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Single use plastic settlers for clarifying cell culture broth, selective removal of dead cells and affinity capture of antibodies on protein A beads

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Single use disposable cell settler
for 1. selective retention of live cells, 2. clarification of cell culture harvest, 3. affinity capture of antibodies, etc.

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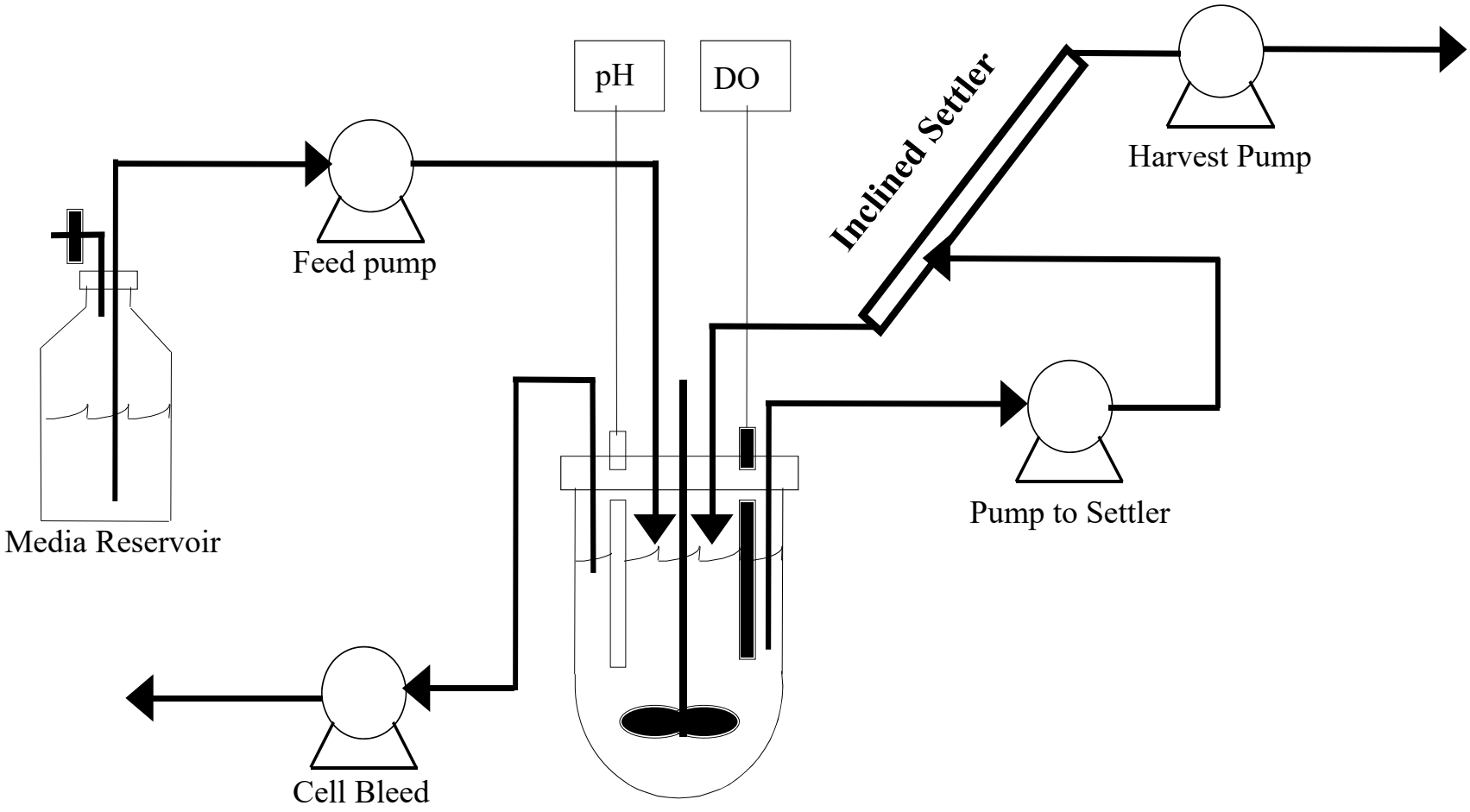
Presentation Outline

