EXPLORING METABOLIC DEMANDS OF HIGH DENSITY CHO-CELL CULTURES

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Key Words: CHO-cells, perfusion, omics, genome scale model, fed-batch

As the world population continues to grow and age, access to medical therapies, like therapeutic monoclonal antibodies (mAb), increases in importance. Chinese hamster ovary (CHO) cells are the preferred expression platform for biopharmaceutical production, such as mAb, due to their ability to generate human-like post-translational modifications and strong legislation background. Unfortunately, biopharmaceuticals production using CHO cells is costly leading to an expensive product. Detailed knowledge of their metabolic demands is still lacking. Hence, increasing understanding would help to develop rational approaches to enhance expression, lower production costs and subsequently make therapies more accessible.

Industrial large scale CHO cell cultures are typically run in fed-batch mode, which is limited by the accumulation of inhibitory by-products. Driven by higher volumetric productivities, shorter residence time of products, industry is shifting towards perfusion cultures. This shift in cultivation mode is accompanied by new metabolic conditions for the cell in form of higher cell densities and different exometabolite levels. Comparison of both culturing modes, as well as different perfusion dilution rates, revealed differences in growth and productivity patterns. Cell densities and cell specific productivities increased from fed-batch to perfusion and from low to high dilution rates. Aiming to progress from pattern observations to a systems biology view, the metabolome and proteome profiles of both cultures modes will be analysed and mapped onto the CHO genome-scale model. Thus, protein expression and metabolite levels will be compared on a genome wide level and provide a metabolic profile, specific for the culture condition. Alterations between metabolic profiles of different culture conditions can then be identified, understood and utilized to reverse engineer variations by using genetic engineering approaches or media design and process optimization.

Overall, this study aims to establish a systems biology approach to better understand the CHO cell metabolism in the aspect of emerging perfusion systems based on comparison of different culture modes.

MODELING THE RESIDENCE TIME DISTRIBUTION OF AN END TO END INTEGRATED BIOMANUFACTURING PROCESS

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Key Words: Numerical simulation, process design, ramp up phase, disturbance propagation

With the advancements in continuous manufacturing focused mainly on the development of individual unit operations, only a few end-to-end integrated continuous bioprocesses (ICB) have been reported. As the scope starts shifting also towards commercial applications, detailed process understanding is required for quality process design, process optimization and developing process control strategies.

We have developed a flexible and modular platform for modeling a residence time distribution (RTD) of an ICB. The platform can be easily modified and extended with new unit operations and additional functionalities. The output model of our platform is capable of describing the RTD in steady-state and rapid ramp up phase. The model also includes description of the mass flow, perturbations in product or buffer species and the propagation of possible disturbances. Furthermore, it is applicable across scales given the different upstream scenarios.

In this presentation our approach towards modeling the RTD will be discussed along with the consideration for a general use-case. The resulting model can serve as a tool for root cause investigations and plays a major role in Quality by Design approaches, process optimization and enabling the implementation of automated process control strategies.

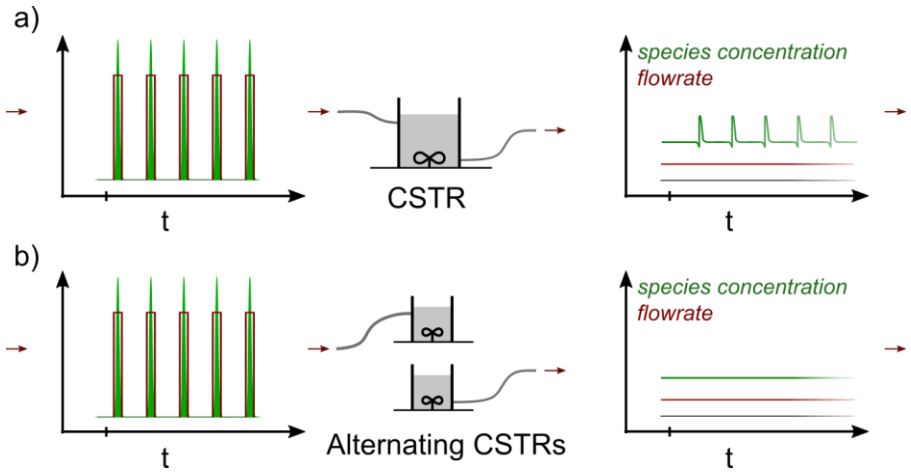

Figure 1 – Semi-continuous unit operations can disrupt the steady flowrate and cause fluctuations in product concentrations. In order to stabilize the flowrate, we can use a CSTR as a surge tank (a). However, during the elution the concentration in CSTR fluctuates. This can be mitigated by using a larger CSTR which would result in broader RTD. The alternative approach with a system of multiple alternating CSTRs (b) overcomes the fluctuations and narrows the RTD, but it increases the complexity of the process. A preferred solution would depend on the operating conditions and the unit operations downstream.

EXPERIMENTAL DESIGN AND SMALL-SCALE MODEL FOR HIGH-PERFORMING PERFUSION MEDIA AND PROCESSES SCALABLE TO 50 L BIOREACTORS

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Cell culture perfusion processes are considered optimal for a truly integrated continuous biomanufacturing pipeline. The nutrient-rich but balanced media should be designed to support very low cell-specific perfusion rates (CSPR) that minimize media consumption but maximize viable cell days and productivities. Optimized processes at low CSPR drastically reduce equipment costs, lab space, and product dilution. Finally, operating at very low CSPR enables running mammalian cell bioprocesses as true chemostat cultures in the future. We demonstrate a general workflow to develop high-performing perfusion media using small-scale models and transferred the process to 50 L scale at CSPR of 20 pL/c/d.

Recombinant CHO cells were evaluated at small scale in shaking tubes. Cells were grown in HyClone™ ActiPro or CDM4NS0 basal media, and optimal spike concentrations of HyClone Cell Boost™ supplements were determined using a DoE-supported workflow. The identified high-performing perfusion medium was applied to ReadyToProcess WAVE™ 25 and XDR-50 bioreactor runs. Different strategies were tested to find the critical minimum CSPR and maximum supported viable cell density (VCD). The obtained product profile was compared between scales, as determined by glycan-, charge-, and size-variant distribution.

Scale-down models were leveraged to define high-performing media and applied to bioreactor runs at constant volumetric perfusion rate, VCD, or CSPR. CSPR values as low as 10 pL/c/d at 2×10^8 c/mL were achieved. These results make high-density perfusion processes suitable for inoculum preparation (N-1) or high cell density cryopreservation. The developed perfusion processes supported steady-state production at constant 5×10^7 c/mL by applying a continuous cell bleed and were scaled to 50 L.

ELUTION PROFILE FROM PERIODIC COUNTER CURRENT CAPTURE STEP AS AN ON-LINE MONITORING AND CONTROL TOOL FOR PERFUSION BIOREACTORS

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Key Words: periodic counter current chromatography, on-line monitoring, continuous capture, integrated bioprocessing, process analytical technology

Current trends in bioprocessing move towards the implementation of more on-line sensors such as Raman spectroscopy for titer monitoring in perfusion bioreactors. However, process performance data from one downstream unit operation can also be used to monitor and control the unit operation directly upstream. Despite several authors demonstrated a successful integration of continuous up-and downstream processes little attempts have been made to leverage the information derived from downstream processing as a real time feedback loop for upstream processing. We have developed a simple and robust approach in which protein A periodic counter current chromatography (PCCC) can function as an on-line monitoring tool for protein titer in continuous upstream fermentations. For a proof of concept, we exploit the fact that performance and binding capacities of state of the art protein A chromatography material do not significantly decrease throughout hundreds of cycles. Therefore, it is possible to predict the concentration of antibodies in the feed material from the elution pool and the volume loaded onto the column. We use the breakthrough curve during the interconnected phase of the PCCC, which is key for this approach. In the interconnected phase, the first column was loaded to 80% breakthrough, and the breakthrough curve modelled for a number of different concentrations in the feed material. Using the breakthrough curve, the time of the breakthrough can be modelled against the increase of product present in the feed stream, allowing the prediction of the concentration of antibody in the perfusion fermentation. This information feedback loop through the integration of PCCC and fermentation into effectively one single unit operation makes the titer determination in the fermentation obsolete, using the PCCC effectively as online monitoring tool itself. Future work after this proof of concept will include the prediction of protein A binding capacities through the lifetime of the resin and determination of accuracy and quantification limits of the interconnected units.

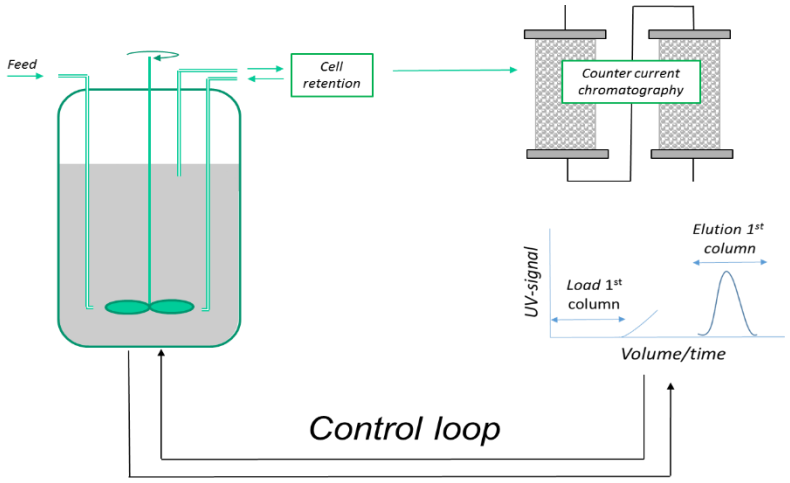

Figure 1 – Titer monitoring by interconnected PCCC. The amount of antibody eluted in each cycle is divided by the volume loaded. This value is used to determine the antibody concentration in the perfusion reactor with a delay of one switch time.

STRAIGHT-THROUGH PROCESS DEVELOPMENT OF UP AND DOWNSTREAM INTEGRATION OF MONOCLONAL ANTIBODIES PRODUCTION USING FLOCCULATION, AEX AND ONE PASS TFF

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Key Words: Protein purification, flocculation, anion exchange chromatography, one pass tangential flow filtration, mAb.

The monoclonal antibody (mAb) market has been presenting a significant growth rate in the last two decades, which increased the interest of biopharmaceutical companies in this product class. Many improvements have been achieved in the upstream processing of mAbs, leading to significant increases in bioreactor titers. However, the production costs are still high, especially due to downstream processing costs, which can represent a major part of the of overall production costs. Traditional mAb platform processes include a very selective, but high-cost Protein A (PrA) affinity chromatography as the first purification step (capture). Several approaches have been recently explored in order to replace PrA chromatography. In this work, we propose a new, low-cost strategy for integrating clarification and capture step for mAbs using flocculation followed by a straight-through process with single-pass tangential flow filtration (TFF) and suspension anion-exchange (AEX) chromatography. First, the recombinant anti-IL8 mAb were produced by CHO-DP12 cells (ATCC, USA) in shake flasks at 180 rpm and 37°C using TC-LECC medium (Xell, Germany). After harvest, cells were flocculated using 5 pg per total cells at pH 6.5, allowing 15 min for settling of cells. Subsequently, the resulting supernatant and a Q-Sepharose resin (GE, Sweden) were pumped in equal amounts to a vessel, where a residence time for AEX adsorption of 15 min was applied, with the aim of allowing contaminants to adsorb to the resin. The resulting supernatant/AEX resin suspension was pumped out of the vessel into a 0.22-µm hollow fiber system (GE, USA). The mAb was recovered in the permeate, whereas the AEX resin remained in the retentate and could undergo elution, regeneration and sanitization for reusing. Two process variations were evaluated (Table 1), which were combined resulted in 6 different process strategies: (i) the ratio of clarified supernatant to AEX resin; (ii) the use of a device for cell/flocs retention named inclined lamella settler (Biotechnology Solutions, USA) and depth filter Clarisolve (Merck, USA), both were placed after the flocculation step to ensure a cleaner supernatant and to allow reducing the cell settling time. The integrated clarification-capture process showed to be simple and fast. Steady-state conditions were obtained during adsorption and filtration for all conditions studied. The average recovery of mAb during the steady-state was 48.5% ± 2%, which means a loss of approximately 3% of mAb product, since it was 2-fold diluted by the 1:1 mix with the resin suspension. However, considering the overall process, from start to final permeate recovery, global yields between 61% and 90% were obtained. These results are mainly related to the void volume of inclined lamella settler. The best global recovery (90.4%) was obtained when the depth filter was included in the process. Regarding impurities removal, in all 6 process strategies evaluated more than 85% of DNA was removed, and approximately 70% of HCP removal could be achieved when depth filter was used. Taking into account that two different supernatant/AEX resin ratios tested, a lower supernatant/resin ratio (41) provided a higher DNA clearance (86 fold), compared to less than one third of this clearance when sample/resin ratio was doubled to 82.

Table 1: Straight-through process variations studied.

Process	Viable cells $x10^6$/mL	Cell viability (%)	Retention device	Ratio Sample/AEX
1	7.3	87.6	None	82
2	11.9	95.2	Settler	82
3	15.1	88.8	Settler	82
4	19.3	80.4	Settler	41
5	12,1	91.9	Filter	41
6	8.3	87.7	Filter	41

HYDROCYCLONES FOR SINGLE-USE PERFUSION APPLICATION

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Key Words: hydrocyclone, perfusion, single-use bioreactor, cell retention device, high cell density

Hydrocyclones (HC) are very compact devices that promote solid-liquid separation under the action of a centrifugal field. Despite the small size, HCs have a large processing capacity and do not suffer from clogging. Therefore, several publications explored HCs as a potential cell retention device in perfusion applications in the last 20 years, but limited to non-disposable lab-scale bioreactors and to relatively low cell densities (up to ~10 million cells/mL).

