Workshop 7- Perfusion Technology: Challenges and Future Strategies

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Special thanks to the grad students Mariana Monteiro (Imperial College London, UK) and Cristina Silva (Polytechnique Montreal, Canada).

Instructions for workshop:
1. Map out what is the current situation and discuss where gaps exist.
2. Solutions needed for the future, address in 3 levels
    - Level 1: What is already coming (low hanging fruit)
    - Level 2: What we should have and could develop (feasible, but takes effort resources, and money)
    - Level 3: Does not exist yet (needs academia, industrial commitment (new thinking, efforts, technology)

CCE pre-conference survey questions on overall status on perfusion usage: see Q60, Q61, Q62, Q63

Session 7 Workshop Questions	Reference CCE Workshop Survey Question
1. Are the actual technical solutions in terms of equipment and media fitting the needs, e.g., cell retention device, oxygenation, culture media, etc.	Q51, Q52
2. Are there hurdles in terms regulatory strategy, e.g., process characterization, tackling long duration of process, that need new ways?	Q53
3. Which strategy should be adopted to decide the process modality, fed-batch vs. perfusion, or hybrid solution, e.g., COGS, product quality, legacy stainless-steel infrastructure, risks, CMO, transfer fed-batch to continuous, etc.?	Q54
4. Which enabling technologies such as PAT, feed-back control, data storage and treatment, do we need?	Q55, Q56
5. Do we have the suitable tools for scale-up and scale-down, and do they give us satisfying information for downstream integration considerations?	Q57, Q58
6. How can we apply continuous culture to non-glycoprotein/mAb modalities?	Q59

1. Are the actual technical solutions in terms of equipment and media fitting needs e.g., cell retention device, oxygenation, culture media, etc.

Challenges

Media:

- Better characterization of scale-up equipment to avoid differences in prepared media
- Variations in RMs lead to media composition changes and process deviations
- Balancing concentration and volumes
- Supporting cell culture costs
- Automated media prep / concentrates
- Optimization of formulation
- Costs
- Storage
- Logistics
- Media precipitation at scale-up (loop and brxtr)

Product Retention / Fouling:

- Sieving decay
- Sieving capacity, sieving differences
    - Vary between suppliers and/or lots leading to variations in process (early fouling, product retention, etc.)
- Multiple sourcing - often single sourced
- Supply chain
- Surface area limitations / scale-down / adsorption of trace materials
- Pore size
- Modality-specific options
- Automation equipment
- Membrane technology advancements
- Cleaning protocols
- Handling multi-devices
- Changing filter one after another

Equipment:

- Probes for long term processing
- Bag design for long term culture
- Hesitancy to adopt
- Regulatory difficulties
- Sparger configurations (gassing limitations)
- Understanding bubble size impact
- Connection/integration to downstream
- Scale-up and scalable equipment / having scalable material
- Variations in retention times may lead to CQA deviations
- Connections and integrations between different devices/ analytical tools
- Continuous process flow rates / filtration rate consistency
- Maintaining steady state VCD
- Ease of setup and operation

- Number of pumps and filters for filtration
- Integration of all unit ops
- Costs and validation
- Bioburden for upstream (sterile connectors)

Other:

- Process definition
- Continuous VI - residence time/ flow paths

Solutions

Level 1: Retention devices: increase the length/width of filter, change pump settings, multi-tank with in-line dilution, redundant systems (multiple filters).

Continuous powder media addition, sieving - use a back-up, Raman for yield / fouling monitoring, monitoring of fouling (TMP or other) for automated filter change, process algorithm for cell volume (not cell density). CFD for media prep vessel characterization

Level 2: Small-scale continuous model, monitoring systems addressing reliability, sourcing membranes. How to calculate sieving, mechanistic model for filter fouling, more probe space, better hVCD monitoring (capacitance works, but needs validation / guidance), foam sensor + algorithm, low shear mixing at high kLa, probe durability (not for long processes and may fail during production)

Level 3: Media recycling, sieving capacity, membrane technology advancements, integration across platforms (standardization), antifoam free processing (or at least reduction)

2. Are there hurdles in terms of regulatory strategy e.g., process characterization, tackling long duration of process, that need new ways?

Challenges

- Batch definition for long processes: no knowledge of which parameters define a batch (i.e., in terms of volume, time, mass)
- No metrics for clones for long perfusion processes: how do they differ from fed-batch clones?
- CPP definition: what parameters to control (vcd, viability, steady-state or not)
- Control strategy: what is the level of required automation (glucose control, raman, feedback)
- High throughput scale-down models not good enough (data generation for regulatory purposes)
- Maintaining aseptic conditions: is it necessary to show that the reactor is aseptic at all times or just show that the reactor is being overall monitored?
- No clear GMP guidelines for perfusion processes (as opposed to fed-batch which is very well defined)

Solutions

- Have the right tools (like scale-down models) available to address limitations/deviations at large scale
- Development of really high throughput scale-down models for perfusion (Ambr system has 24 small bioreactors, but sometimes up to 90 conditions need to be tested)
- Joint effort from industry on perfusion GMP guidelines: get associations more involved (BPOG, Biopharma, …), release actual documents with guidelines

3. Which strategy should be adopted to decide the process modality (fed-batch vs perfusion, or hybrid solution), e.g., COGs, product quality, legacy stainless-steel infrastructure, risks, CMO, transfer fed-batch to continuous, etc?

