

Single-Use Cultivation in a Classical Format:

Reflecting the demand for single-use manufacturing by implementing stirred single-use bioreactors based on classical standards

Dr. Andreas Kocourek, Sartorius Stedim Biotech, Application Specialist, Fermentation

Australia (andreas.kocourek@sartorius-stedim.com)

Overview

- Benefits & challenges of single-use bioreactors
- Single-use bioreactor with "classical" design, such as BIOSTAT CultiBag STR
- Some technical details
  1. Vessel design
  2. Bag design
  3. Impeller design
  4. Aeration/gassing design
- Case studies (mammalian cell culture)
- Summary

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Single-use bioreactors: Benefits

Reduction:

- Contamination risk
- Time for set-up
- Space requirement
- Plant validation
- Investment
- Energy, WFI,...

Increase:

- Flexibility

Remove the need for:

- Cleaning & Sterilization

⇒↑ Process efficiency & ↓ Process Cost

Impact of Single-Use to a manufacturing process: Case Study

Stainless Steel Scenario

Throughput	Batches per year			**204**	**#/yr**
	Annual product mass			**419.6**	**kg/yr**
Capital	Total			**115,685,482**	**$**
	Per Litre of bioreactor			**9,640**	**$/L**
CoG	Cost per gram			**152.2**	**$/g**
L/Batch	**PW**	**WFI**	**Total**	**%**	
Process	0	11,474	11,474	11%	
Cleaning	55,869	37,191	93,060	89%	
Total	**55,869**	**48,665**	**104,534**	**100%**	

Disposable Scenario

Throughput	Batches per year			**226**	**#/yr**
	Annual product mass			**464.9**	**kg/yr**
Capital	Total			**62,286,851**	**$**
	Per Litre of bioreactor			**5,191**	**$/L**
CoG	Cost per gram			**119.1**	**$/g**
L/Batch	**PW**	**WFI**	**Total**	**%**	
Process	0	11,474	11,474	85%	
Cleaning	1,600	450	2,050	15%	
Total	**1,600**	**11,924**	**13,524**	**100%**	

Scenario	**CoG $/g**	**Capital $million**
Stainless Steel	152.2	115.7
Disposable	119.1	62.3
Savings	**21.7%**	**46.2%**

Source: Biopharm Services

Single-use bioreactors: Challenges for upscaling

Challenges during upscaling

- Very complex process
- some parameters proved their usefulness are:
  - same geometry/ratios
  - impeller design
  - tip speed
  - Power input per volume (P/V)
  - Gassing strategy
  - kLa
- Knowledge and experience are nearly as important as the scaling-up parameters

BUT

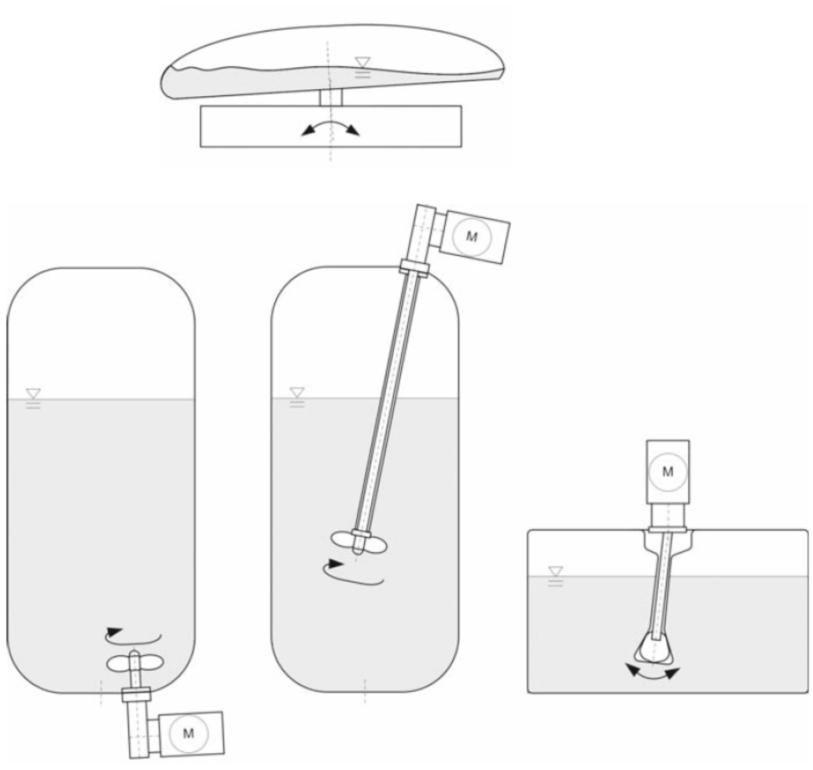
- Variations between reusable and currently existing single-use systems
- Single-use not existing for all volumes

Single-use bioreactors: Difficulties to overcome

Challenges in transfer due to:

- Change of Mixing Technology & behaviour
  - Wave induced mixing
  - Non-classical stirrer position
  - Non-classical impellers
- Gassing strategies
  - Only surface aeration
  - Non-classical spargers

⇒ Need for improvements in comparability reusable and disposable processes



From R. Eibl and D. Eibl; Adv Biochem Engin/Biotechnol (2009) 112: 183–207

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Single-use bioreactors with a "classical" design
Case Study: BIOSTAT® CultiBag STR

Requirements for „classical“ single-use bioreactors: Scaling up towards the new modern production chain

12,5-50 L 50-200 L 250-1000 L

Requirements for „classical“ single-use bioreactors: Scaling up towards the new modern production chain

12,5-50 L 50-200 L 250-1000 L

Requirements for „classical" single-use bioreactors:
control depending on application

Flexibility by Application:

- For R&D, Seed, Pilot and Production
  - Basic & advanced version
  - Heating only or Heating/Cooling
  - Single & Twin set-up
  - Large variety of volumes:
    - » First systems 50L & 200L
    - » scaleable from R&D to large scale production

Requirements for „classical" single-use bioreactors: advanced control with safe data-storage

