

## **PROCESS INTENSIFICATION IN BIOMANUFACTURING DRIVEN BY ADVANCES IN SINGLE USE TECHNOLOGIES**

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Single use technologies now represent the state of the art in biomanufacturing, but still the full potential of a broad application has not been tapped yet. One strategy that captures most of disposable benefits is process intensification. Here single use systems are optimized to achieve similar output levels as traditional stainless-steel facilities but at much lower capital expenditure, smaller footprints and faster implementation times. This presentation gives an overview on current approaches, describes the opportunities and limits of disposables and delivers recommendations to what should be done by suppliers and users in the near future.

# A DEEP DIVE INTO THE PROCESS OF DESIGNING AND DEVELOPING A SINGLE-USE ASEPTIC CONNECTOR

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Key Words: aseptic connector molding materials validation

With the rapid adoption of single-use products over the past decade, connections have become more and more critical to biopharmaceutical applications especially as the applications move into commercial applications. As a result, there have been a drastic increase in opportunities to close the process with the utilization of aseptic connectors. Currently, there are a lot of connector options in the market and each have their own promoted benefits that end users can evaluate. But what is seen in the market is just the finished product and does not tell the story of the process it takes to develop a high quality connector that fits the end user needs.

Though connectors can seem minor when compared to a large single-use bioreactor bag or a pleated, sterile filter capsule there is still a lot of work that is needed to develop a robust, aseptic connector. Several of the items that can go into the design of the connector can be:

- Design functionality of the connector
- Materials selection
- Injection molding design and process optimization
- Prototyping
- Effects of sterilization
- Risk assessments (FMEA's)
- Process validation
- Functional testing

This presentation will dig deeper into the process of designing and developing a connector. Though this presentation will use aseptic connectors as a case study, most of this information should apply to any single-use component that involves molding and assembly. This will not be a commercial presentation to try and lean the audience to a certain company's technology, rather its goal will be to help educate the audience into what goes into developing a connector and considerations and obstacles that a manufacturer will encounter.

## **SPEED UP BIOPHARMA DEVICES' RELEASE TO MARKET**

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Key words: Aging, Polymers, Simulation, Time to Market

It is a challenge in the biopharma industry to attain appropriate qualification protocols for devices. The manufacturers necessitate device storage that lasts for an extended period of time.

Single-use devices may need storage for up to 6 years before their functional lifetime actually starts. In other words, every product completes its unique journey before it can be functionally used by the customer.

At this point, the manufacturer needs to provide a quality certificate that guarantees the device's safety and robustness during its life cycle. Likewise, package material aging information is needed to ensure package integrity, satisfy FDA validation requirements, and provide evidence of sterility and fitness for use over a product's life cycle. To decrease the time necessary for testing prior to commercialization, or in other words to speed up product's market release; manufacturers perform accelerated-aging studies on the product/ package combination. These studies are performed at elevated temperatures, so to simulate the realistic life span of the product. Life span of every product is unique in line with its application requirements and consists of sections such as storage of components, assembly and irradiation, storage of the irradiated product and finally application. Polymers are similar to living organisms and their properties are time, temperature and stress dependent, making the job of simulating the life cycle very complicated and difficult.

As Sartorius Stedim Biotech, we are taking this challenge and adapt our aging methodology to each polymers. Our method will be shared to show how we can speed product introduction to market.

### Biography

Dr. Nazli Gulsine Ozdemir has received her PhD from Kingston University London, UK at the age of 28 and following her graduation she worked as a postdoctoral researcher at University of Bristol, UK. Currently she works as a materials specialist, at Sartorius Stedim Biotech in the United Kingdom. She has published 10 papers as first author in reputable journals. She has also given speeches in many polymer engineering and materials science conferences across the globe.

Nelly Montenay has received a degree in Engineering from the ITECH Lyon Engineering School. She is specialized in polymers science and plastic transformation. She is Platform Manager for Single Use Systems in Sartorius Stedim Biotech Research and Development Group, with 14 years of experience in polymer science, film development and product qualification testing.

## USE OF SUS FOR NON-CONVENTIONAL SYNTHETIC MANUFACTURING PLATFORMS

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The proliferation of SUS has been due to a combination of factors including increased demands on throughput efficiencies as well a growing number of product modalities manufactured at pharmaceutical facilities. Some new modalities (e.g. Anti-Sense Oligonucleotides, Gene Therapy) use predominantly solvent based chemical synthesis processes for upstream manufacturing. As a result, these new modalities present a whole new set of challenges with respect to use of SUS. In these cases, historical challenge agents used to assess the chemical compatibility and performance of SUS may not be representative of the actual manufacturing conditions. Furthermore, as most of the solvents exhibit strong extraction properties, worst-case testing conditions for extractable studies need to be redefined. In this presentation, we will present data on the specific challenges with the application of SUS in Anti-Sense Oligonucleotide manufacturing and propose other material testing and regulatory aspects that may need to be considered when assessing the suitability of SUS systems for these applications.

## POLYMER HYDROGELS: A BIOCOMPATIBLE MATERIAL WITH MULTIPLE POTENTIAL USES IN SINGLE-USE SENSORS

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Polymer hydrogels are crosslinked polymer networks that can absorb large quantities of water without dissolving. When contacting cells, they are relatively biocompatible compared to most other materials, because they are soft, and because they are mostly composed of water. Polymer hydrogels are already widely used as commercial contact-lens materials, and they are also essential elements of most continuous sensors that are currently being developed for single-use bioreactors. All continuous bioreactor sensors require a molecular recognition element that binds to the target analyte of interest (e.g. glucose). In many continuous sensors, the recognition element is an enzyme such as *glucose oxidase* that is immobilized within a polymer hydrogel matrix. In other continuous sensors, the molecular recognition element is itself a “smart” polymer hydrogel. A *smart polymer hydrogel* is a polymer hydrogel that reversibly and autonomously changes its degree of swelling in response to some external stimulus, such as change in temperature, pH, or concentration of a target analyte such as glucose. In this talk, I will review the basic physics and chemistry of polymer hydrogels, and then discuss how hydrogels can be designed for the above-mentioned sensor applications.