Challenges:

- Drivers: Productivity, cost of goods, flexibility, required levels of quality
- Difficulty in evaluating market forces that justify a continuous production: decision based on market supply/demand which can be volatile and hard to predict
- How to decide?
    - Fed-batch is already optimized
    - Identify key products / need to be the right ones
- Decide what stage of the development processes to take to continuous (some companies do it earlier on, other later, it seems to be very product related)
- Defining how to operate continuously (very empirical)
- Ease of scale-up for continuous
- Availability of cell retention devices at scale
- Different modalities for different stages of the process (N-1, N, etc.)
- Defining cell lines / clones that perform well in perfusion
- Filter clogging
- How to continuously permeate
- Media design

Solutions

Level 1: Select host that works OK (but not necessarily optimally). For example, a high producer clone with lower growth rate for steady state, long term perfusion processes

Level 2: Equipment/ Filter supply, need for more suppliers of equipment. Development of high throughput scale-down models for perfusion - screening and process development in a more representative way.

Level 3: Engineering cell lines for perfusion without screening for 100s clones); Cell retention devices, diverge energy from growth to qp; incorporate downstream

(Level 4 “impossible”: forecast market demands)

4. Which enabling technologies such as PAT, feedback control, data storage and treatment, do we need?

Equipment

- Raman
- Biocapacitance
- Integrated HPLC
- Integrated ViCell / Nova Flex

Uses

- Steady state control
- Bleed rate control

Challenges

- Non-standard equipment for single-use TFF integrated: shear effects in flow circuits; availability of options
- Use of PAT to monitor culture broth and define process duration (clone stability, CQAs)
- Monitoring using PAT for filter clogging with ATF and TFF: value black issues with ATF
- Combination of different signals (integrated HPLC, Raman, capacitance) for monitoring/control (titer, bleed rate)
- Excessive media volumes usage: not optimized
- Precision/accuracy of online measurements for feedback control
- Scalability of gravity settlers: mostly of only 100Ls scale (the commercial ones) which are very small and pose a scalability problem
- Feedback loops in Raman Spectroscopy
    - Monitoring and control of nutrient levels. Nowadays mostly used for glucose monitoring and control, but ideally should be applied for monitoring/control of other nutrients, amino acids, metabolites
    - Control CQA from online PAT through feedback control
- Clone stability given perfusion takes longer
- Modelling spectral data and generation of good models based on online signals for development of feed-back control loops
- PAT integration with small scale models / development of better Raman technology for scale-down models (data generation)
    - Currently available with the Ambr system, but limited number of measurements since there is one probe for 24 bioreactors
- Precision and accuracy of online measurements (pH offset for example)

Solutions- main problems to solve: Poor control / prediction as you switch to perfusion

Level 1: Universal N-1 implementation (issues w/ value and know-how); PAT and TMP measurements for filter fouling in long perfusion runs; modelling spectral data for accurate CQA estimation.

Level 2: Guidelines for integrating the large size with connectors to new process; Retrofit guidelines to perfusion process; relation between process parameters and CQA; automation capabilities to switch filters; PAT for OUR estimation that accounts for latency and filtering. PAT for definition of culture duration (use of PAT (based on Raman, capacitance, etc.) to monitor apoptosis markers. Development of models and digital twins for better control of the process to ensure CQA.

Level 3: Control models for closed loop control – understand the levers from proteins to cells; PAT for genetic / clone stability estimation.

5. Do we have suitable tools for scale-up and scale-down, and do they give us satisfying information for downstream integration considerations?

Challenges (N-stage focus)

- High density (+100 mvc/mL) scale-down models: fed-batch/ambr is very desirable, there is no whole understanding of scale-up or down
- Scale-down
    - Scale down of connection between cell retention device and bioreactor difficult to mimic
        - Different pumps and filters, different retention time, differences in shear stress (especially for scale-up)
    - Lack of option of tools integrated to the scale-down model (raman, cell retention device, etc)
    - Difficulties in maintaining flow-rates in scale-down perfusion models
    - Sparger options in scale-down models are limited
- Scale-Up
    - Cell retention device scale-up (cost, retention time, filtration effectiveness)
    - Media precipitation and media preparation
    - Sterile connectors for downstream processing are less available
    - Filter service area
- Cell separator (no sieving prediction) / filtration consistency
- Definition of the process
- Maintaining VCD steady state
- Residency time

Solutions

Level 1: algorithms for cell volume instead of cell density, back-up filters for fouling/automated filter replacement

Level 2: Better models for monitoring / prediction of fouling; lower shear stress with still a high kLa; new membranes; more size-based approaches, development of high-kla/low shear spargers for scale-down models, more options of probes and multi-usage probes

Level 3: Nonfilter based methods - instead of changing the filter, study how to regenerate (while still being chromatography loadable); create an antifoam free process

6. How can we apply continuous culture to non-glycoproteins/mAb modalities?

Challenges

- How to grow more cells / Increase VCD with other cell lines such as HEK293, suspension Vero cells etc
- Develop and assess cell retention devices and pumps for more shear-sensitive cell-lines (HEK293 for example)
- Development of perfusion media for other cell lines

- Stability of the viral stock (to meet CQAs)
- Quality of the product
    - AAV – empty/full capsids – does continuous processes help of not?
- Gene/cell therapies: allogeneic
- AAVs / Lentivirus: downstream bottleneck; COGs
- meat: pumping problem because product becomes solid
- What parts are likely to successfully operate continuously or not

