9-25-2018

Continuous Process Performance Enhancements for 50-500 L S.U.B.s

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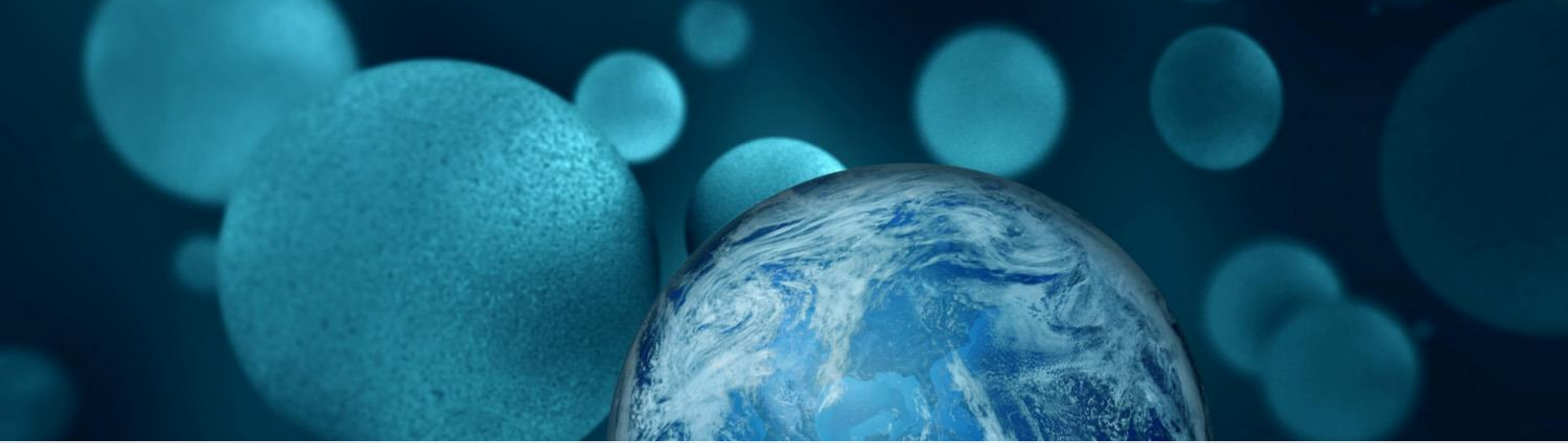

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Continuous Process Performance Enhancements for 50-500 L S.U.B.s

September 25, 2018

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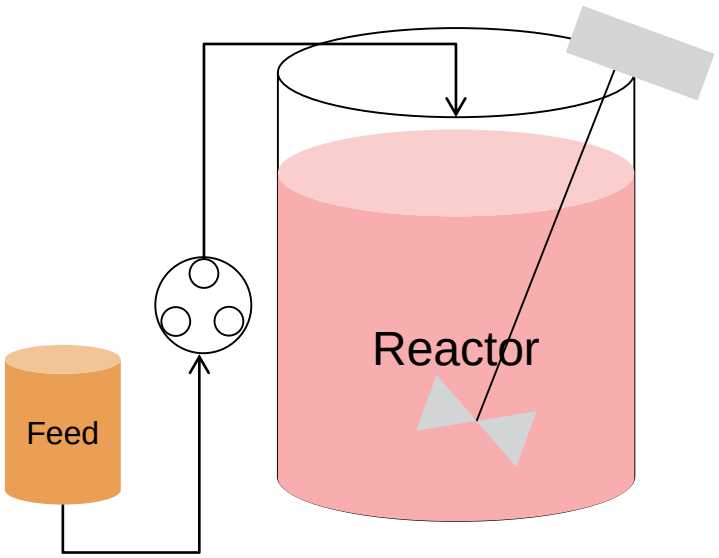
Outline

- Perfusion Overview and Workflow
  - Mass transfer limitations
  - Mixing limitations
- S.U.B. Enhancements
- Results
  - Mass transfer performance
  - Cell culture case study
  - Cell culture mass transfer models

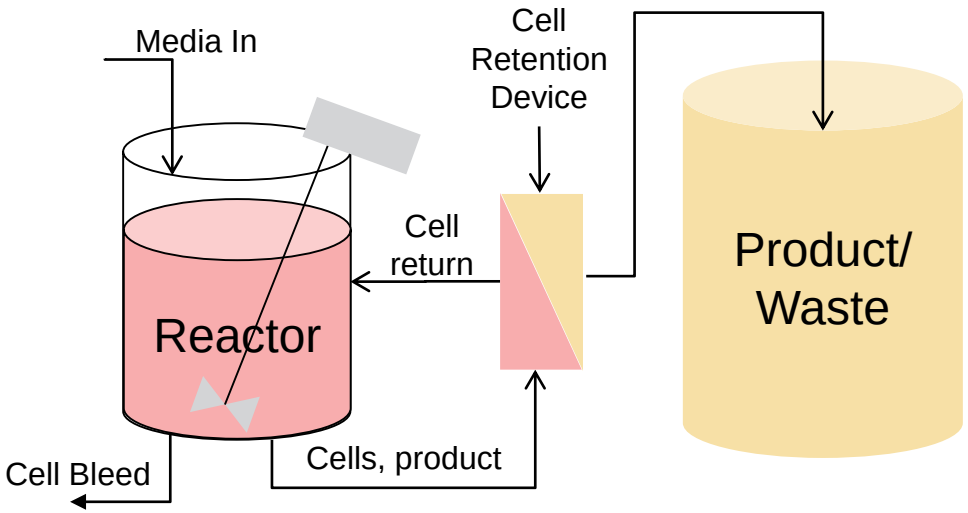
Perfusion vs. Fed-batch

Fed-batch



- Feed addition from D3-end
- Volume change in reactor
- High titers achievable
- Large production vessels (≥2000L)