- Inclined Settler – a selective cell retention device - for achieving high cell densities in perfusion bioreactors
- Novel Compact Cell Settlers – more easily scalable – achieves high cell density in microbial perfusion bioreactors
- Single Use Disposable BioSettler – expanded new applications in Bioprocessing:
  - Selective Cell Retention Device for perfusion bioreactors
  - Clarification of fed-batch cell culture broth
  - Affinity capture of antibodies on protein A beads
  - others in development

Inclined Settler as a selective cell retention device

Batt, Davis & Kompala, Biotech Prog, **6**:458-464,*1990*;
Searles, Todd & Kompala, Biotech Prog, **10**:198-206,*1994*



Inclined Settler – a selective cell retention device

- Simple, passive, robust & powerful technology
- Uses no membrane barriers that can clog!
- Uses no moving parts that can break down!
- Proven at large scale for mammalian cell cultures
- Selective removal of dead cells and cell debris
- Complete recycle of live cells to bioreactor
- High *viable* cell density and productivity
- Perfusion bioreactors can be operated indefinitely
- Not demonstrated successfully for microbial cells

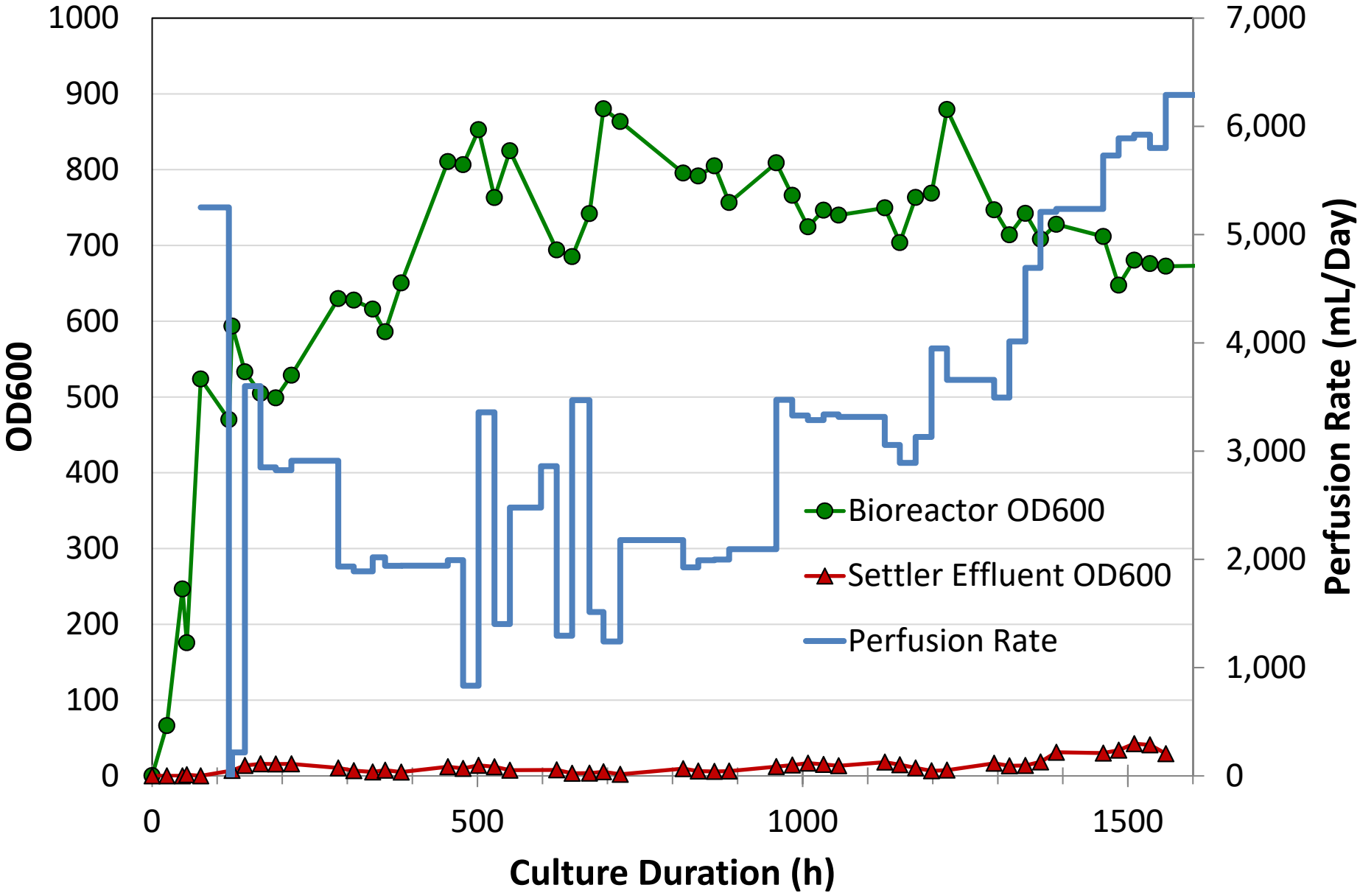
Inclined settler scaled up for a 3000 L perfusion bioreactor (replacing n-1 seed train)
Pohlscheidt, et al. Biotech Prog. (2013)29:222-229, Roche/Genentech, Germany