Solutions

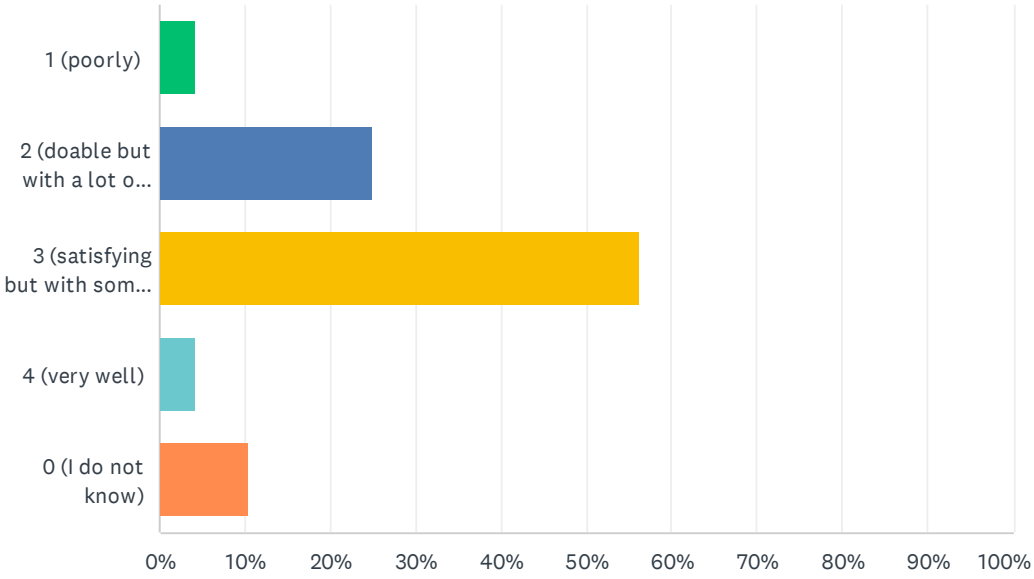
Level 1: Microcarriers / fixed bed – building the technology and make that available to other cells and viruses

Level 2: Media, retention devices that work with different cell types and that have low stress

Level 3: Develop pumps that work with cultured meat

Q51 How well existing technical solutions for perfusion processes, in terms of equipment and culture media / additives, etc., answer to your needs, on a scale 1 to 4 as

Answered: 48 Skipped: 281



ANSWER CHOICES	RESPONSES	
1 (poorly)	4.17%	2
2 (doable but with a lot of gaps, i.e. missing solutions)	25.00%	12
3 (satisfying but with some gaps, i.e. missing solutions)	56.25%	27
4 (very well)	4.17%	2
0 (I do not know)	10.42%	5
TOTAL		48

Q52 Which new technical solutions for your perfusion process do you think are most lacking today or needing improvements, in terms of equipment and culture media / additives, etc.? (List all in decreasing order of your priority.)

Answered: 28 Skipped: 301

#	RESPONSES	DATE
1	1 - robust cell retention device, avoiding/minimizing membrane fouling & maximizing culture length 2 - media depth & balance, to sustain very high VCDs with low CSPR	4/14/2023 3:14 PM
2	Perfusion filters with reduced fouling tendencies	4/14/2023 3:08 PM
3	Equipment is the area needs improvement. We need the ability to continue to increase perfusion mass output (e.g. productivity.) Increased cell mass in the bioreactor (mass transfer optimization?) and/or higher throughput, non-fouling cell separation technologies might be reasonable places to start.	4/14/2023 8:03 AM
4	* Robust cell retention for high VCD processes * Versatile perfusion media balancing growth and productivity at low VVD * Robust solution concepts enabling uptake by smaller players with less risk to reap the benefits * Connectivity between continuous USP and mostly still batch-wise DSP	4/14/2023 5:07 AM
5	Cell retention technologies Bioreactor/equipment design to support very high cell density cultures	4/13/2023 9:58 PM
6	Cell retention devices (filters that don't foul immediately> Upstream/Cell culture equipment for shear sensitive cells/product), perfusion rocking bags that are larger than 200L > automation for feeding > gas supply/demand ability for high density culture	4/13/2023 6:25 PM
7	More robust (lower failure rate) More commonality (instead of so many individual approaches) More scalability (including to smaller, simpler scale down model systems)	4/13/2023 5:27 PM
8	culture media exchange and large volume requirement	4/13/2023 4:25 PM
9	Improved media Robust pumps and filters Enhanced PAT tools to control and monitor process	4/13/2023 4:03 PM
10	Equipment - size of ATF limits reactor volume, even with TFF, reactor volume tends to be limited at 1000L scale. Equipment/facility - liquid management, this also applies to lack of concentrated media blend where we can do inline dilution to reduce hold volume. Equipment - frequent filter fouling	4/13/2023 3:59 PM
11	1) Scale-down models with PAT tools integrated 2) Scalability between high throughput systems versus large scale bioreactors 3) High-performing culture media fit for multiple processes and cell lines 4) Software allows the communication between hardware and automated control. 5) Autosampler 6) Online analyzer 7) Downstream column integration and continuous manufacturing	4/13/2023 3:53 PM
12	Filter fouling Media precipitation	4/13/2023 3:44 PM
13	Adequate scale down models	4/13/2023 4:11 AM
14	1. Lack of availability of perfusion specific media and feed 2. Pre sterilized perfusion units with all pore sizes at lab /commercial scale. 3. Leakage of perfusion filter 4. Sustainable solution for clarification 5. Integration of small /commercial scale bioreactor with perfusion controller.	4/13/2023 1:27 AM
15	1. Lack of availability of perfusion specific media/feed. 2. Pre sterilized perfusion units with all pore sizes at lab /commercial scale. 3. Leakage of perfusion filter 4. Sustainable solution for clarification 5. Integration of small /commercial scale bioreactor with perfusion controller.	4/13/2023 1:27 AM
16	EQUIPMENTS AND TECHNOLOGY	4/11/2023 2:40 AM