## **pH EVOLUTION IN SOLUTION AFTER CONTACT WITH MULTILAYER FILMS AFTER DIFFERENT $\gamma$ -IRRADIATION DOSES AND THUS RECONCILIATION OF pH AND TOC WITH CARBOXYLIC ACIDS DETECTED BY ION CHROMATOGRAPHY**

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**Keywords:**  $\gamma$ -irradiation, Polyethylene, Ethylene Vinyl Acetate, Ion Chromatography, Carboxylic acids

For a number of various uses (storage, mixing, freezing, transportation, formulation, and filling) biopharmaceutical solutions are stored in sterile single-use plastic bags. Material transfers can then occur between containers and contents. These migrations, of different types, depend on the physicochemical characteristics of the material (composition, pH, solubility, viscosity, molecular weight, etc.), the nature of the product (solid, semi-solid and liquid) and the conditions of the material utilization. In the case of single-use polymers,  $\gamma$ -irradiation sterilization of the polymer is often carried out. The interactions could be therefore influenced by the dose and the contact time between the container and the contents.

$\gamma$ -sterilization of single-use systems initiates chemical reactions and complex modifications inside the plastic material. In this study,  $\gamma$ -irradiation doses investigated are up to 270 kGy in order to emphasize the  $\gamma$ -irradiation effect and to better investigate the modifications of commercial PE(Polyethylene)/EVOH(Ethylene Vinyl Alcohol)/PE-film and commercial EVA(Ethylene Vinyl Acetate)/EVOH/EVA film. This study is a part of a global investigation on  $\gamma$ -irradiation on multilayer films Non-specific (TOC, pH, conductivity) or specific (e.g. chromatographic, spectroscopic, gravimetric) analytical methods can be used. several approaches were used to study the impact of  $\gamma$ -irradiation on multilayer films, as ion chromatography to detect and quantify the ionic species, and as pH and conductivity measurements to observe the consequences of the chemical modifications.. There are few references available on the leaching of carboxylic acid species impacting aqueous solutions used in biopharmaceutical applications in contact with plastic single-use systems [1]. Stability studies under accelerated or real-time degradation conditions make it possible to define the shelf life and storage conditions in order to guarantee the quality of the product. The aim of the study is to identify and quantify the acid compounds that can be released from the container under normal conditions of use of the materials: the extractables.

[1] D. Jenke, D and V.J. Barge. Factors affecting the release of extractable acetic acid from multi-layered plastic films containing ethylene vinyl acetate (EVA) and polyethylene (PE) layers. Pharm Outsourcing. 15 (2014) 56-59.

## AUTOMATED FOAM CONTROL IN SINGLE USE BIOREACTORS USING THE SINGLE USE FOAM PROBE

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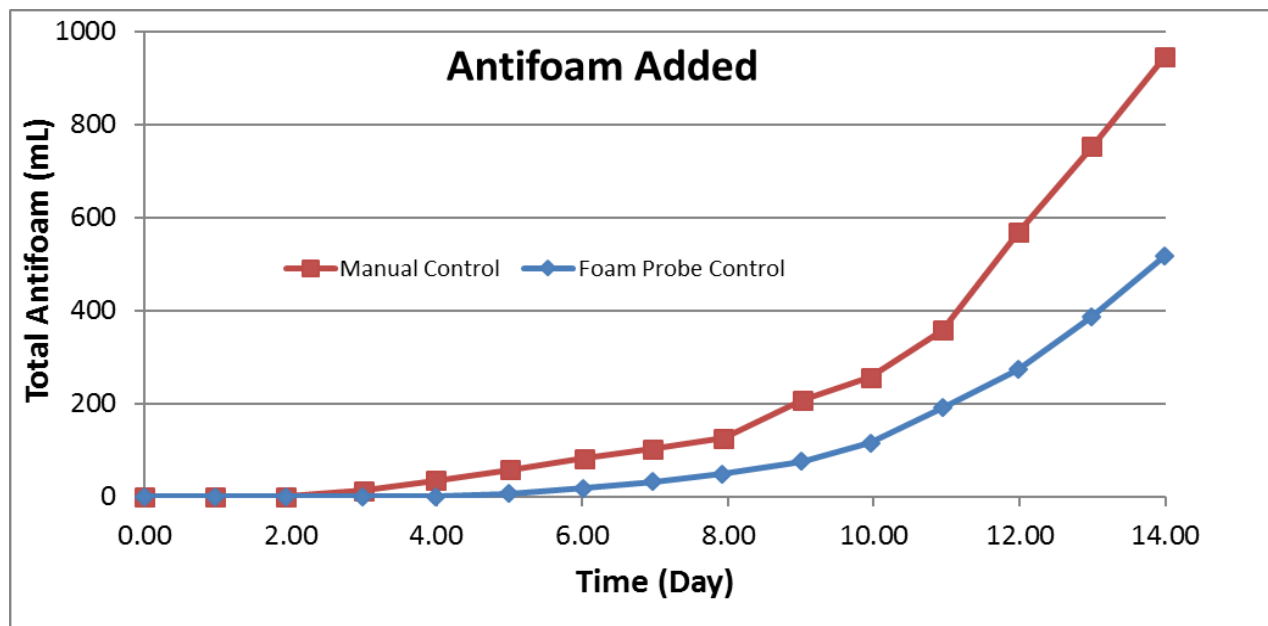
Key Words: Antifoam, Single-use bioreactor, foam probe, S.U.B.

Headspace foam in bioreactors can cause significant issues to a biological process among which are unfavorable metabolic conditions for cells, increased shear from bursting bubbles, cell death/entrainment, mass transfer interference and potential fouling of exhaust filters which can allow a point of entry for contamination or lead to pressure build-up and possible failure of the bioprocess container. This study investigates the efficacy of using a single use foam probe coupled with an automated response of an antifoam addition with an integrated DeltaV controller. Two 50L S.U.B.s run in parallel clearly illustrate differences; the first employing foam control using the foam probe and the second 50L S.U.B. where foam is manually controlled via a dosing interval and manual additions when determined by the operator.

Aggressive fed-batch operating parameters show that a foam probe greatly reduced the amount of foam present and required antifoam additions. This work demonstrates the following:

- Foam probe selection and integration into the S.U.B. bioprocess container
- Optimal control parameters of antifoam addition using real-time feedback from the foam probe and integrated controller

This side-by-side study demonstrates a 47% reduction in the amount of antifoam used. It is commonly accepted that avoiding excess antifoam addition improves downstream processing minimizes risk of foam outs, and thus reduces operator stress, fatigue, and potential for unplanned process intervention. Reducing risk in SUB operation includes use of an automated antifoam based on real time feedback.