1. Vessel design
Classical requirements

Single-Use Bioreactor Design: Bag Holder

- Rounded bottom as classical vessels
- Opening for harvesting port at lowest point
- Side openings for installation of probes
- Filter holders with filter heater for increase process safety

1. Vessel design
Easy handling and time-saving

BIOSTAT® STR Holder Design:

- Disconnectable from control tower for transfer to DSP and connection of another bag holder skid

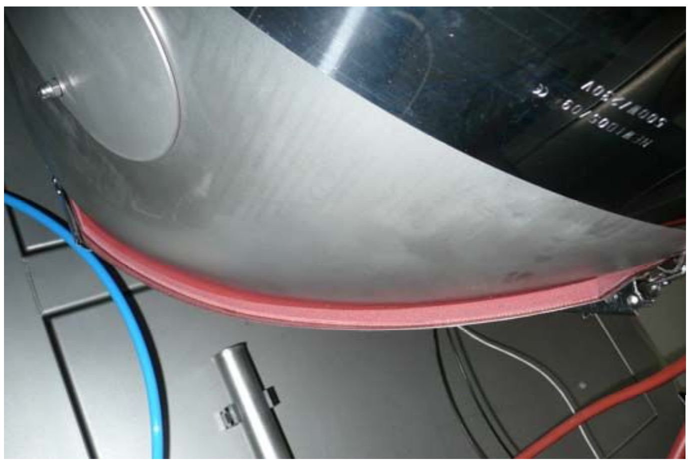
⇒ no time loss due to bag preparation, harvesting, further process steps

⇒ very low operational downtime of equipment

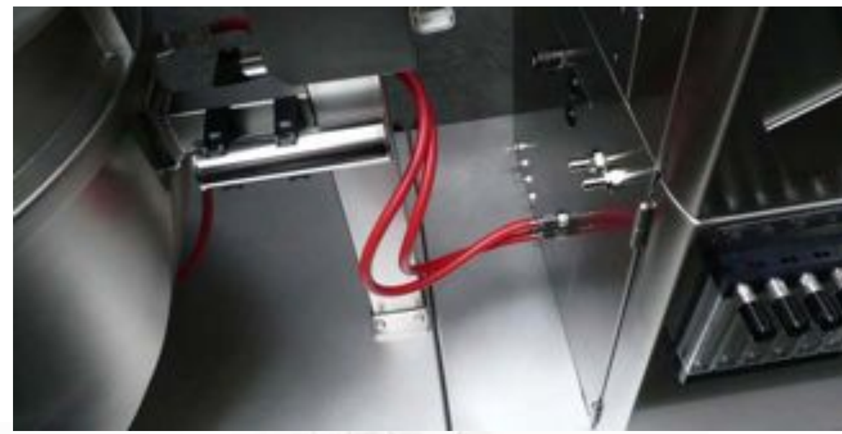
- Double door for easy installation bag

1. Vessel Design
Temperature Control

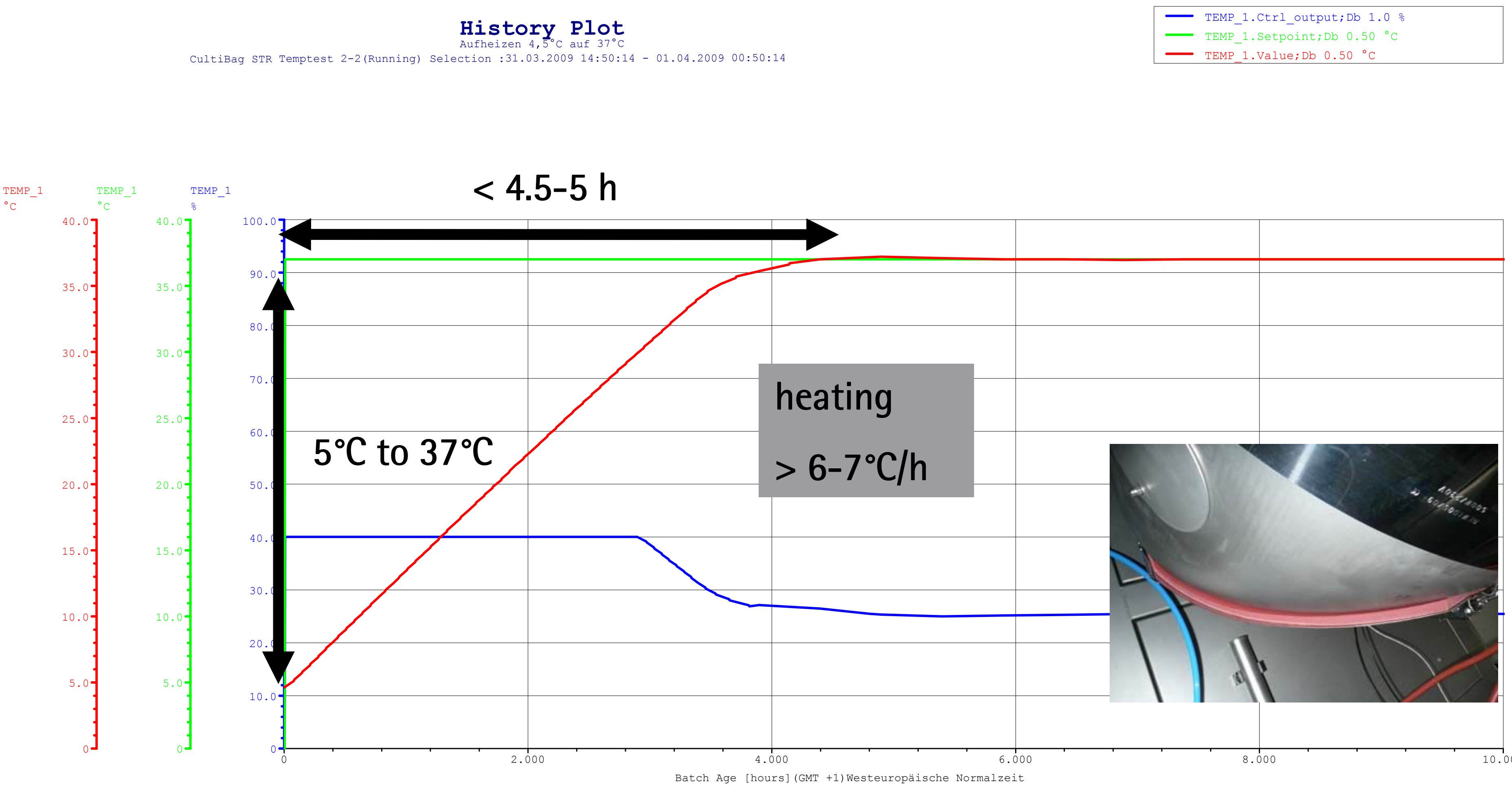
Heating blankets (heating only)