17	Perfusion filters, filter cleaning	4/10/2023 7:13 AM
18	DSD unit operations to efficiently purify higher quantities, such us AEX, CEX, virus filtration and UF/DF	4/6/2023 6:04 AM
19	Scalable cell retention devices that minimize product loss (enveloped virus) for HCD processes	4/4/2023 3:49 PM
20	Robustness of ATF filters	4/4/2023 11:59 AM
21	ATF modules for long term cultivations without product retention or fouling Culture media to support lower CSPR - cost reduction, smaller volumes to manipulate	4/3/2023 1:26 PM
22	ATF filter robustness. Number of cycles which can be performed without pressure increases and eventual clogging.	4/3/2023 11:33 AM
23	Media VCD control (bleed or other) Cell line (creation, selection (rank order during clone selection)	4/3/2023 10:34 AM
24	Variability of pH, viable cell density between In-line sensor and off-line measurement because of high cell density. Difference of sieving rate between lab-scale and pilot-scale.	4/2/2023 8:56 PM
25	cell retention	4/2/2023 11:18 AM
26	A focus on cell lines built for perfusion. The question's phrasing already suggests the lack.	4/1/2023 4:02 AM
27	equipment and data	4/1/2023 3:09 AM
28	Scalable filtration devices	3/31/2023 6:34 PM

Q53 Which hurdles do you encounter in terms of regulatory strategy, e.g. process characterization, tackling long duration of process, etc.? (List all in decreasing order of your priority.)

Answered: 24 Skipped: 305

#	RESPONSES	DATE
1	1 - scale down model that appropriately mimics all aspects including cell retention device performance 2 - batch definition, alignment with health authorities 3 - process characterization approach, especially at AMBR scale (due to lack of biocapacitance capability)	4/14/2023 3:14 PM
2	Process Characterization, Process Stability, Barch definition	4/14/2023 3:08 PM
3	Long process duration add complexity to the entire regulatory strategy. However, recovery (or not) from process upsets/deviations and defining batches are my largest concerns.	4/14/2023 8:03 AM
4	Strategy how to make use of shorter runs and resulting material should the process stall due to a technical issue -> what can still be used?	4/14/2023 5:07 AM
5	Guidance for process characterization > impurities level specifications > overall guidance is lacking	4/13/2023 6:25 PM
6	Process consistency, especially with regard to quality	4/13/2023 5:27 PM
7	Batch definition Compatability strategy Process characterization approaches	4/13/2023 4:03 PM
8	We are not there, yet.	4/13/2023 3:59 PM
9	1. Perfusion process development can be convoluted when too many scale-down models being used while there is a poor comparability between them. 2. The characterization process can be very long especially for steady state process. This can lead to a development time of more than a year. 3. We lack metabolic model for perfusion system. Most of the metabolic profiling are built around fed-batch.	4/13/2023 3:53 PM
10	Process characterization	4/13/2023 3:44 PM
11	NA	4/13/2023 1:27 AM
12	NA	4/13/2023 1:27 AM
13	PROCESS CHARACTERIZATION	4/11/2023 2:40 AM
14	Don't know	4/10/2023 7:13 AM
15	not for intensified fed batch processes	4/6/2023 6:04 AM
16	small scale models are lacking	4/4/2023 11:59 AM
17	Monitoring parameters - how much data is asked? Controlling the process for a long time - how oscillations in the control of parameters can impact on product quality	4/3/2023 1:26 PM
18	Going from traditional fed-batch to perfusion has led to increased protein quality. Less time in the tank before forward processing has shortened the change for degradation of the product to occur. How do we reconcile the improved product quality with legacy fed-batch processes whose products are already in the clinic.	4/3/2023 11:33 AM
19	Bio Comp (feb batch v. perfusion changing platform during clinical trials)	4/3/2023 10:34 AM
20	Process characterization and control strategy: what items are KPI, potentical CPP, and so on.	4/2/2023 8:56 PM
21	longer duration, capability building	4/2/2023 11:18 AM
22	I am not a perfusion practitioner, but I think the effects of long term culturing on product quality and cell genetic stability are issues to tackle broadly.	4/1/2023 4:02 AM

23	data	4/1/2023 3:09 AM
24	N/A	3/31/2023 6:34 PM

Q54 How do you decide the process for the production of your new products, in terms of fed-batch vs. perfusion, or hybrid solution, and which tools do you use to support this decision?