Perfusion



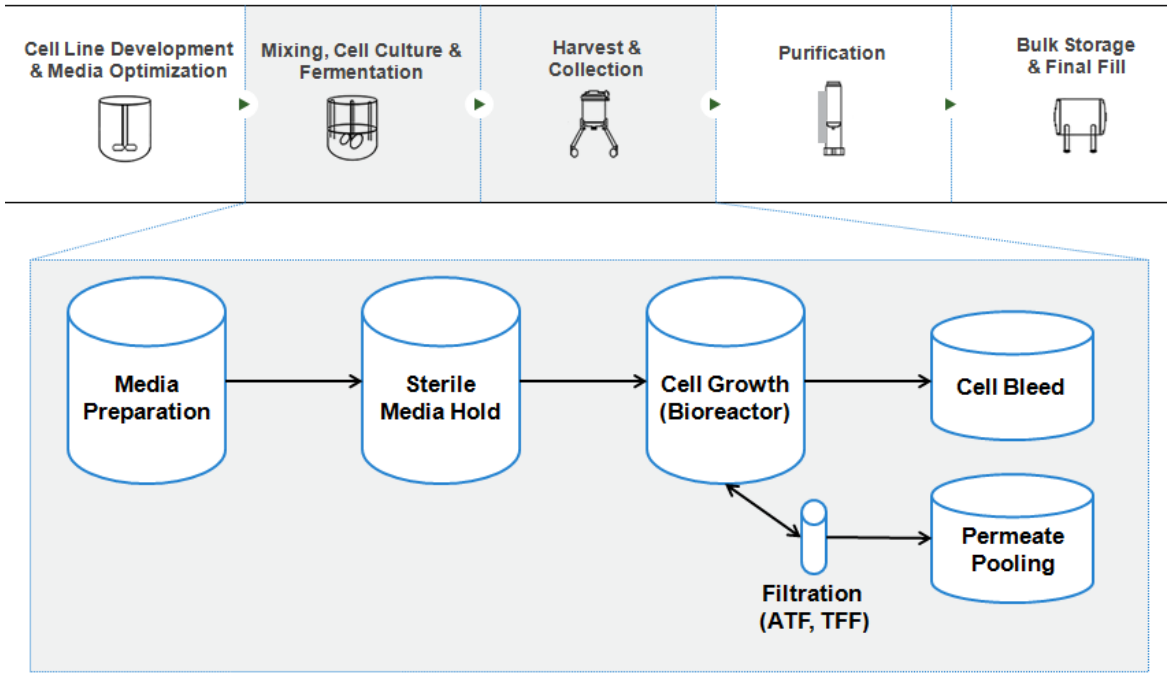
- Continuous filtration for ≥21 days
- Cell retention usually via filtration (0.2-0.65µm)
  - Concentrated Fed-batch uses 30-50 kDa
- Used for:
  - Unstable products
  - Intensified seed train
  - Cell banking

Perfusion Advantages/Challenges

- Advantages
  - Smaller reactors
  - Healthier culture with less waste = predictable product quality
  - Higher cell densities
  - Consistency from steady state production
  - Less frequent bioreactor turnaround
  - Clean harvest stream for DSP
  - Continual harvest ensures some success despite late-stage contamination
  - Cost savings

- Challenges
  - What is steady state?
  - Process control and integration
  - More complex setup (Regulatory)
  - High validation costs
  - Filter fouling leads to culture shutdown
  - Higher gas usage = foaming risk
  - Sensor stability

Perfusion Work Flow



- Thermo Fisher Scientific Offerings:
  - Cell line development
  - Media optimization
  - Media hydration
  - Sterile media hold
  - **Bioreactor production vessels**
  - Product pooling
  - Waste containment
  - Chromatography resins
  - Bulk storage and final fill

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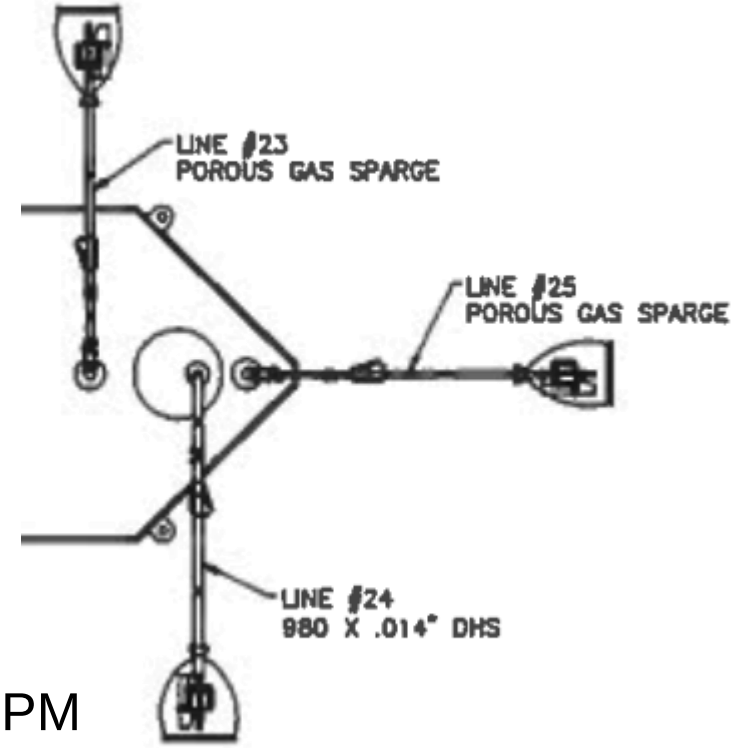
Overcoming Legacy S.U.B. System Limitations

- Custom 500 L S.U.B. configuration
  - Standard impeller (25.1 cm diameter)
  - Standard laser DHS (980 pores, 0.368 mm Ø)
  - 2 ea frit (1 standard, 1 additional)
- Standard Dynamic k_La testing
  - 1 g/L pluronic
  - 3.5 g/L HEPES
  - DHS = **15 slpm**
  - Agitation = **150 RPM** (65 W/m^3)

		Additional Frit (slpm)		
		10	20	40
Standard Frit (slpm)	10	36.5	37.6	38.1
	20	37.6	40.5	42.6
	40	39.6	40.2	41.9