## RESPONSIVE HYDROGEL SENSOR FOR MONITORING ANTIBODY PRODUCTION

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Key Words: Biosensor, Bioproduction, Monitoring, Antibodies, Disposable

Precise control over the biomanufacturing process is crucial for maximizing yield and quality of monoclonal antibodies (mAbs); however, the industry does not have sensors capable of continuously monitoring either mAb yield or quality. Consequently, this production is plagued with poor quality control, reduced productivity, and increased costs. To develop such a sensor, we investigated the use of aptamers selective to human immunoglobulin G (IgG, sub-type of mAbs). First, we investigated the physiochemical properties of six different aptamers that bind to two distinct regions of the protein as well as tested their the binding affinity to human IgG, before and after standard sterilization procedures (autoclave and gamma irradiation), using surface plasmon resonance (SPR, Figure 1). Chemical modification procedures were developed for immobilization of the aptamers onto a biotin capture sensor chip for use in SPR. Based on these results, two aptamers were selected which bind to separate regions of IgG, which have optimal physiochemical properties and have strong binding affinity to IgG. Similarly, the aptamers were modified to covalently bond and incorporate into a hydrogel network creating an IgG-sensitive hydrogel. In the presence of IgG in solution, both immobilized aptamers bind to the IgG molecule and form a new crosslink which subsequently causes shrinking (volume reduction) of the hydrogel [1]. This change in volume is monitored using our patent-pending magnetic transduction technique [2]. The degree of hydrogel shrinkage is measured using a magnetometer chip and fixing a permanent magnet to the hydrogel surface. An electronic reader with the magnetometer transduces the hydrogel response into an electrical signal. Response tests using this setup were performed in four different complex environments including industrial cell culture medium. The results show that this IgG-sensitive hydrogel is stable to autoclave and gamma irradiation and responds to increasing and decreasing concentrations of IgG in various solutions (Figure 2). The magnitude of hydrogel response is used to correlate the change in IgG concentration.

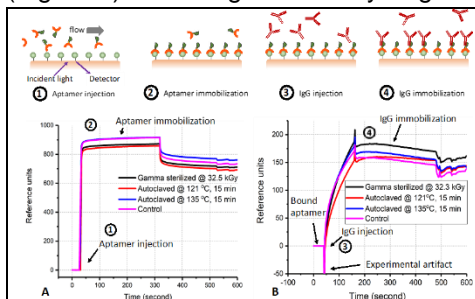


Figure 1 – Surface plasmon resonance (SPR) technique used to determine IgG-binding affinity of selected aptamers before and after sterilization.

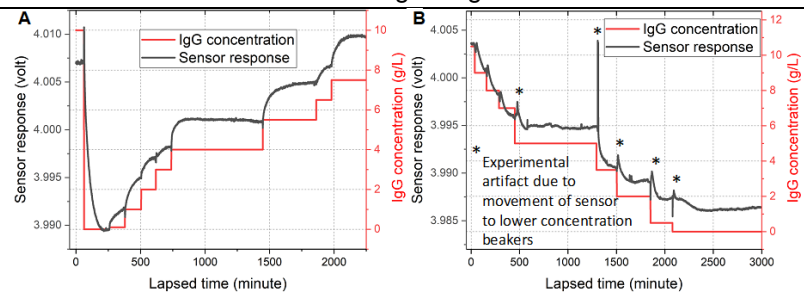


Figure 2 – Demonstration that the IgG-sensitive hydrogel can detect IgG over the entire commercially-relevant concentration range.

### References:

- [1] W. Bai, N. A. Gariano, and D. A. Spivak, "Macromolecular Amplification of Binding Response in Superaptamer Hydrogels," *J. Am. Chem. Soc.*, vol. 135, no. 18, pp. 6977–6984, 2013.
- [2] T. Nguyen *et al.*, "Sensor-array for continuous monitoring of biochemicals for bioprocess control," *2015 Transducers - 2015 18th Int. Conf. Solid-State Sensors, Actuators Microsystems, TRANSDUCERS 2015*, pp. 1684–1687, 2015.



## PROBING FOR SOLUTIONS: EVALUATING NEW PH SENSORS FOR UPSTREAM SINGLE-USE APPLICATIONS

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Key Words: pH probe, Sensors, Single-Use Technology.

The growing field of single-use manufacturing has created a desire for single-use pH sensors that are comparable in performance and operational ease of use to traditional multi-use pH sensors. Some of the current challenges with single-use pH technology include narrower pH ranges, lot-to-lot variability, limited sensor lifecycles, and complicated calibration procedures. Additionally, although sterile connectors allow traditional glass pH probes to be utilized, they have complex assembly procedures that lead to contamination concerns. A wave of new single-use pH sensors are being developed to address and improve upon the aforementioned challenges. Prototype sensors from three different vendors were evaluated to determine their ability to replicate the robustness and reliability of multi-use sensors.

This presentation/poster discusses the evaluation of three new pH sensor technologies with the goal of providing a recommended path forward for pH sensors in upstream single-use processes. One of the sensors evaluated was a solid state “glass-free and calibration-free” pH sensor. The other two sensors were glass probes enclosed in an adapter and built into the SUB. The glass elements remained wetted with a storage solution in the adapter, thus enabling prolonged shelf life of the sensors. A series of experiments were conducted to compare the performance of these sensors against the performance of a traditional glass multi-use pH probe. The experiments included pH buffer range tests (pH 2 – pH 11), drift tests, experiments in non-cell media under normal process parameters (agitation, temperature control, pH control, and sparging), and traditional cell culture runs (including implementation in SUBs). The data from these experiments provide a basis for assessing the performance of each sensor (such as pH measurement range, drift, resolution, scan rate, effect of agitation, effect of sparging, response to changes in pH, and performance in cell culture), and determining whether the sensor is viable for implementation in upstream single-use applications for both development and GMP Manufacturing.

## DATA ANALYTICS AND SOFT SENSORS FOR SINGLE USE BIOPROCESSING

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Key Words: Soft sensors, single use, data analytics

In the last decades, innovative research and engineering brought birth to a plethora of robust and mature process analytical devices. Nowadays, the possibilities to extract chemical-, physical- and biological data from single use processes are manifold and a great quantity of process data is collected on a routine basis. However, novel challenges in the field of data processing and information mining emerged: How can the maximum information content be extracted from the combination of process analyzers? How can big process data be handled and exploited efficiently? And ultimately: How can this information be translated in a business benefit for the manufacturers?

Here, we demonstrate how these challenges can be addressed within the bioprocess lifecycle using innovative mechanistic methods. We present i) novel non-invasive soft sensors for real-time monitoring of single use processes, ii) information mining and process analysis based on the combination of mechanistic models and statistical tools and iii) efficient and scalable process control strategies.