Answered: 27 Skipped: 302

#	RESPONSES	DATE
1	1 - commercial volume & uncertainty 2 - cost of goods	4/14/2023 3:14 PM
2	Volumetric productivity and protein stability	4/14/2023 3:08 PM
3	I am not sure. I am looking for guidance on how to best make this decision.	4/14/2023 8:03 AM
4	* Product requirements (stability, glycoform complexity * Cell line (growth sustainable enough to warrant perfusion?, byproduct profiles) * Simulate expected performance in perfusion to support decision-making (software only or using perfusion mimic)	4/14/2023 5:07 AM
5	Efficiency and Productivity were the main drivers, POC experiments were performed at bench scale (3L bioreactors) comparing the three modes of operations. COGs analysis was performed and the one with the highest efficiency/productivity - which is perfusion, was then chosen	4/13/2023 6:25 PM
6	Fed-batch unless it cannot be made to work in reasonable time frame (6 months - 1 year) in terms of product quality or ability to meet demand. Demand requirements are challenging for cultured meat. Might need to intensify process versus fed-batch. Would intensify only as much as needed for any case. Would hesitate to go to truly continuous production.	4/13/2023 5:27 PM
7	titer	4/13/2023 4:25 PM
8	Product quality Material demand Life-cycle management and patent life	4/13/2023 4:03 PM
9	We are still in the development phase of the perfusion process. However, we have encountered a few cases where the projected commercial demands significantly exceeds the current manufacturing capacity. This is where perfusion can be helpful. I can envision that whether a perfusion process makes sense would be dependent on the demands. Please note that meeting demands can be done via both manufacturing (e.g., scale-up, scale-out) and/or process (improving titer) strategies.	4/13/2023 3:59 PM
10	1. Type of molecules: perfusion for labile molecules 2. Perfusion clones: perfusion instead of fed-batch There is no tools to help us deciding this. Mostly based on the stability data and data from cell line engineering supporting that cells are engineered to work better in perfusion.	4/13/2023 3:53 PM
11	COGs Product stability/fit to production process	4/13/2023 3:44 PM
12	1) Meeting target COAs 2) Plant economics - CoGs	4/13/2023 1:27 AM
13	1. Desired CQAs 2. Plant economics	4/13/2023 1:27 AM
14	PRODUCTIVITY PER BATCH, and COGs, and timeline	4/11/2023 2:40 AM
15	Amount needed to be produced per year	4/10/2023 7:13 AM
16	molecule format, indication and expected market demands try to follow a pipeline approach, benefit from existing experience and infrastructure tool: plant modeling	4/6/2023 6:04 AM
17	Based on product stability, availability of high performance concentrated feeds for the cell line. Scale-down models, DOE	4/4/2023 3:49 PM
18	Strategic, financials, and technical	4/4/2023 11:59 AM
19	Product stability and toxicicity to the producer cells.	4/3/2023 1:26 PM
20	Productivity, complexity (of the process) and product quality.	4/3/2023 11:33 AM

21	We have a single platform.	4/3/2023 10:34 AM
22	This is a hot topic in my company. Molecular assessment (e.g. instability molecule) would be performed, but it is not decided.	4/2/2023 8:56 PM
23	selection guidance based on several criteria (forecasted demand, plant capacity, product stability)	4/2/2023 11:18 AM
24	Cdmo, receives from clients	4/2/2023 2:19 AM
25	N/A	4/1/2023 4:02 AM
26	??	4/1/2023 3:09 AM
27	Business decision	3/31/2023 6:34 PM

Q55 Which enabling technologies such as PAT, feed-back control, data storage / treatment, real time release, etc., do you use?

Answered: 25 Skipped: 304

#	RESPONSES	DATE
1	Biocapacitance - cell density, and bleed feedback control Raman glucose feedback control	4/14/2023 3:14 PM
2	PAT, feedback control, data storage/treatmet	4/14/2023 8:03 AM
3	* feedback control loops for bleeding * comprehensive data capture and analysis	4/14/2023 5:07 AM
4	Feed-back control	4/13/2023 6:25 PM
5	PAT, feed-back control, data historian with convenient search, sorting, and statistical analysis capability	4/13/2023 5:27 PM
6	PAT	4/13/2023 4:25 PM
7	In development	4/13/2023 4:03 PM
8	We are exploring the use of capacitance probe to control some of the processes.	4/13/2023 3:59 PM
9	- Capacitance, DO, pH, temperature - No feedback control - Lucullus for data repository - Mobius(R) Breez microbioreactors for highthroughput testing	4/13/2023 3:53 PM
10	PAT Feed-back control	4/13/2023 3:44 PM
11	NA	4/13/2023 1:27 AM
12	NA	4/13/2023 1:27 AM
13	feed back contraol	4/11/2023 2:40 AM
14	The normal on-line pH, oxygen etc. Looking into more on-line measurements like RAMAN	4/10/2023 7:13 AM
15	rather in development; online viable cell count measurement for monitoring and control	4/6/2023 6:04 AM
16	Not using PAT at this time	4/4/2023 11:59 AM
17	Data storage/treatment	4/3/2023 1:26 PM
18	None currently	4/3/2023 11:33 AM
19	all of the above	4/3/2023 10:34 AM
20	We are investigating PAT technology like Raman. To apply for GMP manufacturing, we are discussing the necessity.	4/2/2023 8:56 PM
21	Capacitance, Raman, UV	4/2/2023 11:18 AM
22	Aber biocap, raman	4/2/2023 2:19 AM
23	N/A	4/1/2023 4:02 AM
24	none	4/1/2023 3:09 AM
25	PAT	3/31/2023 6:34 PM

Q56 Which enabling technologies such as PAT, feed-back control, data storage / treatment, real time release, etc., are most urgently needed? (List all in decreasing order of your priority.)