Even though the absence of moving parts may streamline the HC manufacturing, the performance of solid-liquid separation is highly dependent on the HC internal geometry. Said that, hydrocyclones can be produced by 3D printing, making them a promising alternative for the integration of cell retention devices in single-use bioreactor bags. The performance of hydrocyclones also depends on the attachment configuration to the bioreactor and cell concentration of the feed suspension. In this work, at first rapid batch tests were carried out to evaluate the impact of: (i) cell concentration; (ii) diameter of connector installed in the recirculation loop; and (iii) controlled harvest flow rate enabled by a peristaltic pump (520U model, Watson Marlow). The main response considered was their effect on HC separation efficiency. The stainless-steel HC2015 designed for mammalian cell separation (Pinto et al., 2008) was selected for the preliminary batch tests, and also used as a benchmark for plastic prototypes produced by 3D-printing techniques. Afterwards, the same HC2015 was installed in a 50-L single-use bag (XDR50 Xcellerex, GE Healthcare) specially customized for a perfusion cultivation with a mAb producer CHO cell line.

The stainless-steel HC2015 when operating at 2.3 bar provided a total separation efficiency (E_t) up to 96%, and a centrifugal separation efficiency (E´) of 82% for a CHO cell suspension at 24E6 viable cells per mL Concentrated cells recovered by the underflow port did not show decrease in viability compared to the feed suspension. The reduction of a TC connector size from 19.7 to 12.7 mm resulted in the total filling of the recirculation loop with liquid, disrupting the formation of the desirable umbrella-pattern discharge of the underflow and reducing cell retention. The use of a peristaltic pump to control the overflow flow rate equivalent to perfusion rates of 1 and 2 RV (reactor volume) per day in 40-L bioreactor working volume resulted in a reduction of the E´ values and a consequent increase of cell concentration in the harvest stream. The reduction in the separation efficiency was probably due to a disturbance of the liquid flow pattern inside the HC, since it was observed that the typical gas core coming out from the overflow was absent. These features were taken into account in the HC operation in the single-use 50-L perfusion bioreactor, and a cell concentration of 50E6 cells per mL was successfully achieved with a cell-specific perfusion rate (CSPR) as low as 20 pL per cell per day. The harvest stream consisted of a natural cell bleeding leaving the overflow outlet. Moreover, the lower cell viability and average diameter in the overflow evidenced the preferential retention of viable cells returning into the bioreactor, thus providing a healthier culture environment. A 3D-printed hydrocyclone with equivalent geometry to the stainless-steel HC2015 was made and presented slightly lower separation efficiencies. Further studies proposing materials with a smoother surface and investigating further 3D-printing techniques are currently ongoing.

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FLOW-THROUGH CHROMATOGRAPHY AS A CONTINUOUS AND INTEGRATED PURIFICATION METHOD

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Key Words: Flow-through Chromatography, continuous chromatography, linear gradient elution

Continuous manufacturing is expected to improve the efficiency and economics of protein and other bio-product production processes. However, it is not easy to design and operate the continuous process especially for downstream processing as many unit operations (chromatography and membrane filtration) are involved.
An operation method known as flow-through chromatography (FTC) is considered to be an efficient purification method as the flow is continuous. In FTC, a target bio-product is eluted from the chromatography column without adsorption whereas contaminants are strongly bound. Usually two different modes of chromatography are needed in order to remove various kinds of contaminants. Two FTC columns have to be connected in order to build the integrated continuous process. This is not an easy task since the mobile phase properties (pH, salt, buffer ions) are different for the two columns.

In this study, we investigated how FTC processes can be designed based on linear gradient elution (LGE) data by using our LGE model. As a first model separation system removal of BSA dimer from BSA monomer was chosen. The distribution coefficients of BSA monomer and dimer and the mass transfer data were obtained from LGE experimental data based on the model. Experimental breakthrough of BSA dimer was well predicted by the model simulation. The model simulation also showed that FTC is very sensitive to a small change in salt concentration and/or pH of the mobile phase as well as the mobile phase velocity (see Fig.1). The productivity calculation method was also developed. The second model system was to use two FTC columns for removing multiple contaminants. In this system the efficiency of FTC processes was examined in terms of impurity removal efficiency from the cell culture broth containing monoclonal antibody. It was found that two FTC (anion exchange chromatography and cation exchange chromatography) can remove impurities efficiently when the mobile phase pH and conductivity were properly chosen. It was also shown that the two columns can be connected as a pseudo continuous FTC operation.

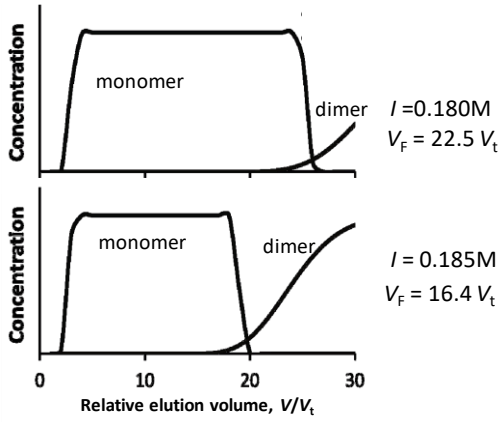

Figure 1 Effect of mobile phase salt concentration I on BSA dimer removable by flow-through chromatography with anion-exchange column (Q-Sepharose HP). V_t :column volume, V:volume, V_F: sample feed volume, I: salt (NaCl) concentration. The curves were calculated with the distribution coefficients as a function of I and mass transfer data determined by LGE experiments.

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DEVELOPMENT OF INTEGRATED CONTINUOUS BIOPROCESSING USING CONTINUOUS COUNTERCURRENT TANGENTIAL CHROMATOGRAPHY (CCTC) PLATFORM FOR CAPTURE AND POLISHING OF MONOCLONAL ANTIBODIES

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Key Words: Continuous, steady-state, chromatography

Several recent studies have demonstrated the potential of using continuous countercurrent tangential chromatography (CCTC) for the purification of monoclonal antibody products. CCTC operates with a flowing resin slurry, exploiting traditional approaches of countercurrent staging to achieve > 10-fold increases in productivity compared to traditional batch columns in a truly steady-state low pressure (<15 psi) unit operation that can be directly integrated into a fully continuous biomanufacturing process. The featured data shows productivity and product quality outcomes from Protein A capture, as well as Anion exchange and Cation exchange polishing steps for purification of a commercial mAb. Another unique aspect of CCTC is the ability to use small particle size resins with improved binding kinetics and better system productivity but without any increase in pressure. The data also features our strategy for integrating the unit operations into a single process train incorporating an integrated in-line sampling strategy. In addition we discuss the potential of this platform to enable efficient processing of sensitive biologics because of significantly reduced residence time (<10 min from binding to elution), as well as rapid in-line buffer adjustments of eluted product.

INTENSIFYING THE MANUFACTURE OF HIPSC THERAPY PRODUCTS THROUGH METABOLIC AND PROCESS UNDERSTANDING

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Key Words: cell therapy, hiPSC-derived cardiomyocytes, hiPSC-derived hepatocytes, three-dimensional culture, perfusion, omics technologies

In vitro differentiation of human induced pluripotent stem cells into specific lineages such as cardiomyocytes (hPSC-CM) and hepatocytes (hPCS-Hep) is a crucial process to enable their application in cell therapy and drug discovery. Nevertheless, despite the remarkable efforts over the last decade towards the implementation of protocols for hPSC expansion and differentiation, there are some technological challenges remaining include the low scalability and differentiation yields. Additionally, generated cells are still immature, closely reminiscent of fetal/embryonic cells in what regards phenotype and function. In this study, we aim to overcome this hurdle by devising bioinspired and integrated strategies to improve the generation and functionality of these hiPSC-derivatives. We also applied robust multi-parametric techniques including proteomics, transcriptomics, metabolomics and fluxomics as complementary analytical tools to support bioprocess optimization and product characterization.

We cultured hiPSC as 3D aggregates in stirred-tank bioreactors (STB) operated in perfusion and used a capacitance probe for in situ monitoring of cell growth/differentiation. After cell expansion, the hepatic differentiation step was integrated by addition of key soluble factors and controlling the dissolved oxygen concentration at various stages of the process to generate populations enriched for definitive endoderm, hepatocyte progenitors and mature hepatocytes. The analyses of hepatic markers expression throughout the stages of the differentiation confirmed that hepatocyte differentiation was improved in 3D spheroids when compared to 2D culture. Noteworthy, these hiPSC-HLC exhibited functional characteristics typical of hepatocytes (albumin production, glycogen storage and CYP450 activity). We also demonstrate the potential of dielectric spectroscopy to monitor cell expansion and hepatic differentiation in STB.

For CM differentiation, we relied on the aggregation of hPSC-derived cardiac progenitors to establish a scalable differentiation protocol capable of generating highly pure CM aggregate cultures. We assessed if alteration of culture medium composition to mimic *in vivo* substrate usage during cardiac development improved further hPSC-CM maturation *in vitro*. Our results showed that shifting hPSC-CMs from glucose-containing to galactose- and fatty acid-containing medium promotes their fast maturation into adult-like CMs with higher oxidative metabolism, transcriptional signatures closer to those of adult ventricular tissue, higher myofibril density and alignment, improved calcium handling, enhanced contractility, and more physiological action potential kinetics. "-Omics" analyses showed that addition of galactose to culture medium and culturing the cells under perfusion improves total oxidative capacity of the cells and ameliorates fatty acid oxidation. This study demonstrated that metabolic shifts during differentiation/maturation of hPSC-CM are a cause, rather than a consequence, of the phenotypic and functional alterations observed. The metabolic-based strategy established herein holds technical and economic advantages over the existing protocols due to its scalability, simplicity and ease of application.
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INCLINED PLATE SETTLERS WITH NOVEL RECEIVER SECTION AS A UNIT OPERATION FOR COMPLEX CONTINUOUS SOLID-LIQUID SEPARATION PROBLEMS

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Key Words: Continuous processing, solid-liquid separation, plate settler, receiver section

Solid-liquid separation has been identified as a bottle-neck in continuous processing, especially regarding precipitate collection[1]. Previously, tangential flow filtration has been proposed as a means of solid-liquid separation that retains precipitate flock structure[2]. We have developed a novel receiver section concept for inclined plate settlers. Inclined plate settlers have been described for cell retention in perfusion processes in the 1990s[3]. Conventional plate settlers suffer from non-ideal flow distribution between the individual plates [4, 5],mixing effects in the receiver section and significant losses of solubilized product during withdrawal of the concentrated suspension. Consequently, conventional plate settlers are not suitable for applications outside of perfusion. We devised a novel receiver section concept employing a system of flow distributors, which overcomes the deficiencies in flow distribution and separation between inflowing suspension and sedimented solids. Furthermore, the novel concept enables wash of the collected solids and thus significantly reduces fluid carry-over into the (intermittent) discharge stream. Thereby, the application range of inclined plate settlers is expanded from cell-retention to solid-liquid separation in general. Both streams, clarified fluid as well as concentrated and washed solids, can leave the system. The separated solids can, but do not have to be returned to the process. Proof-of-concept was provided using a single plate inclined settler. Operation conditions of the inclined plate settler system were optimized for precipitated product harvest. Scale-up is demonstrated from one to multiple plates for cell removal. We demonstrated continuous operation for ~90h and once in steady-state, turbidity was reduced by 95 % at consistently high product yields.

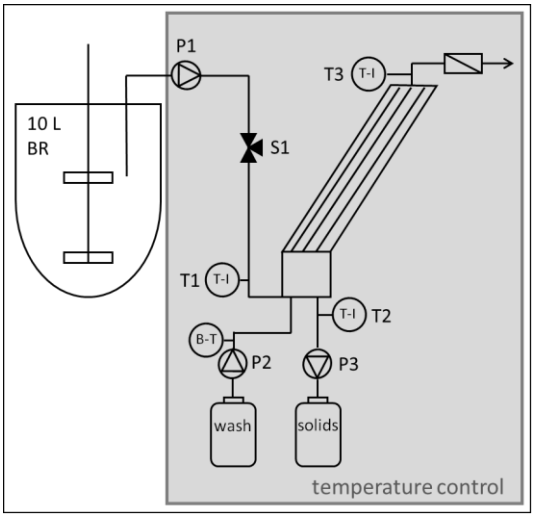

Figure 2 – Continuous cell removal in a cell-culture process with wash of the solids using the inclined plate settler with the novel receiver section concept.

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PERFUSION MICROBIOREACTOR WITH INTEGRATED CELL RETENTION DEVICE

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Key Words: Small-scale, microbioreactor, perfusion, intensified perfusion, cell retention device

Intensified perfusion processes are a key component in Integrated Continuous Biomanufacturing that still faces many challenges. For example, delivering the right amount of nutrients for extremely high cell densities at economically feasible perfusion rates need the right cell culture media and process development. To this end, data sets are generated using high-throughput small-scale models. In the absence of a cell retention device that can work with those small working volumes, strategies that mimic perfusion are being used. For example, a common technique is to use either high density batch or simulation of perfusion in spin tubes or ambr®15[1] using discrete media exchanges. However, the applicability of the data generated in these small-scale models is limited and process development must be supplemented with perfusion bioreactors at benchtop scale. The key point that these small-scale models miss to accurately predict perfusion processes is a cell retention device that enables continuous media exchange while retaining cells in the bioreactor. In this work we will present the evaluation of a perfusion microbioreactor system. This 2mL perfusion microbioreactor has all the requirements to accurately control DO, pH and temperature; it is equipped with a filtration-based cell retention device and optical density sensors that enable the performance of continuous perfusion with automated cell bleed (Figure 1)[2]. We will show cell performance in the perfusion microbioreactor system at 2vvd using a CHOZN®-GS producing a fusion protein (n=3) and a CHO-S producing an IgG (n=2) in steady state with a target viable cell density of 50x10^{6}vc/mL and dynamic perfusion. We will then compare cell growth, metabolites and production to steady state and dynamic 3L perfusion bioreactors and we will review process modifications made during the evaluation, including gas and mixing strategies. Lastly, we will present two case studies using the perfusion microbioreactor system: 1) Evaluation of cell performance in three different media. 2) Determination of minimum CSPR.