Novel Compact Cell Settlers

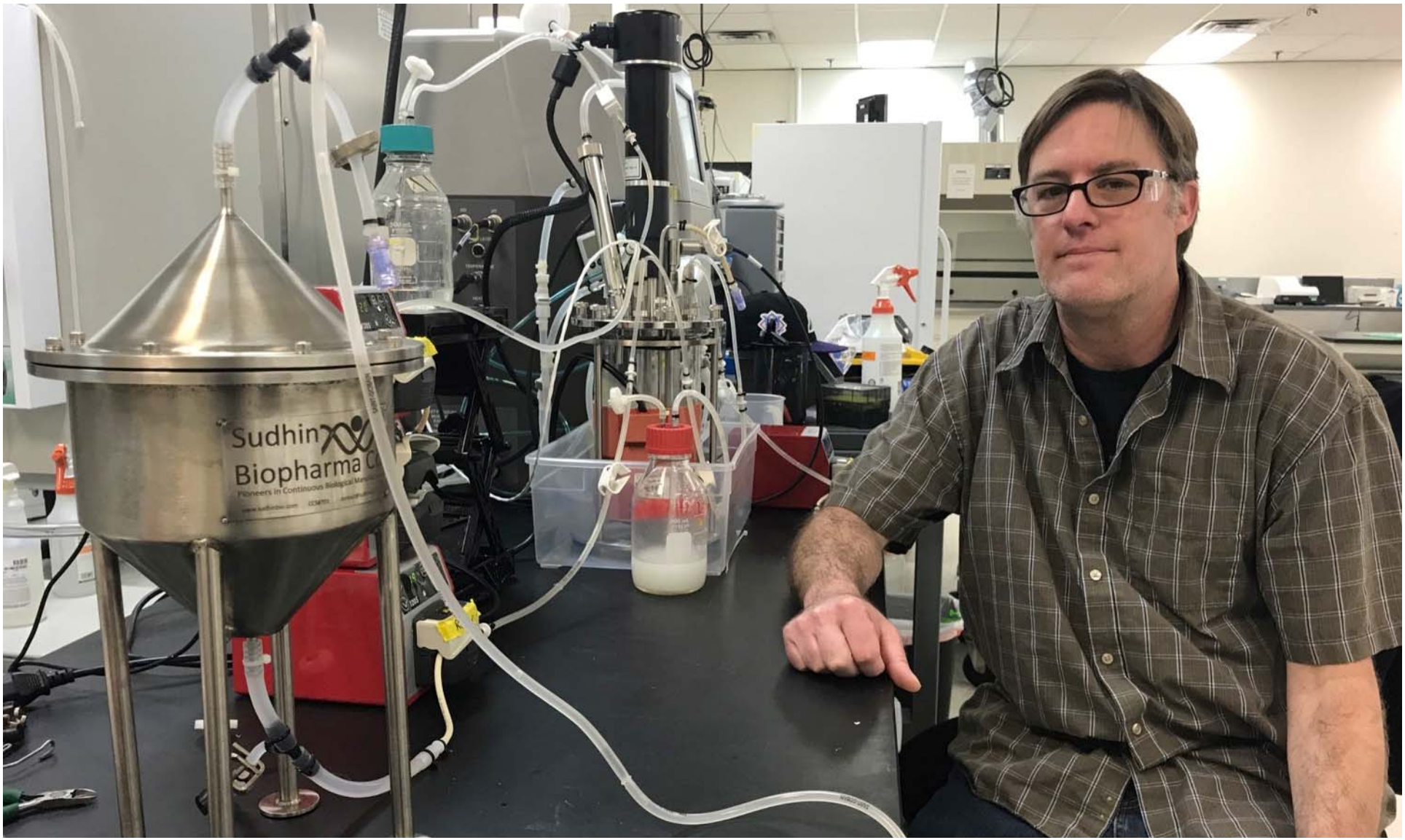
Easily scalable cylindrical & conical design

