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Challenges of scale down model for disposable bioreactors: Case studies on growth & product quality impacts

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Abstract

Despite wide-spread use of disposable bioreactors, there is a lack of well-established scale-down model for larger scale SUBs. Here we report a case of NS0 cell culture process transfer from 2000L stainless steel bioreactor (SST) to 2000L disposable bioreactor (SUB). Initial attempts in trying to grow the NS0 cells in the small scale 2D bags yielded non-satisfactory results, as growth was impacted by bag material type as well as by suppliers of the same bag material type. However, 3D bags of 50L and above proved to be supportive of the NS0 cell line growth.

Even for cell lines that do not have growth issues in SUBs, surprising product quality difference between SUBs and traditional bench top glass bioreactors are still being observed, thus making the bench top glass bioreactors non-ideal as scale down models. We report two cases where glycan profiles of the expressed antibody products show such dramatic differences. In one case, extensive testing of glass bioreactors from various suppliers led to a particular type being able to mimic the glycan profiles from the SUB, whereas in the other case, alternative scale down model had to be identified and the process had to be modified to maintain the glycan profiles when scaling up to the 200L SUB.

Leachables & extractables on SUBs

- Concern on L&E for cell culture is one of the main challenges for SUB implementation
- Impact of L&E for cell culture
  - ➢ Patient safety: toxic effects on patients
  - ➢ Process impact: cell culture performance impacts

▪Not all bags are the same

- Different bags have different materials & are made in different ways
- Even bags with same contact layer material had different impact on growth
  - Other materials, e.g., additives, could have major impact
  - Ex: HyClone's new Aegis5-14 film

Disposable Bag	Contact Layer Material
Hyclone SUB	**ULDPE**
Hyclone Container Bag	**ULDPE**
WAVE Bag	**EVA**
Sartorius RM Bag	**EVA**
Sartorius Flexboy Bag	**EVA**
Sartorius Flexel Bag	**ULDPE**
Sartorius STR CultiBag	**ULDPE**
Shake Flask	**PC**
Millipore Container Bag	**ULDPE**

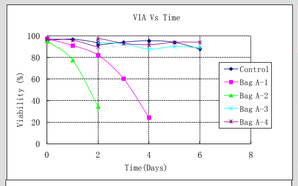
Lack of good scale down models for SUBs

- None of the major suppliers of SUBs offer representative scale-down models of the larger scale SUBs
- 50L SUBs appear to be the most appropriate models to represent 2000L scales. But it is too expensive to be an economical model
- Benchtop glass bioreactors are still being widely used as scale-down models for large scale SUBs.
- However, leachables & extratables can not be tested with glass bioreactors. Product quality impact from SUBs also can not be evaluated with glass bioreactors

Case Study I: growth challenges when transferring process from 2000L stainless steel bioreactor to 2000L SUBs

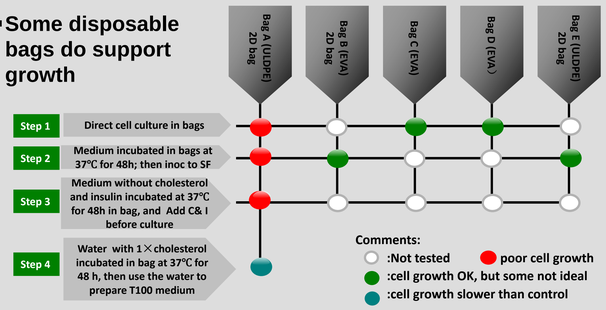
▪Background: NS0 cell line with chemically-defined medium
  ▪Medium contains insulin & cholesterol
  ▪Robust process demonstrated by 2000L SST GMP runs
➢ Objective: transfer & scale-up to 2000L SUB for PhIII trials

▪Challenges in growing NS0 cells in disposable bags



Study name	Study conditions
Bag A- 1	Direct culture in Hyclone 2D bag
Bag A- 2	Media incubation @ 37C, 48hr; then inoc to SF
Bag A- 3	Media w/o cholesterol & insulin incubated @ 37C, 48hr; then add chol & insulin back; inoc to SF
Bag A- 4	Water w/ 1x chol incubated @ 37C, 48hr; then used to prep medium and inoc to SF

▪Some disposable bags do support growth



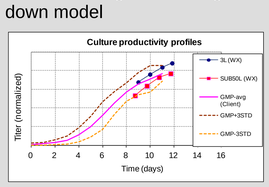
➢ **Various attempts in trying to grow the cells with the 2D Bag A did not succeed**

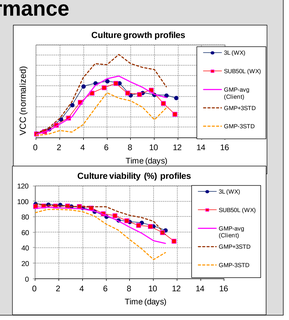
▪Decision to try 50L SUB directly

- Various attempts using vendor A 2D bags did not lead to satisfactory results
- The fact that other bags w/ the same ULDPE material supported growth was encouraging
- 2D bag might not be a good scale-down model of 3D & large volume bags
  - Surface to volume ratio much larger
  - If there are leachables, 2D bag would be worst case scenario
- Two options
  - Try vendor A 50L SUB to see if growth is OK
  - Try vendor B SUB