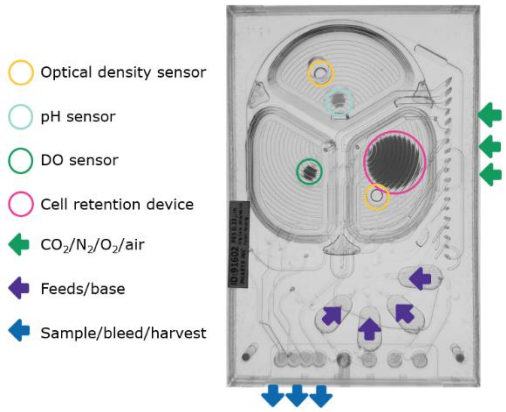

Figure 1 – Erbi Biosystems perfusion microbioreactor

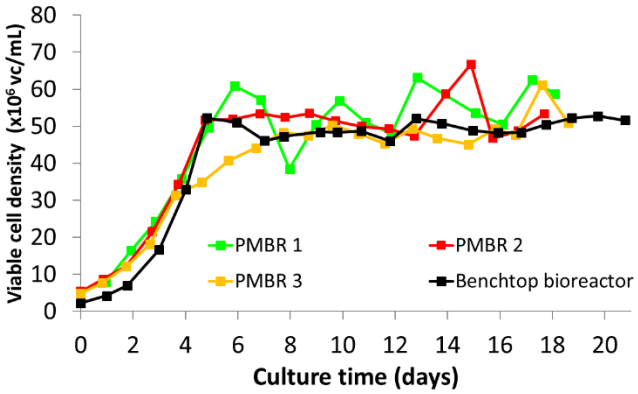

Figure 2 – Viable cell density profile of perfusion microbioreactor (PMBR) experiments compared to a 3L benchtop perfusion bioreactor using a CHOZN®-GS producing a fusion protein

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DECREASING DRUG DEVELOPMENT TIMELINE VIA UPSTREAM PROCESS INTENSIFICATION

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Decreasing drug development timeline via upstream process intensification
A scalable, high-intensity perfusion process was developed at Boehringer Ingelheim, Fremont Inc which is 10x more productive for producing recombinant proteins than comparative fed batch processes in the same 14-day run duration. By eliminating wasteful cell bleed we were able to achieve cell densities up to five times greater than standard “steady state” perfusion culture previously used. In order to sustain such large cell masses at manageable media exchange rates, concentrated media feeds were developed which effectively allow for optimization of nutrient delivery and dilution rate. We believe this system is scalable up to 1kL; the process has already been demonstrated successfully at the pilot scale (100L), where bioreactor productivities averaging over 5 g/L/day have been demonstrated.

We begin development with new cell lines for the high intensity perfusion process by adapting spin-tube and shake flask models that others have used for fed batch. These methods are used to test for important control parameters to allow full development in a 2L bioreactor. AMBR250 bioreactors can be used, though not optimal, as will be discussed. Due to the simplicity of the process design, the integrated downstream is developed at small scale using classical batch chromatographic techniques, including high throughput process development and standard chromatographic steps. The virus inactivation step is developed by accounting for viscosity and titration of the product and buffer in the Protein A elution peak, which differ slightly from product to product. With these simple development techniques, we believe the highly productive process could be commercially viable at Phase I, with limited to no Phase III process development.

VIRUS REDUCTION FILTRATION IN CONTINUOUS BIOPROCESSING: CRITICAL FLUX CONCEPT FOR VIRUS BREAKTHROUGH

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Key Words: Virus Reduction Filtration, Monoclonal Antibody, Critical Flux, Downstream Process

Virus reduction filtration is an integral component of virus clearance strategy in modern biologics production processes. Continuous bioprocessing and process intensification are the buzz words in biopharmaceutical industry due to benefits of cost savings from higher productivity, operational flexibility and better product quality. A typical batch process operates virus reduction filters at constant pressure to maximize the throughput within one shift of process time constraint. However, continuous bioprocessing would demand constant low flux virus reduction operation over extended duration to address the complications of frequent filter switching. Due to relaxation of process time constraint, continuous bioprocessing allows better utilization of filter capacity (L/m^2). However, there is limited understanding around the virus breakthrough mechanism and associated critical process parameters. This poses an interesting question how to define end point for virus reduction filtration while utilizing most of the filter capacity for low fouling feed. What is the effect of flux, differential pressure and total viral particle load on virus clearance performance of commercially available viral filters? Is there a critical flux for viral reduction filters below which they are susceptible to significant virus breakthrough (LRV <4) and if yes, how sensitive it is to filter's property and other process parameters? This study explores the effect of flux, differential pressure and total viral particle load on viral clearance performance of three commercially available filters. These filters are selected to cover the wide range of filter permeability and membrane material. The viral clearance study was performed both in the presence and absence of products.

NOVEL PERIODIC ALTERNATING TANGENGIAL FITRATION HARVEST APPROACH PROVIDES INCREASED VOLUMETRIC PRODUCTIVITY

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Key Words: Upstream, Increased Yield, Periodic Harvest

Perfusion cell culture processes provide opportunities to increase product yield through improved cell growth, increased productivity and extended process duration. Most commonly, perfusion cell culture also leads to a continuous harvest operation and collection of harvested cell culture fluid to be processed downstream.
As an alternative to continuous harvest, we evaluated instead a periodic harvest approach applied to a non-steady state perfusion cell culture process using alternative tangential flow (ATF). In this ATF perfusion process, product is first accumulated in the bioreactor using ultrafiltration for 15 days with the product then being harvested by microfiltration at end of the process. To further extend the culture time beyond 15 days and maximize productivity, we investigated a sequence of five periodic harvests from a single upstream bioreactor run. The periodic harvests were achieved using an ATF configuration in which ultrafiltration and microfiltration hollow fiber filters were stacked in series (Figure 1). The ultrafiltration hollow fiber retains the product while the microfiltration filter allows product to be collected in the permeate. Permeate was only drawn from the microfiltration filter during the periodic harvest cycles while the permeate was drawn from the ultrafiltration filter during the none harvest cycle periods. This allowed for the accumulation of product in the bioreactor between the periodic harvests. Five harvest cycles were conducted over a 24-day perfusion process. Each harvest cycle was collected for a day with the first harvest cycle starting on day 11.

Using a model antibody expressing cell line in the described 24-day extended periodic harvest (X-PH) process, we increased the daily volumetric productivity (g/L-day) 86% relative to the 15-day ATF process in which product is recovered using a single microfiltration at the end of the process. Furthermore, two 24-day X-PH lots would provide 98% more recovered material than three lots of a 15-day process with a single ultrafiltration at the end of the process, in the same 51-day period assuming a three day turn around between lots. The increase in recovered product with the X-PH process is driven by a 48% increase in cell-specific productivity (attributed to periodic harvest cycles) and a 26% increase in integrated viable cell density (IVCD) for the same 51-day period. Finally, product quality analyzed from the X-PH process was both similar among the five harvests and as compared to the 15-day ATF process with single microfiltration harvest at the end of the process.

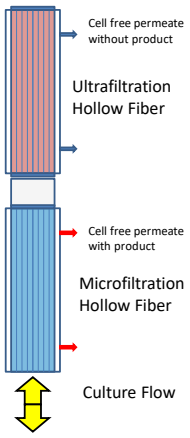

Figure 1 - X-PH ATF filter configuration

MANIPULATIONS OF ANTIBODY GALACTOSYLATION IN A FED-BATCH ADAPTED PERFUSION PROCESS

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Key Words: Next-Generation Bioprocesses, ATF, Fed-batch, Galactosylation

Perfusion processes have demonstrated significantly higher volumetric productivity while maintaining consistent product quality attributes (PQAs) throughout an extended harvest period. With this, a drastic decrease of the bioreactor footprint in future commercial plants can be achieved. Therefore, there are significant efforts in biopharmaceutical industry focused on developing high productivity perfusion process for both future biologics, as well as on developing new generation processes for legacy fed-batch processes.

In this presentation, we will report the adaptation of an established 14-day fed-batch process for a therapeutic monoclonal antibody to a 30-day perfusion process using alternating tangential flow (ATF) technology. In the proof-of-concept perfusion process, a 6-fold viable cell density (VCD) was reached and maintained compared to the peak VCD in the fed-batch process. The productivity increased 3.5-fold as compared to the original fed-batch process after normalization to cell culture duration. The major PQAs from this perfusion process were consistent throughout the harvest period. Most of the PQAs from the perfusion process showed no significant differences from the original fed-batch process. However, significantly higher galactosylation level was observed in the perfusion process as compared to its fed-batch version. In the presentation, we will also detail the perfusion medium and process parameter optimization, which enabled the matching of the galactosylation profile of the last version perfusion process to the established fed-batch process without compromising target VCD, productivities, and other PQA profiles.

DEVELOPMENT OF SCALE-DOWN MODELS FOR VALIDATION OF INTEGRATED CONTINUOUS VIRUS FITLRATIONS

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Key Words: continuous virus filtration, validation, scale-down model

Continuous bioprocessing is becoming more widely adopted in biomanufacturing. While much progress was achieved in upstream processes and downstream chromatography and viral inactivation steps, there is little data published on continuous virus filtration (VF). As continuous manufacturing leads to changes in processes, facilities and equipment, these factors need to be considered within the VF design space. Additionally, since the choice and performance of a virus filter is generally dependent upon the outputs from upstream unit operations, the filter may experience load solutions with fluctuating protein concentrations, salts and impurities, consistent with a chromatography step elution profile. Understanding how continuous processing impacts the performance of a virus filter can lead to developing both integration and validation strategies. In this work, we designed scale-down virus filtration models to investigate the impact of extended process times and dynamic product streams present in continuous manufacturing. We performed long-term PP7-spiked virus filtrations using Planova 20N and BioEX filters. The results show that Planova 20N and BioEX virus filters are capable of effectively (> 4 log) removing bacteriophage PP7 when run for up to one week continuously. Creative methods were successfully implemented in order to overcome long-term PP7 stability and pressure fluctuations. Additionally, both the 20N and BioEX filters were able to successfully process a mock elution peak of increased protein, salt, and bacteriophage concentrations with only an increase in filtration pressure observed during the higher protein concentration peak. Effective virus removal was achieved even under challenging PP7 particle loads (>9.5 logs total). These experiments demonstrated that small-scale viral clearance studies can be designed to model a continuous viral filtration step with specific process parameters. Both Planova 20N and BioEX filters were shown to be robust with respect to extended processing times and fluctuating elution peaks. The integration of continuous virus filtration into continuous biomanufacturing processes is therefore applicable and adaptable; it remains largely process-dependent. Further validation strategies may include mimicking multiple elution peaks in series to allow for a better characterization of the pressure limit of these filters in a continuous setup.

CONTINUOUS INTEGRATED BIOLOGICS MANUFACTURING

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Key Words: Continuous Integrated Biologics Manufacturing

Biosimilars and patent expiry are forcing the biopharma industry to find new ways to maintain competitiveness by ensuring affordability, quality, and delivery performance. Despite great improvements in upstream processing (USP) efficiency, higher titres create downstream processing (DSP) bottlenecks and facility fit issues: Equipment reaches its physical and capacity limits thereby increasing processing time, material consumption and overall cost. Continuous processes have been proposed as a solution to many of these issues as they offer higher productivity while reducing cycle times, buffer/resin usage and required footprint.

A consortium of UK based biopharmaceutical companies, suppliers and not for profit research organisations, funded by an Innovate UK grant, has been created and will investigate how such an integrated, continuous downstream process system can be realised. The system has been constructed and is currently operating at the Centre for Process Innovation at Darlington, UK. The project combines and condenses multiple DSP unit operations to function as one uninterrupted system with integrated analytics and overarching automated control. The aim is to create an operationally-efficient, multi-product platform which replicates the functionality of a larger plant processing 100 L feedstock per day (independent of product titre).

The integrated unit will be tested on several biologic processes demonstrating the system’s potential to enable product changeover, increased facility flexibility and productivity. Significant focus will be given to process validation procedures and the use of low level control to achieve process stability (steady state) and maintain acceptable product quality. This work will lay the foundation for real-time release strategies and replace drug substance release testing.

This presentation will provide an overview of the project and show recently-acquired data from the automated purification of industry-relevant monoclonal antibodies. In doing so, this will highlight the applicability and demonstrate the real-world potential for integrated continuous processing to advance the manufacturing of biopharmaceuticals.

PERFUSION PROCESS FOR THE PRODUCTION OF A NEW, VLP-BASED YELLOW FEVER VACCINE CANDIDATE

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Key Words: Virus-like particles (VLPs), yellow fever virus (YFV), vaccine, process development, perfusion.

Yellow fever (YF) is an acute viral hemorrhagic disease endemic in tropical areas of Africa, Central and South America, which is transmitted by the bite of infected mosquitoes. It is a “historically devastating disease” (Paules and Fauci, 2017) that killed during outbreaks in past centuries, before the introduction of the current vaccine, approximately 10% of the population of cities like Philadelphia (USA) and Barcelona (Spain). According to Garske et al. (2014), YF caused in 2013 78,000 deaths worldwide, which is a disease burden comparable to influenza. In the past few years, outbreaks in Angola (2016) and in Brazil (2017-2018) led to the depletion of the WHO vaccine stockpile and to the introduction of the emergency use of a fractional dose (1/5). Furthermore, the Angola outbreak in 2016 caused the first cases of YF ever to occur in Asia (11 imported cases to China), rising the concern about approximately 2 billion immunologically naïve people who would be at high risk in Asia in case local transmission of the virus starts to occur (Wilder-Smith et al., 2019).