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## **PILOT SCALE IMPLEMENTATION OF A SINGLE-USE, HIGH INTENSITY, INTEGRATED PROCESS SYSTEM**

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Key Words: Single-Use, Pilot, Integrated, Continuous

Pfizer and Boehringer Ingelheim are developing pilot scale systems with entirely single-use flow paths that are fully integrated end-to-end under a single control system from bioreactor through downstream processing. These prototype systems are designed to run continuous processing from the bioreactor to downstream, and periodic processing to the end of the downstream system. This presentation shows the evolution of the systems including some novel single use technologies, details of some high-intensity run results, and offers future single use improvement ideas.

# CONTINUOUS BIOPROCESSING IN SINGLE-USE BIOREACTORS: BEYOND STIRRED TANK-BASED SOLUTIONS

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Key Words: continuous, rocking motion, perfusion, monitoring, hybrid sensors

Continuous bio-processing opens up new possibilities for single-use concepts. Several mammalian cell culture processes are operated in continuous operation using perfusion technologies to not only ensure product quality and avoid product degradation, but also to reduce costs and working volumes. In combination with single-use bioreactors, the effects on production costs are even higher. The introduction of single-use bioreactors in continuous operation for microbial applications also reduces the costs of producing microbial molecules, e.g. if previously rarely used co-cultivation systems are applied.

Although mainly limited to mammalian cell culture processes, single-use bioreactor concepts have been developed that are also suitable for microbial processes. In addition to stirred tank reactors, two-dimensional rocking bioreactors are well suited for fed-batch and continuous cultivation processes, since no dynamic parts have to be integrated into the bag.

Whether for cell cultures or microbial processes, the robustness of the bag material and the quality of the sensors must be ensured during the longer process times in continuous cultivation. Classic electrochemical electrodes, in this case hybrid sensors of a disposable and a reusable part, can be an option to achieve long-lasting operation without compromising data quality. In addition, it is obvious that continuous processes require specific and appropriate monitoring tools to meet regulatory requirements and to detect process disturbances as quickly as possible to adjust dilution rates and product separation cycles. Therefore, the latest advances in optical density measurement and single cell analysis in combination with single-use bioreactor concepts are presented. Some examples are shown of how the construction of a single-use bioreactor including monitoring tools (*on line* and *in line*) enables continuous processes with a suitable robust control option in the case of cell culture and microbial cultivation processes.

Finally, a cost estimate is made for a specific biosimilar production process to demonstrate the potential of suitable continuous bioprocessing with a single-use bioreactor and downstream processing compared to alternative, conventional concepts.

## Literature

1. Junne S, Solymosi T, Oosterhuis N, Neubauer P. Cultivation of cells and microorganisms in wave-mixed disposable bag bioreactors at different scales. *Chemie-Ingenieur-Technik*. 2013;85(1-2). doi:10.1002/cite.201200149.
2. Junne S, Neubauer P. How scalable and suitable are single-use bioreactors? *Curr Opin Biotechnol*. 2018;53. doi:10.1016/j.copbio.2018.04.003.

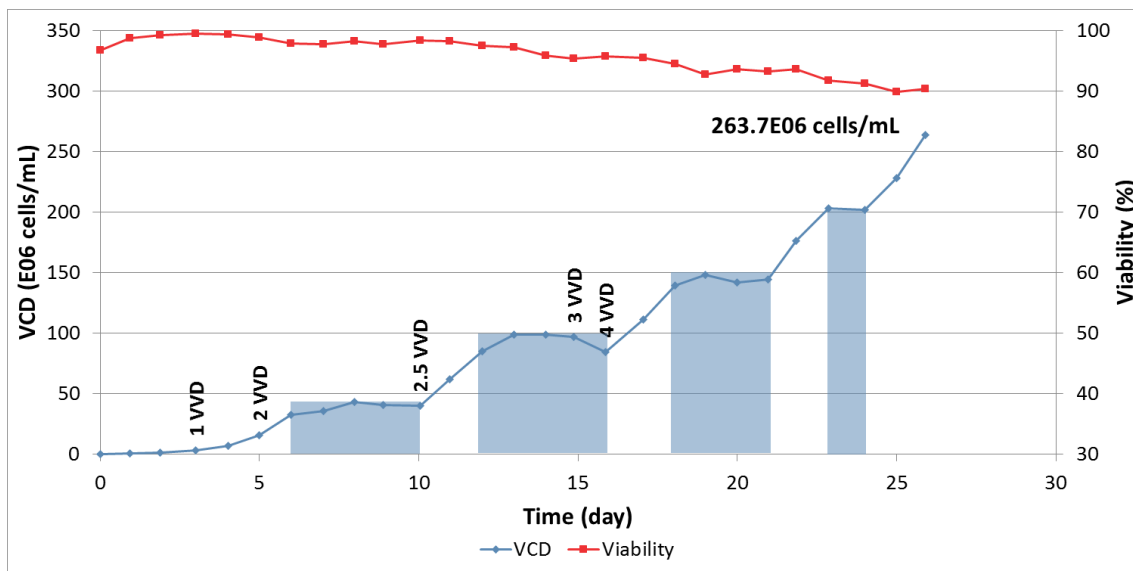
# CONTINUOUS PROCESS PERFORMANCE ENHANCEMENTS FOR 50 L TO 500 L SINGLE-USE BIOREACTORS: A TECHNICAL COMPARISON OF PERFORMANCE CHARACTERIZATION, CELL CULTURE, AND SCALE-UP MODELING

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Key Words: Continuous processing, Single-use bioreactor, drilled hole sparger, Enhanced S.U.B.

Improvements in single-use systems have allowed implementation of high-density cultures in emerging bioprocess workflows while progressive advances in media optimization and improved clone genetic selection have underscored the perceived performance limitations of single-use bioreactors (S.U.B.s). This study presents how strategic enhancements to the sparge and agitation systems of Thermo Scientific™ HyPerforma™ S.U.B.s have revealed the potential for a three- to four-fold improvement of mixing and mass transfer performance compared to legacy SUB designs. This study investigates the following:

- Bioreactor characterization, TruBio™ DeltaV™ controller optimization, online process analytics, and scalability analysis of the S.U.B. when targeting perfusion applications from 50 L pilot scale to 500 L production scale working volumes.
- High-density culture results (>260E06 cells/mL) while maintaining proper operating parameters. New data reveal 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 S.U.B. operating efficiency. Results also include specific suggestions on how to maintain a nearly ideal dissolved carbon dioxide environment, reduce headspace foam generation, and produce lower overall shear levels, thus yielding excellent cell viability.
- The work also demonstrates best practices and the desirable process benefits that can be achieved through reduced technical risk, lower labor, and simplified technical transfer of a completely disposable processing assembly. Further evidence is presented on the advantages of continuous processing when used in high-density seed train intensification or as a compact production-scale bioreactor system operating at reasonable media exchange rates of one to two vessel volumes per day (VVD).