▪1st 50L SUB showed good performance

- Culture performance in 50L SUB was comparable to 3L glass vessel and historical GMP data
- Indeed 2D bag was not a good scale-down model





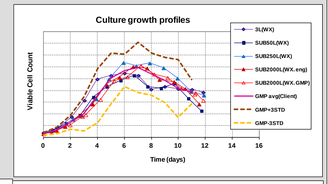
Case Study I (Cont'd)

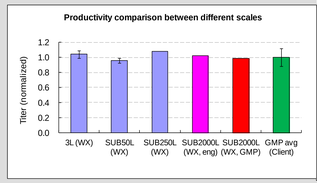
▪Process confirmation at 250L SUB

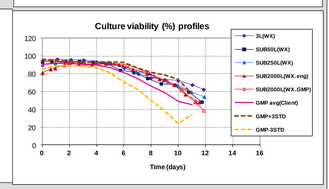
- 250L SUB as last step before scaling up to 2000L SUB
- Process designed to mimic 2000L operation as much as possible
- Good performance at 250L SUB, with full analytical comparability assessment
- Cleared to scale-up to 2000L SUB

➢ **Successful scale-up to 2000L SUB**

- Growth & productivity at 2000L SUB (eng run & GMP run) matched very well with historical GMP data

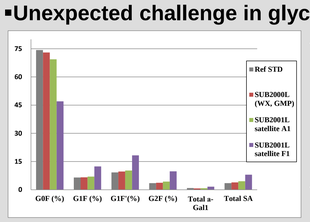






Case Study 2: product quality challenges in bench top glass BR scale down models for 2000L SUBs

▪Unexpected challenge in glycosylation profiles

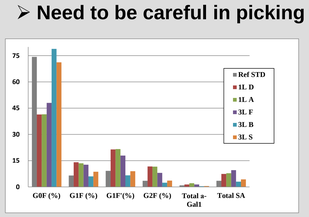


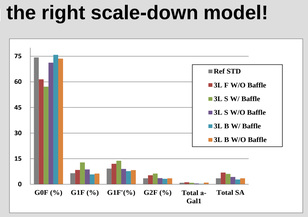
- Satellite cultures of 2000L SUB had dramatic difference in glycosylation profiles
  - One matched 2000L SUB well
  - The other had significant differences
- Other performance indicators were comparable, e.g., titer, growth etc

▪Unexpected challenge in scale-down model transfer

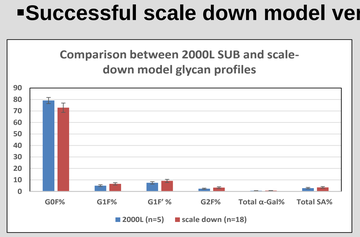
- Significant differences in glycan profiles among different small scale cultures
  - Difference between 1L vs 3L model
- Even among 3L bioreactors, difference remained
  - Glass vessel had same dimensions
  - Agitator diameter different
  - Sparger different
  - Baffle presence or not also made a difference

➢ **Need to be careful in picking the right scale-down model!**





▪Successful scale down model verification

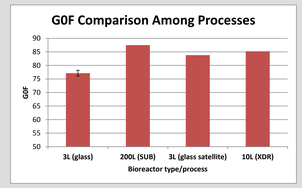


- Picked the BR model that's closest to 2000L SUB data, and also most consistent product quality data

Case Study 3: product quality challenges when scaling up from benchtop glass BR to 200L SUBs (with ambr to rescue!)

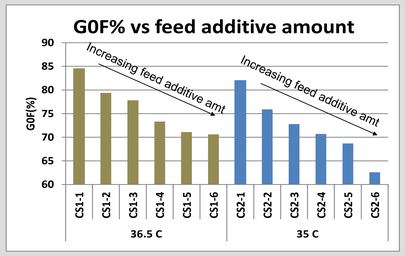
▪Background: biosimilar CHO cell culture process developed in benchtop glass BR
▪Objective: scale-up to 200L SUB

▪Surprise seen when process scaled up to 200L SUB



- Much higher G0F
- ➢ **Failed lot!**
- More data revealed potential difference from plastic vs metal material
- N culture vessel material
- N-1/N-2 culture vessel material

▪Process development to fine-tune glycosylation profile



- Shake flasks and ambr15 microbioreactors (from TAP) were used for new process evaluation (not glass BR)
- Process identified to hit target G0F in ambr
- A single feed additive was shown to be very effective in adjusting glycan profiles

➢ 200L process proposed based on ambr study results

▪Successful scale-up and confirmation at 200L SUB

Sample ID	G0F(%)
Reference STD	69
Old process ambr	84.5
Optimized process ambr	68.7
Old process 7L glass BR	74.0
Optimized process 7L glass BR	40.1
Old process 10L XDR	85.2
Optimized process 10L XDR	64.7
Old process 200L SUB	87.5
Optimized process 200L SUB (n=3)	71.0 +/-2.0
Old process 200L satellite 3L glass BR	83.8
New process 200L satellite 3L glass BR	52.9

➢ Ambr microbioreactor (15mL scale) served as a good scale-down model for the 200L SUBs

➢ With the modified process, glycan profiles between 3L benchtop glass BR and the 200L SUB still had dramatic differences
  ▪ GE's Xcellerex 10L XDR did better than the glass BR, but not as good as ambr

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