The urgent need for a new YF vaccine becomes evident from two major issues concerning the current vaccine, which consists of a live-attenuated virus propagated in chicken embryos: (i) vaccine shortage due to limitations in the manufacturing technology; (ii) rare, but fatal adverse effects. Therefore, this work focuses on the development of a safe, non-replicating YF vaccine, produced by a high-productivity perfusion process.

Stable recombinant HEK293 cell lines constitutively expressing the structural proteins prM (pre-membrane) and E (envelope) of YFV were generated, enabling long-term production and secretion of recombinant virus-like particles (VLPs). FACS (fluorescence activated cell sorting) was used to sort the transfected population for high producer cells and allowed obtaining an enriched cell pool producing significantly higher amounts of VLPs. Small scale kinetic studies under intermittent perfusion (pseudoperfusion) were performed in order to investigate possible feeding strategies and to evaluate the use of short-chain fatty acids as productivity enhancers. Subsequently, perfusion runs were carried out in stirred-tank bioreactors in order to investigate optimal conditions for VLP production, as well as to evaluate different cell retention devices (e.g. inclined lamella settler and ATF-2). Partial retention of the VLPs in the perfusion bioreactor system occurred when the ATF-2 was used. VLPs produced by perfusion were purified by a two-step chromatographic process, and transmission electron microscopy (TEM) images confirmed the expected size and morphology of the VLPs, enabling their use in mouse immunogenicity studies.

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CRITICAL QUALITY ATTRIBUTES (CQAS) OF A THERAPEUTIC ANTIBODY PRODUCED FROM INTEGRATED CONTINUOUS BIOPROCESSING

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Key Words: Perfusion cultivation, Periodic counter-current (PCC) chromatography, Critical quality attributes (CQAs), Charge heterogeneity

The integrated continuous bioprocess provides an innovative way to produce protein drugs with flexibility and efficiency. However, during the long-term cultivation and complicated production, how to ensure the process stability and product quality is critically important. In this study, the monoclonal antibody (mAb) was produced in a bioreactor operated in a perfusion mode utilizing the ATF cell retention system for up to 32 days. The 2L harvest per day starting at day 10 was continuously purified using the 3-column periodic counter-current (PCC) chromatography system. The first protein A capture purification was performed with the dynamic binding capacity of 50% breakthrough around 60 mg mAb/mL of resin (vs 20 mg/mL resin for batch purification) for 120 cycles or 360 column operations followed by a polishing step of mixed mode chromatography for 20 cycles. The process and quality attributes were monitored daily. The results demonstrate consistency in both the purification process and the mAb qualities (in the aspects of product integrity, aggregates, and glycan profile) between PCC and batch purifications. Culture-related charge heterogeneity was observed accompanied by an increase of bioreactor harvest time using both batch and PCC purification processes. In addition, the impurities such as endotoxin and HCP were also monitored while under this high capacity utilization of chromatography resins. By sharing the insights of process and quality attributes, we hope to provide better understanding on the process-related heterogeneity between batch and continuous production and/or purification.

Figure 3 – Integrated Continuous Bioprocessing

PROCESS SIMULATION BASED DECISIONAL TOOL TO EVALUATE STRATEGIES FOR CONTINUOUS DOWNSTREAM BIOPROCESS IMPLEMENTATION – A CDMO PERSPECTIVE

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Key Words: CDMO, continuous process, bioprocess simulation

To maintain a competitive space in the rapidly expanding and highly competitive market, many biopharmaceutical companies are outsourcing to contract development and manufacturing organizations (CDMOs) to accelerate research and development, shorten the time to market, alleviate internal capacity and technical constraints, and reduce risks associated with production [1]. To acquire new and maintain current clients, CDMOs must have strong, diverse technical offerings for development, manufacture, and testing of products with competitive pricing and timelines [2]. Adopting innovative technologies like continuous downstream processing can help debottleneck the process and reduce processing time, which is the most appealing to CDMOs as it translates to an increased number of batches per year. The majority of continuous processing assessments to date have focused on cost of goods and not on the time reduction potential [3-7]. End-to-end continuous downstream processing is not always practical as CDMOs must accommodate a wide range of molecules and processes. Hence, it is imperative to evaluate and customize continuous production based on client needs. Application of process simulation as a decisional tool to select an appropriate downstream processing strategy was evaluated. Two modelling programs were evaluated: BioSolve Process and SuperPro Designer®. Fully continuous and hybrid (continuous Protein A operation only) downstream processing were assessed for a 2000 L fed-batch bioreactor producing 1, 5, and 10 g/L of monoclonal antibody at 40 and 200 kg production demands. Hybrid and continuous processing decreased batch duration by 20% and 60%, respectively. Continuous processing was more favorable for higher titer processes (≥ 5 g/L). The largest cost reductions were observed for 5 and 10 g/L titer processes during 40 kg production. The results highlight the business case for continuous downstream bioprocessing especially at a CDMO. Selection of a processing method will be influenced by a range of factors and the impact can easily be assessed using process simulation. Therefore, it is recommended that CDMOs use process simulation to ensure the most favorable processing strategy is selected.

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HIGH CELL DENSITY OPTIMIZATION STRATEGIES FOR CONTINUOUS BIOPROCESSES USING PERFUSION BIOREACTORS

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Key Words: Intensified perfusion bioprocesses, optimization of bioreactor parameters, dynamic perfusion run

Intensified perfusion bioprocesses enable high cell density cultures with higher volumetric productivity and are a promising alternative to the fed-batch technology most commonly used in current biopharmaceutical production processes. Some of the key challenges when working with extremely high cell density cultures are high oxygen demand, consequent generation of shear stress, and foam by spargers, resulting in technical difficulties to maintain the bioprocesses. Our study demonstrates the optimization of bioreactor parameters and culture conditions enable very high cell densities using perfusion systems for intensified processes. One of the parameter focused in our study is to improve bioreactor performance measuring dissolved oxygen (DO) to determine volumetric mass transfer coefficient (kLa) using static gassing out method in EX-CELL® Advanced HD Perfusion Medium to understand the mass transfer as a function of agitation speed and aeration rate using spargers. We evaluated bioreactor process parameters such as agitator speed, gassing rate, properties of the medium, anti-foam agents, surface active solutes that affect kLa to enable higher cell density suspension cultures while maintaining high viability. Our study showed that aeration rate has larger effect on kLa than agitation rate and gives us tool to predict Kla requirements at specific cell densities in the perfusion bioreactor. With the above mentioned optimized kLa conditions, we made improvements on CHOZN®GS cell line for dynamic perfusion (no bleed) bioprocess and showed how changes in the process can enable the increase of cell density by 3-fold, reaching densities above $250x10^6$vc/mL with 2vvd (CSPR<10pL/cell/d) - while maintaining or increasing viability.

CONTINUOUS PROCESS PERFORMANCE ENHANCEMENTS FOR 50 L TO 500 L SINGLE-USE BIOREACTORS: A TECHNICAL COMPARISON OF PERFORMANCE CHARACTERIZATION, CELL CULTURE, AND SCALE-UP MODELING

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Key Words: Continuous processing, single-use bioreactor, perfusion, S.U.B. Enhancements

Improvements in single-use systems have allowed implementation of high-density cultures in emerging bioprocess work flows. Specifically, advantages of single-use bioreactors have been realized in perfusion applications in high-density seed train intensification or as a compact production-scale bioreactor system. Due to this and additional progressive advances in media optimization and improved clone genetic selection have increased stress on the perceived performance limitations of single-use bioreactors. This study shows integration of the Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) and how strategic enhancements to the sparger and agitation systems have revealed the potential for 3-4X improvement of mixing and mass transfer performance compared to legacy SUB designs. This work includes:

1) Bioreactor characterization and scalability analysis of the S.U.B. when targeting perfusion applications from 50 L pilot scale to 500 L production scale working volumes.

2) High-density culture results (>200E06 cells/mL) while maintaining proper operating parameters using a TruBioTM DeltaV controller and online process analytics. New data reveals specifically how a 50 L S.U.B. equipped with a specialized precision drilled hole sparger (DHS), single use foam probe, and oversized impeller is able to improve overall SUB operating efficiency. Coupled with best practices and the desirable process benefits achieved through automation and control of vital process parameters, evidence is provided as to the advantages of continuous processing in single-use systems.

APPLY ADSORPTION TECHNOLOGY TO SOLVE THE UV SENSOR INSTABILITY OF DYNAMIC CONTROL ON PERIODIC COUNTER CURRENT PURIFICATION SYSTEM

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Key Words: UV sensor, periodic counter current purification system, resin lifecycle prediction

Cell culture media are a source of major nutrients that provide cell growth and synthesis of proteins. Researchers and suppliers use combine media components test continually in an attempt to find more suitable cell culture media for use in continuous cell culture processes. Commercial media components are a black box for downstream purification researchers who cannot predict the effects of their constituents on resin affinity, absorbability, even for interference with UV instrument. To more effectively utilize in dynamic continuous purification system, the loading product percentage of resin absorbability is typically controlled at 40-60% maximum binding capacity. It is often five times more than traditional batch purification. Therefore, the swing of UV percentage caused by color material in the bulk harvest is the key process parameter in the periodic counter current purification system. We compared different commercial quaternary ammonium adsorbent matrix (such as Stabilized Regenerated Cellulose, Agarose and Styrene Divinyl Benzene Copolymer) to observe their effects on the swing of the UV amplitude. The results show that agarose group has better de-coloration efficacy than the other two commercial quaternary ammonium groups.

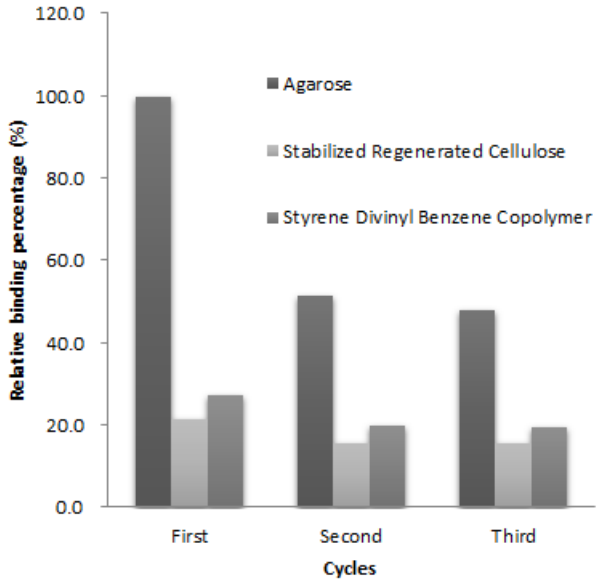

Figure 4 – De-coloration efficacy of quaternary ammonium adsorbent matrix

ACOUSTIC WAVE SEPARATION – A NON-FILTRATION APPROACH FOR CONTINUOUS CLARIFICATION OF PERFUSION CELL CULTURE PRIOR TO CAPTURE CHROMATOGRAPHY

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Key Words: perfusion, clarification, cell, retention, acoustic

Advances in perfusion cell culture have led to cell densities in excess of 100 million cells/mL with product titers similar to those obtained in fed batch (3-5 g/L). This performance has necessitated improvements in the yield and efficiency of the cell harvest and clarification stage to generate a stream of Harvested Cell Culture Fluid (HCCF) for capture chromatography and subsequent downstream processing. This is further driven by the evolution of continuous processes where there is a preference for a continuous feed of HCCF available for direct load to the continuous multicolumn capture chromatography step.

In the present work we report on a novel disruptive and scalable single-use technology for cell retention during perfusion cell culture based on an acoustophoretic separation. Acoustic Wave Separation (AWS) technology exploits the use of low frequency acoustic forces to generate a three-dimensional standing wave across a flow channel. Recirculating cell culture from a perfusion bioreactor enters the flow channel and passes below the acoustic zone. The product-containing stream of HCCF is removed from the recirculating cell culture by passage through the acoustic zone. This yields a well clarified HCCF that can be polished using a small area filter.

We report the continuous cell retention during a perfusion culture of a CHO cell line expressing a mAb. At process development (PD) scale we demonstrate the ability to continuously process CHO cell culture and retain cells at densities of up to 100 million cells/mL, at flow rates of up to 2 bioreactor volumes per day. Since the clarification technology does not involve the use of hollow fiber tangential flow filtration (TFF) we ensure 100% transmission of the mAb through the AWS device. The closed system remains operational for up to 60 days enabling this scalable technology to be suitable for use in clinical manufacture. The post-AWS HCCF is 99% clarified and any residual cellular material can be removed using a small gamma stable membrane filter or directly loaded onto a 0.2 micron filter prior to chromatography. Additionally, no demonstrable adverse effects have been identified for the quality of the HCCF, the product itself, or the viability of the returning perfusion cell culture following cell retention using AWS technology.

AWS technology enables the continuous cell retention from recirculating cell culture withdrawn from perfusion bioreactors in a single-use operation. AWS technology has been shown to perform well at cell densities of up to 100 million cells/mL, so is well positioned to meet the cell retention requirements of emerging higher cell density perfusion processes that are gaining momentum in the biotech space. This novel cell retention approach offers economic benefits in terms of yield improvement as well as eliminating the hollow fiber TFF operation. This offers the advantage of a stable mAb concentration in the HCCF stream during the perfusion process. This facilitates improved process control since the volume of HCCF to load on to the capture columns remains constant which is especially important during continuous multicolumn chromatography. By comparison with hollow fiber TFF, the mAb concentration varies during the cell retention process making an integrated process more complex to control.

PRODUCTION OF ZIKA VIRUS-LIKE PARTICLES (VLPs) BY PERFUSION PROCESSES

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Key Words: Virus-like particles (VLPs), Zika virus, HEK293 cell line, perfusion, ATF-2.