## **LOW-FOOTPRINT, INTENSIFIED, SINGLE-USE PLATFORM FOR THE PRODUCTION OF VIRAL VACCINES**

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Key Words: Single-use, end-to-end processing, perfusion culture, vaccines.

The world is facing an under-supply of some key vaccines due to poor synergies between growing market demands, the need for global epidemic preparedness and aging production models. In this light, funded by a grant from the Bill & Melinda Gates Foundation, Univercells has developed an affordable, ultra-low footprint platform designed to produce up to 40M doses per year of sIPV vaccines. The process makes use of a reduced footprint, single-use perfusion cell culture as well as chained filtration and clarification steps in order to (a) reduce batch time, (b) increase equipment utilization and ultimately (c) intensify operations. The result is a drastic reduction in Cost of Goods (CoGs) by taking advantage of a single-use bioprocessing architecture.

One of the key challenges in achieving this goal is the industrialization and subsequent intensification of adherent cell cultures for the production of viruses. Perfusion cell cultures in stirred tanks are typically associated with a large number of operations and complexity in process development. Addressing these challenges, Univercells has developed a single-use fixed bed bioreactor which addresses the latter. The other key challenge is in reducing the footprint required to clarify and purify the resulting concentrated product stream.

This talk will demonstrate how Univercells has optimized single-use technologies to design a fully continuous and automated production process, integrating both USP and DSP steps within a confined and contained low-footprint facility. Based on sIPV as a case study, we will present how this micro-facility is aiming to achieve up to a 10-fold reduction in CoGs while delivering high robustness, quality and safety.

## UNDERSTANDING THE SCIENCE BEHIND LIQUID LEAK AND MICROBIAL INGRESS MECHANISMS AS THE FOUNDATION FOR SINGLE-USE CONTAINER CLOSURE INTEGRITY (SU-CCI)

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Key Words: SUS, Integrity, Liquid leaks, Microbial Ingress, Maximum Allowable Leakage Limit (MALL), Cell & Gene Therapy, USP<1207>, CAR-T, autologous

This presentation describes a scientific approach to establishing a relation between liquid leakage and microbial ingress mechanisms in single use plastic containers and developing the appropriate physical integrity testing methods and specifications.

With the expansion of Single Use Systems (SUS) in all process steps of commercial manufacturing, integrity failure can significantly impact drug safety, availability and costs. The use of closed systems in cell & gene therapy can support the reduction of production costs when moving the manufacturing process from class B to class C or even D environment. More significantly, in very critical autologous applications, like e.g. CAR-T cell therapy on terminally ill patients, a single bag integrity failure can result in the loss of the only possible batch, followed by the death of the patient. As current approved sterility test methods take longer than the shelf-life of the cell preparation, additional integrity testing for risk mitigation can help to support the final cell product release before injection to the recipient. A combination of an integrity test of the bioprocess containers with a quantitative, real-time PCR of a product sample (e.g. on mycoplasma) can provide a strong indication that the cell preparation has not been contaminated.

During lentivirus production, operator safety is of great concern as the components are derived from viruses that target human cells, and feature an inherent risk for insertional oncogenesis. Assuring that the virus is contained by technical means is favorable to relying on organizational or personal means, such as use of safety equipment and personal hygiene, as it should present lower risk.

Consequently, there is an increasing industry scrutiny on SU CCI, raising the need to develop good science behind liquid leakage and microbial ingress and appropriate physical integrity testing technologies.

The authors will first review the emerging industry association initiatives and introduce an integrated quality by design (QbD), material science and process control approach as the prerequisite to SU-CCI.

The presentation will then describe how applying good science can help determine the maximum allowable leakage limit (MALL) under which no product leakage and no bacteria ingress occur with SUS under various fluids and process conditions. The understanding of liquid leakage and bacteria ingress mechanisms also enables the validation of robust liquid leak tests and microbial aerosol challenge which are both correlated to the detection limits of physical integrity testing methods.

The authors will conclude with the development of highly sensitive deterministic integrity testing technologies, such as gas tracer detection and pressure decay, which are able to detect the MALL determined during the scientific study. The Helium based Supplier Integrity Test (SIT) for instance is able to control the finished products with a detection limit of 2µm and is correlated to both, liquid leakage and microbial ingress, under all tested process conditions.

Audience take home messages:

- QbD, QRM, Process Control and Quality Control ensure CCI along the entire production cycle
- Understand the science behind SUS films' behavior and the determination of the Maximum Allowable Leakage Limit (MALL)
- Integrity testing technologies can detect the MALL in all parts of complete SUS assemblies at both the supplier and point of use and can be correlated to a bacterial challenge
- Use of closed systems in combination with an appropriate integrity assurance strategy can support cost reduction and risk mitigation for product release in cell & gene therapy applications.

# AUTOMATED APPROACHES TO PROCESS DEVELOPMENT AND MANUFACTURE OF HUMAN T-CELLS AND MESENCHYMAL STEM CELLS USING SINGLE-USE BIOREACTOR TECHNOLOGIES

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**Key Words:** Cell and Gene Therapy, hMSCs, T-cell Bioprocessing, Automation, Single-Use Technologies

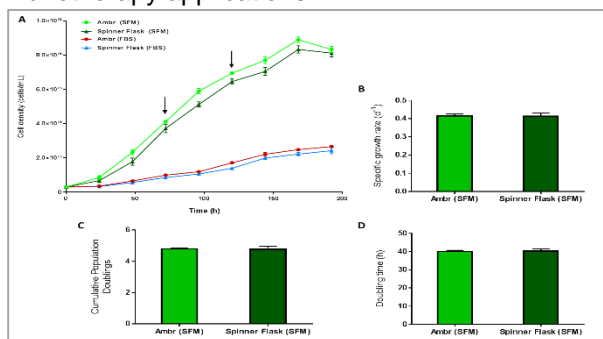
Improvements to process development and manufacturing technology will have a significant impact in reducing the overall costs associated with the manufacture and scale-up of cell and gene therapies. Small-scale models, including single-use technologies such as microbioreactors, play a critical role in this regard as they reduce reagent requirements and can facilitate high-throughput screening of process parameters and culture conditions.

This talk will demonstrate the amenability of the automated, single-use systems such as the ambr15® and ambr250® platforms for adherent human mesenchymal stem cell (hMSC) culture and suspension T-cell culture. We have also demonstrated that such systems can be used for effective bioprocess development of both a microcarrier and suspension cell line process, with data being validated in larger-scale studies.