Answered: 20 Skipped: 309

#	RESPONSES	DATE
1	PAT	4/14/2023 3:08 PM
2	Real time release could probably represent the largest economic benefit (if done without a need of offline testing) which could enable investment in PAT and feedback control to further improve processes.	4/14/2023 8:03 AM
3	* Data collection/mathematical models enabling prediction of imminent process failure to stop on time	4/14/2023 5:07 AM
4	PAT> feed-back control > real time release > data storage/treatment	4/13/2023 6:25 PM
5	Beyond ones listed to answer 5 --- hard to say. Probably not real time release.	4/13/2023 5:27 PM
6	real time CQA - HPLC - autosampling and data integration	4/13/2023 4:37 PM
7	PAT Modeling and release	4/13/2023 4:03 PM
8	- Raman spectroscopy - Glucose control - Amino acid control - Cloud-based data storage and online analysis/visualization tool - Prediction modeling	4/13/2023 3:53 PM
9	Real time release PAT Feed-back control	4/13/2023 3:44 PM
10	PAT, feed-back control	4/13/2023 1:27 AM
11	PAT Feed-back control	4/13/2023 1:27 AM
12	PAT	4/11/2023 2:40 AM
13	On-line measurement of biomass (volume), glucose, product titer, product quality	4/10/2023 7:13 AM
14	Which ones are really needed and have a clear business advantage? Except for project specific challenges	4/6/2023 6:04 AM
15	There is a need to monitore more parameters online and to store data properly	4/3/2023 1:26 PM
16	Continuous processing and capabilities of the downstream process to keep up with perfusion processes.	4/3/2023 11:33 AM
17	PAT: Glucose control, Process monitroing Data storage / Treatment	4/2/2023 8:56 PM
18	N/A	4/1/2023 4:02 AM
19	??	4/1/2023 3:09 AM
20	Protein conc Media Components Cell densities and viabilities	3/31/2023 6:34 PM

Q57 Which equipment’s/tools do you use for scale-down of perfusion USP, and for scale-down of integrated continuous USP-DSP?

Answered: 21 Skipped: 308

#	RESPONSES	DATE
1	2-L bioreactors and AMBR-perfusion	4/14/2023 3:14 PM
2	Ambr250 HT Perfusion System	4/14/2023 3:08 PM
3	As an upstream person, we use bench top bioreactors. I personally have not participated in USP-DSP scale downs yet.	4/14/2023 8:03 AM
4	* Ambr 15 perfusion mimic * Spin Tubes * Cell Insights simulation of fed batch and perfusion data of clones	4/14/2023 5:07 AM
5	Repligen TFDF - The best out there when compared to what's on the market including ATF -- for viral vectors perfusion production	4/13/2023 6:25 PM
6	Scale -down ---- cross flow filtration, ATF at smallest available scale, off-line countertop or floor model centrifugation (once per day or so) with return of resuspended cells.	4/13/2023 5:27 PM
7	Ambr250, PAK, 3L bioreactor	4/13/2023 4:03 PM
8	3L, 50L, and 200L scales with ATF. ambr250 perfusion system. We don't do continuous USP-DSP at this point.	4/13/2023 3:59 PM
9	- Mobius Breez - TPP spintube - 3L Applikon bioreactors	4/13/2023 3:53 PM
10	Lab-scale bioreactors	4/13/2023 1:27 AM
11	Benchtop bioreactors	4/13/2023 1:27 AM
12	TFDF	4/11/2023 2:40 AM
13	Scale-down of perfusion USP: ambr-15 mL in chemostat mode: Exponentially increasing In-flow and Discontinuous Out-flow (EIDO) - I present a poster on it at the conference. Scale-down of integrated continuous USP-DSP: Not very low scale, a real perfusion scaled bioreactor connected to a programmed down-stream system	4/10/2023 7:13 AM
14	ambr250 and small scale glass bioreactors with focus on N-1 perfusion	4/6/2023 6:04 AM
15	scale-down USP: shake-tubes, Ambr	4/4/2023 3:49 PM
16	Decrease bioreactor size or perform intermittent perfusion in shaken tubes.	4/3/2023 1:26 PM
17	5L bench-top STR with Repligen ATF, Sartorius Ambr250 HTwith perfusion modules, Ambr15 with vessel centrifugation	4/3/2023 11:33 AM
18	1L Bioreactor expected a scale down model for perfusion.If possible, we would like to use ambr 250.	4/2/2023 8:56 PM
19	HT lab scale systems	4/2/2023 11:18 AM
20	I'm very interested in this question.	4/1/2023 4:02 AM
21	home made	4/1/2023 3:09 AM

Q58 Which approaches do you use for scale-up of perfusion USP, and for scale-up of integrated continuous USP-DSP?

