Level 8 Cell Culture Processing (BIO08045)

Lecture 6 – "Bioreactors – Stainless Steel & Single Use Bioreactors"

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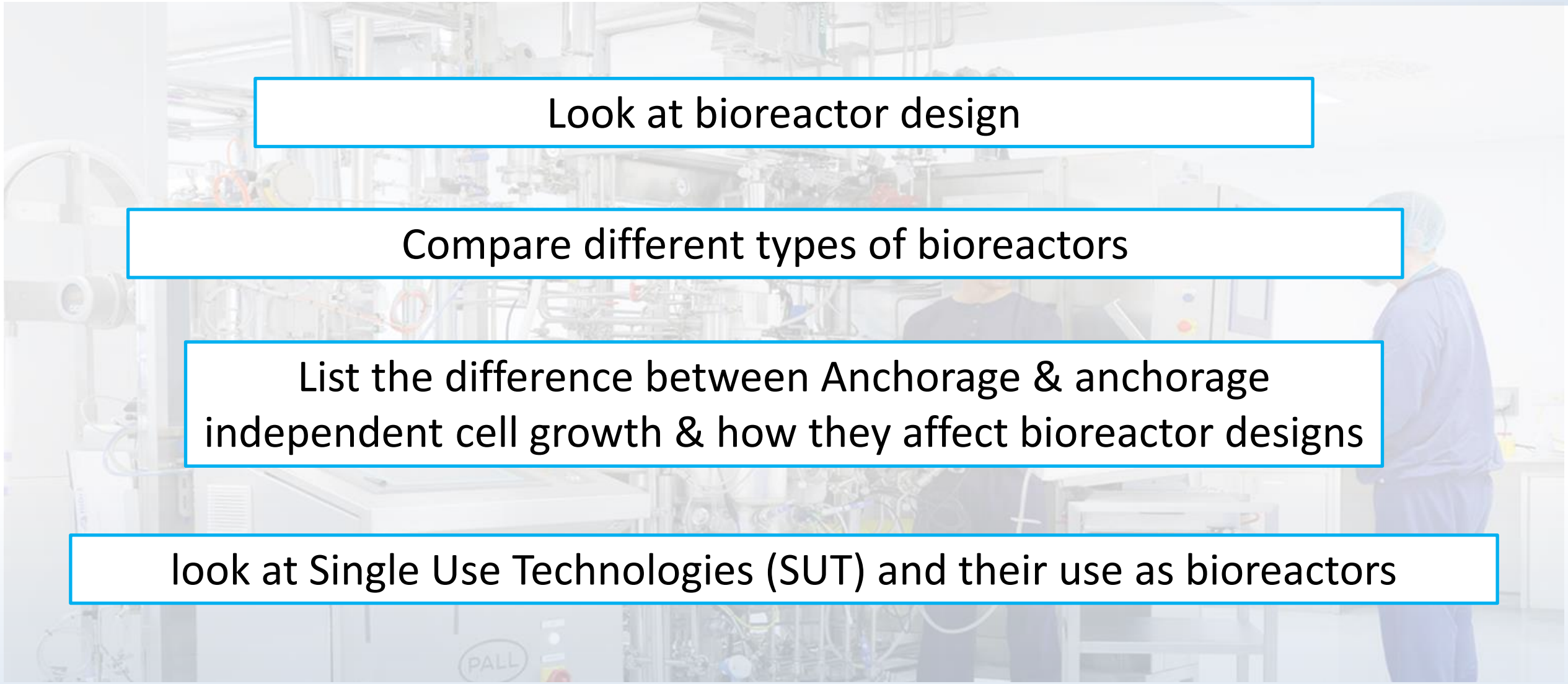
Learning Objectives



Look at bioreactor design

Compare different types of bioreactors

List the difference between Anchorage & anchorage independent cell growth & how they affect bioreactor designs

look at Single Use Technologies (SUT) and their use as bioreactors

Reading Material

- Links available in Moodle

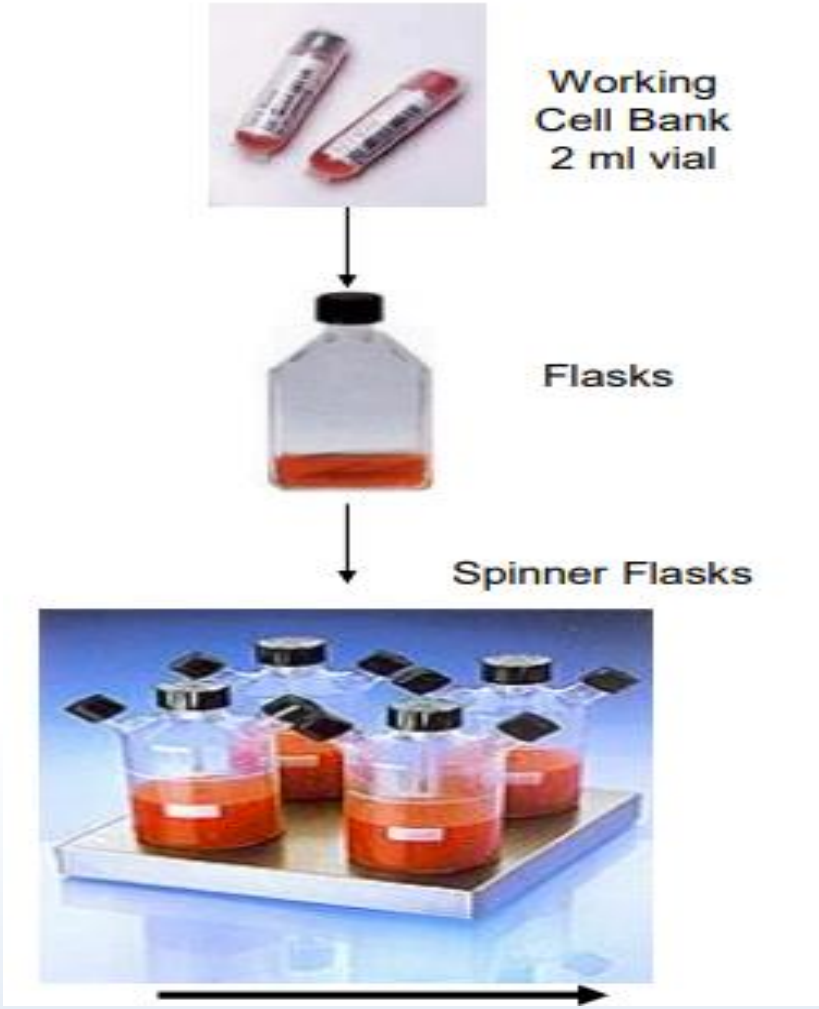
1. Corning® Roller Bottles Selection and Use Guide
2. Bridging the Gap from Reusable to Single-Use Manufacturing with Stirred, Single-Use Bioreactors: A Development Approach Based on the Gold Standard Davy De Wilde, Ute Noack, Wolfgang Kahlert, Magali Barbaroux, and Gerhard Greller BioProcess International April 2009 Supplement p36-41
3. Single-Use Bioreactors for the Rapid Production of Preclinical and Clinical Biopharmaceuticals Heidemann, R. et al. BioPharm International October 2014 p. 22 – 34 www.biopharminternational.com
4. Single-use in the biopharmaceutical industry: A review of current technology impact, challenges and limitations Adriana G. Lopes 2015 Food and Bioproducts Processing Vol.9(3): p. 98 – 114

Lecture Topics

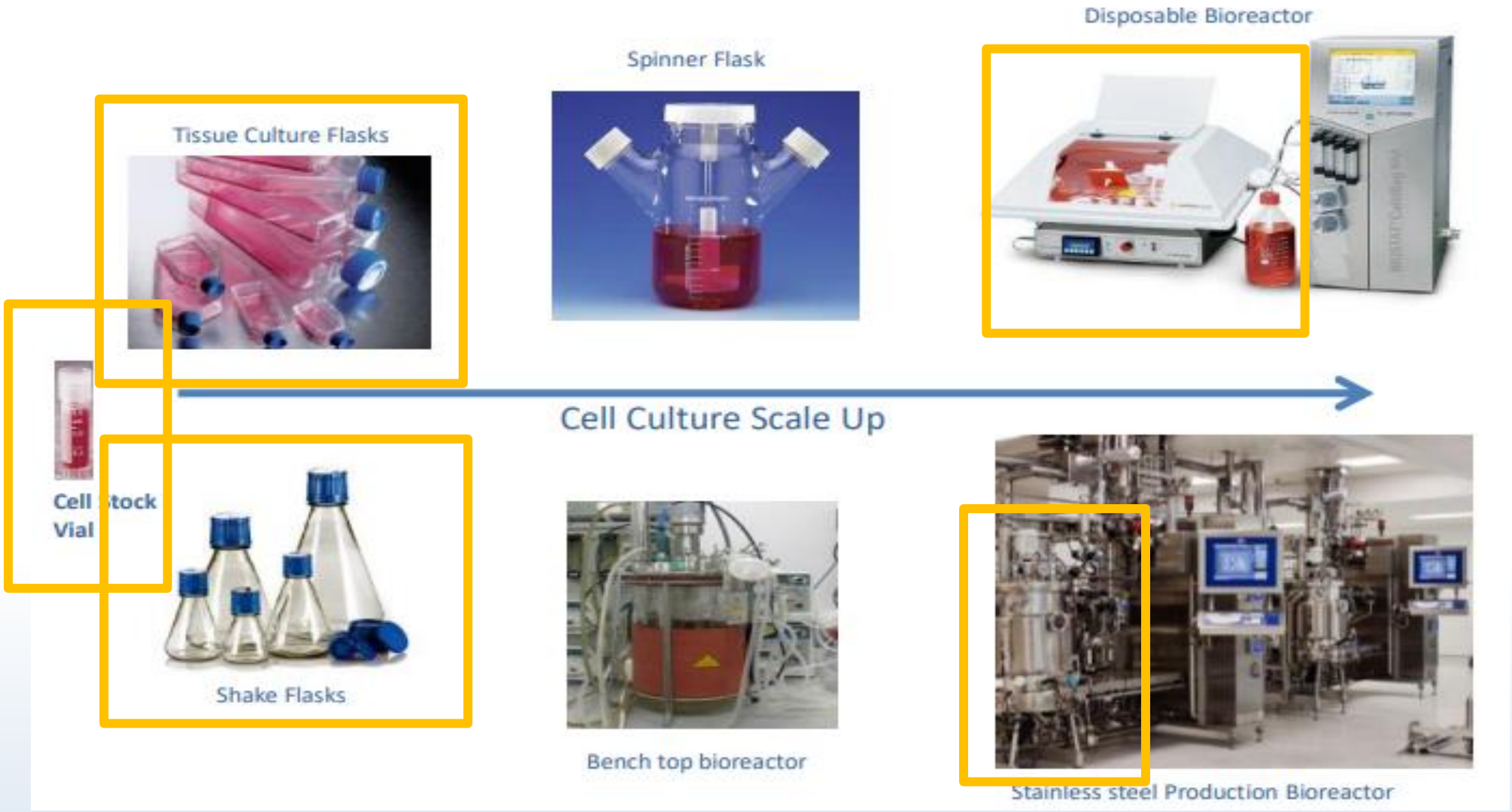
- Challenges of Large-Scale Cell Culture
- Anchorage Dependent Cell Culture Systems
- Anchorage Independent Cell Culture Systems
- Disposable Technologies
- PAT Solutions in the Biopharmaceutical Industry

Typical scheme for mammalian process

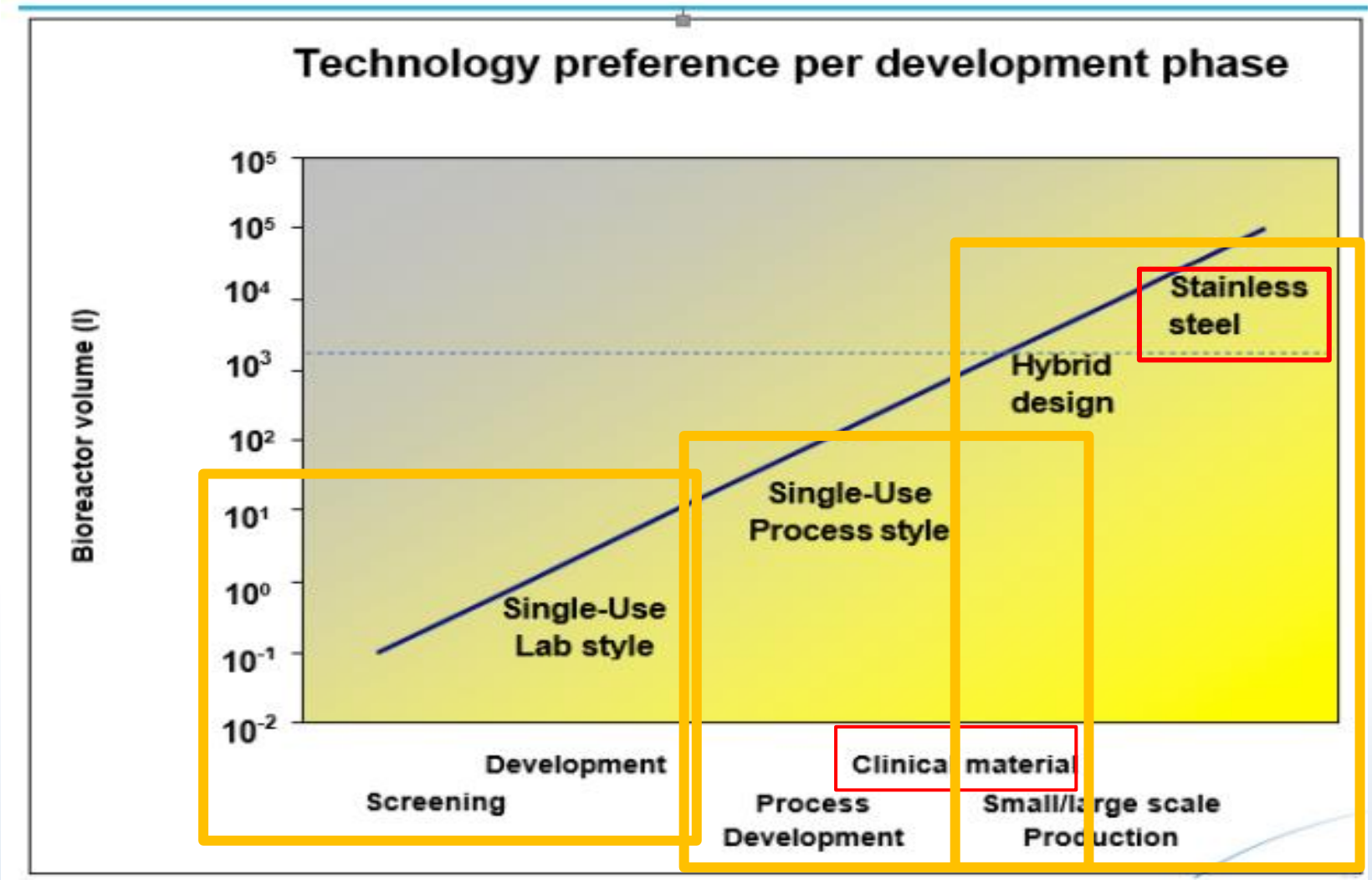
- Thaw vial into flask
- Once the flask is confluent, you have several options
  - Shake flasks (200mL – 2L)
  - Spinner flasks (200mL –1L)
  - Wave Bioreactors
  - Bench scale bioreactors (1 -5L)
- Inoculate cell expansion train