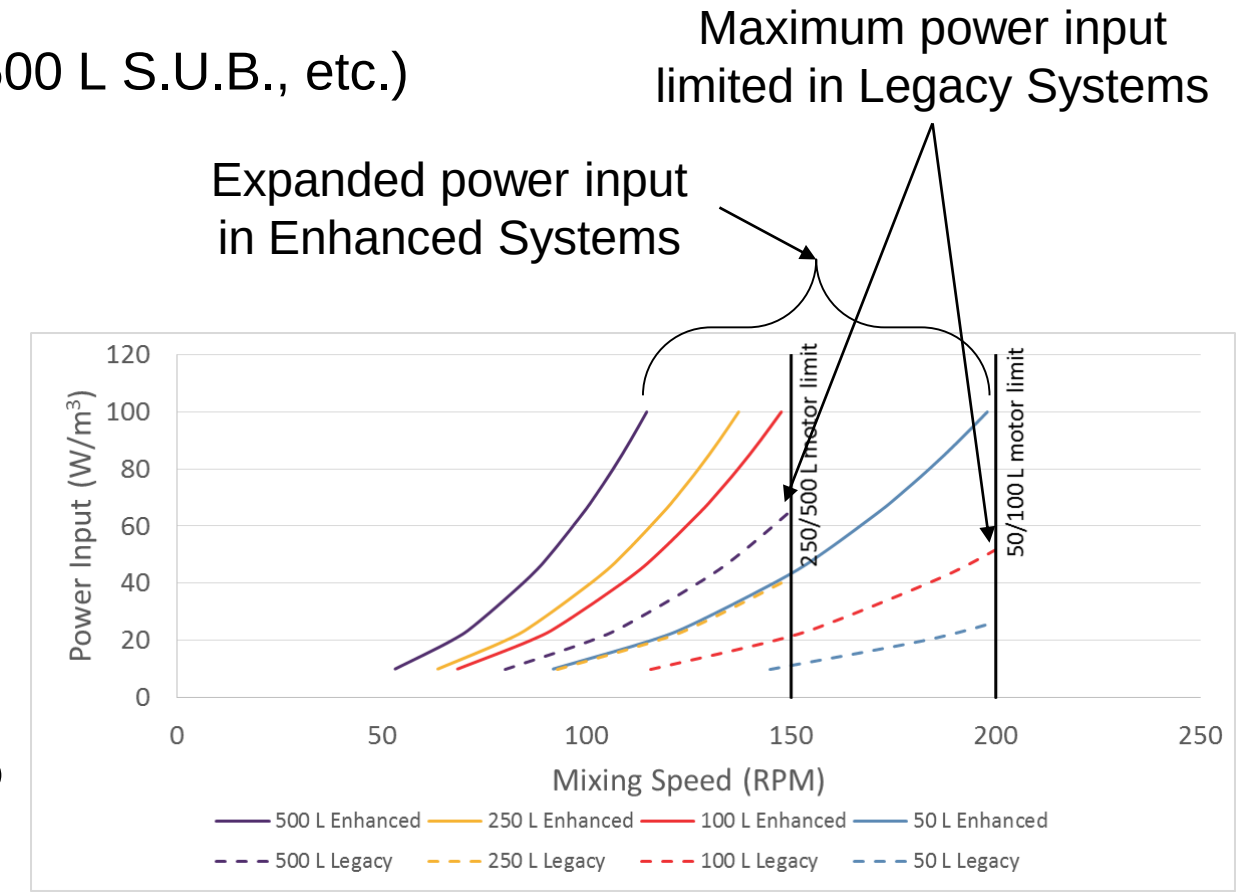


- Limited benefit from running frits at extreme flow rates even at max RPM
  - Combined air sparge of 95 slpm (0.19 VVM) = ~42/hr
  - Second option would be to increase DHS sparging rate

S.U.B. Mixing Enhancements

- Implement upsized impeller (1000 L impeller in 500 L S.U.B., etc.)

	S.U.B. Size	50	100	250	500
	Max Motor RPM	200	200	150	150
Standard Impeller	Diameter (cm)	11.11	14.6	20	25.1
	Speed at 20 W/m^3 (RPM)	182.5	145.8	117.1	101.1
	Max PIV at max RPM (W/m^3)	26.3	51.6	42.0	65.4
Upsized Impeller	Diameter (cm)	14.6	20	25.1	32.1
	Speed at 20 W/m^3 (RPM)	115.7	86.3	80.2	67.1
	Max PIV at max RPM (W/m^3)	**103.2**	248.9	130.8	223.7

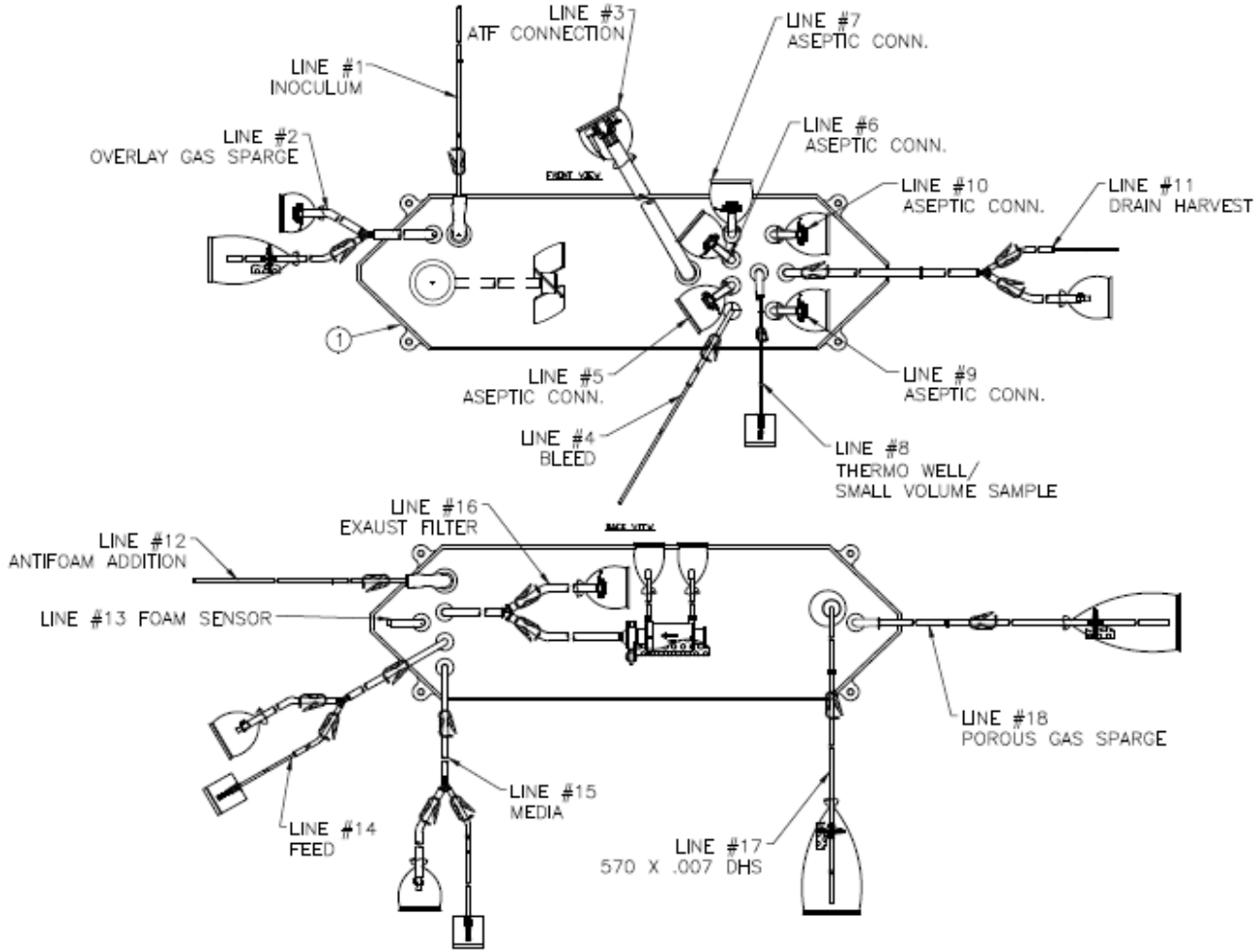


All systems meet objective of 100 W/m^3

Note: Safely within the qualified design space of the 1000L/2000L S.U.M. (rated up to 350 RPM).