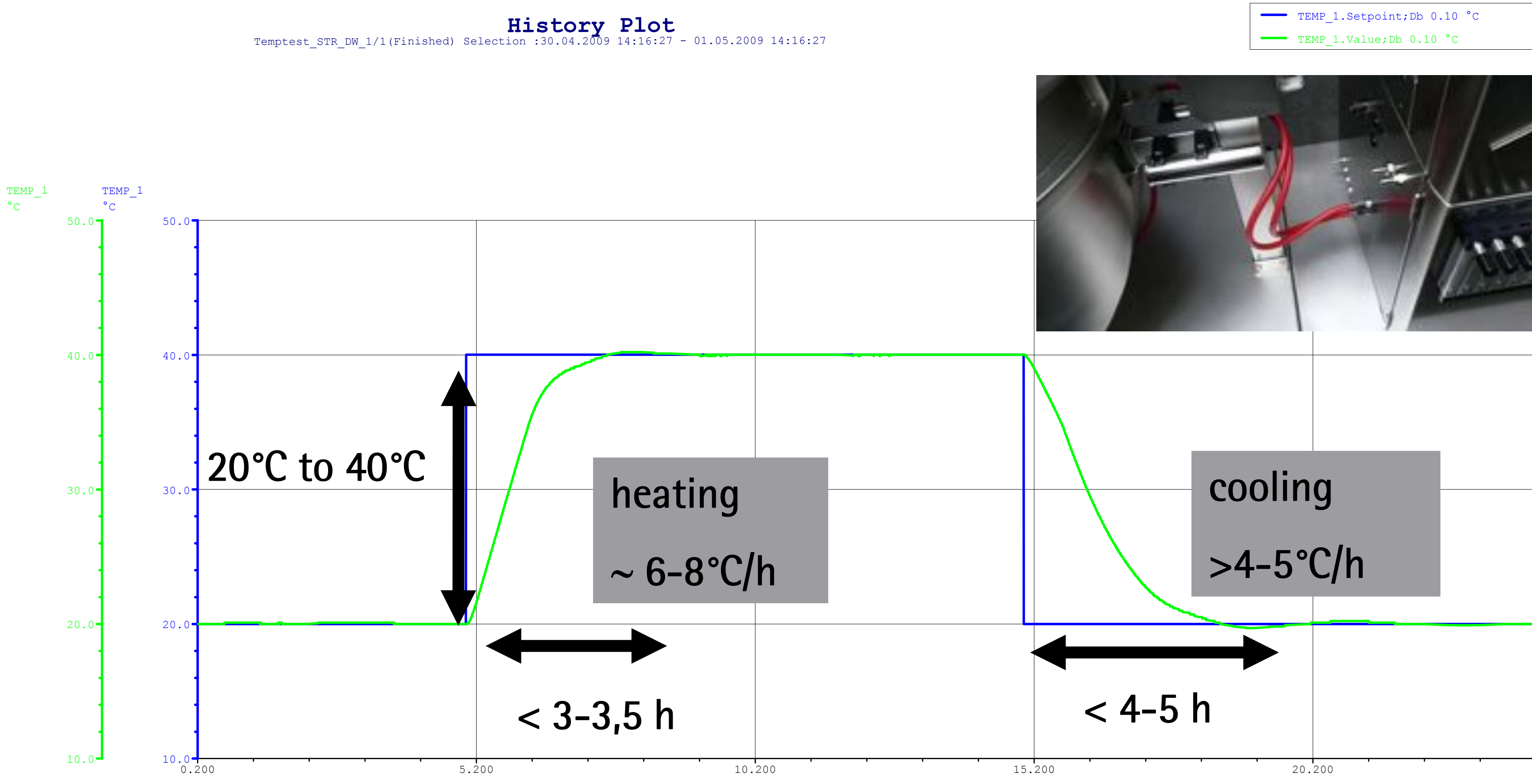
Double wall (heating and cooling)