Answered: 18 Skipped: 311

#	RESPONSES	DATE
1	Constant Flux	4/14/2023 3:08 PM
2	Traditional bioreactor scaling approaches are used to ensure proper mass transfer and performance of the culture. For the cell separator, flux, crossflow and external residence time are the primary concerns.	4/14/2023 8:03 AM
3	* Ambr 15 perfusion mimic * Cell Insights simulation of fed batch and perfusion data of clones * MVDA-type analyses and derived prediction models	4/14/2023 5:07 AM
4	still deciding	4/13/2023 6:25 PM
5	Scale up --- ATF, cross-flow filtration, Centritech, gravity sedimentation, spin filters No continuous USP-DSP	4/13/2023 5:27 PM
6	Subs, PAKs	4/13/2023 4:03 PM
7	We haven't scale our perfusion up to MFG scale. However, we are using flux to assess scalability.	4/13/2023 3:59 PM
8	Provide data and knowledge-sharing for the scale-up application team to run their pilot scale bioreactors (50L - 200 l). Analyze the data to take action on what next to be done.	4/13/2023 3:53 PM
9	Constant P/V for bioreactor and constant flow per fiber for perfusion	4/13/2023 1:27 AM
10	constant p/v and flow per fiber for USP	4/13/2023 1:27 AM
11	?	4/11/2023 2:40 AM
12	USP: Flows (of course) of harvest and bleed, perfusion filter area USP-DSP: Flows and column sizes	4/10/2023 7:13 AM
13	current focus rather on N-1 perfusion	4/6/2023 6:04 AM
14	We rely on the CDMO to do an initial tech transfer to their bench scale (2-10L) and trust that they know their equipment enough and have the experience to scale-up the process in their plant.	4/3/2023 11:33 AM
15	At the moment, pilot scale 50L is used.	4/2/2023 8:56 PM
16	same as fedbatch	4/2/2023 11:18 AM
17	N/A	4/1/2023 4:02 AM
18	model building	4/1/2023 3:09 AM

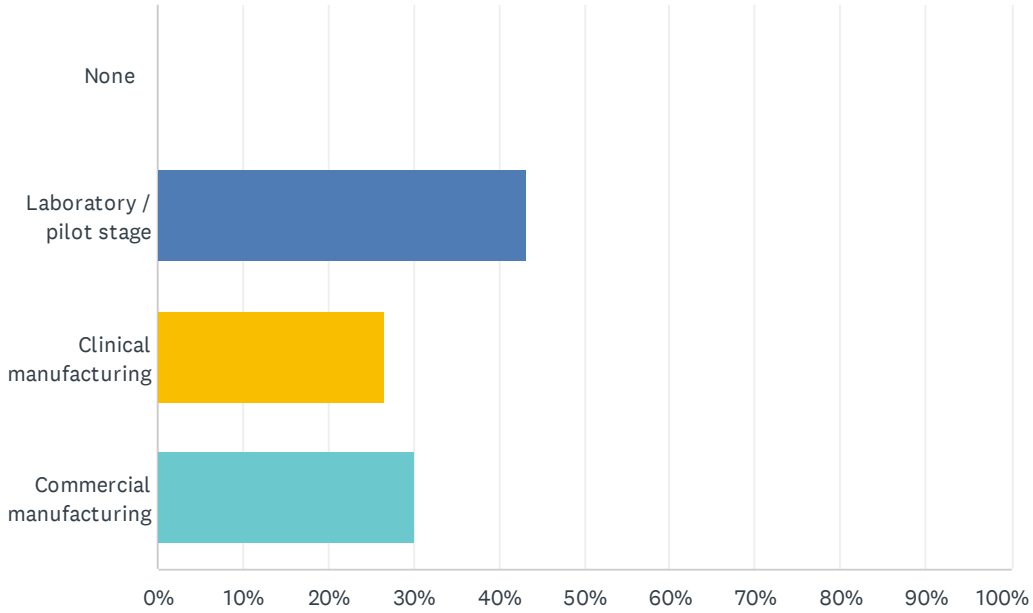
Q59 Do you apply continuous culture to non-glycoprotein/mAb modalities, and for which ones (if you can disclose)?

Answered: 20 Skipped: 309

#	RESPONSES	DATE
1	Almost all programs in pipeline are mAb or mAb-related products (multi-specifics, ADCs, fusion proteins etc). There are some products which may be sensitive to residence time in bioreactor which may benefit from perfusion	4/14/2023 3:14 PM
2	No comment.	4/14/2023 8:03 AM
3	N/A	4/14/2023 5:07 AM
4	We are a gene therapy company, viral vector	4/13/2023 6:25 PM
5	Not yet. Maybe cultured meat.	4/13/2023 5:27 PM
6	Yes	4/13/2023 4:03 PM
7	NA	4/13/2023 3:59 PM
8	We do mAb-producing CHO cell line using perfusion.	4/13/2023 3:53 PM
9	NA	4/13/2023 1:27 AM
10	NA	4/13/2023 1:27 AM
11	CGT- NOT THERE YET	4/11/2023 2:40 AM
12	mAb	4/10/2023 7:13 AM
13	no	4/6/2023 6:04 AM
14	viral vaccine, viral vector	4/4/2023 3:49 PM
15	Virus like particles - zika and yellow fever Spike protein (SARS-CoV-2)	4/3/2023 1:26 PM
16	No, only mAb processes at the moment	4/3/2023 11:33 AM
17	There are no plan.	4/2/2023 8:56 PM
18	N/A	4/1/2023 4:02 AM
19	yes	4/1/2023 3:09 AM
20	No.	3/31/2023 5:45 PM

Q60 For industrial participants, what stage is your company in adopting continuous concepts?