S.U.B. BPC Perfusion Enhancements

1. Enhanced DHS for each S.U.B. size
   - Increased DHS efficiency (smaller pore/bubble)
   - Boost capacity (0.2 VVM @ <15m/s GEV)
   - Frit optional in all S.U.B. designs
2. Upsized impeller in each S.U.B. size (n+1)
3. SU foam sensor for automated antifoam dispensing
4. Port for cell bleed – maintain cell density in perfusion cultures
5. 1” ID ATF/TFF connections available



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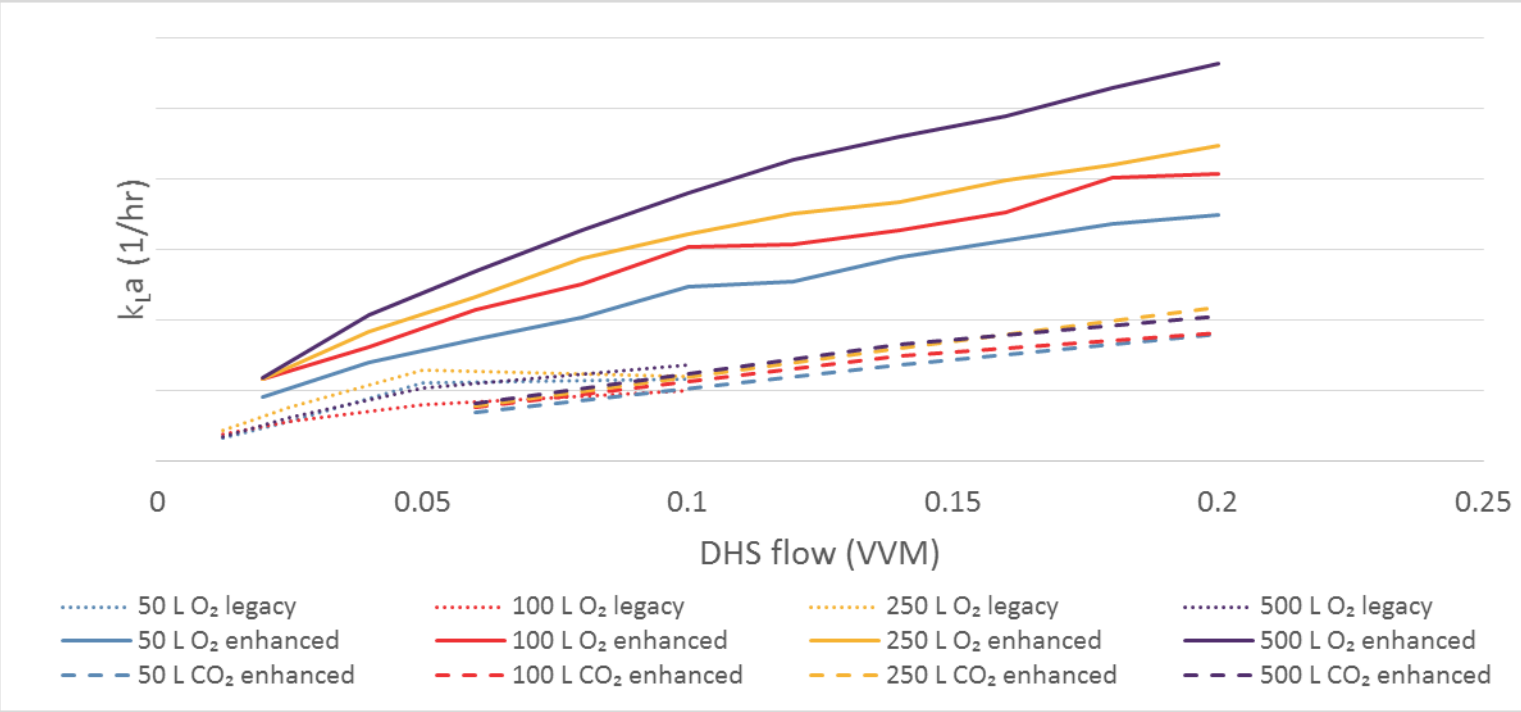
Characterization Results with Enhanced Sparging and Mixing



50L Perfusion S.U.B. Case Study Setup

	Components/Conditions	Setting
Cells/Media	Cell Line	CHO-DP12 (Supplied by Repligen)
	Seed Density	0.4 x 10^6 cells/mL
	Target Cell Density	Various up to maximum
	Media Composition	CD OptiCHO media + 100 ng/mL LR3 + 4 mM Glutamax
	Base	N/A
	Antifoam	Antifoam C (10,000 ppm simethicone stock)
	Glucose	450 g/L stock
Bioreactor	Bioreactor W.V.	40 L
	Temp	37°C
	DO Setpoint	30% (O_2/air through DHS)
	pH	7.0 ± 0.2 (CO_2)
	Air	Headspace sweep at 2 slpm
	Agitation	107-184 RPM 20-100 W/m^3
	Antifoam dosing	Sensor-based antifoam addition
	Glucose Feed	Maintain 1-3 g/L with constant feed
	Cell Bleed	Sensor-based cell bleed (ABER)
ATF	ATF System	ATF6SU
	ATF Filter	F6:RF02PES 0.2 micron PES HF
	Filter SA	2.53 m^2
	Perfusion rate	Day 0-3: None Day 3-26: variable depending on cell density/nutrient demands, 1-4 VVD (40-160 L/day)
	Flux	0.67-2.67 LMH
	ATF Rate	19.2 LPM
	Shear Rate	2264 s^{-1}

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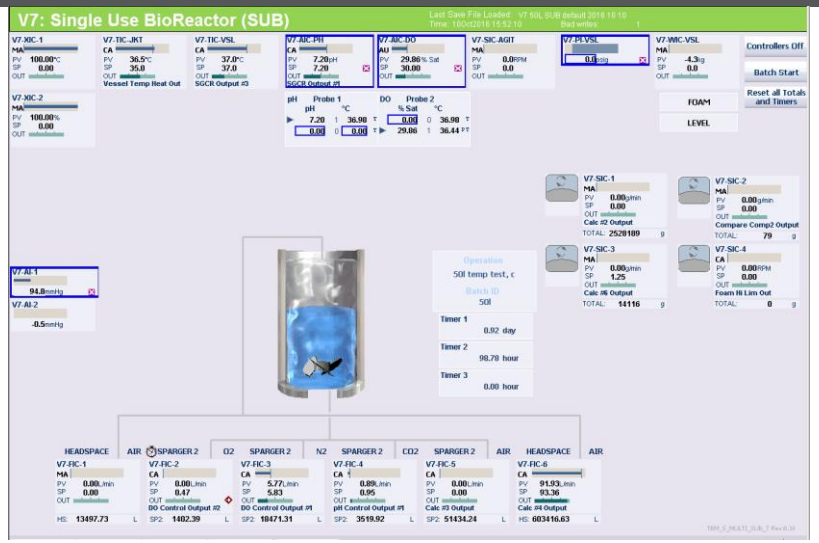
Perfusion Process Automation

- Standard
  - DO (TruFluor DO)
  - pH
  - RTD
- Foam sensor linked to antifoam pump
- Cell density sensor linked to cell bleed pump
- S.U.B. load cells linked to media pump (Heaviside equation)



$$\text{Feed} = 2 \cdot \text{Target} \cdot \left(\frac{1}{1 + \exp^{-2 \cdot k \cdot x}} \right)$$