1. Vessel Design
Temperature Control with heating blanket (only heating)

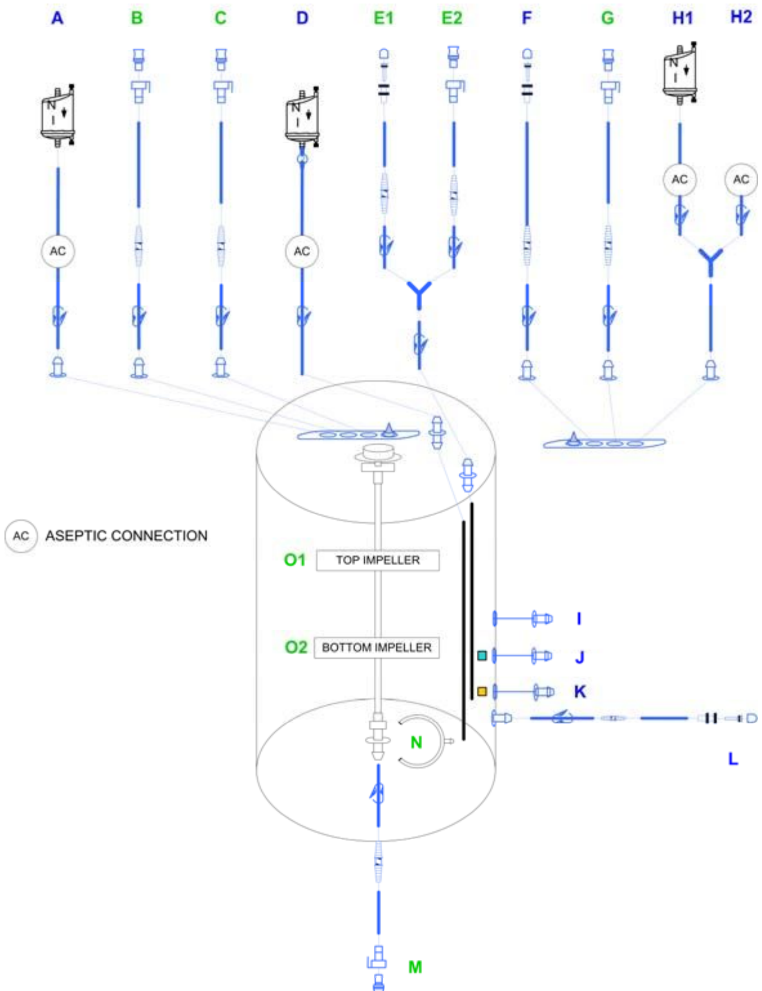


1. New development: Vessel Design
Temperature Control with double wall (heating & cooling)



2. Bag Design

2. Improved Bag Design: sterility, safety & longterm stability Flexibility in plastic



CultiBag STR Design:

- Multilayer film structure, Stedim 40
- Pre-installed stirrer
- Ports for harvesting, sampling, probes, addition, aeration.
- Disposable sensors pre-installed
- Tubing and connectors: Application based / customized solutions
- Increase of safety via second exhaust filter connection
- Height/Diameter ratio (TV): 2:1
- Height/Diameter ratio (WV): 1.3:1

3. Impeller design
Flexibility of mixing

- Type of impeller:
  - 2 x 3-blade segment impeller
  - 2 x 6-blade disk impeller
  - Combination
- Design is a copy of the classical impellers
- Impeller/Bag diameter ratio: 0.38

3. Impeller design
Summary Mixing Studies 200L

liquid volume [l]	agitation [rpm]	mixing time [s]
2 x 3-blade segment impeller		
130	50	26
130	100	18
130	150	12
200	50	24
200	100	16
200	150	11

→ very efficient mixing!!!

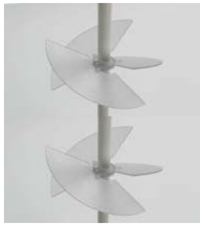
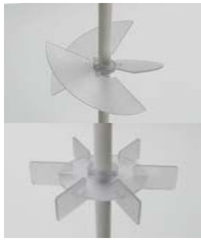
3. Impeller design
Summary Mixing Studies 200L

liquid volume [l]	agitation [rpm]	mixing time [s]
2 x 6-blade disc impeller		
130	50	20
130	100	15
130	150	13
200	50	25
200	100	18
200	150	16

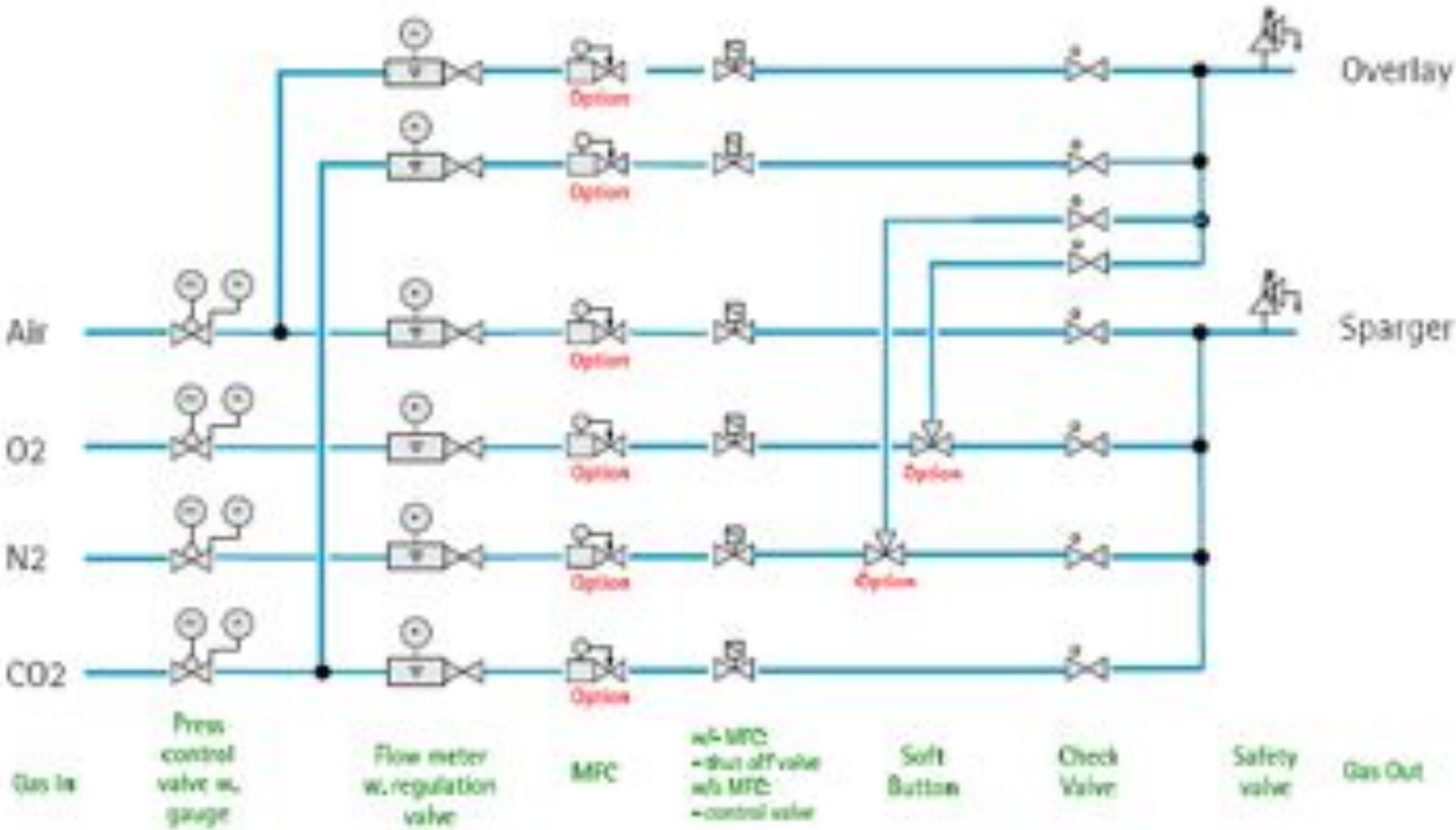
→ very efficient mixing!!!