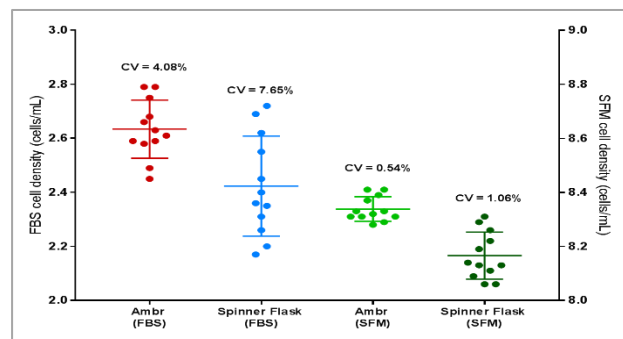
For the hMSC studies, adhere culture on microcarriers was achieved through a combination of strategies including adapting the free suspension design of the vessel to improve the suspension and mixing of the microcarriers. A more effective cell attachment method was also developed by using only 50% of the final working volume of medium for the first 24 h combined with an intermittent agitation strategy. These improvements led to a reduction in the initial lag phase which in turn resulted in > 150 % increase in viable cell density after 24 h compared to the original process (no agitation for 24 h and 100 % working volume). Using the same methodology as in the ambr15®, similar improvements were obtained in larger scale spinner flask studies.

This improved bioprocess methodology, which was developed for a serum-based medium process, was applied to a serum-free process in the ambr15; this resulted in > 250% increase in yield compared to the ambr15 serum-based process. The use of the ambr15, with its improved control compared to the spinner flask, reduced the coefficient of variation on viable cell density in the serum containing medium from 7.65% to 4.08%, and the switch to the serum free medium further reduced these to 1.06% and 0.54% respectively. The combination of both serum-free and automated processing improved the consistency more than 10-fold compared to the initial manual, serum-based spinner flask work.

Similar work has been undertaken with the ambr250®, where a new single-use bioreactor vessel was designed to improve adherent microcarrier culture and has demonstrated improved growth of suspension T-cells for immunotherapy applications.



**Figure 2 – Growth kinetics of hMSCs for serum-free (SFM) and fetal bovine serum (FBS)-based media in both the ambr15 and spinner flasks with data showing (A) the viable cell density, (B) specific growth rate, (C) the cumulative population doublings and (D) the doubling time. Data show mean  $\pm$  SD, n = 8.**



**Figure 2 – Extent of viable cell density variation in the ambr15 and spinner flask for both serum-free (SFM) and fetal bovine serum (FBS)-based cultures. Cell density values for FBS are aligned with the left y-axis and the SFM values with the right y-axis. Data show coefficient of variation (CV), n = 8.**



## **ENGINEERING SCALABLE MANUFACTURING OF HIGH-QUALITY HUMAN MSC FOR CELL THERAPY: FROM UP TO DOWNSTREAM PROCESSING INTEGRATION TO CELL PROTEOME CHARACTERIZATION**

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**Key Words:** cell therapy, product characterization, scale-up, single-use technology mass spectrometry,

Human mesenchymal stem cells (hMSC) are relevant cell therapy products for autologous and allogeneic therapies. To deliver the required cell numbers and doses to therapy, scaling up production and purification processes (at least to the liter-scale) while ensuring high purity, viability and maintaining cells' critical quality attributes (CQA) and functionality is essential.

Therefore, the aim of this work was to prove scalability of an integrated streamlined bioprocess compatible with current good manufacturing practices (cGMP) comprised by cell expansion, harvesting, volume reduction and washing unit operations using human mesenchymal stem cells (hMSC) isolated from bone marrow (BM-MSC) and adipose tissues (AT-MSC). Single-use technologies were adopted at different steps of the manufacturing workflow to support process integration and scale-up.

BM-MSC and AT-MSC expansion and harvesting steps were scaled-up from spinner flasks to 2 L single-use stirred tank bioreactor using synthetic microcarriers and xeno-free medium, ensuring high cellular volumetric productivities ( $50 \times 10^6$  cell.L<sup>-1</sup>.day<sup>-1</sup>), expansion factors (14 - 16 fold) and cell recovery yields (>80%).

For the volume reduction and washing steps, flat sheet cassettes (FSC) and hollow fiber cartridges (HF) were compared showing a fairly linear scale-up, with a need to slightly decrease the permeate flux (30 - 50 LMH, respectively) to maximize cell recovery yield. Nonetheless, FSC performed better allowing recovering 18% more cells after a volume reduction factor of 50 without compromising cell's CQA of viability, identity and differentiation potential.

"Omic" tools in combination with standard analytical assays allow for a better cell characterization, increasing product and process understanding and are thus fundamental for process development. Thus, alongside the standard quality assays for evaluating hMSC's CQA, a proteomics workflow based on mass spectrometry tools was established to characterize the impact of processing on hMSC' CQA. Overall, through sensitivity, robustness and throughput, this type of workflow provided the identification of specific signatures of the final product. Therefore, it proves to be essential to understand the cells' final quality as well as to evaluate the impact of manufacturing at different stages of processing.

The authors acknowledge UniMS – Mass Spectrometry Unit team (ITQB-NOVA/iBET, Oeiras, Portugal), iNOVA4Health Research Unit (LISBOA-01-0145-FEDER-007344), and Fundação para a Ciência e Tecnologia (FCT, Portugal) for funding the project CARDIOSTEM (MITP-TB/ECE/0013/2013), and the grants SFRH/BD/51940/2012 (MIT-Portugal), SFRH/BD/52302/2013, SFRH/BD/52481/2014, SFRH/BPD/86513/2012.

## CREATING COMMERCIAL MANUFACTURING OPPORTUNITIES FOR REGENERATIVE MEDICINE BY INTRODUCING CLOSED, AUTOMATED SOLUTIONS WITH SINGLE-USE PRINCIPLES

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Key Words: Single-Use, Closed, Automated, Commercial Manufacture, Cell Therapy

Cell and gene therapy, with growing proof of efficacy in varied indications, has reached an inflection point resulting in a steep increase in the investment of time and money by established companies and disruptive startups. With rapid growth comes the need to quickly and economically manufacture therapies that maintain a consistent, high-level of quality and, in turn, can support commercial manufacturing. This is especially true when looking at patient specific cell therapies that require rapid change over of equipment and benefit little from traditional sterile barriers (i.e. filters and heat inactivation) or economies of scale. Counter-flow centrifugation (CFC) presents an intriguing technology that, when implemented onto a closed and automated system, provides a platform for upstream processing of cell therapies by incorporating multiple unit processes and mitigating the risk imposed with frequent equipment changes.