Cell Culture Processing



“Disposables”- Application Options



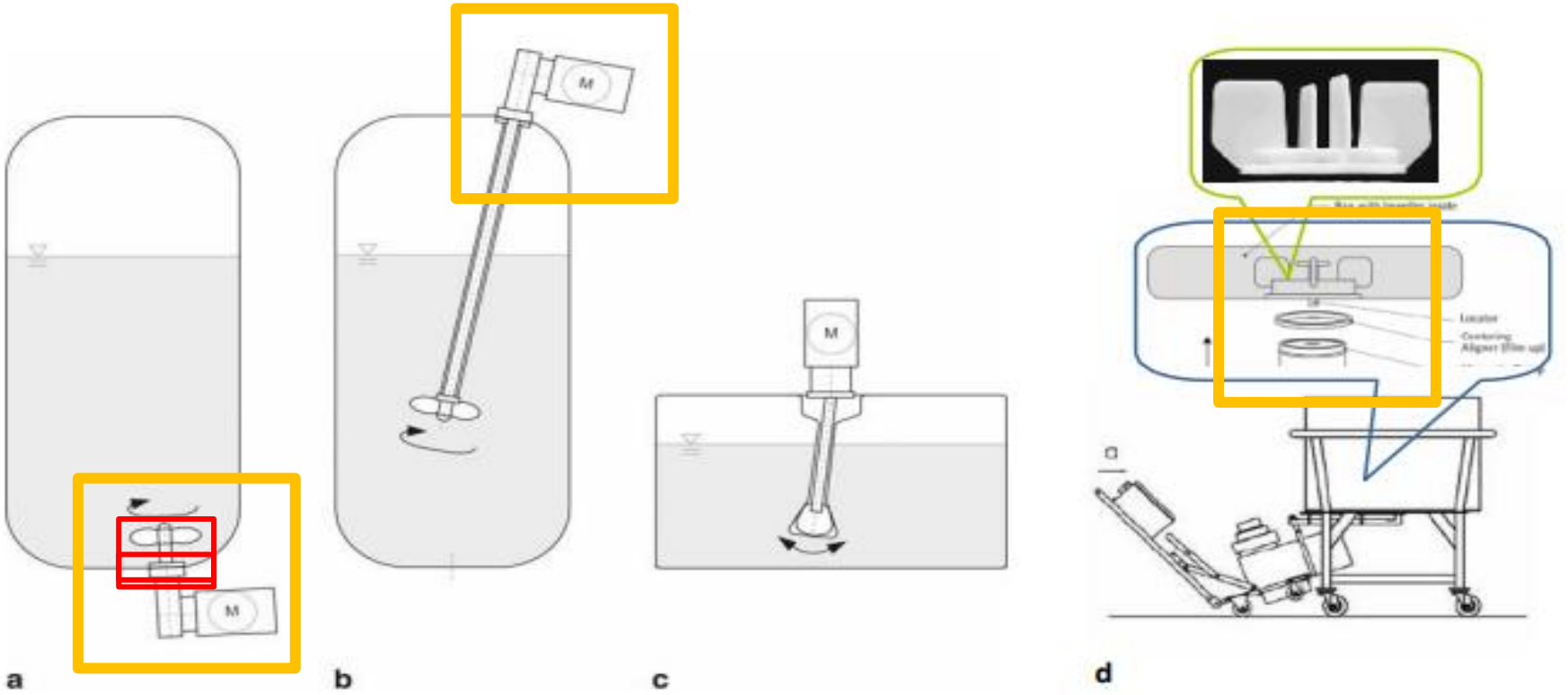
Bioreactors

- Bioreactor – a controlled environment for the culture of pure cell cultures. **Key issue – aseptic control.**
  - At small scale (shake flasks) cells are sub-cultured frequently and the culture is controlled by cell kinetics.
  - At large scale, more complex control is required to ensure optimum cell growth and productivity at large volumes in a contamination free environment.
- **Preventing contamination** – rigorous application of aseptic technique.
  - At large scale, use stainless steel bioreactors or single use bioreactors where any connections / additions to be made must be contamination free.

Limitations of Cell Culture Scale Up

1. **Supply of nutrients** - larger volumes require more media for longer growth periods and the feed strategy must be optimised.
2. **Oxygen** - at lab scale, the initial oxygen concentration is sufficient. In large scale bioreactors, O_2 must be delivered to the culture and aeration becomes a critical parameter.
3. **Mixing of cells in suspension:**
   - Shake flasks rotated on an incubator.
   - Bioreactors require agitation and aeration. However, mammalian cells have no cell wall and can be damaged due to shear forces of agitation which limits agitation speeds.
4. **pH and temperature control** are more complex in bioreactors.

Examples for mixing/agitating systems



a. XDR™-Disposable Stirred Tank Bioreactor, e.g. XCELLEREX

b. S.U.B. Single-Use Bioreactor, e.g. Hyclone-ThermoScientific

c. ’Pad-Drive ™ Disposable bioreactor, e.g. Artelis-ATMI Life-Sciences

d. LevTech-System, floating agitator and superconductor drive, Stedim-Sartorius

Mixing and Agitation

Mixing characteristics – comparisons of technologies - limitations

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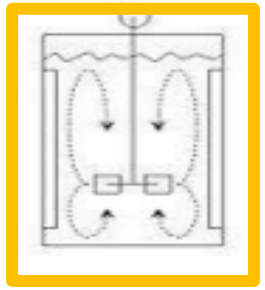
Turn-over times: min/hr

Hose diameter/pump rate



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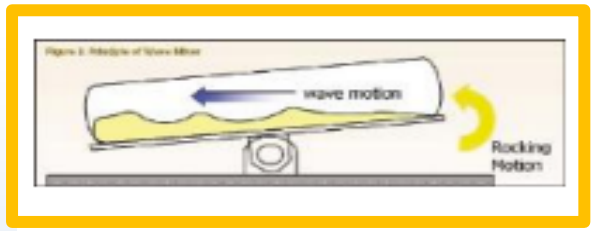
Turn-over times: ≈ min(s)

Agitator design & speed / pump rate



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Turn-over times: < min.

Tilt angle / Mechanical design

Notes: Mixing characteristics could be acceptable; but mass transfer requires superior performance

Choice of System Can Depend on Cell Type

1. **Anchorage dependent cells** require a surface to attach to so the focus is on **increasing surface area to volume ratio** e.g.
   - Cell factories.
   - Roller bottles.
   - Hollow fibres.
2. **Anchorage independent cells grow in suspension** – here the focus is on increased volume e.g.
   - Stirred tank bioreactor (STB).
   - Airlift bioreactor.
   - Wave bioreactor : disposable.
3. **Hybrid systems**: elements of attached and suspension e.g. Microcarrier bead systems.

Lecture Topics

- Challenges of Large-Scale Cell Culture
- Anchorage Dependent Cell Culture Systems
- Anchorage Independent Cell Culture Systems
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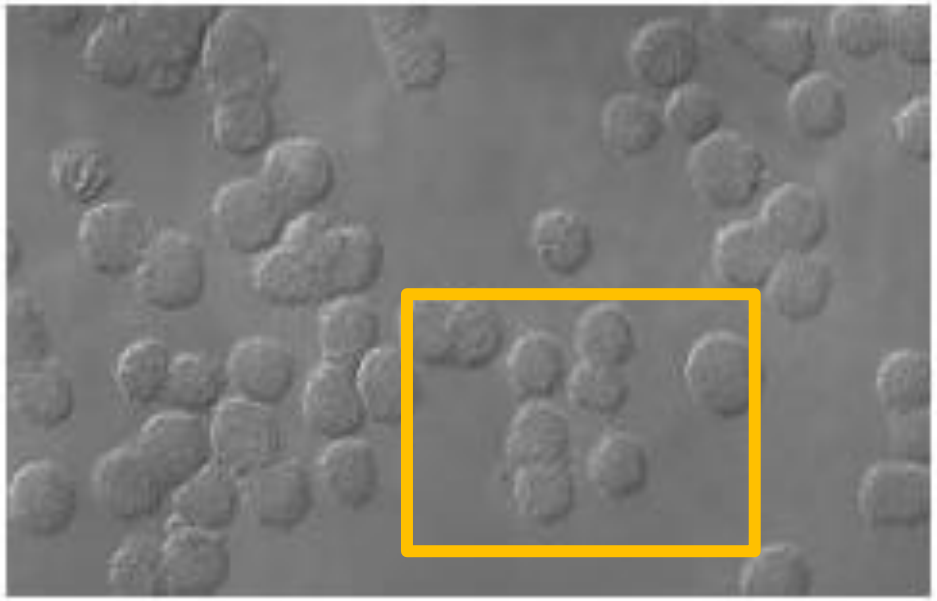
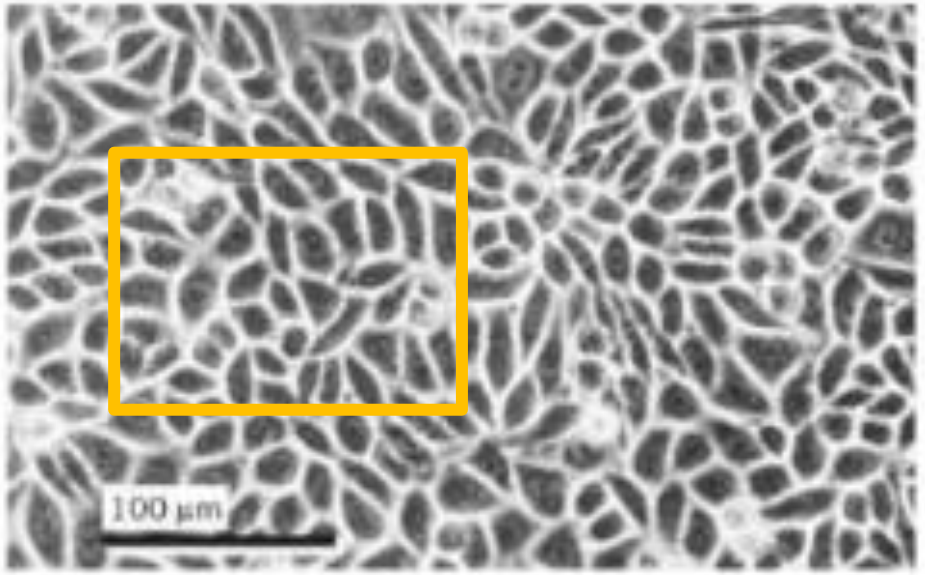
- PAT Solutions in the Biopharmaceutical Industry

Morphology



Anchorage independent growth
NSO cells



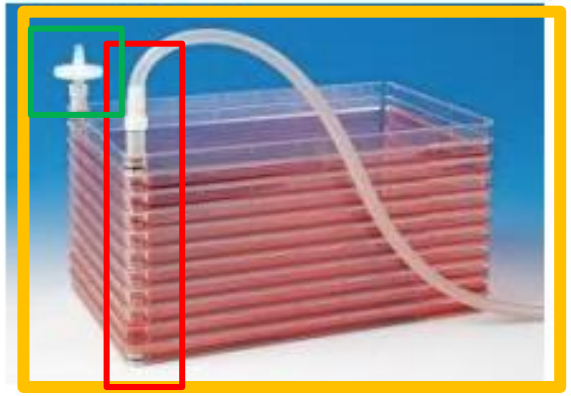
Anchorage dependent growth
BHK cells

Cell Growth Profile

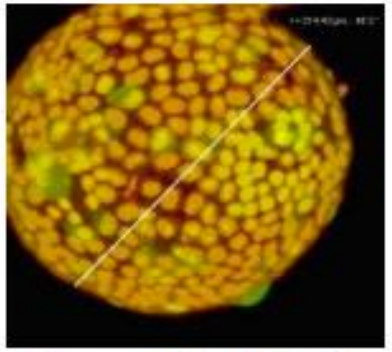
- Adherent cells require a surface to attach to so the focus is on increasing the surface area to volume ratio.