3. Impeller design 200L
Tip speed & power input

agitation [rpm]	tip speed [m/s]
50	0.59
100	1.18
150	1.77

agitation [rpm]	power input per volume [W/m³]	type of impeller
50	2.2	
100	17.5	
150	71.0	
50	4.0	
100	32.2	
150	132	

4. Aeration/gassing design
Flexibility in gassing

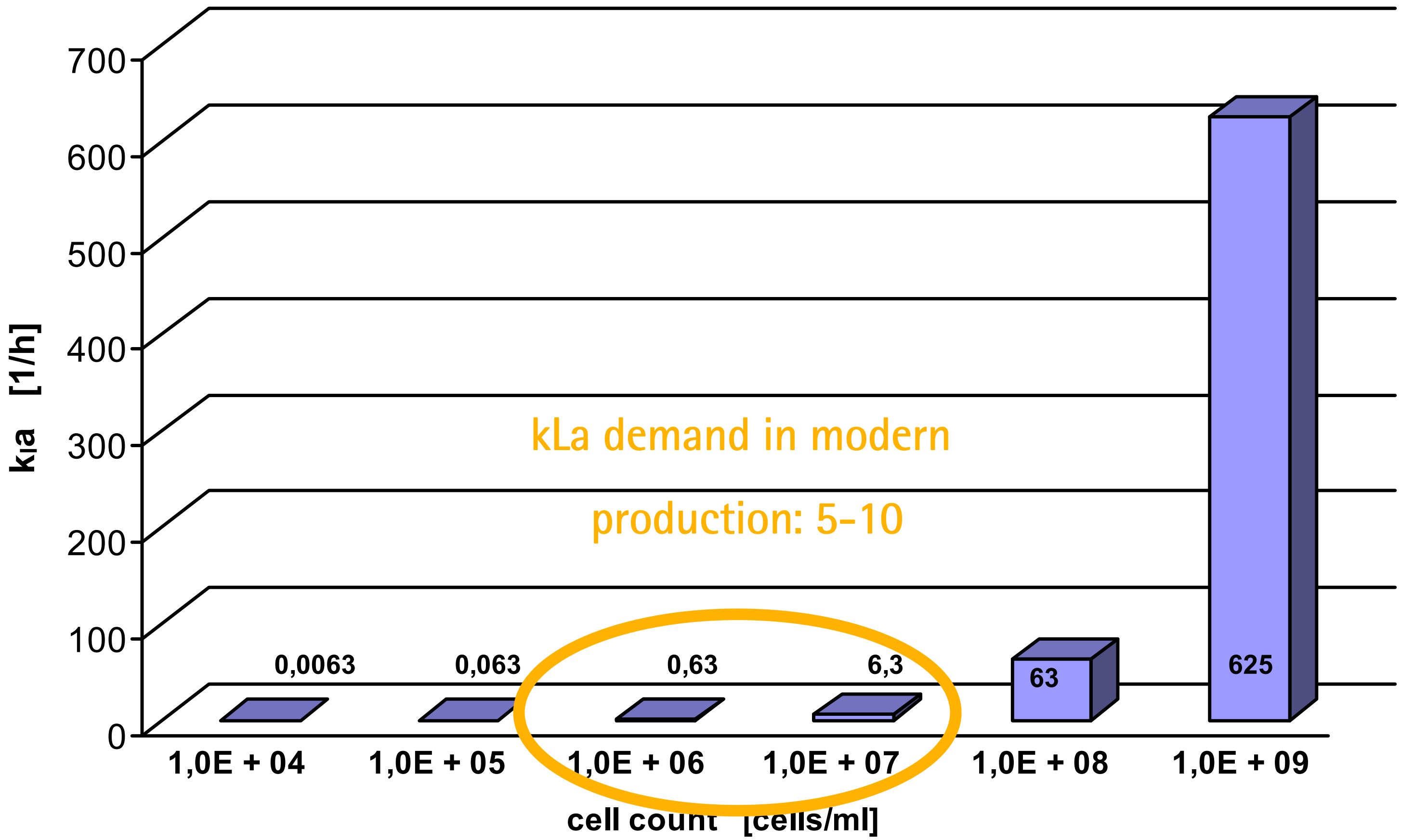