Zika virus (ZIKV) emerged as a major international public health concern in 2015 and rapidly spread to more than 80 countries in Africa, Asia and the Americas. ZIKV infection has been shown to cause Guillain-Barré syndrome in adults, as well as severe congenital malformations in fetuses from as much as 42% of infected mothers (Brasil et al., 2016, doi:10.1056/NEJMoa1602412). While no ZIKV vaccine becomes approved for human use, periodic outbreaks will continue to occur in endemic regions and the risk of spreading to non-endemic regions will continue to exist, especially because ZIKV persists in body fluids for very long time after infection and can be transmitted via the sexual route.

Among many different vaccine platforms currently under study, virus-like particles (VLPs) are a promising alternative for the development of vaccines, since three-dimensional structures, constituted by recombinant structural proteins of the virus but lacking the viral genome, are able to display the antigen in a repetitive pattern, triggering a robust immune response.

In this work, we investigated the production of Zika virus-like particles by both intermittent and continuous perfusion processes, using a recombinant HEK293 cell pool previously generated in our laboratory, which constitutively expresses the VLPs. In order to improve production levels, we first enriched the recombinant cell pool for high producers by means of fluorescence-activated cell sorting (FACS). Using this FACS-enriched cell pool, small-scale shake flask studies showed that intermittent perfusion (also known as pseudoperfusion) with daily medium exchange enhanced viable cell density by 3.5 fold and VLP titer by 4 fold when compared to batch cultures. Continuous perfusion in a controlled stirred-tank bioreactor was carried out using an ATF-2 unit as cell retention device. A steady-state viable cell concentration of 25-30 × 10^6 cells/mL was maintained at a cell-specific perfusion rate (CSPR) of 50-60 pL/cell/day. VLP titers inside the bioreactor were higher than in the harvest, evidencing product retention by the ATF hollow fiber, especially from day 14 of cultivation on.

Our results show that the use of cell lines constitutively expressing zika VLPs, cultured in stirred-tank perfusion bioreactors, represents a promising system for the production of a VLP-based Zika vaccine candidate. This process could potentially be more cost-effective than traditional viral vaccine platforms based on batch production of whole viruses, especially considering that VLPs can be produced in lower biosafety level plants, and that perfusion systems are characterized by higher volumetric productivities, reduced bioreactor sizes, smaller plant footprint and lower investment costs when compared to batch processes.

INTEGRATION OF UPSTREAM AND DOWNSTREAM FOR A HYBRID CONTINUOUS PROCESS DEVELOPMENT AND MANUFACTURING FOR A STABLE MONOCLONAL ANTIBODY PRODUCED IN CHO CELL CULTURE

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Key Words: CHO cells, Perfusion N-1, Medium optimization, intensified fed-batch, Multi-column capture, Integrated pool-less polishing

Process intensification by continuous operation has been successfully applied in the chemical industry, when batch processes matured several decades ago. Fully integrated upstream and downstream continuous processing has also shown great potential for increased productivity and reduced cost in biomanufacturing using mammalian cell culture. After a few decades of development, continuous or perfusion cell culture has demonstrated for manufacturing of labile proteins or low-titer processes. Due to significant challenges implementing fully integrated continuous biomanufacturing and the fact that fed-batch cell culture has not yet matured, fed-batch cell culture and batch chromatography steps are still predominant for stable protein manufacturing in the industry. In comparison to perfusion cell culture, continuous or semi-continuous downstream processing for stable monoclonal antibodies (mAbs) has developed within less than a decade. Due to a high titer, e.g., 8-10 g/L, already achieved via fed-batch cell culture, which challenges the processing capacity for batch downstream commercial manufacturing, the demand of continuous chromatography operation dramatically increases. Here, we present a case study developing a hybrid continuous upstream and downstream as our next generation process for production of a stable mAb. For upstream, we implemented N-1 perfusion seed, which significantly increased the seeding density for fed-batch production. After media and process parameter optimization, the product titer for the intensified fed-batch process with high-seed increased more than 100% over the original fed-batch process. It should be noted that the original fed-batch process was optimized and used for clinical manufacturing at 1000-L scale. For next generation downstream, we developed multi-column chromatography for Protein A step, automated VI step and integrated pool-less polishing chromatography steps with increased productivity and reduction in resin requirement, buffer consumption and processing time. The next generation process with perfusion N-1 seed and continuous chromatography steps has been scaled up in 500-L bioreactor, and now has been demonstrated for full implementation in a GMP manufacturing facility at the 2000-L scale. We will present full set of data to compare the original optimized batch process at 1000-L scale and the next generation process at 2000-L scale for the stable mAb production using CHO cell culture. We believe that the hybrid continuous process is relatively easy to develop and implement in GMP manufacturing with significantly higher productivity than conventional fed-batch process for now, while the hybrid continuous process lays a good foundation for us to further develop and implement fully continuous upstream and downstream process in manufacturing with even higher productivity in future.

CONVERSION OF BIOMANUFACTURING PROCESSES FROM FED-BATCH INTO INTEGRATED CONTINUOUS: STRATEGY, METHODS AND CASE STUDIES

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Key Words: fed-batch, continuous, cell culture, platform, conversion

Integrated continuous manufacturing offers great productivity advantages over the traditional fed-batch and therefore reduces production scale, facility footprint, and manufacturing costs. Recent advancement in process and control technologies, equipment capabilities, and automation has paved the way for the biopharmaceutical industry to start adapting processes to continuous manufacturing for clinical and commercial products. As we shift to these approaches, strategies and methodologies need to be established for introducing and developing continuous processes. Ideally, a platform continuous process would be established. Aspects to consider for the platform include cell line, cell culture media, process equipment, and a framework of process parameters that are all tailored to fit continuous manufacturing. Once established, the platform will allow shortened process development timelines, easy adaptation from one molecule to another, and improved efficiency of tech transfer into GMP manufacturing.

For processes that are in late clinical development or in commercial manufacturing, with an existing fed-batch cell culture process, a process conversion strategy should be established. In these cases, existing development and characterization of the original fed-batch process can be leveraged for unit operations that are constant or similar from fed-batch to continuous. The platform continuous process can be adapted, and toolboxes can be created during the product-specific process development.

The ultimate goal is to deliver robust continuous cell culture manufacturing processes with faster timeline and less development cost. This presentation will illustrate the strategy taken in Novartis with a few case studies.

CONTINUOUS DOWNSTREAM PROCESS DEVELOPMENT FOLLOWING QUALITY BY DESIGN PHILOSOPHY

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Key Words: Continuous DSP, Process Development, QbD

Continuous process technologies are available for essentially every step in a fully integrated continuous downstream processing platform. Most of these technologies have a significantly higher throughput and specific productivity than their batch equivalent process steps. The consequence is that the amount of material required to run experiments during continuous process development work is significantly higher than in batch process development.

High throughput experimentation platforms have been established for performing design of experiments studies for many batch downstream processing steps, but for the equivalent continuous bioprocessing technologies this doesn't exist. This limits the amount of different process variations that can be tested as part of the process characterization studies.

In this presentation, we will analyze a continuous monoclonal antibody purification platform, using the Quality by Design approach to identify the key process parameters and critical process parameters. Based on the process knowledge and process design information available for the various continuous bioprocessing technologies, we will analyze how these critical process parameters translate into a continuous bioprocessing platform for monoclonal antibodies.

EVALUATION OF AMBR® 250 PERFUSION BIOREACTOR SYSTEM AS A MODEL FOR HIGH-THROUGHPUT PERFUSION CELL CULTURE PROCESS DEVELOPMENT

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The efficient development and delivery of high-quality therapeutic products necessitates the need for high-throughput process development (HTPD) tools. In recent years, fed-batch process development timelines have been significantly reduced as industry has implemented fully automated mini bioreactor systems such as the ambr® 250 HT. More recently, as continuous processing for biologics has gained traction, driven by novel technology and economic pressure to significantly improve monoclonal antibody (mAb) production over the standard fed-batch process, more efficient, cost effective, and environmentally sustainable mAb processes are expected. Traditionally, perfusion process development work requires a combination of deep-well plates and small-scale stirred tank bioreactor (STR), both of which are labor intensive and time consuming. The established ambr®250 HT platform has recently been integrated with perfusion capabilities to enable rapid continuous perfusion process development (PD). In this work, we present the results from our assessment of the automated disposable perfusion bioreactor system for high throughput upstream PD activities, including clone selection and process optimization. In addition, several high stress conditions were also examined here to identify optimal operation ranges for the current system. The studies conclude that ambr® 250 perfusion reactor is able to generate process performance and product quality profiles equivalent to bench-top bioreactors for a high cell density perfusion process.

UPSTREAM PROCESS INTENSIFICATION USING FROZEN HIGH CELL DENSITY INTERMEDIATES

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Typical seed train operations start by thawing of a single vial followed by several expansion steps. Reaching sufficient absolute cell numbers for production bioreactor inoculation is time-consuming and reduces plant flexibility. Besides long ramp up times, open cell culture operations are a major source of process variability. High cell density cryopreservation (HCDC) is a method of freezing cells in bags instead of vials and at higher cell densities. This offers the advantage of decoupling expansion and production: both steps can be separated in space and time. Room classification could be decreased due to fully closed processing and reproducibility increased due to a reduction of manual handling steps. Furthermore, these frozen seed train intermediates allow global distribution from a central expansion facility to decentralized global production facilities. Besides from advantages in production, these HCDC bags can be used in process development to ensure equal starting points in experimental setups.

In this study, we developed a single-use bag assembly that supports closed filling, freezing, thawing, and inoculation. Before using the bag application, relevant parameters for this process from filling to inoculation were evaluated in vials with different cell lines. We found that the DMSO concentration for optimal freezing must not be higher than 7,5%. Furthermore, direct freezing at -80 °C instead of using a controlled rate freezing method is possible. Maximum concentration of DMSO in cell cultures should not be higher than 0,5 % when cryopreserved cells in bags are used for inoculation. For the idea of seed train intensification, we tested increasing freezing cell densities from 10 to 100 million cells/mL showing comparable growth. Functionality test of this HCDC method in comparison to vials was demonstrated in 4,2 L bioreactors simulating a manufacturing process. Applicability of this cryopreservation technology has been demonstrated using different bioreactors, perfusion systems, and various CHO cell lines.

VALIDATION ASPECTS IN THE COMMERCIALIZATION OF INTEGRATED CONTINUOUS BIOMANUFACTURING

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Sanofi has been operating continuous processes for production of recombinant proteins for over two decades. Development of continuous processing unit operations has ever increasing attention across the biotechnology industry, including end-to-end continuous processing. The development of robust continuous processing steps enables integration of continuous unit operations. Recently, as part of second generation programs, integrated continuous biomanufacturing are being implemented for GMP manufacturing. This presentation will discuss aspects of equipment qualification and process validation that robust process development can facilitate.

DIRECT INOCULATION OF A PERFUSION BIOREACTOR WITH A FROZEN INTERMEDIATE SEED TRAIN

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Key Words: High cell density culture, frozen intermediate seed train, perfusion, direct inoculation

Flexibility in cell culture manufacturing via a reduction in the process duration is a key strategy to execute cell culture manufacturing campaigns quickly in a multi-product facility. A major bottleneck is the seed train, which can add weeks to the timeline of the production culture. Seeding production bioreactors with a direct, cryopreserved CHO cell inoculum could possibly eliminate the need for a lengthy continuous seed train and provide other numerous benefits.

Previous efforts have shown that high-density (HD) cell banking can be an effective means to reduce the number of seed-train steps required and also improve operational success in seed-train processes. This study demonstrates that it is possible to remove the entire seed train during routine operations by using an intermediate frozen seed train. This involves cultivating cells to high cell density in a perfusion bioreactor, and cryopreserving cells in multiple disposable bags. Each run for a manufacturing campaign would then come from a thaw of one or more of these cryopreserved bags directed inoculated in the N-1 bioreactor.

The data gathered during the development and optimization of the different steps during the generation of a frozen intermediate seed train using various approaches and technologies, will be presented. As well as the extensive data set that has been generated to demonstrate that the new process scheme delivered the same performance as the conventional seed train process.

A VIRUS HARVEST UNIT (VHU) FOR THE CONTINUOUS HARVESTING OF LENTIVIRUS FROM SUSPENSION CELL CULTURES

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For virus particles produced by cell culture systems, free intact virus particles as well as virus particles associated with host cell membranes or other viral particles and damaged virus fragments will be present. To reduce the amount of host cell fragments and/or virus aggregates it is critically important to reduce the shear stress applied to the cell culture during the harvesting step as much as possible. This is mainly accomplished by utilizing a low-shear Virus Harvest Unit (VHU™) with large pore size membranes (5-15mM) and by utilizing a low-shear pump. As shown in Table I, the VHU™, is able to clarify the broth containing the viral vectors from less than 500 Nephelometric Turbidity Units (NTU) down to less than 16 NTU. In contrast the turbidity of a broth harvested by centrifugation from a batch is around 50-90 NTU and the turbidity of the harvest stream from an acoustic filter (Biosep) is 33 NTU. These results suggest it may be possible to eliminate the secondary clarification steps when using the VHU unit as the virus harvesting process mode.

Process Mode	Turbidity (NTU)		
	Broth	Post-Harvest device	clarified
Batch	50-90	N/A	Target: ~5
VHU	<500	<16	N/A
Biosep	N/A	33	13

Table 1. Comparison of nephelometric turbidity (NTU) between batch harvesting and continuous harvesting devices such as the Virus Harvest Unit (VHU) and the acoustic filter (Biosep).

DEFINITION OF A PLATFORM CONTINUOUS CAPTURE SCALE DOWN MODEL AND LINK TO SCALE-UP FOR MONOCLONAL ANTIBODY CLINICAL MANUFACTURING

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Key Words: platform continuous capture, monoclonal antibody purification, small scale model, cleaning, scale-up

Definition and scale-up of a batch chromatography process is based on a few main variables such as linear velocity, column loading, and bed height, which are scaled proportionally to column volume. Continuous chromatography consists of multiple columns with column loading and washes/elution/regeneration occurring simultaneously. The definition of a small scale method for continuous chromatography can be extremely complex due to the extensive number of method variables. Limited knowledge exists for development of a scale down and up strategy for continuous chromatography. This abstract should provide some insight into case studies on integration of continuous operations and scale-up, which is one of the themes of the integrated continuous biomanufacturing (ICB) conference.