6 – 10 x more settling area for the same footprint

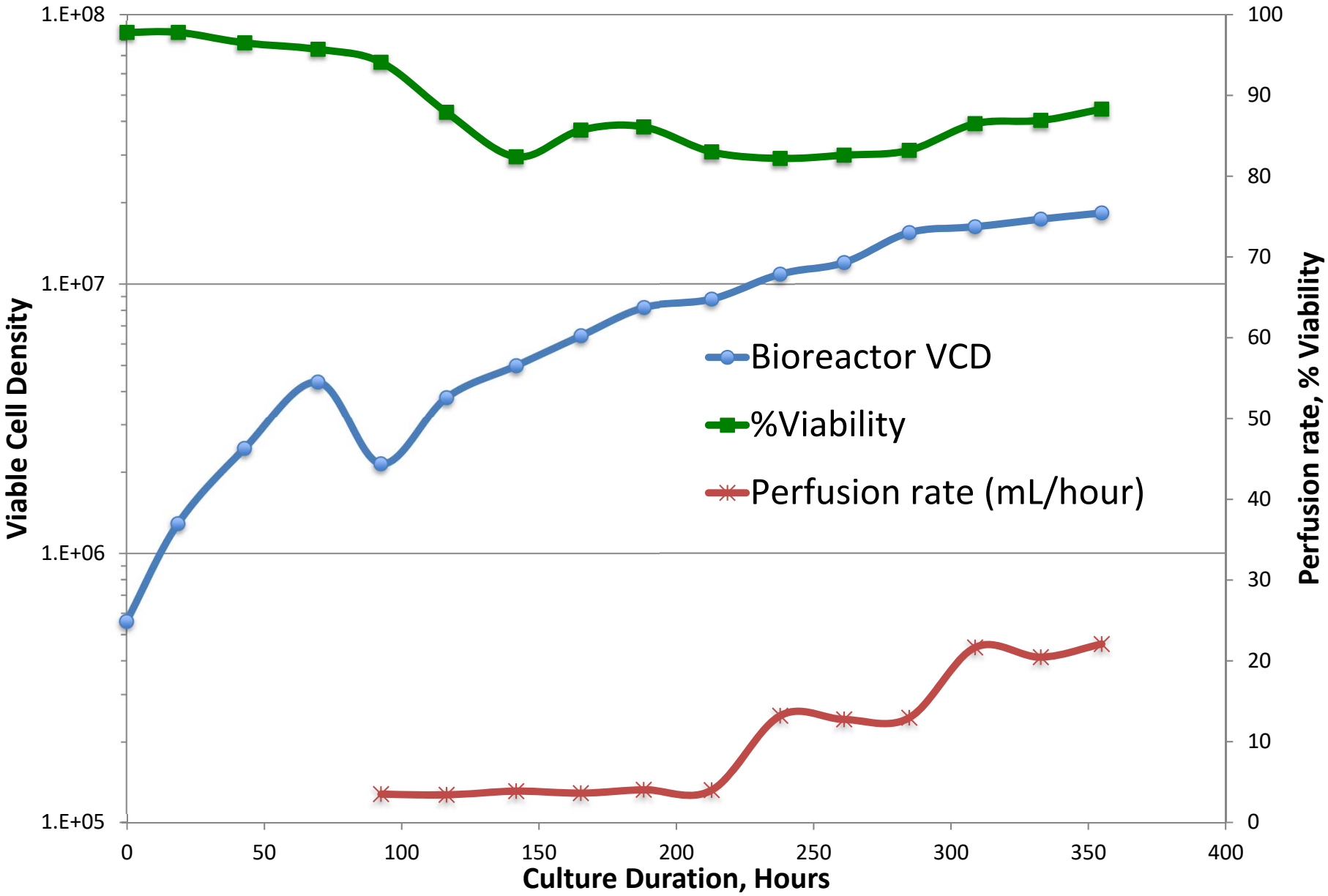
Novel Compact Cell Settlers – Prototype 4