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Cell density balance (probe-less)

$$V_R \cdot \frac{dX_R}{dt} = V_R \cdot \mu \cdot X_R - Q_B \cdot X_R$$

$$\mu = \frac{\ln(X_2) - \ln(X_1)}{t_2 - t_1} + \frac{Q_B}{V_R}$$

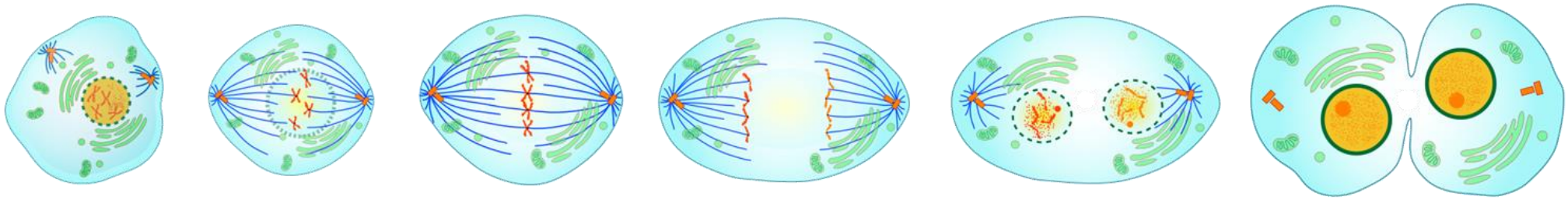
$$Q_B = \left(\mu - \frac{\ln(X_2/X_1)}{t_2 - t_1} \right) \cdot V_R$$

Equation

- Change in cell density = effective growth – cell bleed
- Rearrange, integrate, solve for **Q_B** to obtain bleed rate
- Best to average growth rates over multiple days

Nomenclature

- V_R = reactor working volume
- X_R = cell density
- dX_R/dt = cell density change over time
- μ = cell growth rate
- Q_B = reactor bleed rate



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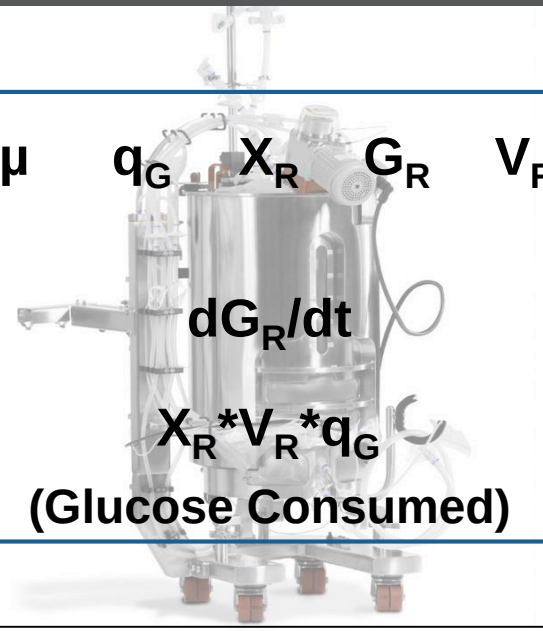
Glucose Mass Balance Equations



$Q_G = ?$ | $Q_G \cdot G_G$ (Glucose Feed)

$Q_M = Q_P + Q_B - Q_G$ | $Q_M \cdot G_M$ (Media Feed)

μ q_G X_R G_R V_R

dG_R/dt

$X_R * V_R * q_G$

(Glucose Consumed)

$Q_P \cdot G_P$ | $Q_P = V_R \cdot D$ (Permeate)

$Q_B \cdot G_R$ | $Q_B = (\mu - \ln(X_T/X_R)) \cdot V_R$ (Cell Bleed)

Cell Growth: $$V_R \frac{dX_R}{dt} = V_R \cdot \mu \cdot X_R - Q_B \cdot X_R$$

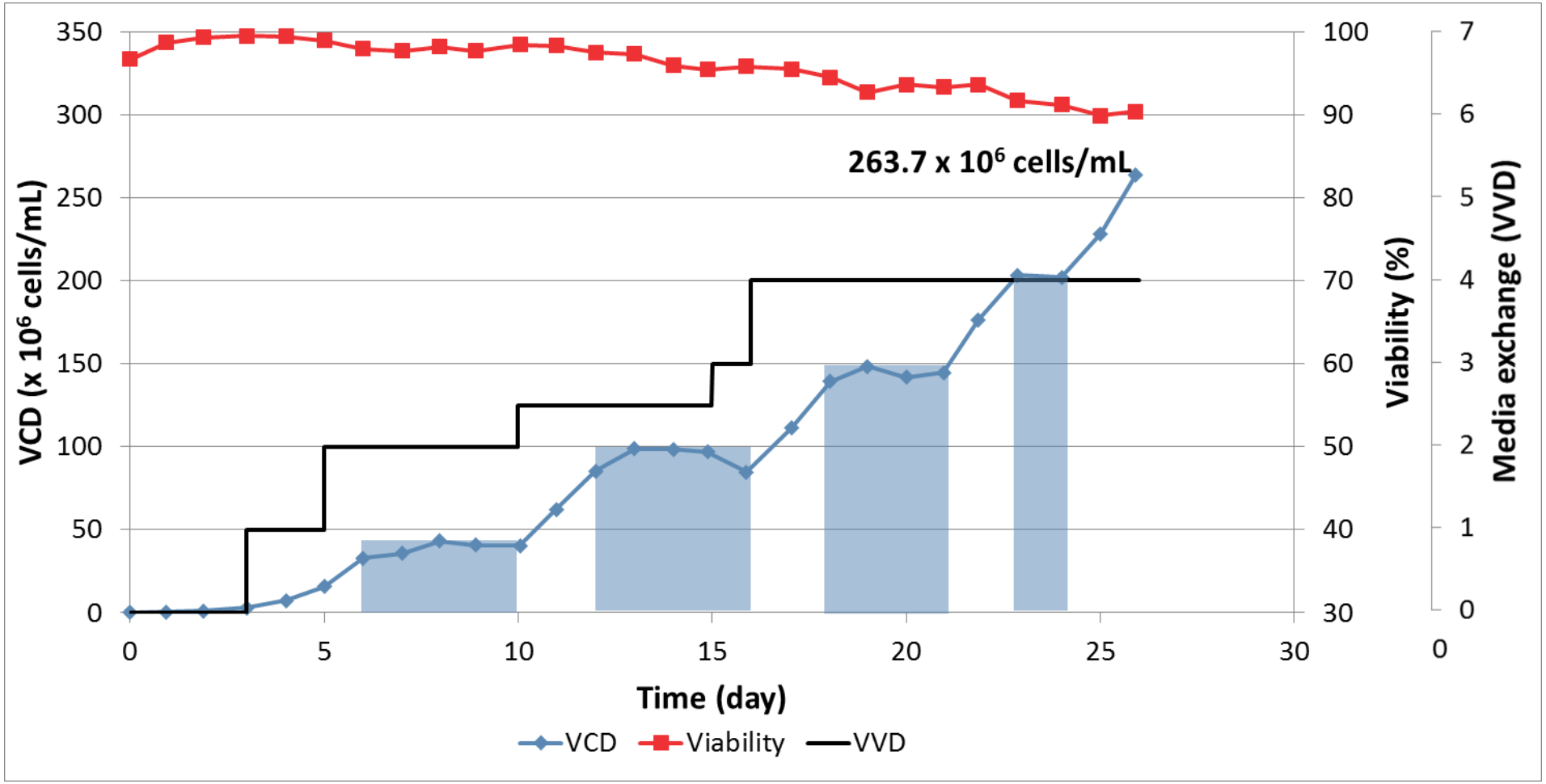
Overall Glucose Consumption: $$\frac{dG_R}{dt} = Q_M \cdot G_M + Q_G \cdot G_G - Q_P \cdot G_P - Q_B \cdot G_R - X_R \cdot V_R \cdot q_G$$