4. Aeration/gassing design
Sparger design

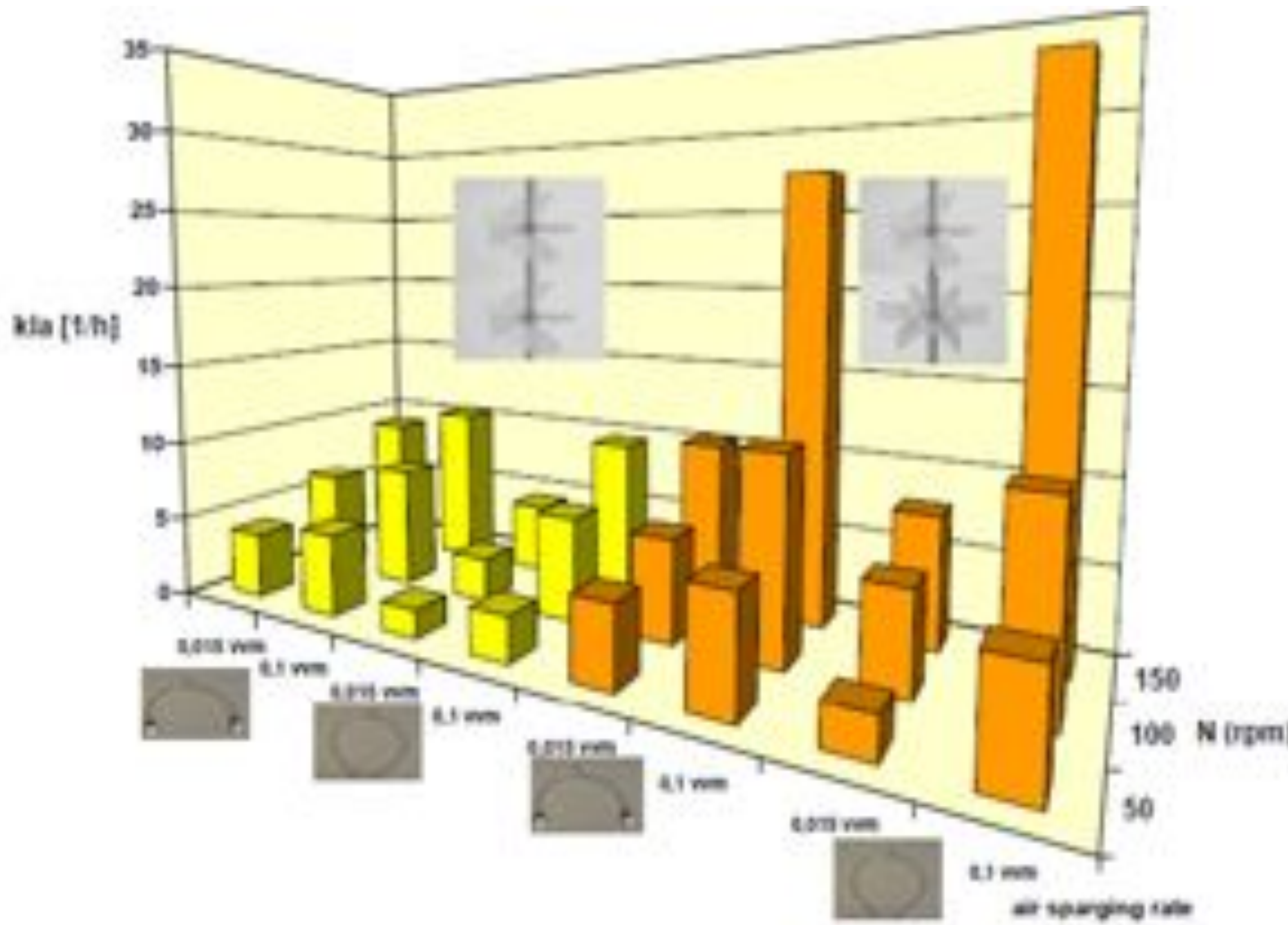
µ-sparger (PE; 18–45 µm)

ring sparger (PC; 0.8 mm)

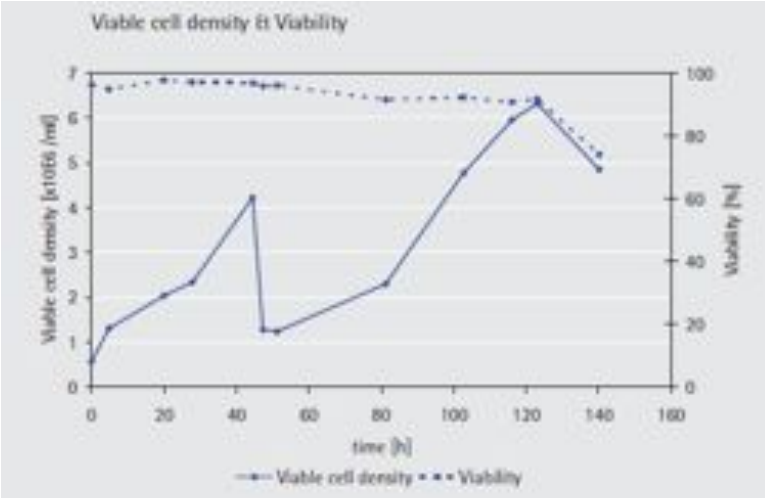
4. Aeration/gassing design : Theoritical calculation kLa vs cell density (CHO cultivation example)