This presentation will describe a strategy for definition of a platform continuous capture scale down model and scale-up pathway. The platform continuous capture step utilizes periodic counter-current chromatography (PCC) for operation of affinity chromatography in a semi-continuous manner. A scale down model for the PCC step was defined and simplified to the following three ranges of harvested cell culture fluid (HCCF) titers: ≤ 2 g/L, 2.5-8 g/L, and 8.5-13 g/L. For each of the three titer ranges, the following variable setpoints are changed based on the specific HCCF titer range: step linear velocity, number of columns, column size, and ΔUV. After these setpoints are inputted into the algorithm, PCC method variables, such as sample loading flowrate, loop time, number of loops and cycles, throughput (g/L/hr), and time cycle, will populate to finish the method design.

This PCC scale down model was utilized to scale-up to a bioreactor range of 500-2000L. Quality results showed a good correlation between scale down model and scale-up data. Additional parameters for the 2000L scale-up run included assessment of cleaning and drug substance stability. The cleaning results of the continuous chromatography skid showed passing bioburden, endotoxin, and conductivity. Drug substance stability was also maintained for a year, which was the study duration. This data set proves the PCC small scale model data is representative of the scale-up quality results. In addition, targets such as skid cleanability and DS stability met specifications, which supports the scale-up package for implementation of a platform continuous capture step into a purification process for clinical mAb manufacturing.

OPTIMIZATION STUDY ON PERIODIC COUNTER-CURRENT CHROMATOGRAPHY (PCC) INTEGRATED IN A MAB DOWNSTREAM PROCESS

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Key Words: PCC, Integrated continuous downstream, Multi-objective optimization, Antibody purification.

In the biopharmaceutical market, there is an increasing pressure to reduce prices. Nowadays, a big part of the manufacturing cost is due to the product purification. A big effort has been made in this matter to reduce costs, and continuous downstream processes like periodic counter-current chromatography (PCC) have gained interest in the last years due to a higher productivity and resin utilization compared to a batch process. Herein, a PCC process is integrated with a virus inactivation and two ion-exchange chromatography steps (polishing steps) for the purification of mAb at lab scale, and multi-objective optimization is used to compare several process configurations using two resins with different particle sizes.

The optimization was based on a mechanistic model of the breakthrough curve in the protein A capture step. The breakthrough curve and the length of the product recovery determined the scheduling of the PCC process, since the loading of the capture step had to be at least so long as the time that takes to purify the product. Residence time during the loading of the capture column and cycle time were selected as decision variables, and productivity and resin utilization were the objective functions, while yield during the loading of the capture column was set as a constraint. A set of optimal solutions were obtained (Figure 1). In the solutions with high productivity, the residence time was lower, and as a result, the breakthrough curve was flatter. Therefore, the PCC cycle ended earlier to avoid product loss, which led to a decrease in resin utilization. The opposite happened for the solutions with higher resin utilization.

The process configuration affected the optimization by setting a lower bound on the cycle length. Therefore, several process configurations were compared: only the capture step in one system, the complete purification in series in one system, and the same process in parallel in two systems. In Figure 1, the effect of the process configuration on the Pareto front is shown. Experimentally, the process was integrated and run simultaneously in two systems, which were centrally controlled by an external software called Orbit. In general, the resin with smaller particle size had a higher dynamic binding capacity at all residence times, which allowed to obtain a better compromise between productivity and resin utilization.

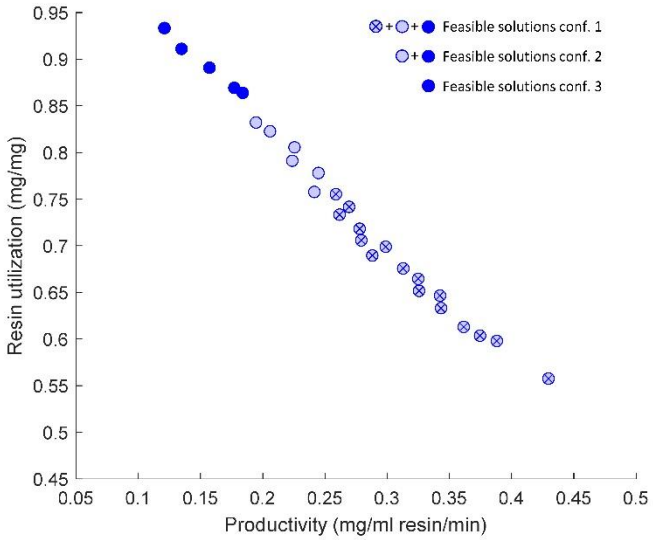

Figure 5. Pareto front of PCC optimization in three different process configurations: (1) Only capture step in 1 system, (2) PCC + Polishing steps in 2 systems, (3) PCC + Polishing steps in 1 system.

FLOW-VELOCITY PROGRAMMED CHROMATOGRAPHY AS AN ALTERNATIVE METHOD FOR INCREASING THE EFFICIENCY OF CONTINUOUS- OR INTEGRATED-CHROMATOGRAPHY PROCESSES

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Key Words: mAb, capture chromatography, continuous chromatography, flow programming

Solvent (mobile phase) programming is most commonly employed for controlling adsorption/desorption in chromatography (linear gradient elution or stepwise elution). For gas separation, temperature- or pressure-swing adsorption is frequently used. Although flow-velocity is another important parameter, which affects both the dynamic adsorption capacity (DBC) and the resolution, it is seldom used as a programmed operating variable. The one exception is the standard 4-zone simulated moving bed (SMB) chromatography, in which the flow-velocities of the 4-zones are different. Several researchers have already shown that DBC can be increased by using two different flow velocities. However, a rational method for determining the optimum flow velocity program has not been established. Moreover, application of this method to periodic counter-current (PCC) chromatography or connected flow-through chromatography (FTC) has not been attempted yet. In this study, we have developed a flow-velocity gradient method for analyzing the breakthrough curves of proteins in ion-exchange or protein A chromatography (Figure 1). The data were obtained at various different gradient slopes. The obtained curves were analyzed based on a model considering mass transfer (pore diffusion) and non-linear isotherm. Then, numerical simulations were carried out in order to find the optimum flow-velocity program for improving the efficiency. This method was further applied to PCC and FTC (Figure 2). The effect of flow programming on productivity and cost reduction has also been examined in both batch and continuous configuration in capture chromatography of mAbs by simulation of the process models. Experimental verification was also carried out using monoclonal antibody samples in the filtered cell culture liquid.

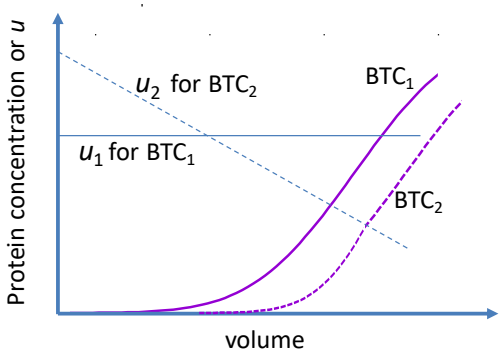

Figure 1 - Breakthrough curves at constant flow velocity (BTC_1) and at programmed flow velocity gradient (BTC_2)

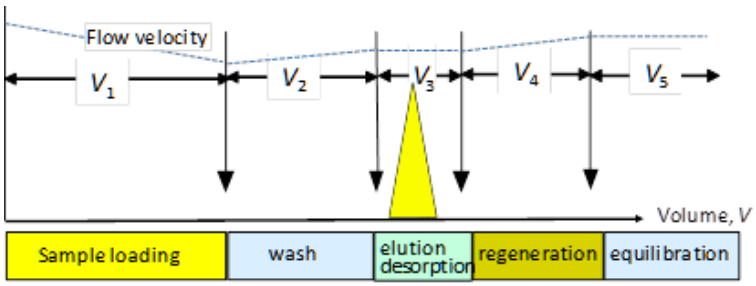

Figure 2 - Typical capture chromatography operations with flow-programming.

CONTINUOUS AQUEOUS TWO-PHASE SYSTEM EXTRACTION USING OSCILLATORY FLOW REACTOR

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Key Words: continuous downstream; aqueous two-phase systems; oscillatory flow reactor

Biotechnology has allowed the development of completely new products from health to food industry. The quality of the process since cell culture until formulation is crucial in their application. Nowadays, almost all biological products are still produced entirely in batch mode, which brings some drawbacks that can be overcome, operating in continuous mode. The continuous downstream processing can bring many advantages such as: automation and integration of unit operations, lower operator interaction with the equipment and consequently lower chance of errors; possibility of recycling buffers and auxiliary material; improvement of product quality, due to the greater uniformity of the process time; and increase of the process productivity.
The usage of aqueous two-phase systems (ATPS) have been proven as an efficient operation for the clarification and purification of biological products, but despite this fact ATPS have had a limited use at large scale. On the one hand, ATPS extraction uses aqueous solutions that provide an ideal environment for biological molecules, on the other, the high salt concentrations and the cost of some polymers are the main drawbacks that make this process less cost-competitive comparing with others; however, the majority of ATPS extractions are still done in batchwise mode.

Oscillatory flow reactor (OFR) is a type of tubular/channel reactor that has been used in processes as liquid-liquid reaction, polymerization, flocculation and crystallization. One of the most important features of this type of reactor is the uniform mixing that is provided by the combination of the periodically spaced restrictions and the oscillatory motion of the fluid. Therefore, the continuous mode and the particular characteristics of OFR could be the answer for a more cost-competitive ATPS extraction.

a) b)

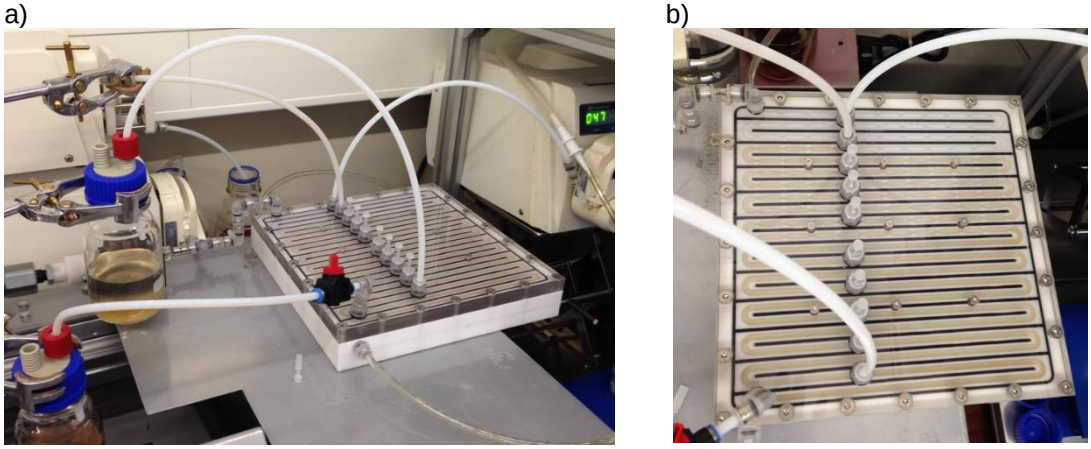

Figure 1 – Continuous ATPS extraction apparatus (a) and oscillatory flow reactor detail (b).

In this work, the properties of different polymer-salt ATPS operated in a continuous oscillatory mode are being studied and compared with the batchwise type, highly described in the literature. The OFR will then be explored for the continuous ATPS extraction as a primary clarification step in the purification of food enzymes and antibodies, Yields and the purification factors obtained with the OFR will be compared with the batchwise operations, envisaging a more cost-effective process.

ANALYTICAL AND DATA STRATEGY FOR CONTINUOUS DOWNSTREAM MANUFACTURING

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Key Words: PAT, automated sampling, data management, real-time visualization

As advances emerge in developing continuous biomanufacturing processes, there is an increased need to deploy PAT tools to characterize, monitor, and control key quality attributes and a criticality to have a data infrastructure to support the immense amount of information being generated. While the desire for these tools exists in traditional batch processing, in a continuous operation, these become a requirement to ensure consistent product quality and enable proactive approaches in maintaining performance. The ultimate goal is to deploy PAT tools to reliably provide real-time information on product and process impurities throughout the entire operation. However, in its current state, there is a reliance on a mixture of inline, at-line, and offline technologies. By identifying the time criticality of CQAs, efforts can be focused on where to prioritize real-time measurements or instead, quicker or more automated testing for a subset of analytics. This work describes the application of this approach in the development of small-scale, compact in-line UV instruments to measure real-time protein concentration and in the integration of an automated sampling system with at-line and offline instrumentation for in-process impurity characterization.

Introduction of these PAT tools add to the complexity of the data infrastructure as it introduces requirements for platforms capable of supporting spectral data, chemometric model deployment, spectral instrument management, and time-alignment of discrete data. With the vast amount of information produced in a continuous environment, interface and analysis tools need to be developed so that any end-user can digest data into a format that easily allows them to gain insight into an ongoing batch. This work will highlight the data architecture of the continuous platform, with a focus on software tools selected for aggregation and real-time data visualization. The capabilities of these software packages were demonstrated through a proof-of-concept study using single-pass tangential flow filtration (SPTFF) as a model unit operation, which allowed integration of continuous, spectral, and discrete data. These tools allowed scientists to go from viewing real-time data across multiple, equipment-specific software to one consolidated interface, which in turn reduced time spent in compiling data for analysis and reporting. In addition, advanced capabilities of deploying model predictive control in SPTFF were demonstrated to show the application of a closed loop process control in continuous manufacturing.