If $$\frac{dG_R}{dt} = 0 \text{ and } \frac{dX_R}{dt} = 0$$

Then $$Q_G = \frac{[D \cdot G_P + \mu \cdot G_R + X_R \cdot q_G - (D+\mu) \cdot G_M] \cdot V_R}{(G_G - G_M)}$$

Nomenclature
Q = flow
G = [glucose]
μ = growth
q_G = gluc consumption
V = volume
X = [cells]
D = perfusion rate

Cell Culture Results – 50 L S.U.B. Cells



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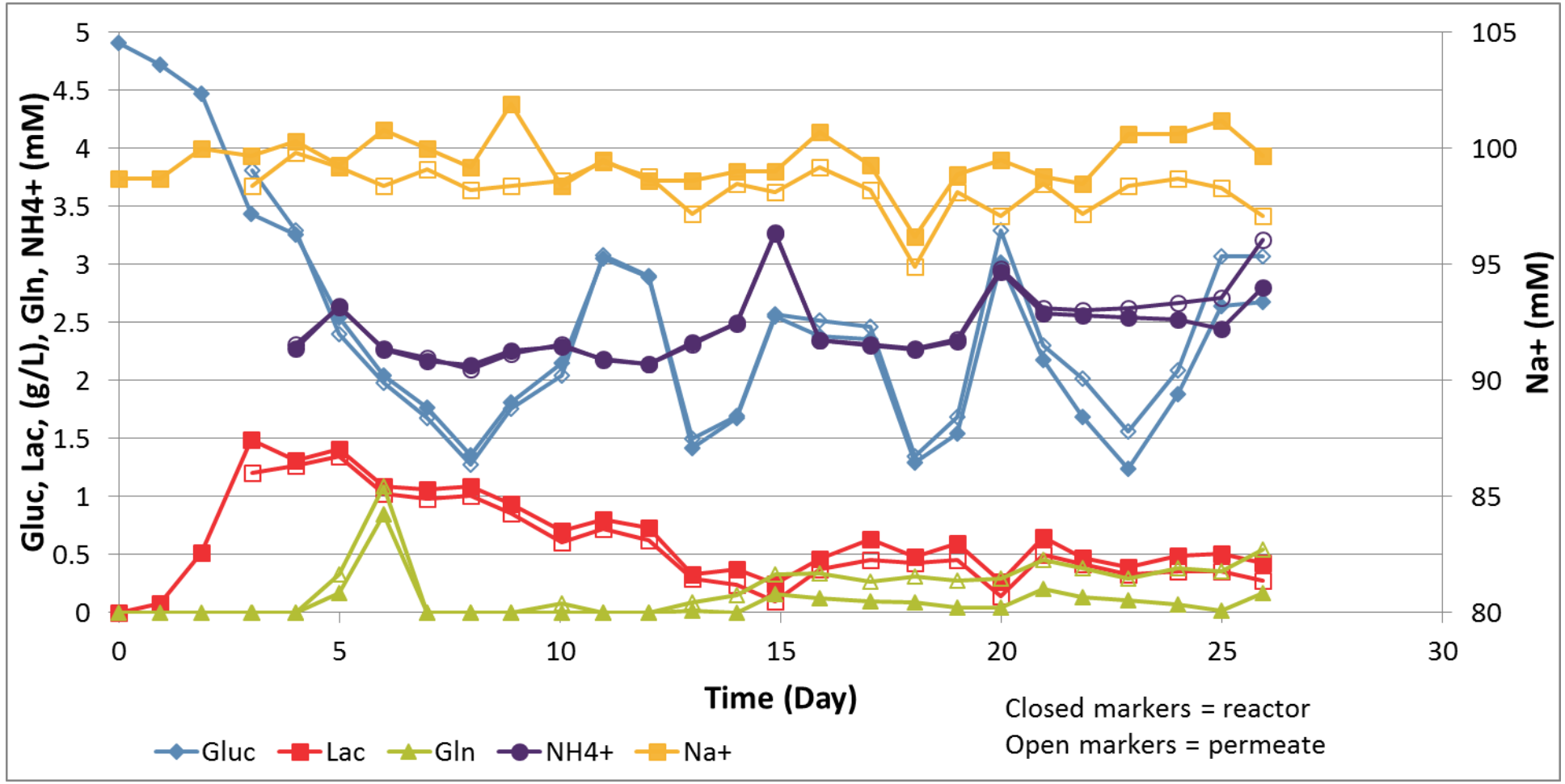
Cell Culture Results – 50 L S.U.B. Culture Parameters