4. Aeration/gassing design : Summary kLa studies based on different impellers, stirrer speeds & flow rates



Case study 1: CHO Cultivation



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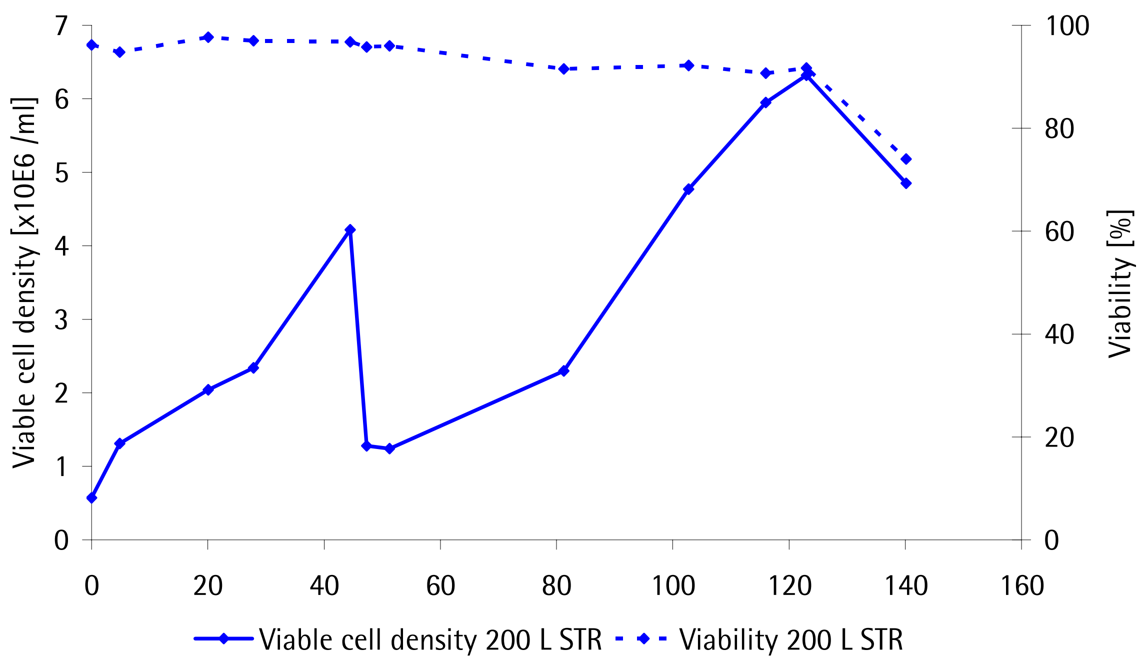
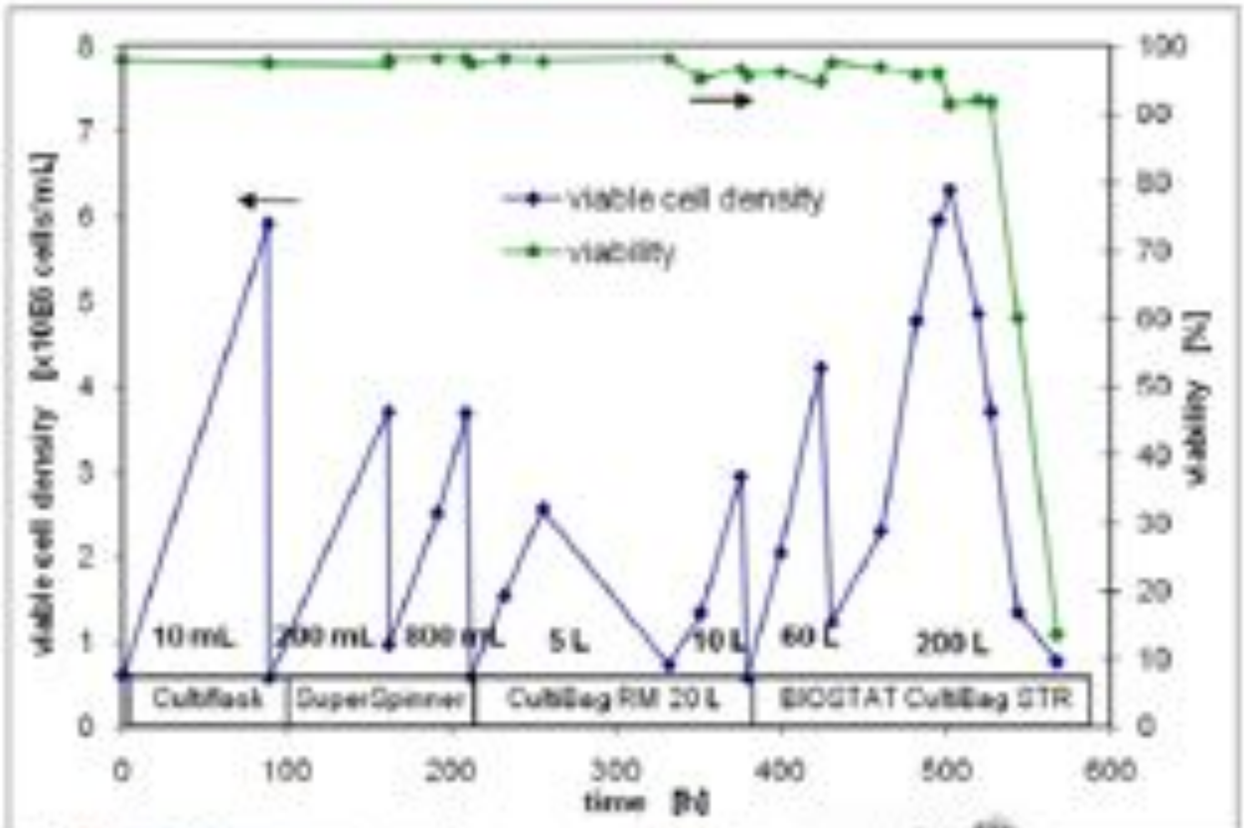
Case Study 1
CHO clone DG44 ST1-6 in Serum free chemically defined media

Viable cell density & Viability

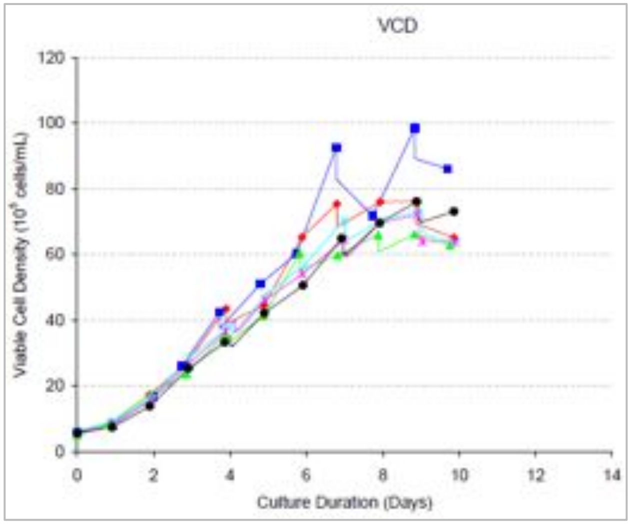


⇒ **No differences in cell density and viability between BIOSTAT® CultiBag STR and classical reusable bioreactor** (typical 6-7 x 10E6 cells/mL for this clone & media)

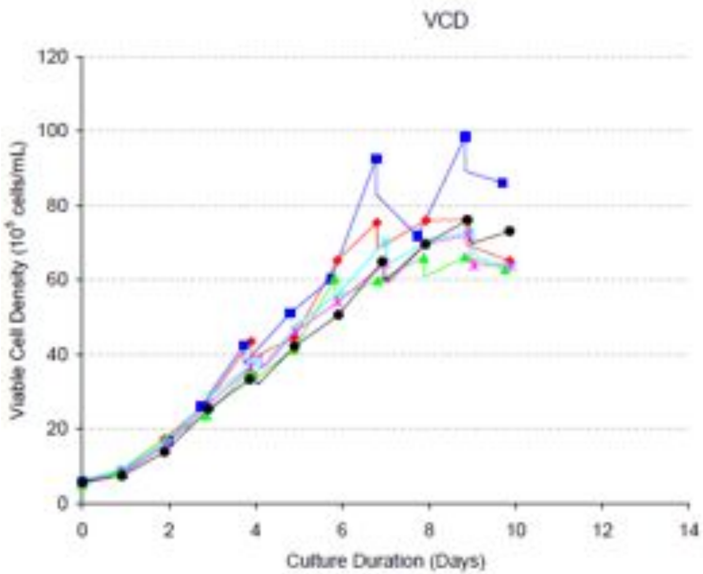
Case Study 2
"Disposable Factory" Upstream