ACCELERATED PROCESS DEVELOPMENT FOR INTEGRATED END-TO-END BIOLOGICS MANUFACTURING

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Key Words: Integrated; Speed to clinic; Straight-through purification; Multi-product

With the exception of monoclonal antibodies, biologics typically require bespoke manufacturing processes that vary widely in the type of and number of unit operations. This constraint leads to custom facility designs and unique strategies for process development for every new molecule. To enable flexible, multi-product manufacturing facilities and to reduce the speed to clinic for new molecules, streamlined manufacturing processes and associated strategies for process development are needed. We have developed a bench-scale, integrated and automated manufacturing platform capable of rapidly producing a variety of recombinant proteins with phase-appropriate quality for early development[1]. The system comprises three modules for fermentation via perfusion, straight-through chromatographic purification, and formulation. To facilitate the production of multiple products on the same system, we have also developed a holistic strategy for process design to manufacture new products in as few as twelve weeks after obtaining the product sequence. While upstream process development in our host (*Pichia pastoris*) has been relatively straightforward, there are not many tools currently available for developing fully integrated straight-through chromatographic processes. Therefore, we developed an *in silico* tool for the prediction of fully integrated purification processes based on a one-time collection of host-related data combined with conventional high-throughput chromatographic screening data for each new target molecule[2]. We used this tool to develop fully integrated, end-to-end production processes for three molecules (hGH, IFNα-2b, and G-CSF) with at least 45% fewer steps than traditional processes. While our *in silico* tool allows for rapid resin selection, it may not predict the optimal process for each individual molecule since it is based on conventional high-throughput screening techniques which seek to optimize each chromatographic step independently rather than optimizing a fully integrated, multi-column process. To address this limitation, we have also developed a DoE-like framework for the optimization of fully integrated purification processes once the resins have been selected. First, a series of range finding experiments are carried out on each individual column, similar to conventional screening but with limited analytics. Next, we carry out fully integrated (multi-column) testing of the proposed operational area with more extensive analytics, including host cell protein, DNA, and yield measurements. We use this methodology to develop optimized processes for the end-to-end production of a variety of single domain antibodies with high yield and purity. Further, we present a method for predicting the optimal operating conditions for a new molecule within the same class based only on its biophysical characteristics, reducing the timeline from sequence to early stage, phase-appropriate product to only six weeks. Using these holistic strategies for process development, we have produced over ten different recombinant proteins on our manufacturing platform including enzymes, cytokines, singe domain antibodies, and vaccine subunits. We believe that such integrated strategies for process design could enable the rapid translation from sequence to early stage clinical development of products for a variety of molecules and potentially allow clinical testing of a greater number of high quality molecules for vaccines and biopharmaceuticals.

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2. Timmick, S. M. *et al.* An impurity characterization based approach for the rapid development of integrated downstream purification processes. *Biotechnol. Bioeng.* 1–13 (2018). doi:10.1002/bit.26718

THE NEVOLINE™ MANUFACTURING SYSTEM: INTENSIFICATION & INTEGRATION OF UPSTREAM AND DOWNSTREAM PROCESSING IN A LOW-FOOTPRINT, AUTOMATED PLATFORM FOR VIRAL PRODUCTION

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Key Words: Single-use, automation, continuous production, vaccines

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The world is facing an under-supply of some key vaccines due to poor synergies between growing market demands and aging production models.

In this light, we have developed a proof of concept of a vaccine manufacturing platform aiming at increasing availability and affordability of vaccines - the NevoLineTM system.

This simulated continuous and automated platform integrates both USP1 and DSP2 processes and is encapsulated into an isolator, making it a self-contained production unit (6m²).
The technology relies on a single-use, high-density fixed-bed bioreactor operated in perfusion chained with downstream filtration, clarification and polishing steps to (a) decrease batch time, (b) reduce equipment utilization, (c) optimize utilities consumption and (d) intensify operations. By optimizing single-use technologies we are able to drastically reduce CAPEX3, CoGs4 and footprint and increase production capacity. Such manufacturing platform can easily be implemented into flexible facilities with simplified infrastructure, increasing adaptability in production and capacity for record time-to-market.

This study will present the platform proof of concept on Vero line and trivalent inactivated polio vaccine (sIPV) production, achieving low CoGs (0,28$/dose for a trivalent sIPV) and large capacity. The presentation will feature the description of engineering development, but also results of cell growth, infections and product quality, as well as a description of the CAPEX, CoGS and capacity calculations. This manufacturing platform is undergoing sIPV process scale-up and pre-clinical bulk production.

The NevoLine system is expected to produce any type of viral vaccine at a very low cost and large capacities to face global health challenges.

[1] Upstream
[2] Downstream
[3] Capital expenditure
[4] Cost of goods

INTEGRATION OF XCell™ ATF PERFUSION WITH SINGLE COLUMN CAPTURE CHROMATOGRAPHY FOR PRODUCTION OF MONOCLONAL ANTIBODIES

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Key Words: XCell, ATF, Perfusion, Continuous, Chromatography

Perfusion cell culture processes have demonstrated higher productivity than fed-batch due to the ability to generate and maintain high cell densities over long process durations. When using a perfusion system, like the Repligen XCell™ ATF, a clarified cell free stream of product is continuously generated and is ready for downstream purification. Similarly, continuous or semi-continuous capture chromatography of monoclonal antibodies (mAbs) can provide significant advantages over batch processes such as higher productivity, improved product quality, lower equipment footprint, and reduced purification cost. Integrating a perfusion system with a continuous downstream purification system could achieve all these advantages over a batch system. Multicolumn based chromatography systems (MCC) have been widely evaluated and show tremendous benefit when integrated with perfusion processes for continuous purification of mAbs. However, adoption of MCC within the biotech industry has been slow due to complexity of the systems which can be challenging to set-up, qualify and come with a higher risk of process failures. In this work, we have combined a single capture chromatography column containing a high capacity Protein A resin, Purolite Praesto® Jetted A50, with a Repligen XCell™ ATF perfusion cell culture system. The performance and productivity of this single column capture integrated system were compared with MCC approaches. The high performance single-column system simplifies the downstream capture process and is scalable to > 5 kg mAb per day process without the need for complicated MCC equipment or operation.

MODEL-BASED EVALUATION AND PROCESS DEVELOPMENT OF CONTINUOUS CHROMATOGRAPHY

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Key Words: Continuous chromatography, Model-based design, Protein separation, Antibody.

Multi-column periodic counter-current (PCC) chromatography has been proposed as continuous capturing technology to improve the process productivity and resin capacity utilization, as well as reduce buffer consumption and equipment footprint, which is applying for monoclonal antibody (mAb) capture with Protein A affinity resin. Due to the complexity of continues chromatography, some mathematical framework should be developed to aid the process development.

For continuous process, more factors should be considered and non-linear and phase-based relationships have to be faced as shown in Figure 1, which would certainly trouble the process development. To better understand and describe continuous capturing process, an integrated approach was developed to combine the Equilibrium Dispersive Model of column, General Rate Model of resin, as well as the Equilibrium Adsorption Model for fitting and predicting breakthrough curves, as well as the continuous process models for evaluating the operation conditions. Considering process productivity and capacity utilization together, a working window could be proposed for continuous chromatography. The model-based approach developed were tested with different PCC modes, including twin-column CaptureSMB from ChromaCon, 3/4-column PCC from GE Healthcare and multi-column BioSMB from Pall. A software platform with the interactive interface was also developed to easily input the data and visually output the results. In addition, artificial intelligence method was introduced to improve the data treatment, and different PPC modes could be evaluated in a fast way. The results indicated that model-based approach could aid the process development and promote the target-orientated process design for continuous process.

This work was supported by the National Natural Science Foundation of China and International Science & Technology Cooperation Program of China.

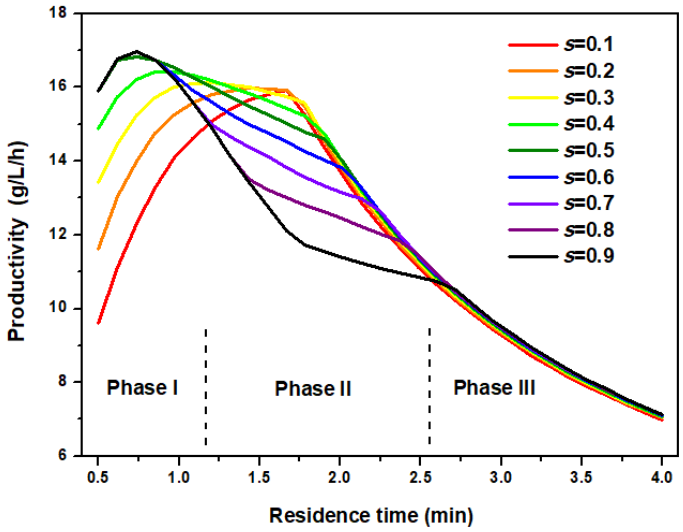

Figure 6. Productivity of continuous process with twin-column CaptureSMB at different residence times and breakthrough point control.

DEVELOPING NEW PERFUSION CAPABILITIES FOR AMBR® MICRO AND MINI BIOREACTORS

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Key Words: automated, high throughput, single-use, perfusion, bioreactor

In recent years a strong trend towards intensified and continuous biopharmaceutical processing has gathered momentum, enabled by key cell culture technologies such as ATF and TFF. However, small-scale application has been limited to traditional benchtop bioreactor formats that are manually intensive, relatively low throughput and costly to operate. Automated high throughput single-use bioreactor systems have transformed fed-batch cell culture bioprocess development over the last decade and new capabilities to support perfusion culture in these micro and mini bioreactor formats could facilitate and accelerate an industry wide transition to intensified and continuous perfusion cell culture processes.

Working in close collaboration with biopharm industry development partners, the design of the ‘ambr 250 high throughput’ bioreactor system has been modified to include hardware, software and single use components required to operate up to 24 parallel bioreactors with ATF or TFF cell retention modes. Iterative prototype testing with development partners has resulted in a novel ambr 250 system design capable of operating for extended culture durations and supporting high cell densities. In addition, a new Generation 2 ambr 15 cell culture system has been developed and demonstrated with technical capabilities facilitating perfusion mimic applications. Case studies will be presented on the utility of new ambr 15 system features for perfusion mimic (20-40M cell/ml) via cell settling and centrifugation methods, together with a range of industry case studies and novel performance data for the new ‘ambr 250 perfusion’ system (24 parallel perfusion cultures; >30d; >100M cell/ml; 0.25 vs. 5L; fully automated VCD control).

As previously established with ambr systems for fed-batch processes, the new Generation 2 ambr 15 and ambr 250 perfusion systems together have the potential to provide the biopharm industry with a step change in perfusion process development capacity, enabling high throughput bioreactor screening and DoE optimization approaches for accelerated perfusion process development.

LEVERAGING SANOFI INTENSIFIED ICB PLATFORM TO ENABLE EARLY PROCESS DEVELOPMENT FOR A LABILE AND HARD-TO-EXPRESS MOLECULE

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Keywords: ICB, intensification, labile molecule, early-stage

Within the biopharmaceutics industry, tremendous progress has been made in the implementation of early development antibody platforms to achieve high volumetric productivity and consistent product quality for novel therapies. More recently, development of new modalities provide opportunities for advancing exciting new therapeutic possibilities. However, many of these modalities present new upstream and downstream development challenges, e.g., low expression, labile molecules, low recovery, and unreliable product quality. The resulting additional development requirements increase the timelines for demonstrating Proof of Concept and may even prohibit certain therapeutic candidates from reaching the clinic at all.

The Sanofi ICB platform provides opportunities to increase productivity and improve product quality, enabling manufacture of new entities previously inaccessible. Here, we present a case study of such a situation, in which the ICB platform is applied to an early-stage, labile, hard to express molecule produced from non-CHO mammalian cells. A combination of upstream and downstream high-throughput technologies have been incorporated to rapidly define a process sufficient for first-in-human studies. Process intensification enables adequate material generation within an acceptable number of batches for both development and clinical manufacturing. This case study demonstrates the strategy of using intensified perfusion platform for non-antibody modalities to support a diverse portfolio for our evolving industry.

DEVELOPMENT OF SCALE DOWN MODELS FOR PERFUSION BIOREACTOR MEDIUM OPTIMIZATION

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Key Words: perfusion, media, scale-down model

Due to the complex nature of balancing >50 individual media components, the development and optimization of bioreactor medium for high performing perfusion bioreactors is a resource intensive, multivariate problem that greatly benefits from the availability of predictive high through-put scale-down models that simulate the bioreactor system. For that purpose, both a 10 mL long-term block model and 50 mL shaker tube model were developed and optimized to settings that balance oxygen transfer, culture health, and productivity. The long-term block model was limited by the volume needed for culture sampling; as a result, the shaker tube model was developed with a 7.5x increase in working volume. This shaker tube model was then applied to adequately characterize cell nutrient consumption profiles and subsequently inform medium development through multivariate design of experiments (DOE). Within two rounds of studies in the scale-down models, Regeneron's first-generation perfusion medium formulation achieved approximately 100% increase in productivity compared to the initial medium. The improved nutrient strategy optimized in shaker tubes translated to several cell lines in the benchtop and pilot scale bioreactor perfusion system, indicating the predictive capabilities of the small-scale model. These results highlight the benefits of using small-scale models to shorten development time for perfusion process implementation.

INTRODUCTION OF INTENSIFIED PROCESSES INTO THE CLINICAL SUPPLY CENTER

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Key Words: PROCESS INTENSIFICATION, CONNECTED PROCESSING, GMP

The requirements for the production of monoclonal antibodies have changed significantly over the past few years. High titer processes and flexible utilization of single use and stainless steel equipment are currently state of the art in bioprocessing. The new goals is to manufacture the same or even more product within a shorter production time while maintaining the same level of product quality.