Tissue culture flasks

Cell factories

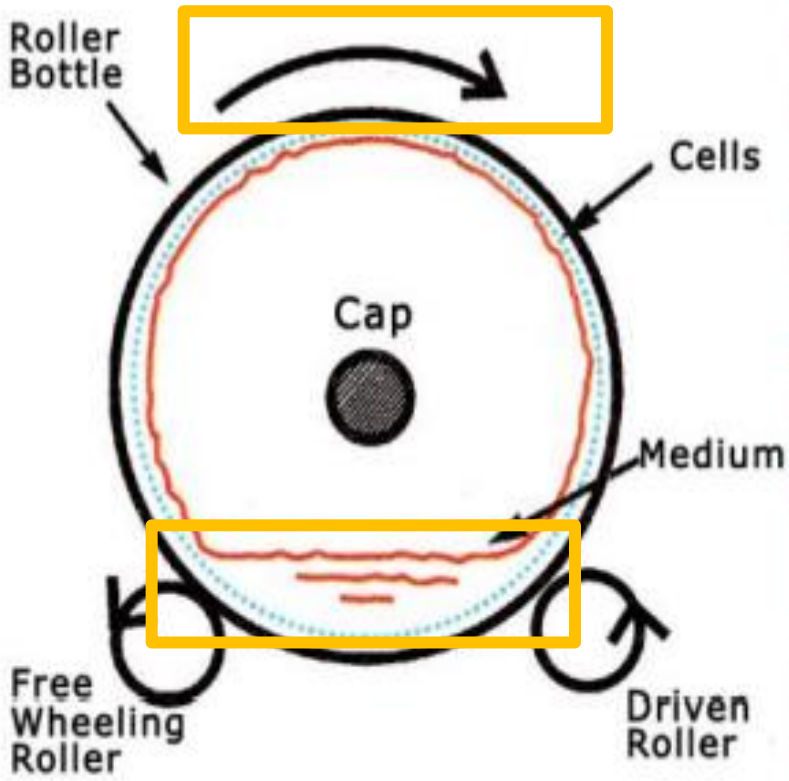


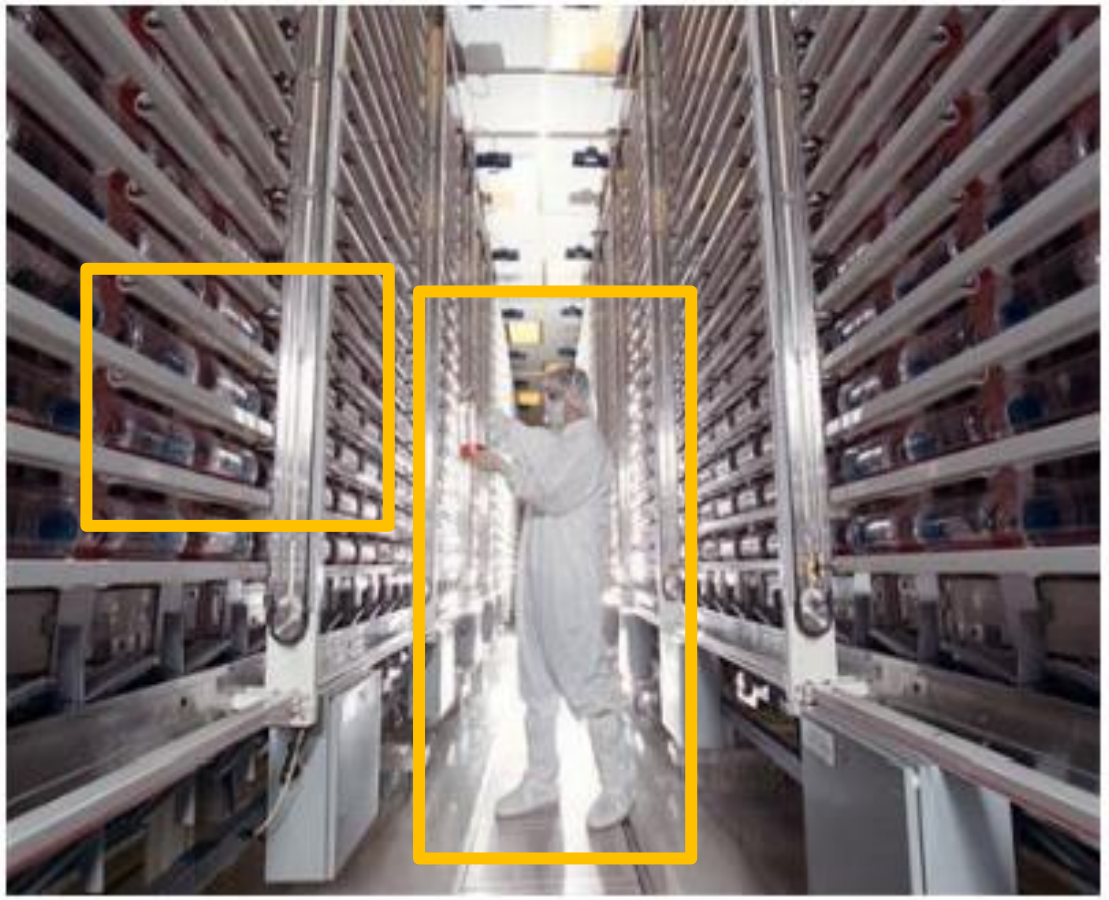
Roller bottles



Microcarrier beads

Roller Bottles





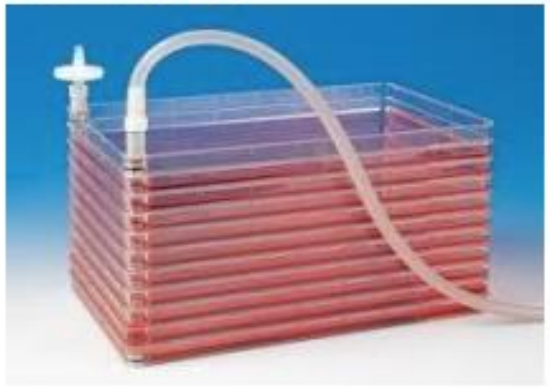
Industry scale manufacturing using roller bottles

Cell Growth Profile

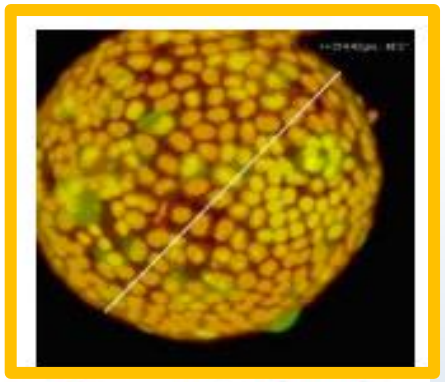
- Adherent cells require a surface to attach to so the focus is on increasing the surface area to volume ratio.

Tissue culture flasks

Cell factories



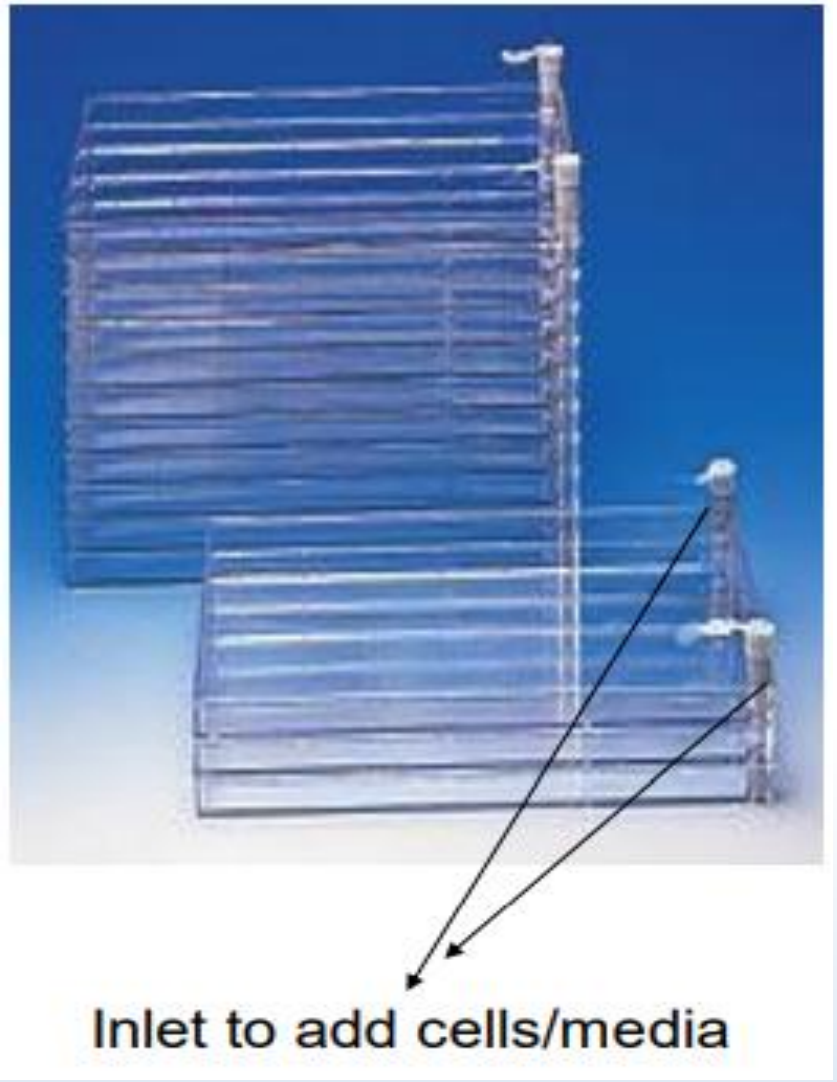
Roller bottles



Microcarrier beads

Cell factories for adherent cells

- **Cell factories:** simplest system for scale-up of adherent cells: **used in production of vaccines and interferon.**
- Series of rectangular dish like units **with surface area of 632cm² (vol 200mL).**
- These can be bought in stacks of 1, 2, 10 and 40: **Max surface area of 25,280cm² (max volume of 8 litre).**
    - Connected at corners by vertical tubes – medium can only flow between compartments when unit is upright

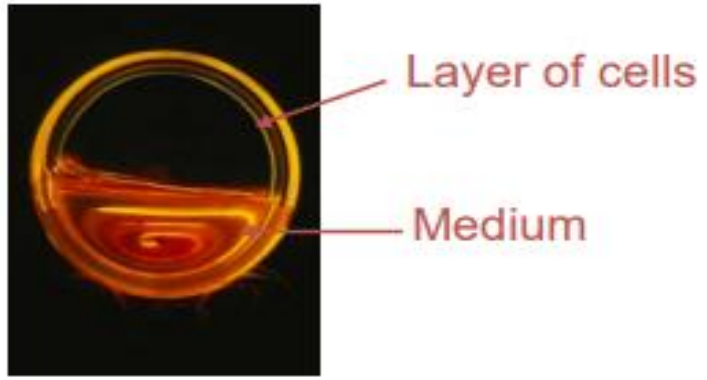


Roller bottles for adherent cells

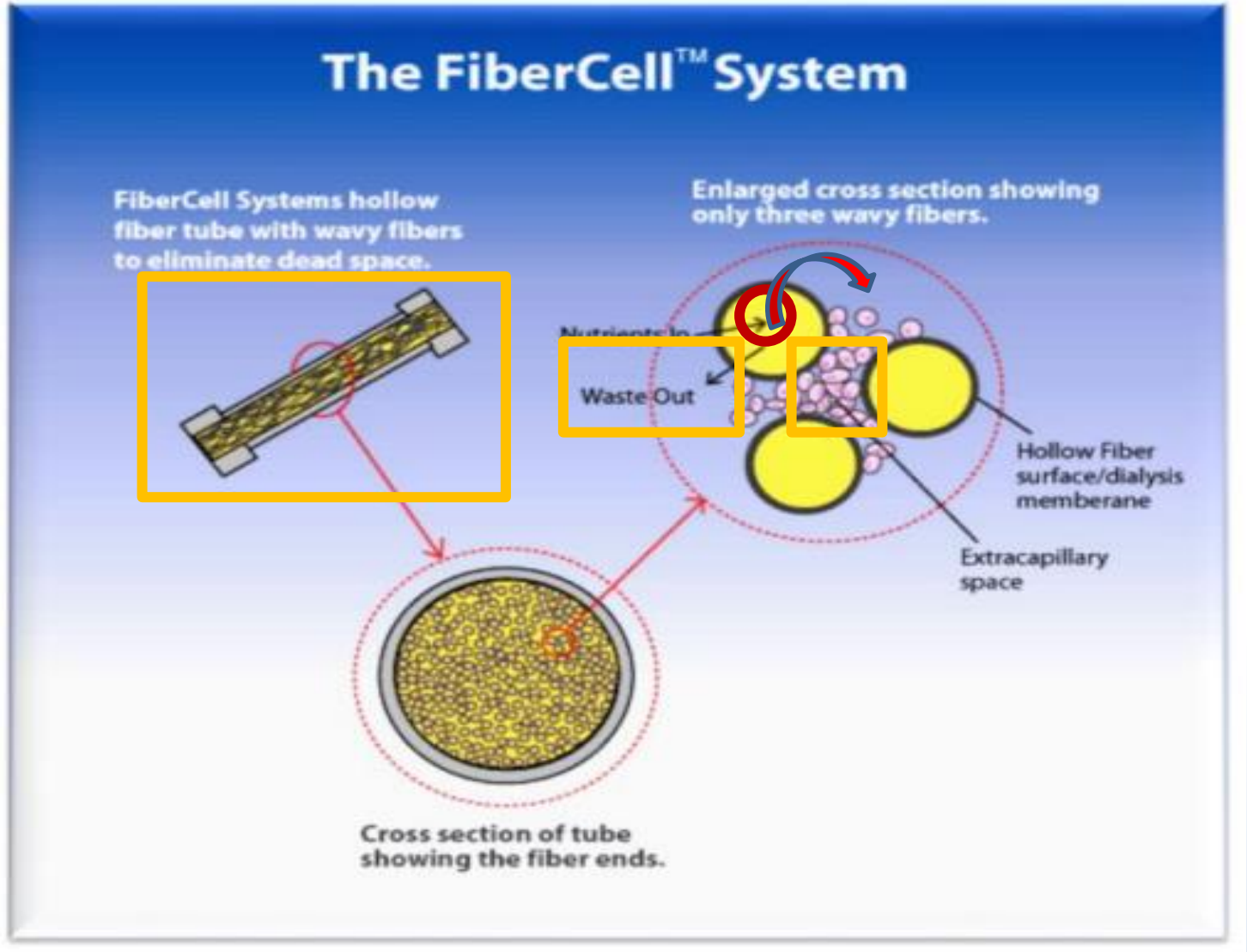
- Roller bottles – Large round plastic bottles with a **surface area of 1050 - 4200cm² - volume from 100mL to 1000mL.**
- Used for vaccine production as good for harvesting cells:
  - Cells grown in bottles, then infected with virus.
  - Collect cells and media, destroy virus with chemicals, purify and use for injection.
- Also used commercially for production of EPO by a small number of companies.