Perfusion bioreactor of P. pastoris, with compact cell settler #4 fully packed
OD600
Perfusion Rate (mL/Day)
Culture Duration (h)
Bioreactor OD600
Settler Effluent OD600
Perfusion Rate
0
100
200
300
400
500
600
700
800
900
1000
0
1,000
2,000
3,000
4,000
5,000
6,000
7,000
0
500
1000
1500

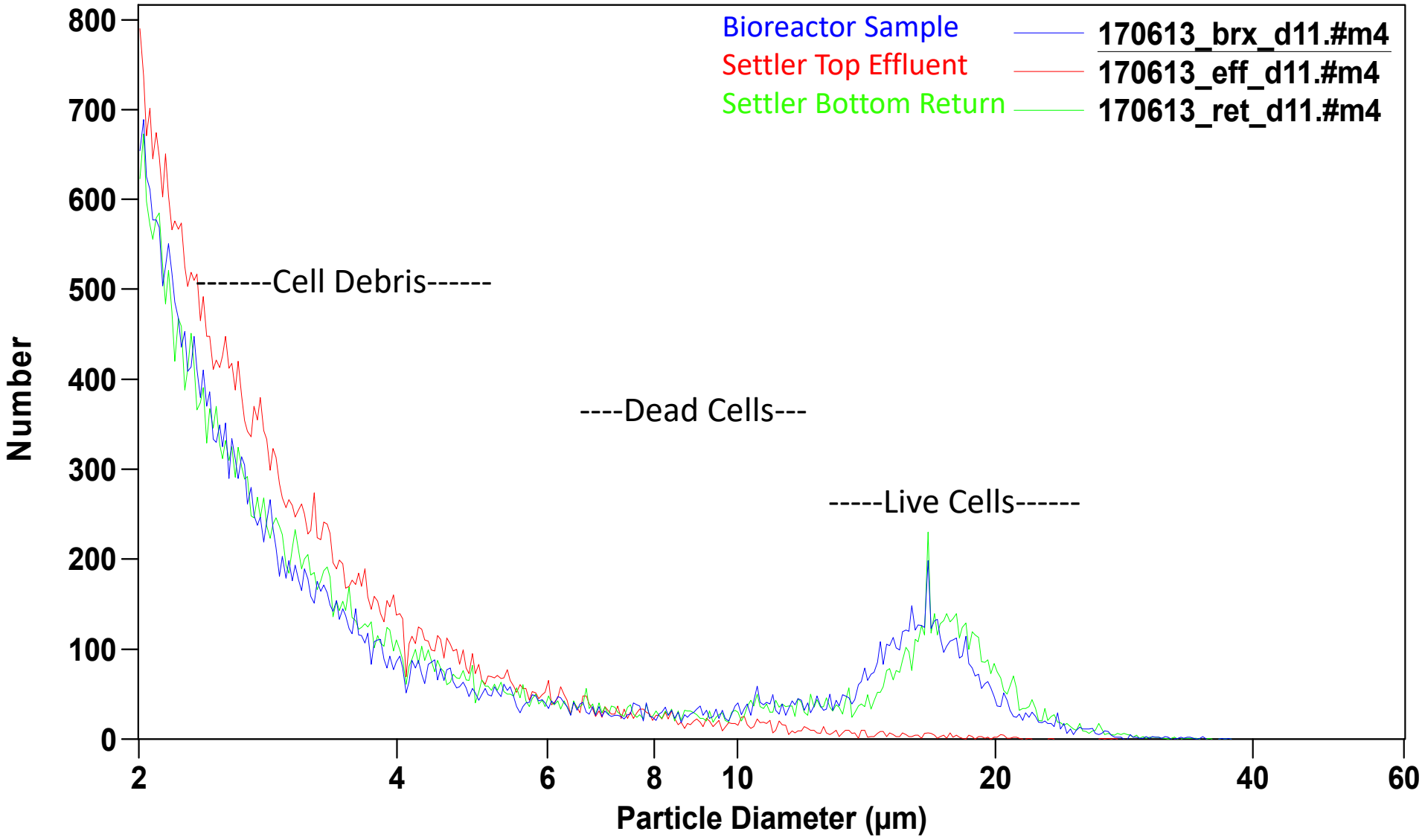
6” diameter Compact Cell Settler attached to a 1.2-liter Celligen CHO Cell Bioreactor