New, intensified and connected processes in the upstream and downstream departments introduced with new technologies are making these visions come true.
Implementing new technologies especially under GMP conditions poses a lot of challenges.
Suitability of established analytical methods needs to be demonstrated (e.g. cell count).
Work flows and new standard operating procedures need to be in place for the high volume media and buffer preparations required for intensified processes.

Finally, new and existing equipment needs to be integrated into the GMP production area with a special focus on safety, i.e. ensuring that the building remains structurally sound, e.g. floor load capacity.
The first intensified drug substance batches comprising new technologies were successfully produced in a 1000 L - 2000 L scale in the clinical supply center at Roche in Penzberg. This presentation aims to illustrate opportunities and challenges of process intensification and connected processing in a clinical supply setting, and how to deal with it in a GMP environment.

The start of the new intensified processes in a commercial facility is only a heartbeat away.

DEVELOPMENT OF AN N-1 PERFUSION MEDIUM TO INTENSIFY SEED TRAIN OPERATION

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Seed train expansion of cells before the final production step is often time-consuming and a major source of process variability. For the intensification of seed train operations there are several opportunities discussed across the biopharma industry today. One of the possibilities is to operate N-1 bioreactors in perfusion mode to shorten timelines and improve bioreactor utilization. In this work, we investigated the influence of using an expansion medium especially designed for N-1 perfusion to gain optimal results in the whole manufacturing campaign.

For screening and designing an N-1 perfusion expansion medium, a scale down model which represents a typical production campaign, including the seed train, was established. Expansion medium prototypes were combined with different production media in the final production step, e.g. EX-CELL® Advanced HD Perfusion as medium designed for high-density perfusion, and Cellvento® 4CHO Medium and 4FEED as exemplary fed-batch process.

After determining a suitable expansion medium formulation, the prototype was evaluated for solubility and streamlined to ensure a simple hydration and robust supply chain. Afterwards, results were confirmed using a simulated manufacturing process using benchtop bioreactor systems, showing that the positive impact of the expansion medium on the final yield is present both when using perfusion or fed-batch as final production stage.

Our results indicate that using the right companion medium in seed train expansion - specifically designed for the purpose - can prepare the cells optimally for the final N-stage and increase productivity while using low CSPRs. Combining these findings with the application of a perfused N-1 step in the manufacturing campaign leads to a great opportunity for the intensification of the whole upstream process.

A CDMO PERSPECTIVE TOWARD THE IMPLEMENTATION OF CONTINUOUS BIOPROCESSING STAND-ALONE AND INTEGRATED OFFERINGS

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Key Words: Perfusion, continuous chromatography.

The challenge involved in integrating unit operations for continuous bioprocessing is a significant impediment to implementation of the technology in the industry. The benefit of continuous bioprocessing can be better understood when the components of the technology are analyzed under multiple factors including modalities, protein quality attributes and stability, specific productivity and overall cost-benefit of implementation and operation of the technology. Contract Development and Manufacturing Organizations (CDMO) need to provide a portfolio of offerings that cover the needs of diverse groups and process needs. For example, processes with lower productivity and unstable molecules can benefit from a perfusion system while more stable molecules with high productivity may need to focus on the benefits of a continuous capture to address a potential bottleneck on the downstream.

In this work, we present Catalent's road map and rationale of the step-wise approach we are using toward the implementation of continuous bioprocessing. Our approach will facilitate the integration of the appropriate technology components tailored for each process in a timely and cost-effective manner. Modeling and simulation data will be presented to support the soundness of the approach using selected stages in a typical process for the production of monoclonal antibodies.

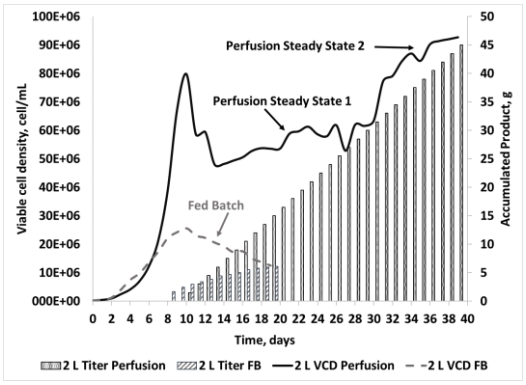

Figure 7 – Optimization of Steady State conditions for increased productivity in a perfusion bioreactor

As an example of the optimization of independent components of the technology, we will present results from experimental studies developed at bench and pilot scale. Figure 1 shows the results obtained when process parameters in the bioreactor were manipulated to achieve different steady states while maintaining titer and product quality for a production perfusion bioreactor.

Additional studies at bench and pilot scale will be presented to demonstrate proof of concept of other components including N-1 perfusion and continuous capture.

The results are allocated to present a combination of scenarios that will guide the use of specific and combined components of the continuous bioprocessing technology based on modality and process needs.

SMALL-SCALE END-TO-END MAB PLATFORM WITH A CONTINUOUS AND INTEGRATED DESIGN

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Fully continuous manufacturing of therapeutic proteins is an emerging trend in the biopharmaceutical industry. Integration of the upstream and downstream processes and conversion to continuous manufacturing leads to increased productivities, decreased equipment size, improved product quality, and overall reduced production costs. An integrated continuous bioprocess (ICB) from the early stages of process development can accelerate the transfer of a new drug candidate to commercial manufacturing. The process presented in this work is a proof-of-concept of an end-to-end monoclonal antibody (mAb) production platform at small scale. It was implemented by a 200 mL ATF perfusion bioreactor, integrated with a single lab-scale chromatography system, performing all purification steps for the mAb from the cell culture harvest in a continuous way. The downstream process consisted of a periodic twin-column capture with protein A resin, followed by a virus inactivation step, a cation exchange step in bind-elute mode, and an anion exchange step in flow-through mode. MAbs were produced for 17 days in a high cell density perfusion culture of CHO cells and purified continuously with a recovery yield of up to 60 % by the following downstream train. A 5-log reduction of host cell protein levels revealed that impurities were sufficiently removed. A consistent glycosylation pattern of the purified product was ensured by the steady-state operation of the process. With this proof-of-concept, we demonstrated the technical feasibility of a fully continuous end-to-end process with a compact design, integrating several unit operations in a single chromatography station and using small-size equipment for the upstream and downstream operations. The work presented here can become a useful development tool for the future of continuous bioprocesses.

NOVEL AMINO ACID FEEDING STRATEGY IN PERFUSION CULTURES TO ENHANCE MONOCLONAL ANTIBODY PRODUCTION

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Amino acids represent an essential source of nutrients in all the cell culture media. The concentration of each individual amino acid in the media has a huge impact on the performance of the cell culture. Traditionally, their concentrations in commercially available basal media have been determined in laborious studies using batch and fed-batch experiments. Hence, the resulting amino acid composition in the culture media is optimized based on the metabolic requirements of a specific cell line in a batch process. These requirements are likely to be different when the cell line is changed and the medium is used in perfusion processes. In order to optimize the performance of a perfusion culture by means of increasing cell specific productivity, decreasing byproduct formation and minimizing the cell specific perfusion rate, we developed a screening procedure for different media with distinct amino acid compositions in pseudo-perfused spin tubes. The results obtained from a monoclonal antibody expressing CHO-K1 (GS) cell line in these media revealed significant differences in amino acid uptake rates, and led to diverse metabolic behaviors. Cell specific productivities varied in a range of 35 % and ammonium production was even completely ceased under certain conditions. The lactate production rates differed widely and was mostly influenced by the seed cell density. From the results of an initial screening we designed a modified medium to target the amino acid consumption to a desired metabolic state with maximized antibody productivity and minimized byproduct formation. The performance of the medium was assessed with a perfusion culture in an ATF bioreactor system, where the amino acid consumption was precisely controlled to the targeted value by the appropriate feeding of amino acids. This strategy can be potentially widely applied across various cell lines to define the optimum concentration of amino acids or other components in perfusion media.

CONTINUOUS ANTIBODY CAPTURE STEP BASED ON MAGNETIC BEADS

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Key Words: magnetic beads, integrated mAb capture, purification, downstream-bioprocess, monoclonal antibody

The integration of a perfusion culture process with periodic counter chromatography currently draws a lot of attention in industry, with the goal of generating more efficient and flexible processes at reduced footprint. Purification process based on magnetic beads has been used for decades for analytical purposes. It shows interesting potential and begins to gain interest in the biopharmaceutical industry for fed-batch processes. This type of separation has characteristics that can facilitate continuous process integrated to perfusion operation. However integrated mAb capture using magnetic beads has not yet been published.
In this study we have designed novel system and process for the affinity purification of monoclonal antibodies (mAbs) using magnetic beads based on work we performed in a pilot-scale set-up[1]. High capacity magnetic protein A agarose beads (LOABeads PrtA) were used with a prototype separation system for this development. In a proof-of-concept, the prototype set-up was tested with supernatant generated from perfusion process. As shown in Figure 1, the integrated process had a rapid adsorption of the mAb within 2 hours. It provided an overall adsorption of 94% and a yield of 87% (Figure 2). The unique use of high capacity magnetic beads together with this novel prototype system for continuous separation showed promising results, which can provide an efficient way for the integration of up-stream and down-stream process.

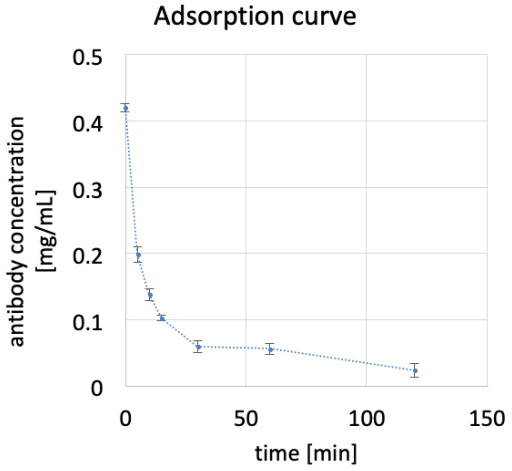

Figure 8 – Adsorption curve of mAbs in integrated magnetic bead affinity capture

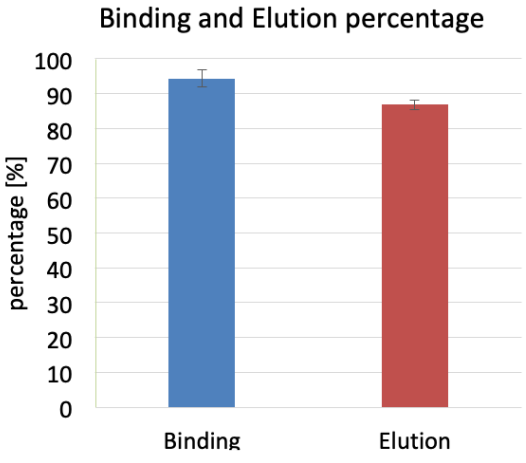

Figure 2 – mAbs binding and elution obtained in integrated magnetic bead affinity capture

1. Brechmann, N. A.; Eriksson, P.-O.; Eriksson, K.; Oscarsson, S.; Buijs, J.; Shokri, A.; Hjälm, G.; Chotteau, V., Pilot-scale process for magnetic bead purification of antibodies directly from non-clarified CHO cell culture. *Biotechnology Progress* 0, (0).

IMPLEMENTATION OF AN INTEGRATED CONTINUOUS DOWNSTREAM PROCESS FOR A MONOCLONAL ANTIBODY PRODUCTION

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The biopharmaceutical market is driving the revolution from batch to continuous manufacturing (CM) for higher productivity and lower cost. In this work, a bench-scale fully integrated continuous downstream process for monoclonal antibody production was established and successfully scaled up to 200 L scale. The process includes a continuous proteinA step, a viral inactivation step, a batch-wise cation exchange and anion exchange step, a batch-wise viral-filtration step, and a single-pass UF/DF step. An inline protein quantity monitoring system was designed to control protein loading mass on cation exchange column. All the steps were connected through surge tanks and integrated by DeltaV™ automatic control system. The setup was tested for the continuous production of a mAb, and the overall production process can be finished within 24 hours (Figure 1). Reproducible performances and product quality were observed over 3 lab batches (Figure 2). The process was then successfully scaled up to pilot plant (200 L, fed batch) with consistent results. The viral clearance study and lifetime study on Protein A step was designed with scale down model. This work demonstrates the feasibility and advantage of applying integrated continuous process in monoclonal antibody production and may provide a reference for large scale manufacturing.

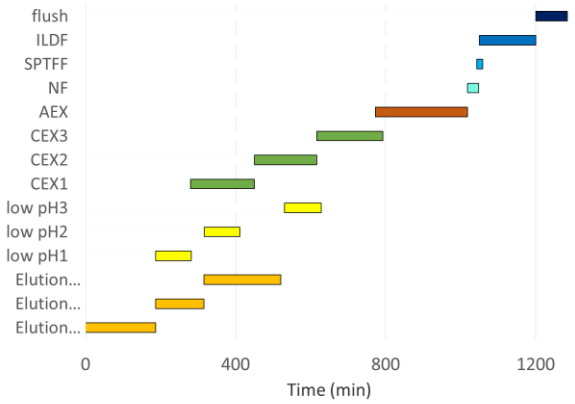

Figure 1. Unit operation time of continuous downstream process

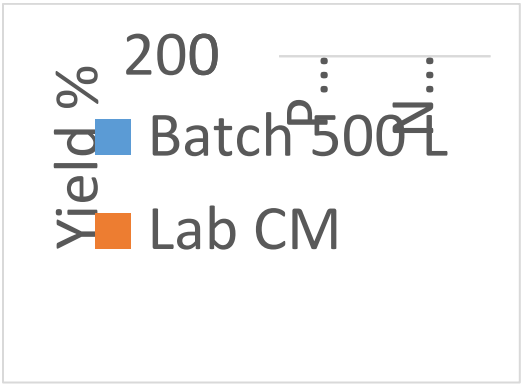

Figure 2. Comparison of recovery yields among 500 L scale batch, lab scale CM and 200 L scale CM