Growth of Cells in Roller Bottle

- Cells are added to the bottle and attach to inside.
- Bottle is then rolled on its axis and cells are bathed in culture medium.
- Constant and gentle agitation of medium (5-50 rev/hr).
- Major advantage is that geometry of bottle not much different from growing cells in plastic flasks.
- Usually add in O_2 into bottle, pH maintained by buffer HEPES.
- Large facilities (30,000 1L bottles) use robotic feeding systems.



Hollow fibre system

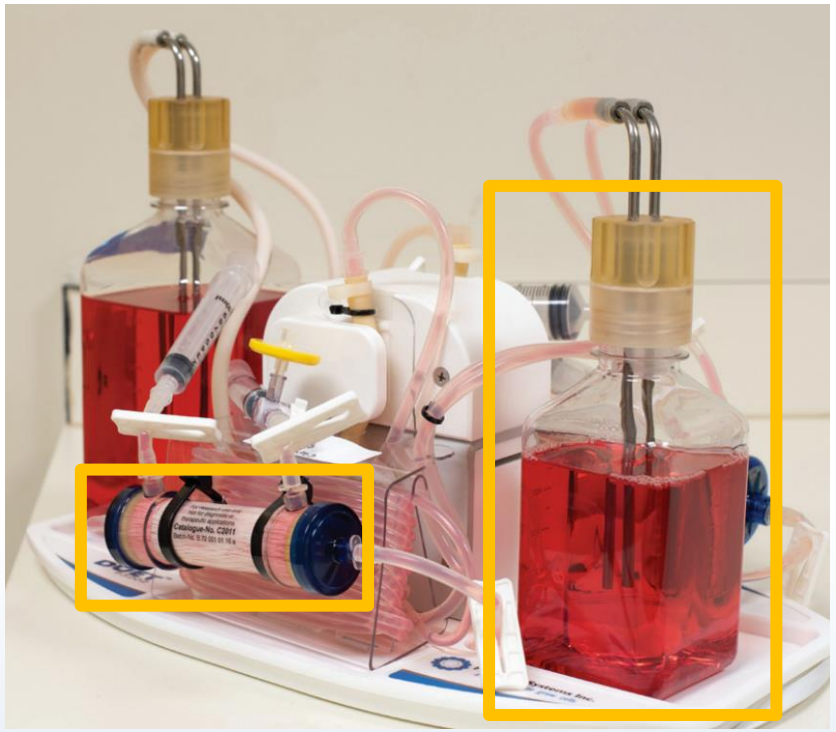


Hollow Fibre System

- Hollow fibers - small tube-like filters approximately **200nm in diameter:**
  - Fibers are sealed into a cartridge shell (many fibers)
- Cell culture medium is pumped through the end of the cartridge and flows through the inside of the fiber while the cells are grown on the outside of the fiber.
- Fibers create **a semi-permeable barrier of defined molecular weight cut-off (MWCO)** between the compartment in which the cells are growing and the medium is flowing.

Hollow Fiber Apparatus

- Modelled after the mammalian circulatory system.
- Hollow fiber cell culture offers the most *in-vivo* like manner to grow cells in any laboratory.
- Splitting of the cells is not required.
- Cultures can be maintained for many months of continuous production.
- If product is a secreted protein, it will be retained in the extra-capillary space.
- Concentration will accumulate up to 100 times higher than with conventional flask or roller bottle.



Anchorage Dependent Cell Growth

- Systems are not well suited to significant scaling – tend to be labour intensive.
- Better suited to smaller batch production e.g. low volume drugs manufacture, pilot-scale studies, production for clinical trials.
- Industry generally prefers use of anchorage independent growth systems e.g. stirred tank bioreactors (STBR) – stainless steel of disposable bag systems, wave bag systems:
  - Offer greater scalability
  - Well defined and understood technologies

Lecture Topics

- Challenges of Large-Scale Cell Culture
- Anchorage Dependent Cell Culture Systems
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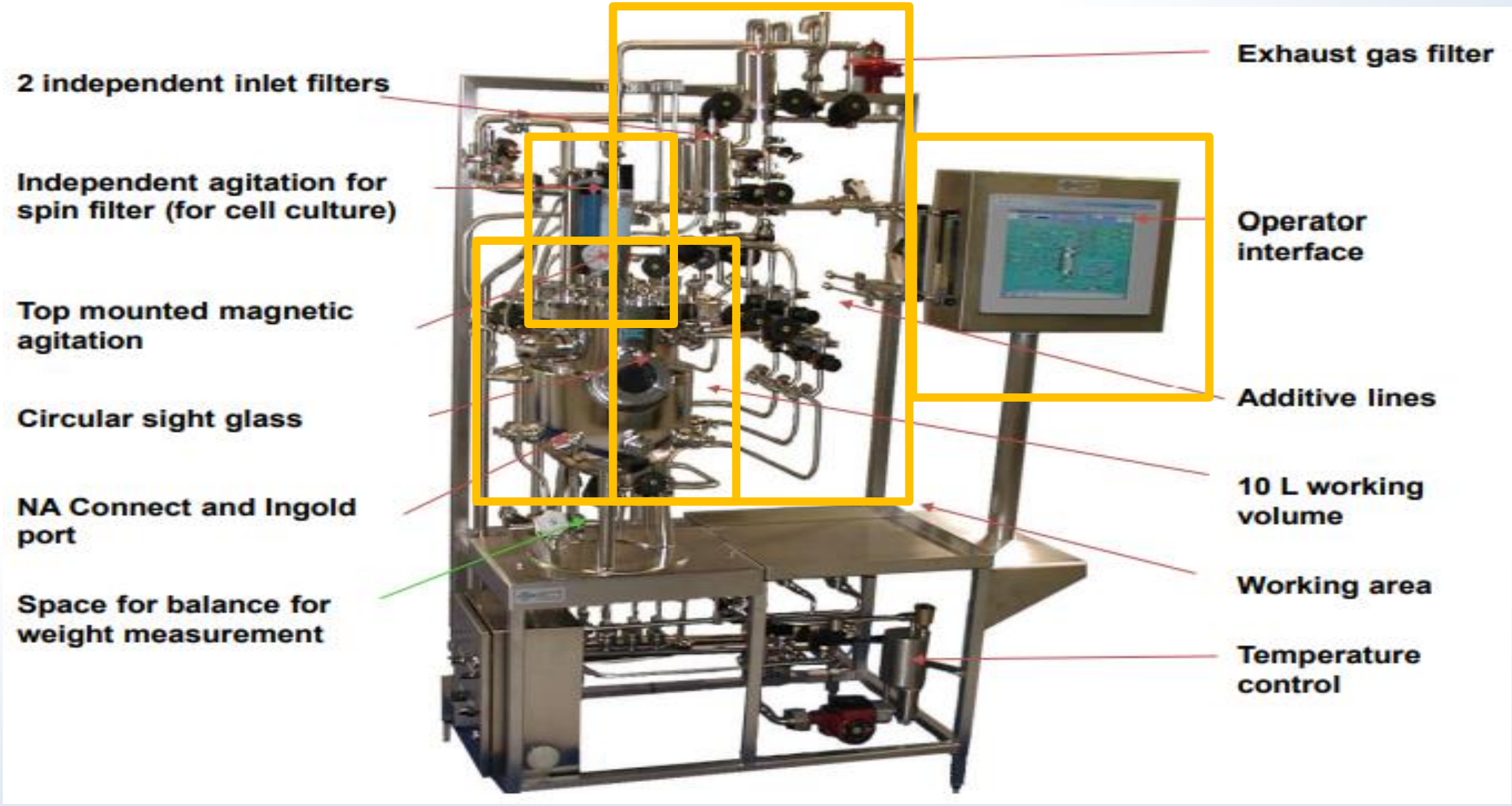
Anchorage Independent Cells

- Generally grown in *STB : regarded as the system of choice for production of biopharmaceuticals:
  - CHO or BHK cells adapted to grow in suspension.
  - Hybridoma cells (NSO) grow naturally in suspension.
- However, certain modifications to STBs are required for mammalian cell culture (as opposed to microbial culture):
  - Different agitation and aeration.
  - More stringent sterile conditions (closed system).
  - Careful monitoring of environmental conditions.
- Many other systems have also been looked at for mammalian cells including airlift bioreactor.
- ** Developed for bacterial cells : referred to as fermentation*

General Bioreactor System



Bioreactor –v- Fermenter Design

Top stirrer, magnetic agitator

H:D ~ 2:1 total volume

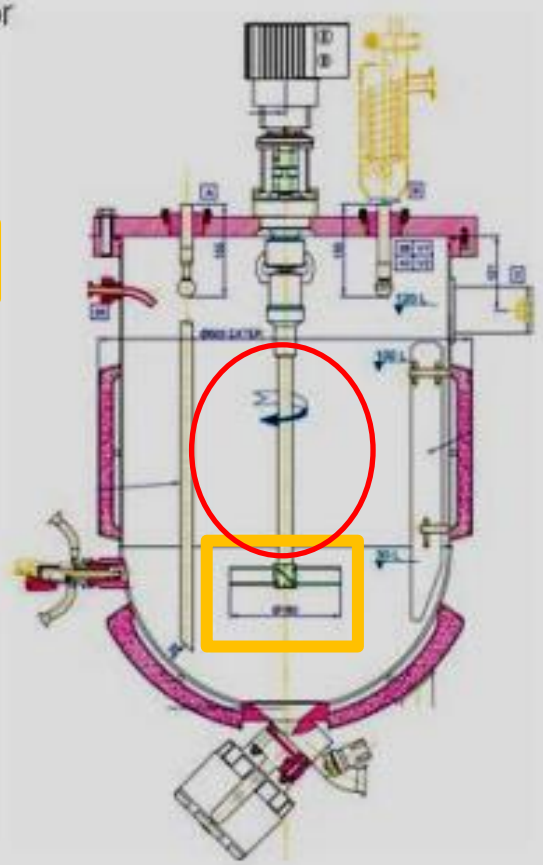
H:D ~ 1:1 working volume

Baffles 0, 1 or 2

Marine type impeller

Jacket extended to bottom

Vessel sterilised empty



H:D ~ 3:1 total volume

H:D ~ 2.2:1 working volume

Baffles 3 or 4

Multiple Rushton and pumping impellers

Jacket designed for cooling efficiency

Vessel sterilised full

Baffles

Bioreactor –v- Fermenter Design

Top stirrer, magnetic agitator

H:D ~ 2:1 total volume

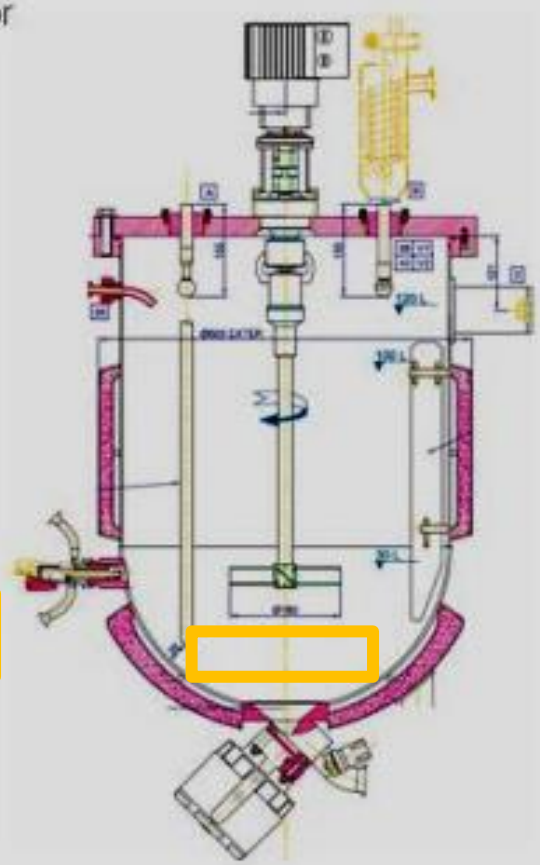
H:D ~ 1:1 working volume

Baffles 0, 1 or 2

Marine type impeller

Jacket extended to bottom

Vessel sterilised empty



H:D ~ 3:1 total volume

H:D ~ 2.2:1 working volume

Baffles 3 or 4

Multiple Rushton and pumping impellers

Jacket designed for cooling efficiency

Vessel sterilised full

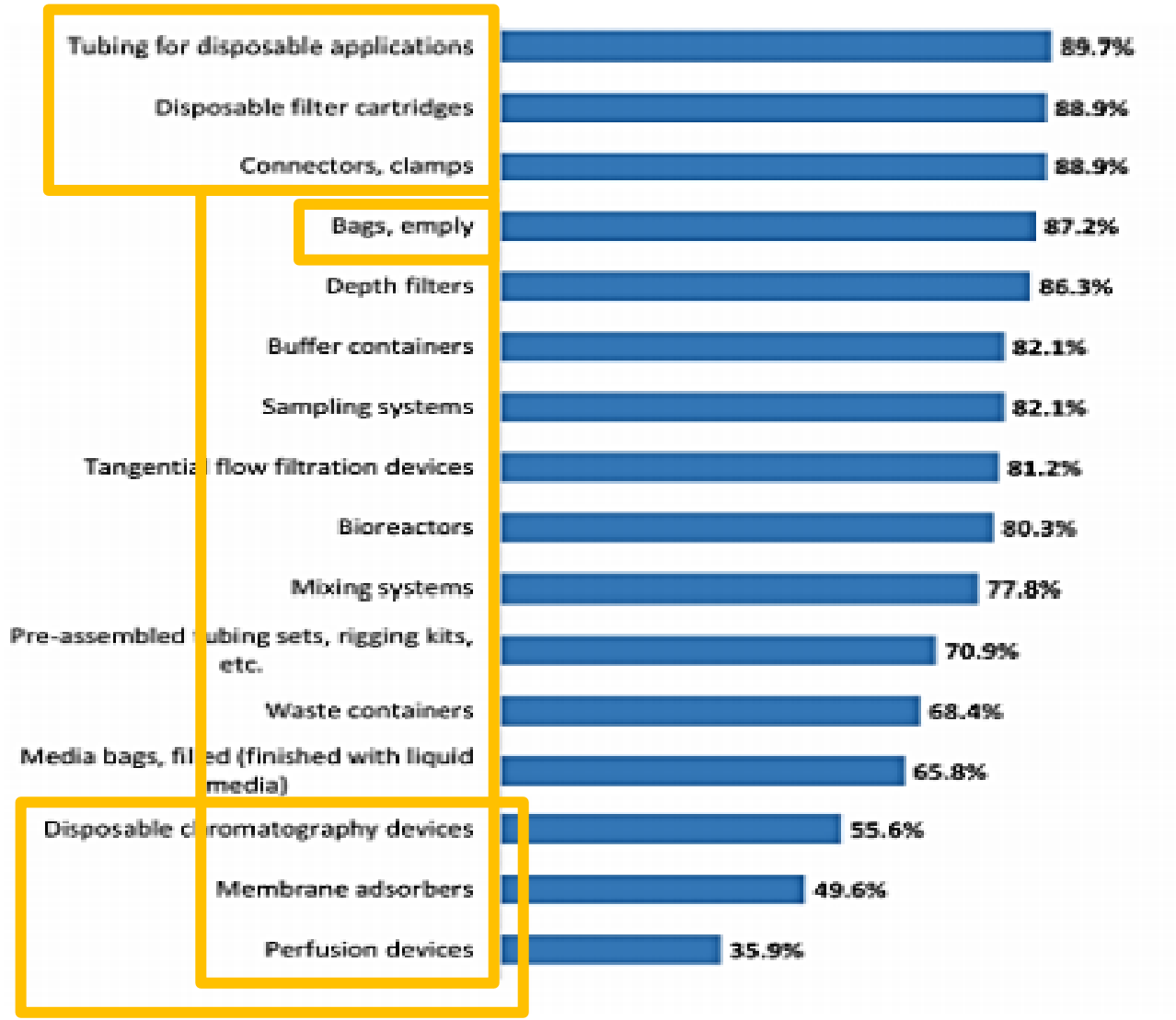
Traditional Manufacturing Processes

- Stainless Steel Equipment
- Expensive tanks, piping, valves, filter housings, etc.
- Limited polymers - gaskets, seals, hoses, filters
- High capital cost with long implementation schedule
- High risk of cross contamination & bioburden
- Repeated high cost of cleaning and sterilization
- Downtime, maintenance