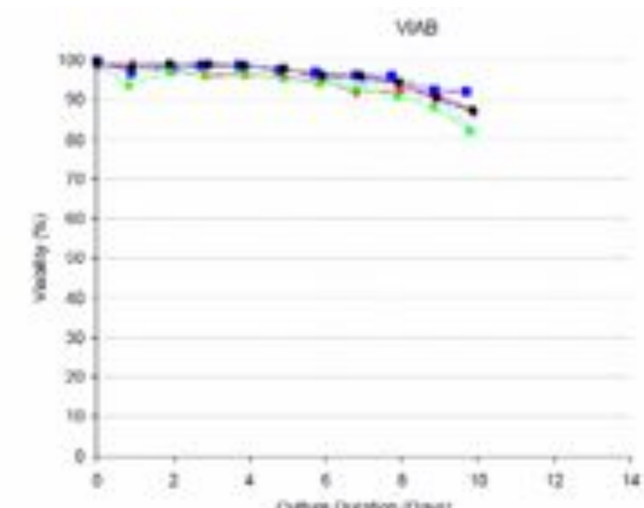


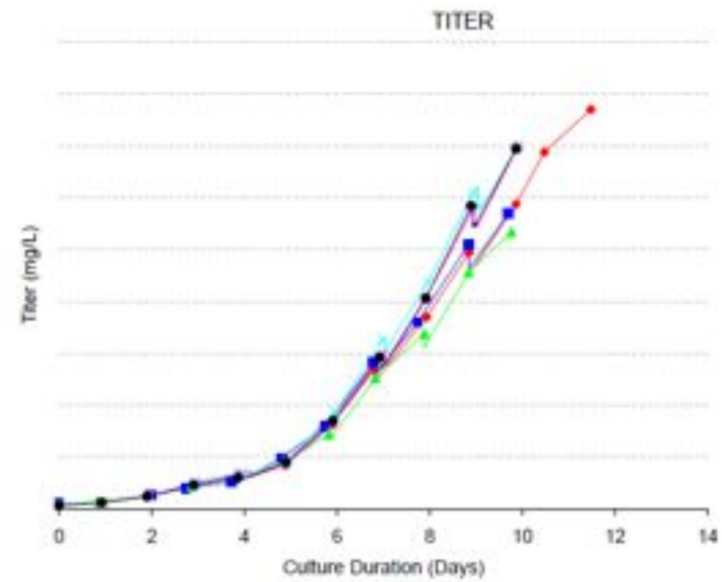
Case study 3: CHO Cultivation (Customer X)



Case Study 3 (Customer X) CHO Cultivation







⇒ **Comparable Results**

Summary: „Classical" single-use bioreactors

Benefits for upscaling when utilizing single-use bioreactors with a classical design

- **Scale-up parameters identical to classical process**
  - tip speed, power input, agitation, k_La,...
- **Easy optimization during the complete process**
  - ⇒ User can implement his experience from classical processes into single-use applications
- **Easy transfer to 5-10,000L stainless steel reactor due to comparability in design**
  - ⇒ Reduction of time, need for optimization & cost during scale-up

Future of modern pharmaceutical manufacturing for upstream processes

- Completely single-use stirred bioreactor
- Advanced control and data-storage
- Classical design on all critical parameters

- Easy transfer from reusable to single-use process
- Easy transfer from single-use to reusable process
- Scaleability -> from R&D to production scale

Thank you for your attention.

Any Questions?

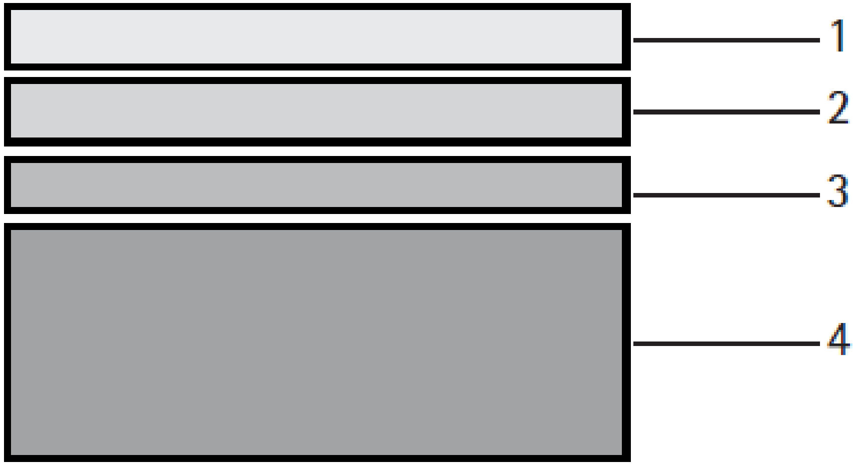
Dr. Andreas Kocourek, Sartorius Stedim Biotech, Application Specialist, Fermentation

Australia (andreas.kocourek@sartorius-stedim.com)

2. Bag Design
Stedim 40

1 Polyethylene terephthalate (PET)

acts as a light, strong and clear protective outer layer. PET provides robustness..



2 Polyamide (PA)

increases durability and strengthens the bag..

3 Ethyl Vinyl Alcohol (EVOH)

acts as the main gas barrier.

4 Ultra-Low Density Polyethylene (ULDPE)

acts as the fluid contact layer. The S40 Polyethylene material is in compliance with usual pharmacopoeias and provides a clean, inert and highly chemical resistant contact layer.