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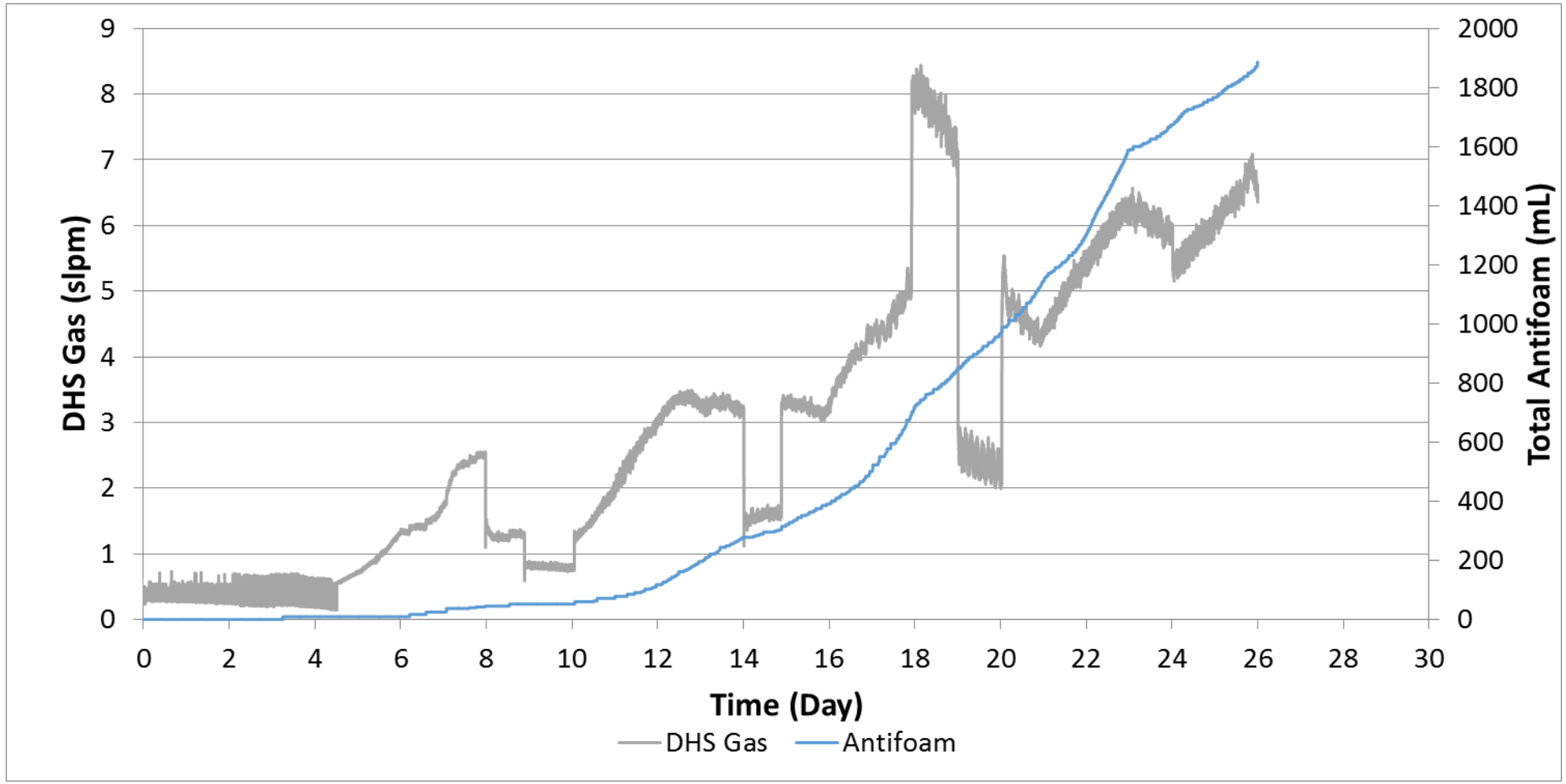
Cell Culture Results – 50 L S.U.B. with Antifoam Control Loop



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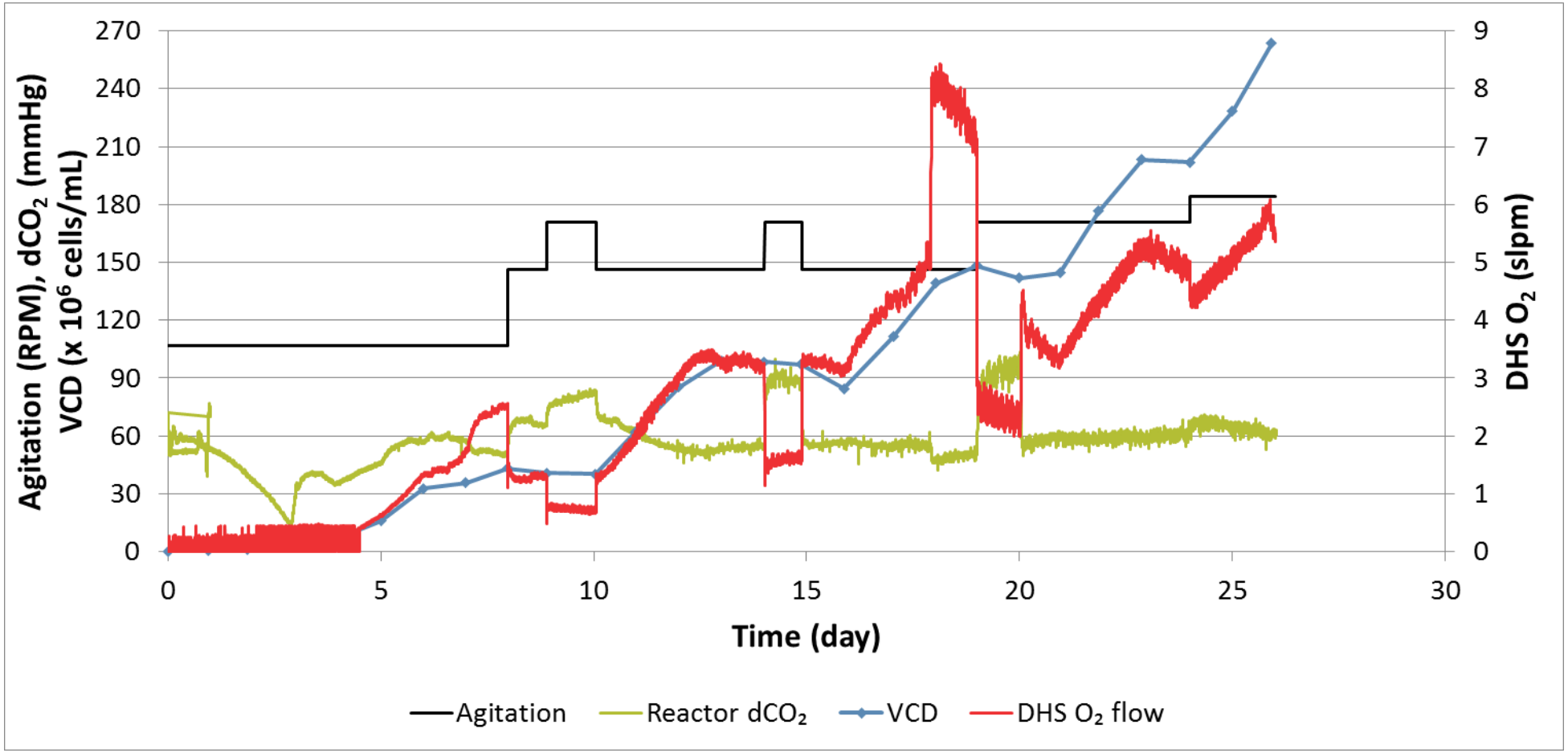
Cell Culture Results – 50 L S.U.B. Gassing, Agitation



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Mass Balance Models – Cells = f(gassing, mixing)