Lecture Topics

- Challenges of Large-Scale Cell Culture
- Anchorage Dependent Cell Culture Systems
- Anchorage Independent Cell Culture Systems
- Disposable Technologies
- PAT Solutions in the Biopharmaceutical Industry

Unit Operational Use of Disposables



SOURCE: BioPlan Report and Survey of Biomanufacturing Capacity and Production, 2017, Preliminary Results

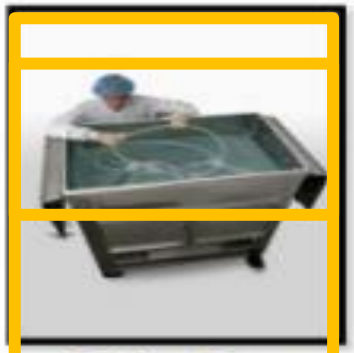
← **Pictured Left:** *Applications in Biopharmaceutical Manufacturing: % Using Single-Use Products (including all stages of R&D or Manufacture, 2017).*

Note: quantities for these products were not indicated in the survey question, only any incidence of usage at any stage. Thus, while any usage and the number of purchasers for these products may be increasing substantially, these data do not provide information on the volume of sales increases.

Examples of Current Uses in the Industry (GMP)

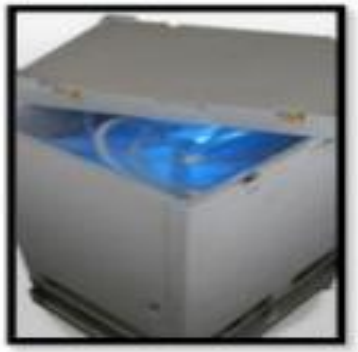
Hydration

Media Prep



Buffer Prep

Mixing

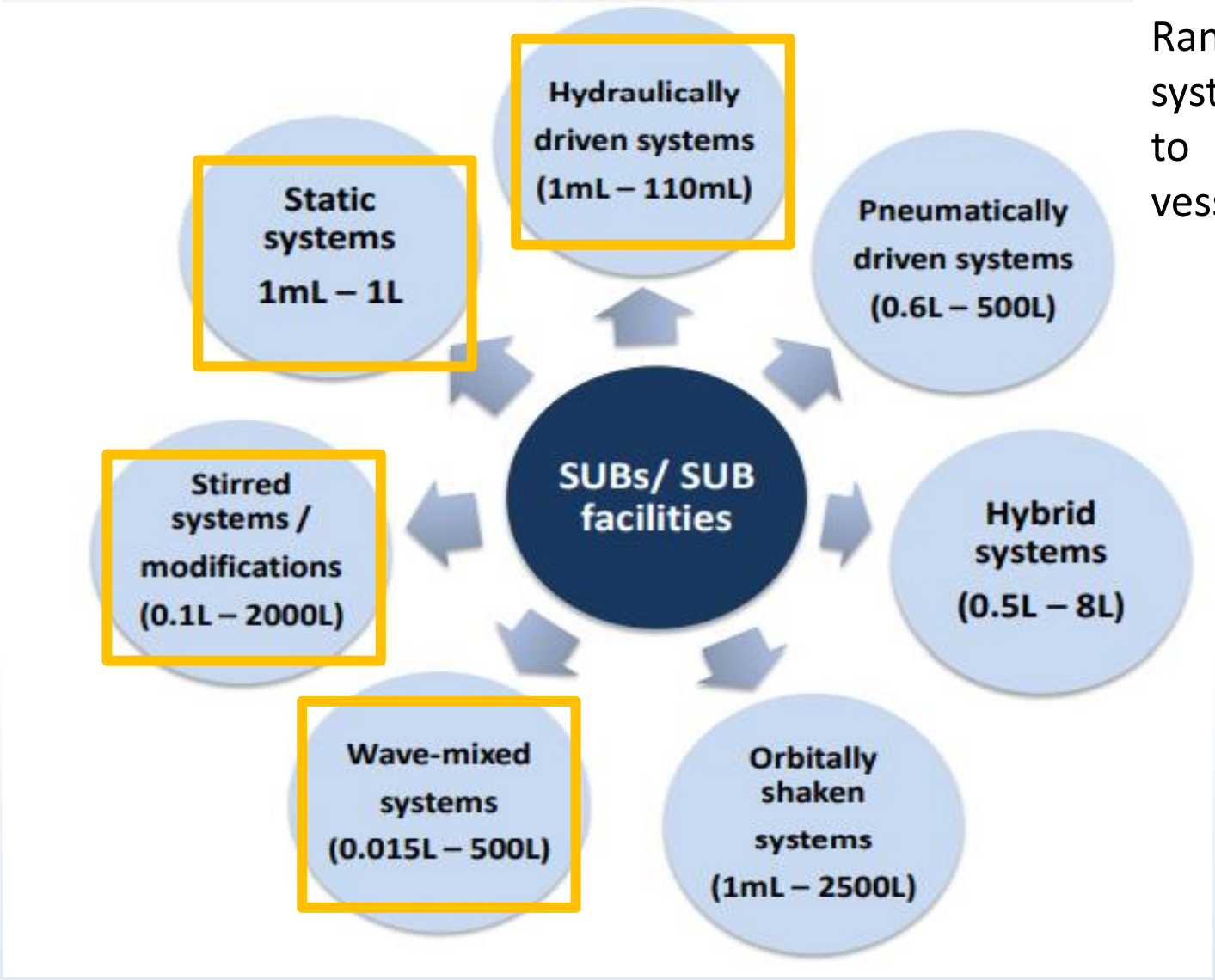


Transportation, Storage & Shipping

Substantial savings in Labor, Water, turn around time for set up break down.
May aid in major debottlenecking efforts

Courtesy Thermo / Millipore

Single Use Bioreactors vs Stainless Bioreactors



Range for miniaturized systems for scale-up studies to full scale production vessels .

SUS vs SS Comparison Table

Parameter	SU	SS	Comments
Energy	++	--	Less steam, less heating, ventilation, and air conditioning
Labour	+/-	+/-	No clean in place (CIP), no sterilize in place (SIP) but a lot of manual activities for preparation of manifolds (less automation)
Material	+	-	Less CIP media, fewer spare parts
Consumables	-	+	Higher cost for single-use material
Maintenance	++	-	Less complex equipment, lower effort for preventive maintenance
Turnover time	+	-	Much faster, no CIP/SIP of unit operation systems
Supply chain	--	+	Some items with long lead time, higher dependency on certain suppliers
Quality	+	+	Extractables and leachables to be addressed, lower risk of cross contamination
Success rate	+	+	Applicability of SU 98 %, 1-2 % failure rate (mostly due to leaks)
Risk of microbial contamination	+	-	Closed systems are much easier to generate (e.g. weldings of tubings)

SS –v- SUT Technology

- Stainless Steel Technology
- **Advantages**
  - Well established and understood
  - Complete ownership
  - Available in large capacities
  - More advanced measurement & control
  - Fixed piping may be used as a structure to implement other technologies
- **Disadvantages**
  - Inflexible infrastructure
  - Cumbersome and routine CIP
  - High utility costs
  - Contamination can still occur
  - Problems occur in piping
  - Higher maintenance requirements, systems & utilities

- Single Use Disposables Technology
- **Advantages**
  - Speed and ease of deployment
  - Reduced cleaning
  - Increased plant flexibility
  - Contamination less common
  - Fast changeover resulting in greater batch/facility/year
  - Reduced capital expenditure for equipment and facility
- **Disadvantages**
  - Capacity limitations – not always suitable for large-scale manufacture
  - Requires ongoing consumables usage forecast
  - QbD needs to consider extractables and leechables study data
  - Damage to SUT bags can impose leakage

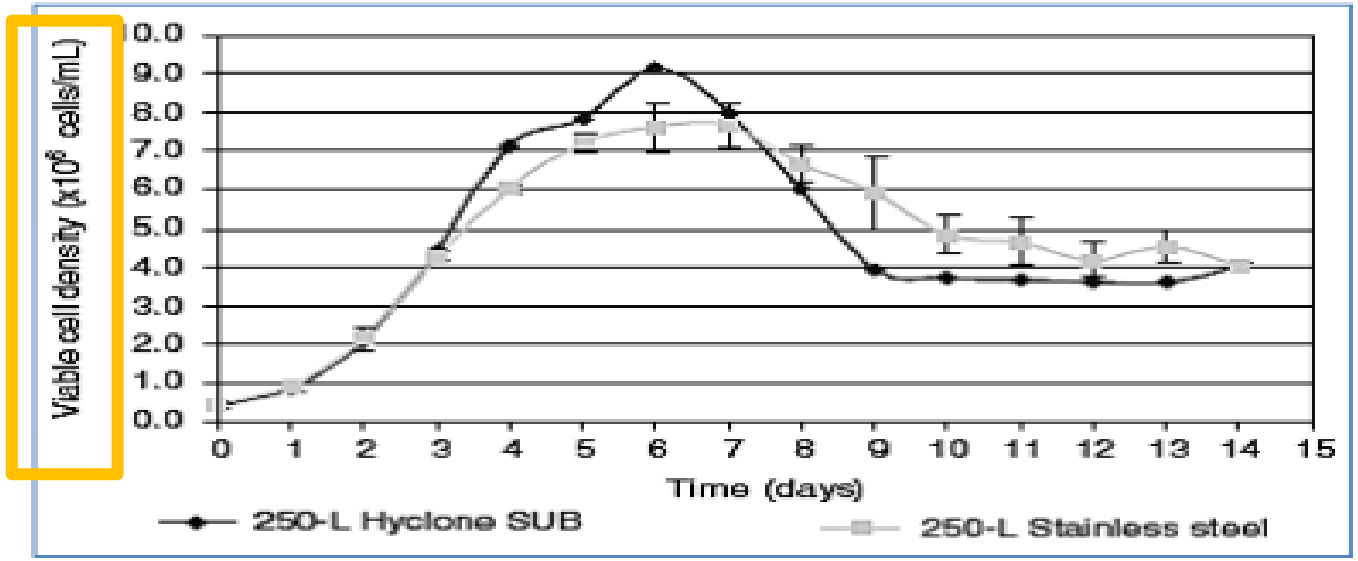
Why Choose Disposables

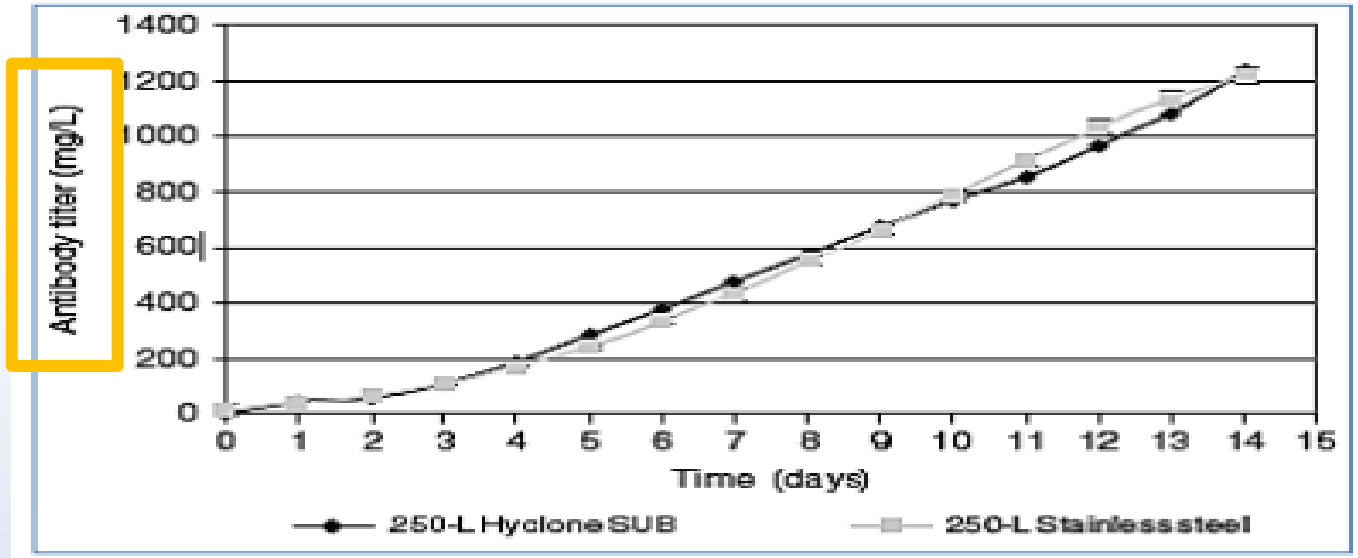
- 2015 industry survey on trends associated with Single-Use Technology.
- 'Top Concerns' for why biopharmaceutical manufacturers are choosing to increase their use of disposables:

1.	Eliminating cleaning requirements:	88.9%
2.	Reduce time to get facility up and running:	87.5%
3.	Reduce capital investment in facility & equipment:	86.3%
4.	Faster campaign turnaround time:	83.2%
5.	Decrease risk of product cross-contamination:	80.6%
6.	Flexibility of a 'modular' approach:	78.6%

SOURCE: BioPlan Report and Survey of Biomanufacturing Capacity and Production, 2015

SS –v- SUT for Cell Performance





Comparison of cell density between a 250-L Hyclone SUB and a 250-L stainless steel bioreactor.