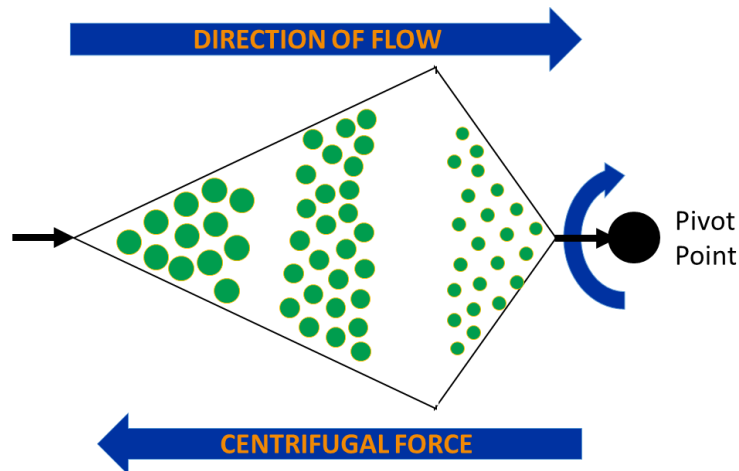


Figure 1. Illustration of counter-flow centrifugation and how it separates objects on a basis of size and density.

The CFC technology under development through a partnership by Hitachi Chemical Advanced Therapeutic Solutions (HCATS) and Invetech uses single-use processing kits with dynamic fluid paths to address the challenges of commercial cell therapy manufacturing. This CFC device has shown the capability to perform platelet wash steps with 99% efficiency and retain 100% of the mononuclear cells. The platform can then harvest a concentrated volume of cells or shift directly into an elutriation protocol to separate hematopoietic cell populations. By utilizing single-use disposables, this platform sees a large cost and time reduction from the large lot number of patient specific therapies. The CFC device provides an effective process solution that exploits single-use technology to meet the commercial manufacturing needs of regenerative medicine.

This work will highlight the benefits of moving to closed, automated solutions for reduction in cost, increase in robustness, increase in scale potential, increase in efficiencies across the therapy lifecycle will highlight important factors in considering a move to automated, closed solutions for individual therapies, utilizing the CFC technology as an in-depth case study.

## **POLYMER INTERFACES AND BIOPHARMACEUTICALS: CHEMISTRY, DESIGNS AND CHALLENGES**

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The complex interactions between biological components and polymer materials has an extensive technical history. Virtually every surface property has been invoked as being important to biological interfacial response: texture, roughness, topology, porosity, hydrophilic, hydrophobic, polar, apolar, (non)-wetttable, non-fouling brushes, surface mobility, rigidity, flexibility, crystalline versus amorphous, aspect ratio. Few surface properties alone, however, provide consistent, global technical solutions to vexing biomedical technology problems, particularly with cell culture, blood, plasma, microbial milieu, and protein solutions. Bio-interface materials performance must therefore be tailored specifically to each application. Short-term contact use (minutes/hours) has different materials interface requirements than long-term (days) use; globular proteins have particularly difficult needs not readily satisfied by any materials solution. Viable biologics interfaces (i.e., fresh blood harvests, cell cultures) must also consider selective gas permeability, leachables, and sterilization issues. Film properties, lamination, cutting, chemical stability, sealing and handling issues are additional considerations for single-use materials. Lastly cost-of-goods and materials economics must be considered, especially for single use technologies. No one-size-fits-all surface solutions currently satisfy all bio-interface materials needs.

This talk will review design principles, dogma and actual polymer chemistries to modulate, modify and manipulate polymer surfaces in contact with biological components. Several polymer surface properties will be discussed with regard to their physical chemistry in aqueous media. Traditional and recent developments in non-fouling interfaces and polymer approaches and their hypothesized influences on biophysical interactions with proteins and cells will be presented.

## **SINGLE-USE EQUIPMENT FOR HANDLING AND MANUFACTURING OF HIGHLY POTENT APIS**

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The industry embraced single-use technology early on after first off the shelf solutions were brought to the market. Initially focused on cell cultures and cell culture products, the use of single-use production solutions expanded over the last decade into other selected technology areas.

The bioconjugates custom manufacturing offering of Lonza has about a 10 years history. While glass and stainless steel based manufacturing equipment dominated in the beginning, the face of bioconjugates manufacture changed in the meantime quite dramatically. Only 7 years after launch of the new market offering, we achieved first successful end-to-end production out of single-use equipment.

Handling of bioconjugates and especially the toxin payload poses a major challenge. The concept of product (patient) safety, a focus in biopharmaceuticals production, is to be amended by the containment and safe working environment aspect. Advantages, caveats and remaining challenges will be discussed.

## PARTICULATE CONTAMINATION IN SINGLE-USE SYSTEMS: REAL VERSUS PERCEIVED RISK

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Key Words: Particles, Particulates, Risk, Single-Use

Certainly final drug products must be “essentially free” of visible particulate contamination and visual inspection systems must meet USP 790 criteria. In addition, final drug products must meet USP 788 limits for sub-visible particles. It is however important to distinguish final drug product standards from requirements for single-use process containers and equipment, even though it is common to claim single-use systems (SUS) “meet USP 788 requirements”. USP 788 does not describe a method for determination of particulate counts in SUS process containers and equipment (1).

Visible particles are “visible” and thus a visual indicator of SUS quality, and consequently sometimes lead to visceral reactions and the perception of major or even critical risk to product safety. However, guidance from PDA TR66 (2), ASME BPE-2016 (3) and the BPSA (4) published in the last few years provide valuable information on assessment of particulate risk in SUS processes. In most situations where SUS are currently applied, filtration and purification steps occur downstream, which essentially reduces the risk to zero for transfer of particulate contamination from SUS to the final drug product. However, any applications of SUS after final filtration (such as in aseptic processes or final filling operations) present significant risk to drug substance or drug product. So is risk to final drug product from SUS an essentially a binary situation: Prior to final filtration low risk, and after final filtration high risk?

While assembly of SUS is a “clean build” process usually done in ISO 7 classified cleanrooms, incoming components and cleanroom processes such as cutting, welding and human assembly are unfortunately not particulate-free with current SUS manufacturing technologies. In addition, visual inspection of SUS components and assemblies is nowhere near 100% effective at detecting visible particles, especially for large complex assemblies or stirred tank reactor systems. Sartorius is currently implementing a “Visible Particle Test” (VPT: liquid extraction and microscopy) for process monitoring and continuous improvement efforts. Thus while most SUS manufactures strive to minimize particulate contamination, absence of particulates remains a goal but is not a currently feasible SUS specification.