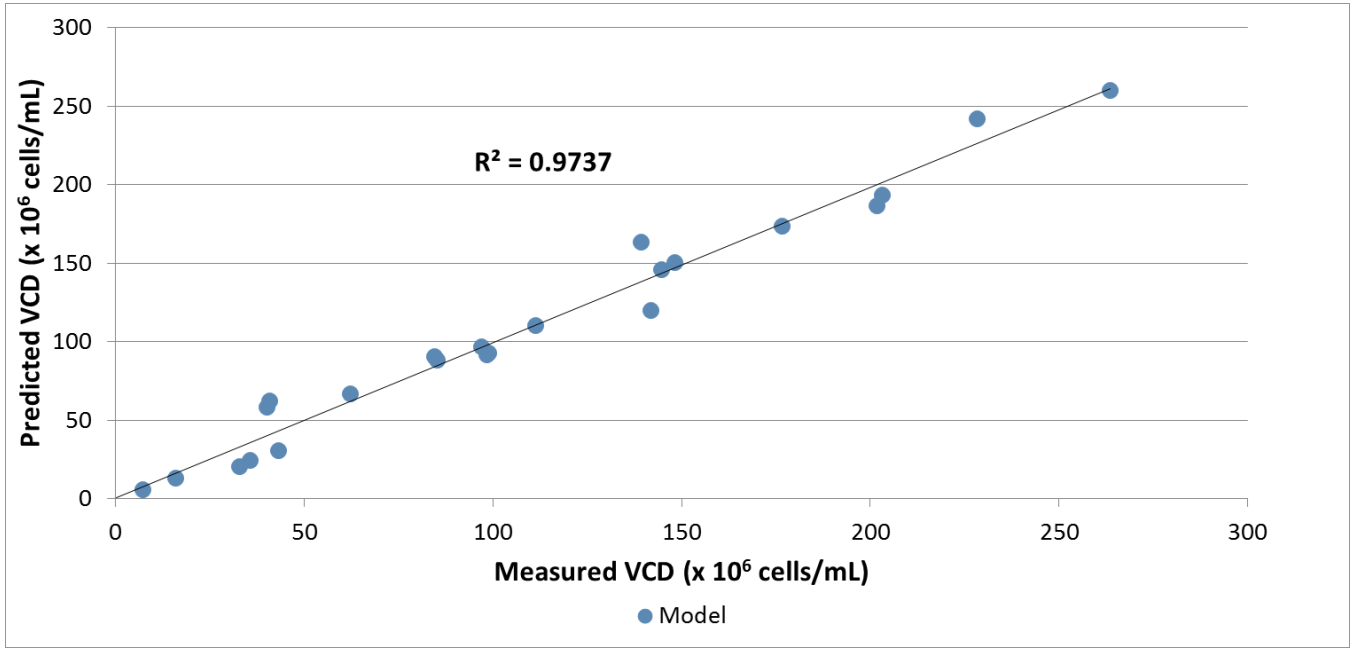
- Can we build an equation to model sustainable cell concentration based on mixing and gassing?

- $OUR = q_{O_2} \cdot C_X$
- $OTR = k_L a \cdot \left(C^*_{O_2} - C_{O_2}\right)$

$$C_{O_2} = pp_{O_2,air} \cdot SP$$

$$C^*_{O_2} = \left(\frac{F_{O_2} \cdot pp_{O_2} + F_{air} \cdot pp_{O_2,air}}{F_{TOT}}\right)$$

$$k_L a = \alpha \cdot PIV^{\beta} \cdot vvm^{\gamma}$$

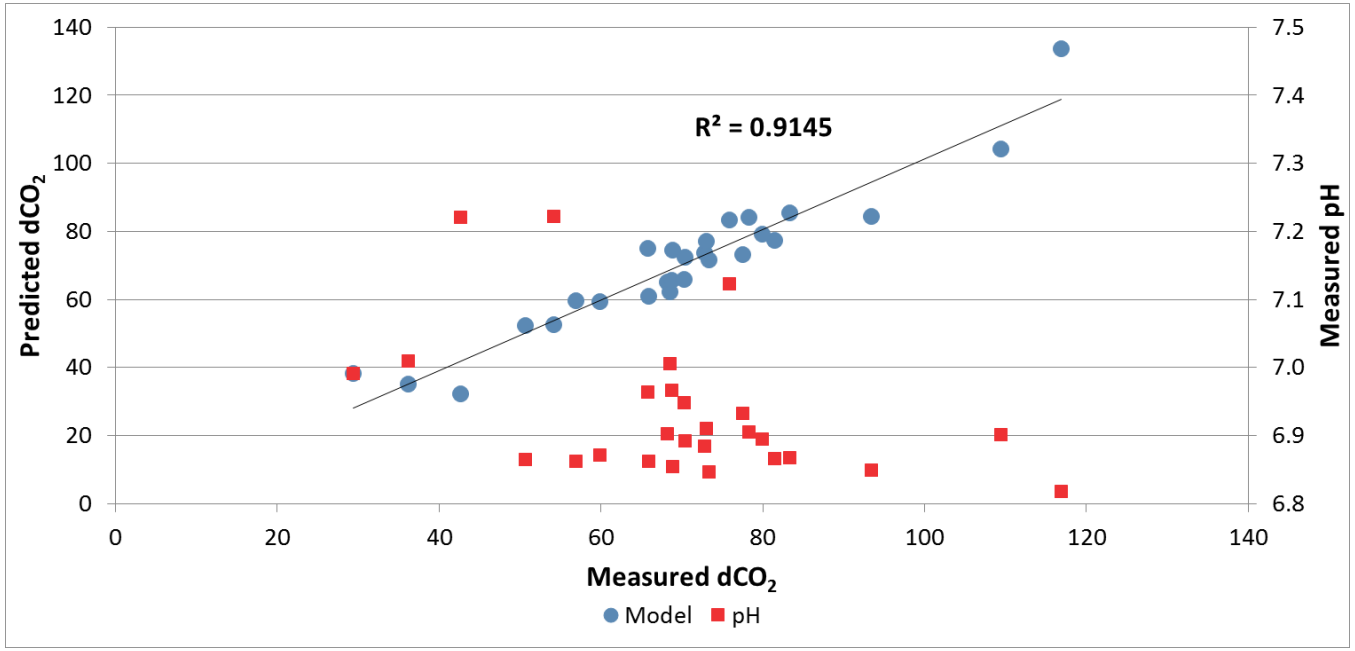


- If $OUR = OTR$

- $\therefore \; q_{O_2} \cdot C_X = \alpha \cdot PIV^{\beta} \cdot vvm^{\gamma} \left(\frac{F_{O_2} \cdot pp_{O_2} + F_{air} \cdot pp_{O_2,air}}{F_{TOT}} - pp_{O_2,air} \cdot SP\right)$

Mass Balance Models – CO_2 = f(acids, base)

- pH = baseline pH – acid buildup + base buildup
- Acid/base accumulations
  - CO_2 concentration – controllable ($k_L a_{CO_2}$)
  - Lactate
  - Ammonium
  - Ammonia
- $k_L a_{CO_2} = \alpha \cdot PIV^{\beta} \cdot vvm^{\gamma}$
- $\therefore CO_2 = \frac{pH}{k_L a_{CO_2} \cdot (acid\ buildup) \cdot (base\ buildup)}$



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Conclusions

- S.U.B. enhancements lead to 3-4 fold increases in oxygen mass transfer from legacy vessels
- Scalability of S.U.B.s
- High degree of automation vital to perfusion success
- Achieved >260 x 10^6 cells/mL in cell culture
  - Room for more oxygen mass transfer at all scales
- Highly automated process controlling DO, pH, dCO_2
- Predictive mass transfer models based on culture data
- Further work to be done optimizing media and exchange rates