Material Classification in Bioprocess

	Single-Use	
Expendable Laboratory Equipment	**Simple Peripheral Equipment**	**Equipment For Unit Operations/Platform Technology**
Vent and liquid filters	**Aseptic transfer systems**	**Bioprocess containers**
Test and centrifuge tubes	2D, 3D, Bags, Bag manifold systems and bag handling sys.	Bioreactors
Syringes	Connectors and Tri-clamps	Centrifuges
Protective Clothing	Flexible tubing	Chromatography system
Pipette and pipette tips	Fittings moulded	Deep Filter reference
Petri Dishes	Liquid containment bag	Freeze- thaw system
Microtiter plates	Closure containers, stoppers, protective caps	Isolators
Flasks	Tank liners	Membrane absorbers'
Culture containers	Valves	Micro filters, ultra filters devices
Analyzer sample caps		Mixing system
		Pumps

Recommended reading

- Study the article on Recommendations for Extractables and Leachables Testing alongside this section.
  - Recommendations for Extractables and Leachables Testing by the Extractables and Leachables Subcommittee of the BioProcess Systems Alliance.
    - Part 1: Introduction, Regulatory Issues, and Risk Assessment. Published in BioProcess International 5(11): p36-49 (December 2007).
    - Part 2: Executing a Program. Published in BioProcess International 6(1): p44-53 (January 2008).
- **Pay particular attention to the nature of Extractables and Leachables, their potential consequences and the development of methods to detect and minimise their impact.**

Capacity and Industry Trends - Volume

- Overall, currently the industry generally considers 2,000L to be an optimal size, in terms of cost-effectiveness for mammalian cell culture bioreactors.
- SS bioreactor-based systems at the ≥1,000L scale are becoming costly and too much work in terms of upfront investment and infrastructure, compared to single-use systems.
- SUTs are already significantly beating out fixed SS systems on economics and flexibility, particularly for manufacture of R&D and clinical supplies.
- The trend going forward is to use multiple disposable 2,000L bioreactors.

SOURCE: BioPlan Report and Survey of Biomanufacturing Capacity and Production, 2015

Economies of Scale

- “Estimate that a typical Mab manufactured in a 2,000-L single-use facility costs 25–30% less than one manufactured in a stainless-steel facility at the same volume.
- At volumes beyond those achievable with single-use systems, economies of scale can make the stainless-steel system preferable”.

- http://www.engconf.org/staging/wp-content/uploads/2015/10/C-E-News-Vol.-93-No.-46-Cover-StoryB.pdf

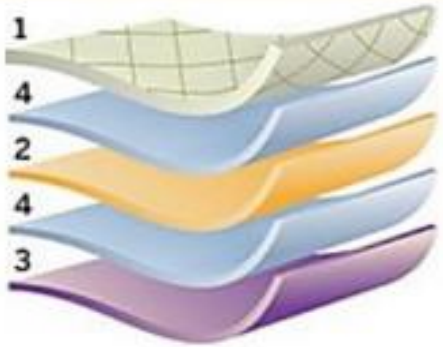
Capacity and Industry Trends - Titer

- Current industry yields are trending at >3 g/L, typically 5 to 6+ g/L:
  - Most mAb manufacturers will likely be rather satisfied with consistently attaining yields on the order of 3 g/L.
  - At least until DSP operations improve to better handle higher-yield bioreactor output, including resolving aggregation and other problems associated with higher titers.
  - Thus, 3 g/L has become a reference standard for SUT system-based mAb manufacture at ≥1,000- 2,000 L scale.
- A single 1,000L SUT (fed-batch or equivalent more continuous culture) production run can provide ~3,000 g of protein per run:
  - Even at a low potency of 100 mg/dose, this provides about 30,000 doses.
  - Presuming this is a relatively inexpensive antibody, selling for only ~$1,000/dose, this provides $30million revenue.

SOURCE: BioPlan Report and Survey of Biomanufacturing Capacity and Production, 2015

Single-Use Technologies for Upstream Bioprocessing

Materials of Construction



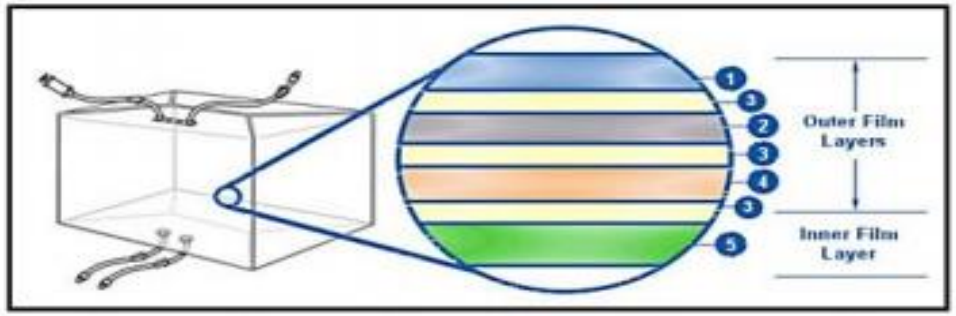
Layer	Thickness	Typical materials	Function
1 Outer	20–150 µm	Polyethylene, nylon, polyesters, polyamides	Abrasion & puncture resistance, strength, feel
2 Barrier	5–50 µm	Ethylene vinyl alcohol	Prevent transmission of O_2 and CO_2
3 Product contact	50–300 µm	Various densities of polyethylene	Biocompatibility, strength, sealing
4 Tie	Various	Modified polyethylene	Bonding between layers

PEELING BACK THE LAYERS

Single-use bioreactors are made of polymer films with multiple layers that serve different functions. The most common polymer in the films is polyethylene.

SOURCES: GE Global Research and other companies

http://www.engconf.org/staging/wp-content/uploads/2015/10/C-E-News-Vol.-93-No.-46-Cover-StoryB.pdf



Requires understanding of many factors such as:

- Film design and composition
  - Outer Layer, e.g. EVA, ULDPE
  - Inner Layer, e.g. PE, EVA
- Testing of raw materials and final film
  - Outer layers may also impact compatibility and E/L of final film

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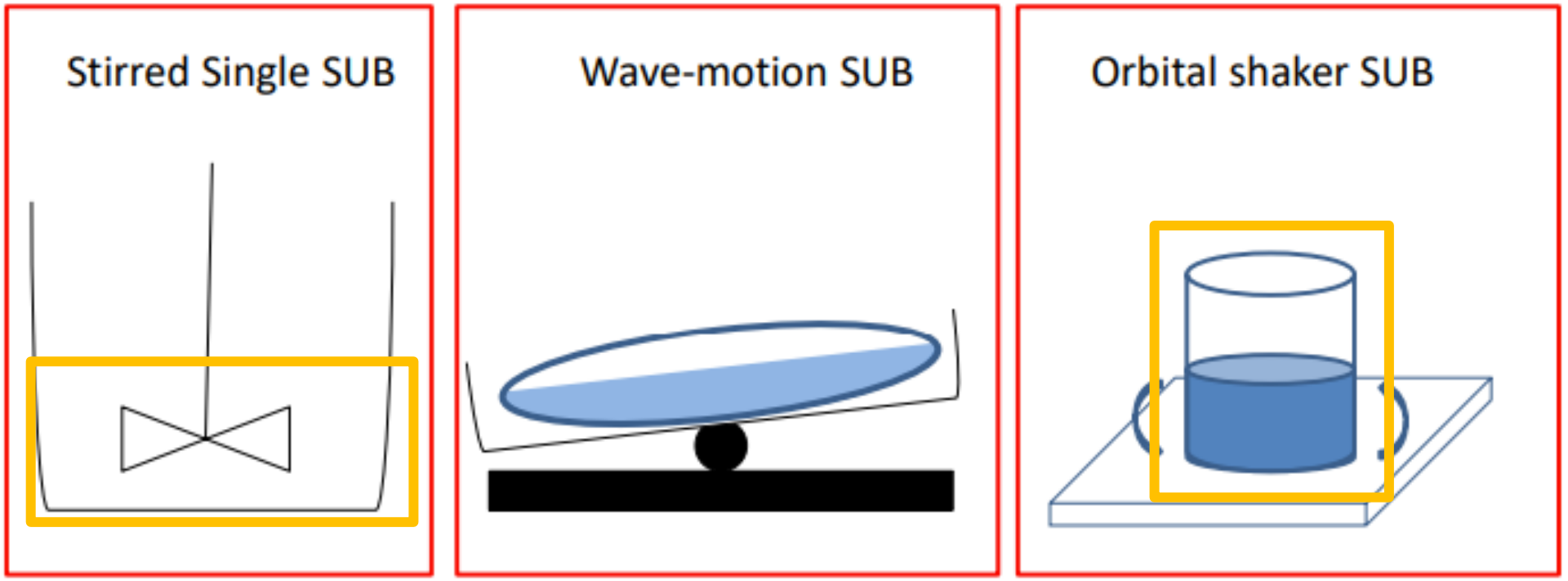
Single-use Bioreactors Categories (SUB)



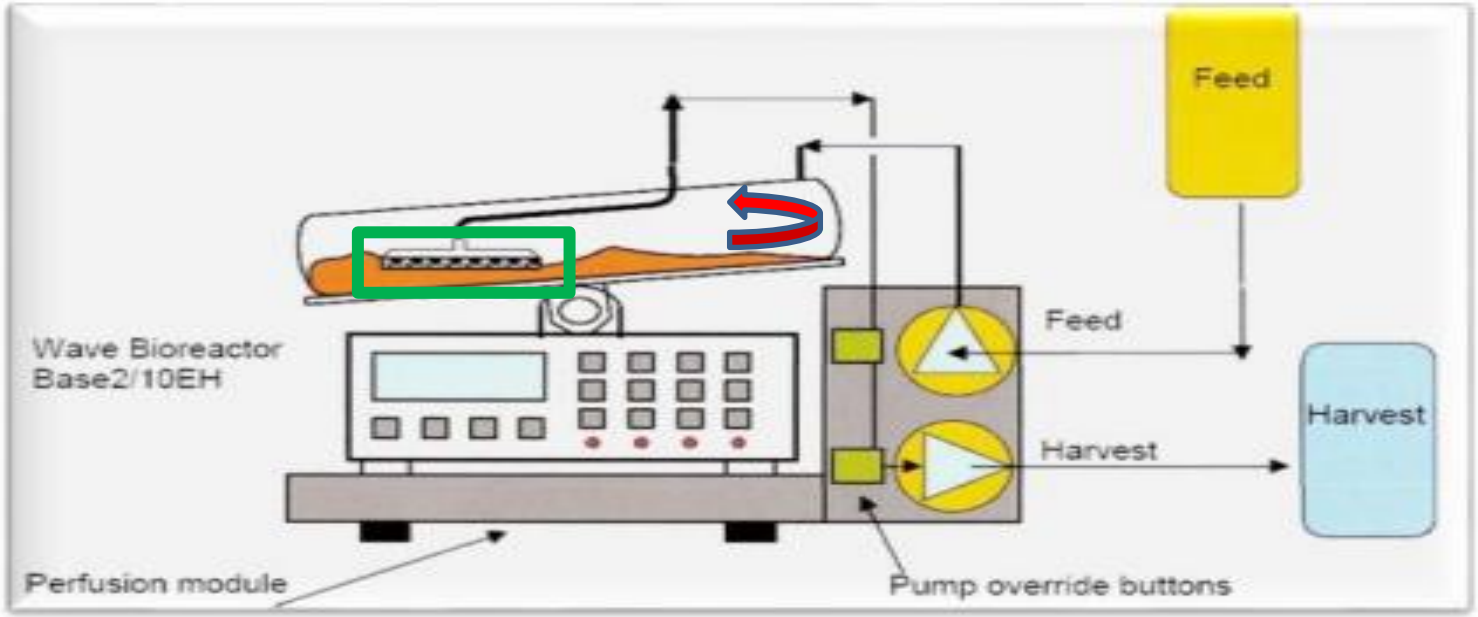
Wave-motion SUBs

Vendor	Name	Working Range	Temp. Control	Reported k_La	Cell Cultures	Sensors
GE Healthcare	WAVE Bioreactor	50 mL to 500 L	Heater Pad	10-30hr^{-1} [9,6]	Batch hybridoma[4] cells 22.3 x 10^5, μ_{max} : 0.053hr^{-1} Perfusion hybridoma[4] cells 20.1 x 10^6 μ_{avg} : 0.017hr^{-1} Perfusion CHO-line[8] 3 x 10^7	Optical single-use or conventional pH and DO sensors
Lonza	CELL-tainer	250 mL to 150 L	Incubator cabinet Integrated cooling plate	>600hr^{-1} : Conditions: 10 L, 42 rpm, max circle angle [1]	Batch, Vero[3]: 0.9 x 10^6 , μ_{max} : 0.027hr^{-1}	Disposable pH and DO sensors.
Applikon	AppliFlex	5 - 25 L[7]	Temperature control[7]	4-8hr^{-1} [5]	Animal and plant cells[6]	Classic sensor pH, DO[7]
Sartorius Stedim	BIOSTAT CultiBag RM	50 mL to 300 L	Electrical resistance heater plate. Cooling by ambient air only	22.0 hr^{-1} Conditions: 2L system, 43 rpm, max angle[2] 6.0 hr^{-1} Conditions: 120 L, 43 rpm, max angle[2]	Batch, Vero[3]: 0.8 x 10^6 , μ_{max} : 0.017hr^{-1}	Single-use optical chemical pH and DO sensors

1. (Nico M.G. Oosterhuis and Berg, 2011), 2. (Terry Burns et al., 2009), 3. (Thomassen et al., 2011), 4. (Tang et al., 2008), 5 (Eibl et al., 2010b), 6. (Eibl and Eibl, 2011), 7. (ApplikonBiotechnology, 2008), 8. (Leigh N. Piece and Shabram, 2004), 9. (Terrier et al., 2007)

Wave-type Bioreactor Design

Wave-type Bioreactor Design *Cont/d*



- Sterile disposable plastic bag called a Cellbag that is placed on a special rocking platform.
- Rocking motion of this platform induces waves in the culture fluid.
- Waves provide good mixing and oxygen transfer.
- Requires no cleaning or sterilization.
- Initially developed for 1-20L : Recently developed 500L.