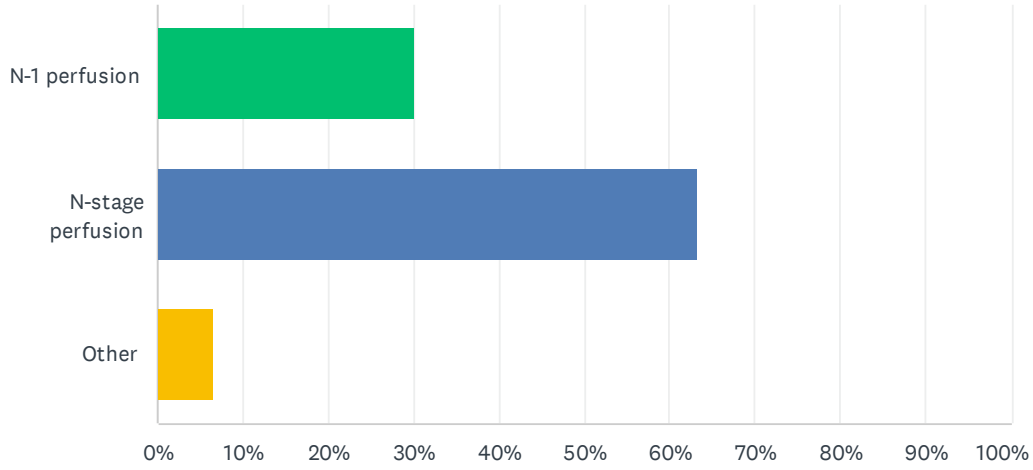
Answered: 30 Skipped: 299



ANSWER CHOICES	RESPONSES	
None	0.00%	0
Laboratory / pilot stage	43.33%	13
Clinical manufacturing	26.67%	8
Commercial manufacturing	30.00%	9
TOTAL		30

Q61 or industrial participants, what forms of continuous has your company adopted?

Answered: 30 Skipped: 299



ANSWER CHOICES	RESPONSES	
N-1 perfusion	30.00%	9
N-stage perfusion	63.33%	19
Other	6.67%	2
TOTAL		30

Q62 If your company has adopted continuous, what were top three reasons that convinced management? What were the top three challenges?

Answered: 17 Skipped: 312

#	RESPONSES	DATE
1	Top 3 reasons: commercial volume projections, COGs, manufacturing footprint Top 3 challenges: infrastructure build, technical effort to convert fed-batch to perfusion during late development, regulatory acceptance across multiple countries	4/14/2023 3:14 PM
2	No comment/I am not sure. Continuous was established prior to my arrival.	4/14/2023 8:03 AM
3	Reaons: 1) Customer demand 2) Increased profitability 3) Improved flexibility Challenges: 1) Check suitability of process first: is it the right process choice? 2) Robust processes at high cell numbers (yields & PQA) 3) Efficient integration of USP and DSP	4/14/2023 5:07 AM
4	Efficiency, Productivity and Quick timeline need for clinical needs	4/13/2023 6:25 PM
5	labile products (classic)	4/13/2023 5:27 PM
6	Pro: Small footprint. Flexible batch size. Reduction of COGs. Con: Complexity of process that needs significant initial investment. Regulatory challenges.	4/13/2023 4:58 PM
7	Titer improvement - a single most important factor, from process perspective, to reduce COG/g. Also to meet demands.	4/13/2023 3:59 PM
8	Reasons: 1. Stable product quality 2. Continuous process 3. High volumetric productivity thus lowering cost as a whole compared to fed-batch Challenges: 1. Technical expertise 2. Lacking available PAT tools for control 3. High media consumption	4/13/2023 3:53 PM
9	COGs, automation / process robustness, & flexibility Automation, connectivity, & real time release	4/13/2023 3:44 PM
10	NA	4/13/2023 1:27 AM
11	NA	4/13/2023 1:27 AM
12	PRODUCTIVITY, COGS (SHORTER TIME, WITH SAME PRODUCTIVITY)	4/11/2023 2:40 AM
13	Quality and space-time-yield (g/L bioreactor/day)	4/10/2023 7:13 AM
14	Better COGs and small facility footprint	4/4/2023 11:59 AM
15	1. Overall product yield 2. Product quality 3. Reduced time in plant / unit product	4/3/2023 11:33 AM
16	Reason: Developability improvemnt for instability molecules Challenge: Risk reduction for application because batch process is already fixed.	4/2/2023 8:56 PM
17	flexibility	4/2/2023 11:18 AM

Q63 If your company has evaluated perfusion, but never adopted, what were the top three reasons?

Answered: 14 Skipped: 315

#	RESPONSES	DATE
1	No comment/I am not sure. Continuous was established prior to my arrival.	4/14/2023 8:03 AM
2	N/A	4/14/2023 5:07 AM
3	We've evaluated and we are adopting perfusion.	4/13/2023 6:25 PM
4	Got batch and/or fed-batch to work	4/13/2023 5:27 PM
5	NA	4/13/2023 3:59 PM
6	n/a	4/13/2023 3:53 PM
7	NA	4/13/2023 1:27 AM
8	NA	4/13/2023 1:27 AM
9	LACK IN EQUIPMENT AVAILABILITY AND RESOURCES ASSISTANCE FROM INDUSTRY/VENDORS/MFG	4/11/2023 2:40 AM
10	N/A	4/10/2023 7:13 AM
11	This technology requires adjustment of existing facilities (if not newly built) which is major change in optimized FB settings	4/6/2023 6:04 AM
12	We have adopted perfusion at the laboratory scale, but have yet to transfer the process. Our top concerns are: 1. Expertise and experience of a CDMO to successfully transfer and run the process at manufacturing 2. Overall increase in complexity and modes of failure over tried and true FB. 3. Increased cost both in development at bench scale and COG at the manufacturing scale	4/3/2023 11:33 AM
13	Perceived cost benefits Speed of build (modular, small, disposable)	4/3/2023 10:34 AM
14	There are no instability molecules at the moment.	4/2/2023 8:56 PM