Particle contaminants may lie within the interior surfaces of SUS (in the fluid contact path), may be embedded within bag films or plastic components, or lie on the exterior surfaces of SUS. Particulates fall into two general categories: intrinsic (particles from SUS manufacturing process and component materials) and extrinsic (particles from human operators or the environment). Extrinsic particles potentially contain microbiological or viral contamination. These classifications of location and particle type lead to different assessments of risk. One concern are potential “secondary effects” of particulate contamination. Particle contamination could potentially nucleate protein aggregation. Particles embedded in SUS films or plastic components, or on the interior surfaces of the SUS assemblies could potentially leach out chemicals or release microbiological or viral contamination into the bioprocess fluids.

In this presentation, the topic of particulate contamination risk is approached holistically and scientifically using literature data along with calculations. The goal of the presentation is to gain feedback from end users, and to facilitate the discussion between suppliers and end users based upon real rather than perceived risks.

(1) Particulate Contamination in Single-Use Systems, J. D. Vogel and K. Wormuth, Bioprocess International, 15(9) 2017

(2) Application of Single-Use Systems in Pharmaceutical Manufacturing, PDA Technical Report No. 66, 2014

(3) Bioprocessing Equipment, ASME BPE-2016, American Society of Mechanical Engineers, 2016

(4) Recommendations for Testing, Evaluation and Control of Particulates from Single-Use Process Equipment, Bio Process Systems Alliance, 2014

# COMPARISON OF SINGLE-USE AND STEEL FERMENTERS FOR KLa AND AGGRESSIVE MICROBIAL CULTURES

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Key Words: Single-use, Fermentor, kLa, culture

Purpose built single-use fermenters enable production facilities to utilize the single-use technologies instead of traditional stainless steel fermentor vessels without modifying their existing procedures. The manufacture lead time of the single-use fermentor hardware is a fraction of traditional vessels. As each single-use bioprocess container ships sterile and validated, reducing down time between cultures and allowing for more production volume in less space. Here we demonstrate kLa studies and aerobic cultivations with up to 2vvm gas flow in comparison of the single-use fermentors to traditional stainless steel fermenters. These single-use fermentors covering 6L-300L working volumes are specifically designed to meet the performance requirements of dense, rapidly growing microbial cultures while offering the benefits of quick process setup, reduced contamination risk, and high production quality of the original single-use bioreactor.

Study	Results	316ss	SUF
<b>Rapid growth and high protein expression</b> ( <i>E. coli</i> , 120 OD <sub>600</sub> by 12 hrs, IPTG induction)	Equivalent titer and yields 200 g/L WCW	100 L	30 L, 300 L
<b>Hi density growth and protein expression</b> ( <i>E. coli</i> , 240 OD <sub>600</sub> , IPTG induction)	Equivalent yields 9 g/L titer scale-up pre clinical production	1 L, 5 L, 100 L	30 L, 300 L
<b>T7 RNA polymerase</b> ( <i>E. coli</i> , IPTG induction, constant temperature)	Yielded higher DNA modifying enzyme activity	10 L, 300 L	30 L, 300 L
<b>RNAse inhibitor</b> ( <i>E. coli</i> , difficult-to-fold protein)	Identical protein folding conditions created. Higher activity in S.U.F.	10 L	30 L
<b><i>E. coli</i> plasmid production</b> (32 shift to 43°C for induction in 20min)	Equivalent plasmid yields and ~70 OD.	10 L	30 L
<b><i>B.subtilis</i> Protein A/G</b> (secreted protein)	Equivalent yields, ~37 OD with 1.5 g/L target protein.	10 L, 300 L	30 L, 300 L
<b><i>P. pastoris</i> / FastAP</b> (yeast, secreted protein)	90g/l DCW, 300 g/L WCW, 600 OD, Equivalent protein yields, higher activity in SUF	7 L	30 L, 60 L, 300 L
<b><i>S. Cerevisiae</i></b> (yeast, secreted)	2X protein yield in S.U.F., Same 160 OD	10 L, 300 L	30 L, 300 L
<b><i>T. album</i> / Proteinase K</b> (fungus, secreted native protein)	11 day process. Identical protein yield per substrate.	10 L, 300 L	30 L, 300 L
<b>Thermoinducible expression</b> ( <i>E. coli</i> , induction by increasing temp to 42°C)	TCU causes slower heat induction than steam. Equivalent product yield and solubility.	10 L, 300 L	30 L, 300 L

Table 1 – Summary of tech transfer results from steel fermentor into single-use fermentor.

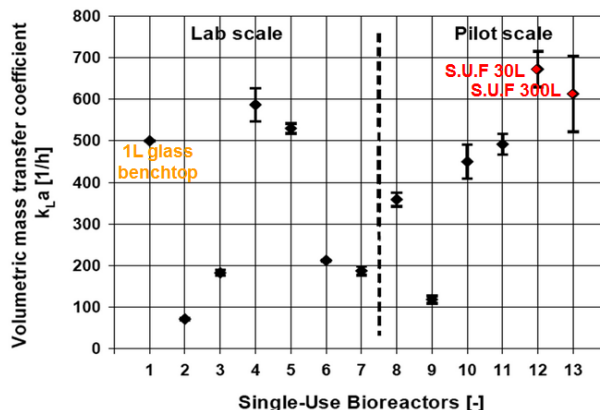


Figure 3 – k<sub>L</sub>a in different single-use bioreactors at laboratory and pilot scale (ISBN: 978-3-89746-171-0).

## ENVIRONMENTAL IMPACTS OF SINGLE-USE

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Key Words: LCA, sustainability, end-of-life, environment, disposal, pollution

The issue of plastic materials in our environment has been of recent interest. The biotechnology community has responded by examining the effects of single-use systems employed in biomanufacturing. It is natural to emphasize a particular set of environmental concerns over others, and especially the more visible ones. But, in supporting truly sustainable manufacturing systems, it is important to consider all relevant types of pollution or environmental stress. The Life Cycle Assessment (LCA) is a science-based approach for evaluating the environmental impacts, benefits, trade-offs, and burden shifts of a process in an objective format. It considers production process' materials, equipment and facilities over their entire life cycle, "from cradle to grave." GE Healthcare has performed a second, extended LCA study of biomanufacturing that considers additional equipment scale, product types, production modes, and installation placements. It compares traditional stainless steel, single-use, and hybrid facilities in the production of Monoclonal antibodies (MAb) and Adenovirus vaccines (Adv), across the full process train including upstream and downstream operations. It includes the effect of many new parameters, including 1) such regional distinctions as shipping distances and power and water sources, and 2) various end-of-life disposal options.