Wave-motion SUB Comparison

- Different geometry.
- Lower scalability range.
- Low shear stress.
- Simple setup.
- Lower oxygen transfer rates.
- Well used in seed trains:
  - Use in perfusion mode-high viable cell conc.

Stirred SUBs

Vendor	Name	Working Range	H:D Ratio	Impeller Position	Impeller Type	Bag Load	Sensors	Aeration
Xcellerex	XDR	4 to 2000 L	1.5 : 1[#]	Bottom	3-pitched blades, 15° off centre[&]	Top or Bottom with automation	Conventional pH, DO, CO2 & biomass probes	Sintered disks or drilled hole
Sartorius Stedim	BIOSTAT CultiBag STR	12.5 to 1000 L	1.8 : 1	Overhead	2 x 3-pitched blades or 2 x 6-blade disk	Front-doors	Optical single-use or conventional optical sensors for pH and DO.	Traditional ring and micro sparger
Thermo Fisher Scientific	Hyclon Single Use Bioreactor S.U.B	25 to 2000 L	1.9 : 1	Overhead	Off centre Direct drive 3-pitched blades	Top[$]	Optical single-use or conventional optical sensors for pH and DO.	Dual sparger Porous frit sparger Open pipe
Millipore Merck	Mobius CellReady	1 to 200L	2.0 : 1[#]	Bottom[*]	4-pitched blades, 13° off centre[*]	Front-doors	Optical single-use or conventional optical sensors for pH and DO.	Dual sparger microsparger open pipe

: XDR-50 has an aspect ratio of 2.5 : 1 and the 3 L Mobius CellReady has an aspect ratio of 1.8 : 1. & : impeller for both XDR-50 and the XDR-10 is positioned centre at 90°. * : 3 L Mobius CellReady has a 3-blade marine scoping impeller positioned overhead. $: Door for front access on 1000 L Hyclone SUB

Xcellerex XDR Disposable Bioreactors

- XDR disposable bioreactor systems are available with maximum working volumes of 200L, 500L, 1000L & 2000L.
- Disposable components include the reactor bag liner, agitation impellor, dissolved oxygen and pH probes, sparge element and tubing.
- Mixing: 0 - 350 rpm, bottom drive, magnetic coupling.
- Jacketed bag container standard for heating & cooling.

ABEC 6000L Disposable Bioreactors

Nucleo Bioreactor Design

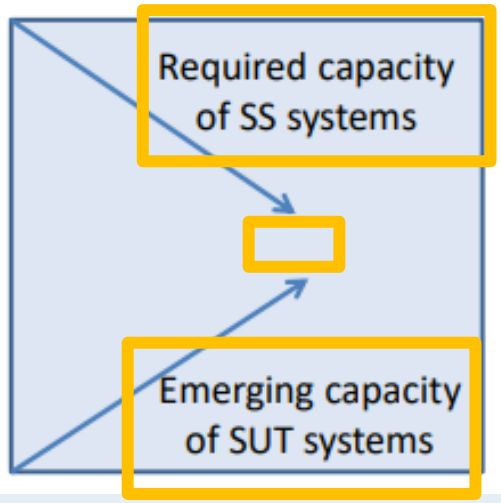
- Combination of ATMI and Pierre-Guerin technology.
- Design based on the ATMI Pad-drive single-use mixing technology, providing good mixing efficiency, reduced shear stress, and good oxygen transfer rates.
- Complete range of standard vessel sizes ranging from 25 to 600 litres, associated accessories.
- The system has an effective combination of rotor speed, sparger in motion, gas flow rate and cubical vessels, providing effective gas transfer rates.

Comparison-Future Scale demand

- Increasing productivity for cell lines.
- Multiple stirred SUBs more suitable option?

Volume (L)	20000	12000	6000
Productivity (g/L)	3	5	10
Harvest/Batch (days)	21	21	21
Process recovery	80%	80%	80%
Product per batch (kg)	48	48	48
No. batches (per yr)	16	16	16
Total product (kg/yr)	768	768	768

Theoretical example of scale requirement of a Mab product based on productivity

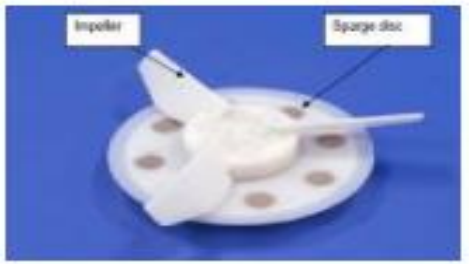


Comparison - Agitation & Aeration

- Bottom mounted vs Overhead Agitation.
- Turn down ratio.
- Blade design.
- Baffles – Rare.

- Two spargers - Aeration and CO_2 stripping.
- Traditional cleaning issues eliminated.
- kLa still lower than traditional bioreactors.

XDR



Hyclone S.U.B

Mobius CellReady

BIOSTAT CultiBag STR

images courtesy of Merck Millipore, Xcellerex, Thermofisher and Sartorius Stedim.

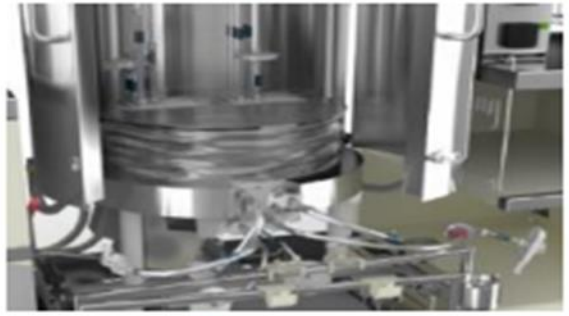
Comparison-Setup and Sensors

- Large size = setup more complex.
- Front versus top loaded bioreactor bags.
- Overhead agitators = setup more complex.
- Rigid base for Mobius CellReady plastic bag.

- All stirred SUBs offer both options for single-use and reusable probes/sensors.
- Mobius SensorReady –external circuit, configured to sensor req.

Hyclone S.U.B. 1000L

Mobius CellReady 200 L



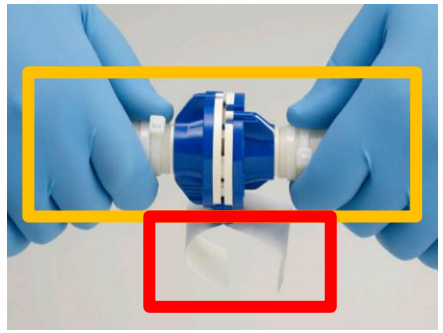
Mobius SensorReady

images courtesy of Merck Millipore and Thermofisher

Types of Connectors

Quick Connect

Sterile Tubing Welder



Sterile Tubing Connector (KleenPack)

Lynx connector

Steam Through Connector

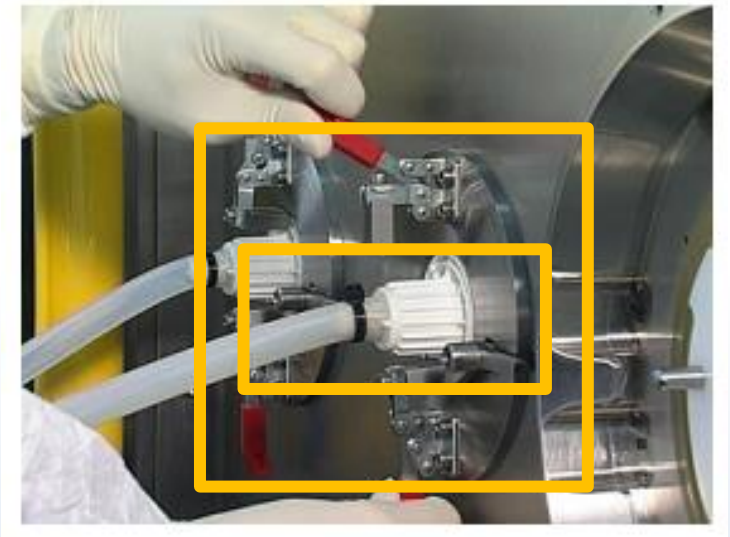
Steam Through Connector

Millipore S2S

"Wave" Tube Welder

Single Use Coupling & Sampling Systems

- Storage of process media is the key area where the single use technologies have made great strides:
  - Especially from the mixing system to the single use storage bag.



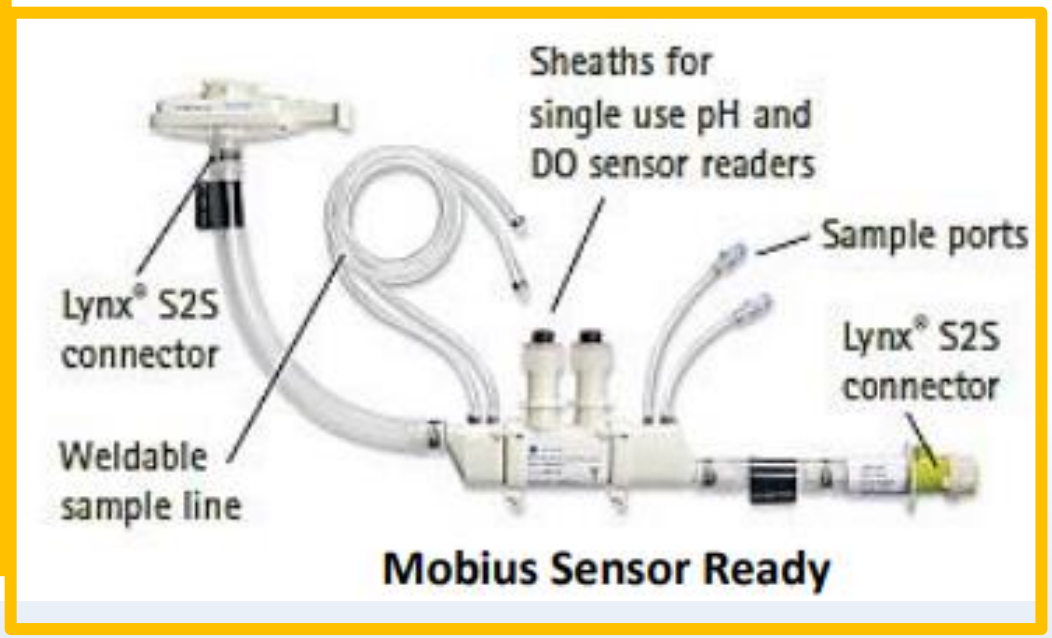
http://www.aseptictech.com/ASEPTIC/Images/connector.jpg

- Single use sampling system -advantage due to the sterility and sensitivity of collecting samples, mostly syringes are used, this mode remains a single use process and could only be replaced with online, in real time where PAT is in place.

Sterile Sampling (sigmaaldrich.com)

Single use Sensor Systems

- Monitoring of the bioreactor parameters is achieved through sample collection and analysis.
- Single use sensors are presently built in with the disposable bioreactors, with the necessary ports for collecting data present.



[See the Bioprocess, Be the Bioprocess (genengnews.com)]

Use of Disposable Technology

- **Disadvantages of System:**
  - The validation of the system may be more complex – need to establish repeatability of the system performance.
  - Concerns re. Leachables / Extractables.
  - Environmental Considerations – disposability, non-renewable resources.

2x1000L New Facility Example of SU vs. MU Investment Costs

Pictured below: Comparison of investment costs for Single-Use (SU) and Multiple-Use (MU) facilities.

Investment	MU facility T€	SU facility T€	(MU – SU) difference T€	(MU – SU) difference %
Process and clean utilities	9555	6018	–3537	–37
Automation and instrumentation	4778	3034	–1774	–37
Process piping and isolation	4778	825	–3953	–83
Building, HVAC, electrical and black utilities	5706	6090	384	7
Engineering cost	7445	4790	–2655	–36
Offices and laboratories	3500	3500	0	0
Start-up cost	6832	6941	109	2
Total investment cost	42594	31198	–11396	–27

Multiple-Use Option
•Capital $ 42 M

Single-Use Option
•Capital $ 31M

Choosing the SU option saves 11M (27%) in investment costs

2x1000L New Facility Example of SU vs. MU Running Costs

Pictured below: Comparison of running costs per year for Single-Use (SU) and Multiple-Use (MU) facilities.

