologies.

The harvested amount of IgG increased with time with a cell specific productivity comparable or higher than batch culture. The final IgG amount harvested was 600 mg in this 150 mL bioreactor even though the cell specific productivity of this cell line was low, i.e. 1.7 pg/cell/day. Importantly no retention of IgG was observed in the polymer matrix.

8 List of abbreviations

Amm = Ammonium
DO = Dissolved oxygen
Glc = Glucose
Gln = Glutamine
Glu = Glutamate
IgG = Immunoglobulin G = antibody molecules
Lac = Lactate
LAF = Laminar air flow hood
MVC/ml = Million viable cells per millilitre
RPM = Revolutions per Minute
RV/d = Reactor volume per day, 1RV = 150 mL
SUB = Single-Use-Bioreactor
WC = water column (pressure indicator in the matrix)

9 Acknowledgements

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