Investment	MU facility T€/year	SU facility T€/year	(MU – SU) difference T€/year	(MU – SU) difference %
Total investment cost (T€)	42594	31198	−11396	−27
Running cost				
Total labor	2993	3150	157	5
Raw material	756	756	0	0
Consumables	2640	3984	1344	51
Utilities	434	423	−11	−3
Waste	10	16	6	60
Maintenance and insurance	1316	852	−464	−35
Total running cost	8149	9181	1032	13

Multiple-Use Option
- Running Cost 8M

Single-Use Option
- Running Cost 9M

It costs 1M more per year to run SU facility, due to consumables

Roche Disposables CMO

Economic Drivers – Labor

Preparation Time

– ~40 hours for Non-Disposable Inoculum Process
- Process all spinner flasks for one production run
- Clean, perform cleaning verification (including testing), assembly, process, and post-use clean
- Documentation and review of all paperwork

– 3-5 hours of prep time for completely disposable process

➢ *Overall, ~20% cost savings (labor and overhead) per run for the Single Use Bioreactor process as compared to the Stainless Steel Bioreactor process*

SOURCE: Avid as Disposable Biologics Manufacturing Partner, Slides, 2015

Economic Drivers – Labor

- Turnaround time:
  - – Stainless Steel is 5-7 days
    - Break down and CIP
    - SIP
    - Quality Control testing
    - QA Release for next run
  - – Single Use is 1-3 days
    - QA release

Lecture Topics

- Challenges of Large-Scale Cell Culture
- Anchorage Dependent Cell Culture Systems
- Anchorage Independent Cell Culture Systems
- Disposable Technologies
- PAT Solutions in the Biopharmaceutical Industry

Reading Material

- Links available in Moodle
  1. Busse, C. et al. Review - Sensors for disposable bioreactors. Eng. Life Sci. 2017, 17, 940–952

Instrumentation

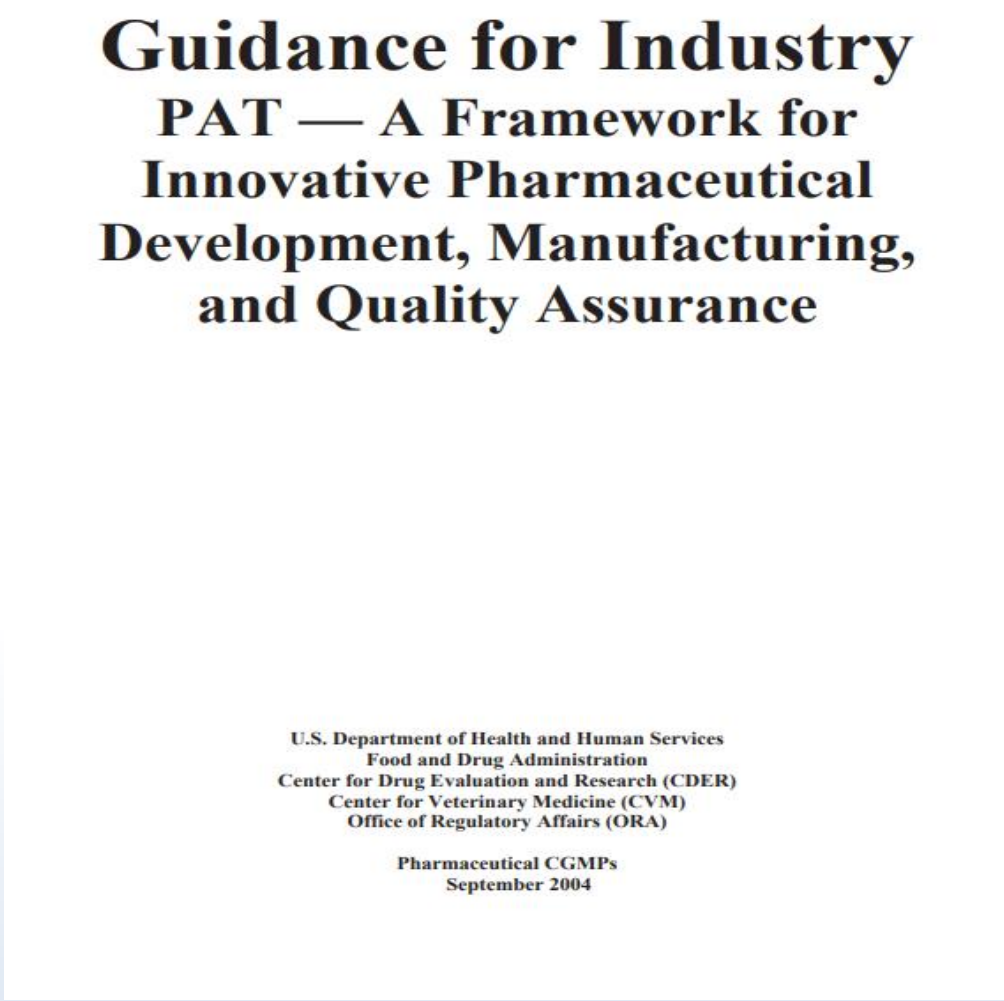
	Physical Parameter	Classical process equipment design	Disposable Technology Single use design
L	Level	Scale / Weight, Radar	Scale, Weight
P	Pressure	Pressure transmitter	Disposable sensor integrated in bag or manifold; coupled to re-usable transmitter Transmitter detached to system via membranes
T	Temperature	Pt100: Thermometer	Similar sensor technology; placement of probe: - Inserted in 'pocket' / 'shaft' or - Access via sterile pipe connector
W	Weight	Scale; External load cells	Scale; External load cells

Instrumentation

	Physical Parameter	Classical process equipment design	Disposable Technology Single use design
F	Flow	Mass flow meters; Turbines; Rotameter	Liquids: - Disposable sensor integrated in bag or manifold; coupled to re-usable transmitter - -Inline measurement with flow metres prior to sterile filter - -Indirect via weight increments (balances) - Gas - gas flow meters prior to sterile filter
Q	pH	Conventional electrode	Optic or conventional via sterile pipe connector
Q	pO2	Conventional electrode	Optic or conventional via sterile pipe connector
Q	Conductivity	Conventional electrode	Optic or conventional via sterile pipe connector
S	Frequency (agitator)	Conventional	Conventional (installed on drive)

PAT opportunities

- Reducing production cycle times.
- Right first time quality (RFT).
- Preventing rejects, scrap, re-processing.
- Managing variability (improving energy and material use and increasing capacity).
- Facilitating continuous processing (improve efficiency).
- Increasing automation to improve operator safety and reduce human errors.
- **Real time release.**


Guidance for Industry

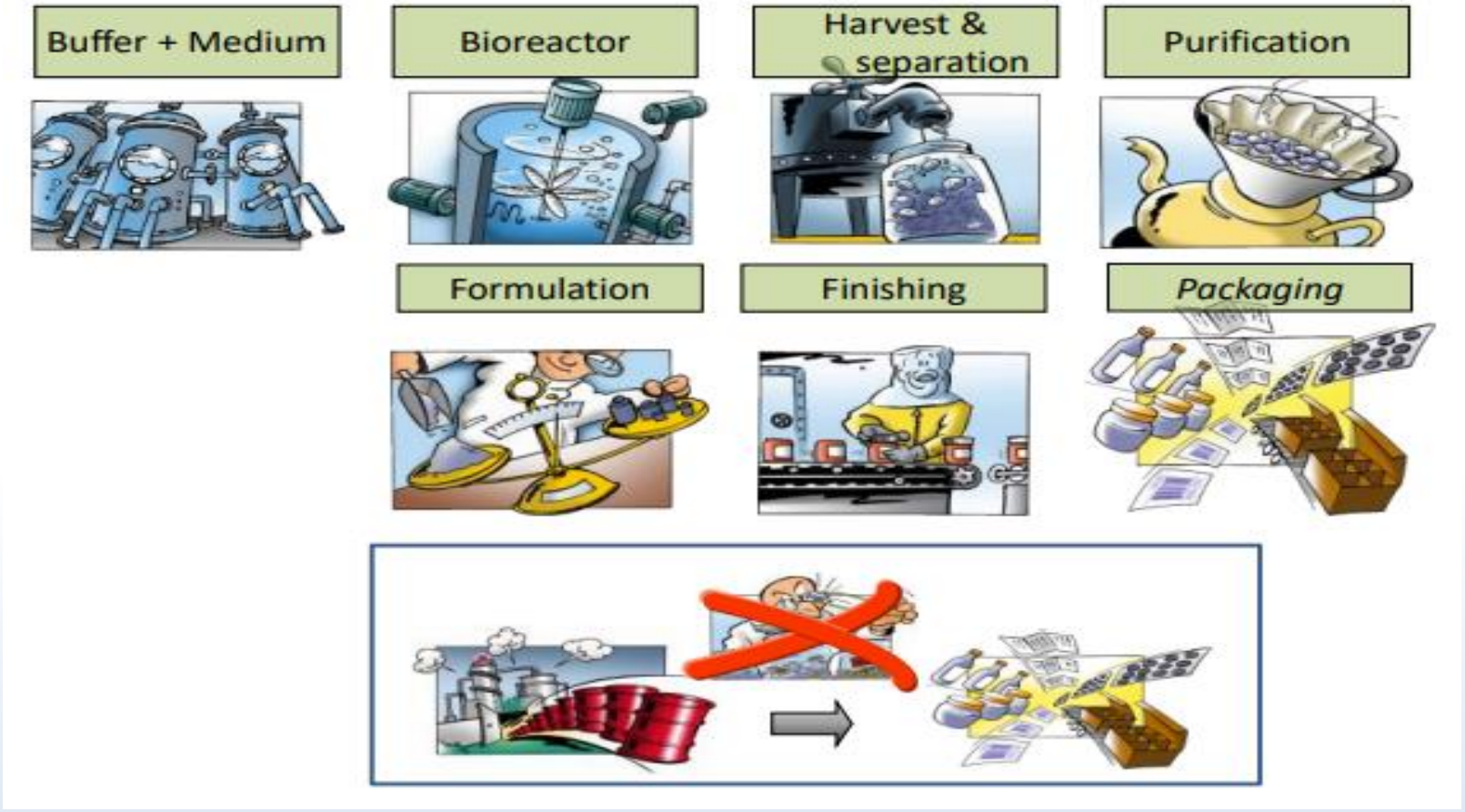
PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)
Office of Regulatory Affairs (ORA)

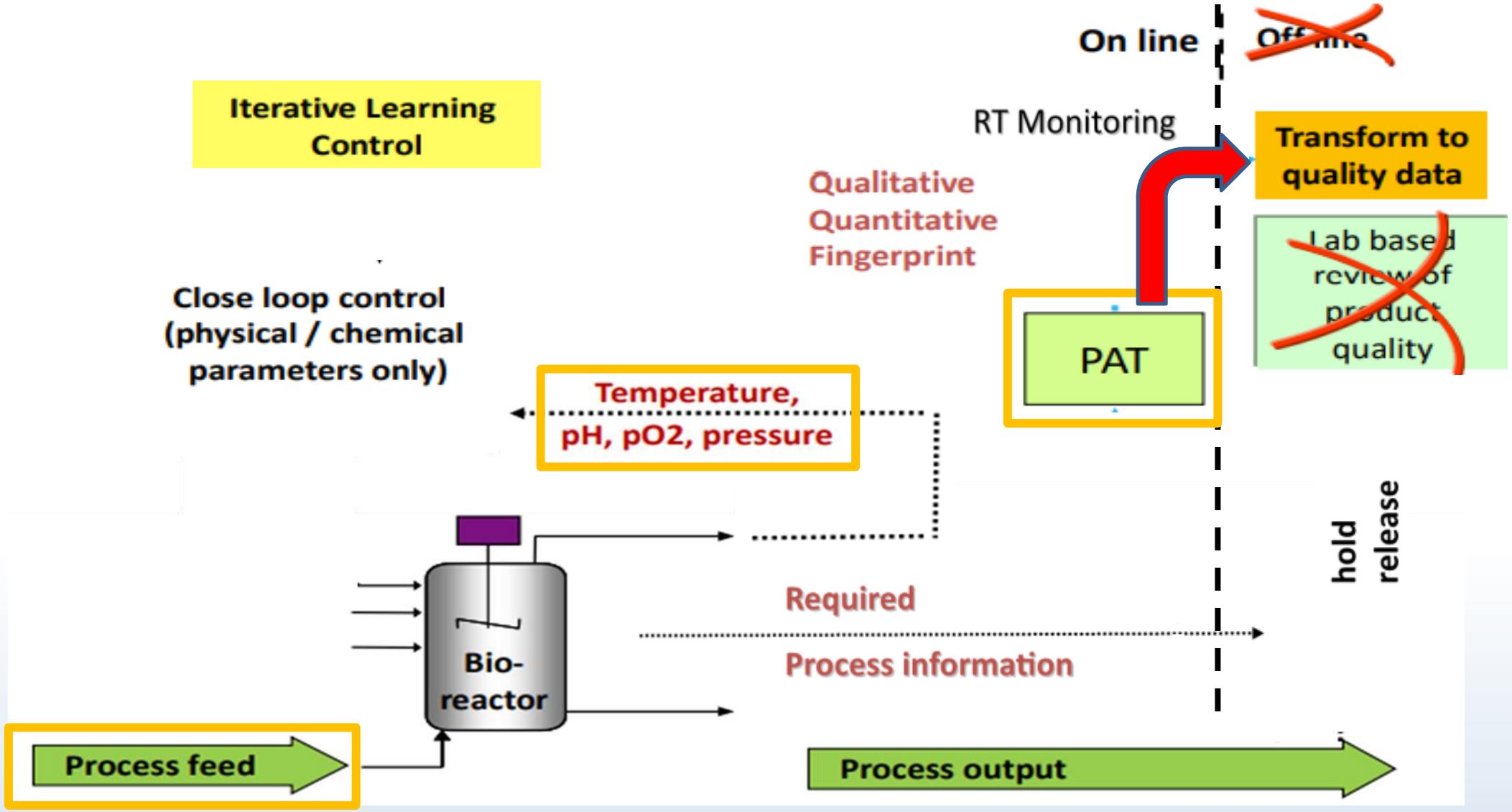
Pharmaceutical CGMPs
September 2004

Ultimate step – Real Time Product Release

- Production and release of pharmaceuticals, without final tests.
- Quality is based only on review of process characteristics.



Real Time Product Release



Summary Points

- Bioreactors, irrespective of scale, provide controlled environments for the aseptic growth of cells and sterile product formation.
  - Key design considerations include cell type, product volume, mode of operation, productivity targets and quality of product.
- Growing trend towards use of disposables:
  - Faster batch changeovers.
  - Greater flexibility during scale-up.
  - Minimises costs of maintaining sterility.
  - Reduced capital costs.
- Concerns include: validation can be more complex, leachables & extractables, environmental considerations.

Questions?

Sample Questions

- Explain the difference between anchorage dependent and anchorage independent cell growth. Which is generally preferred for therapeutic protein production and why?
- Single-use bioreactors are increasing in popularity across the industry. What advantages do they offer over fixed stainless steel bioreactors? Comment also on their limitations.
- What is PAT and what is it hoped that it can achieve for the industry? Give 2 examples of how or where it might be advantageous to introduce PAT.