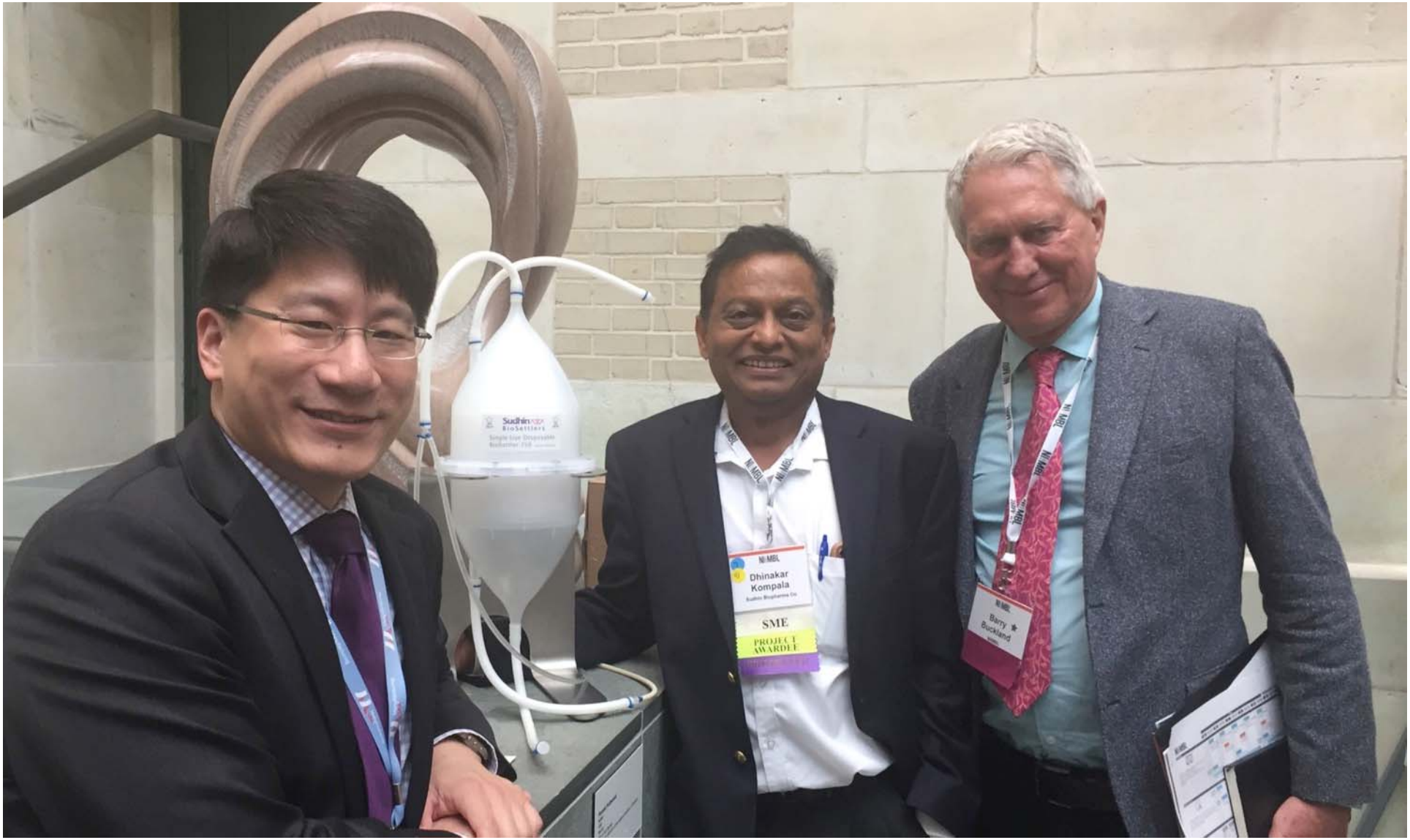
Perfusion Bioreactor of CHO cells with our Novel Compact Cell Settler as the Selective Cell Retention Device



Size distribution of cells in samples from Bioreactor, Settler Effluent & Return

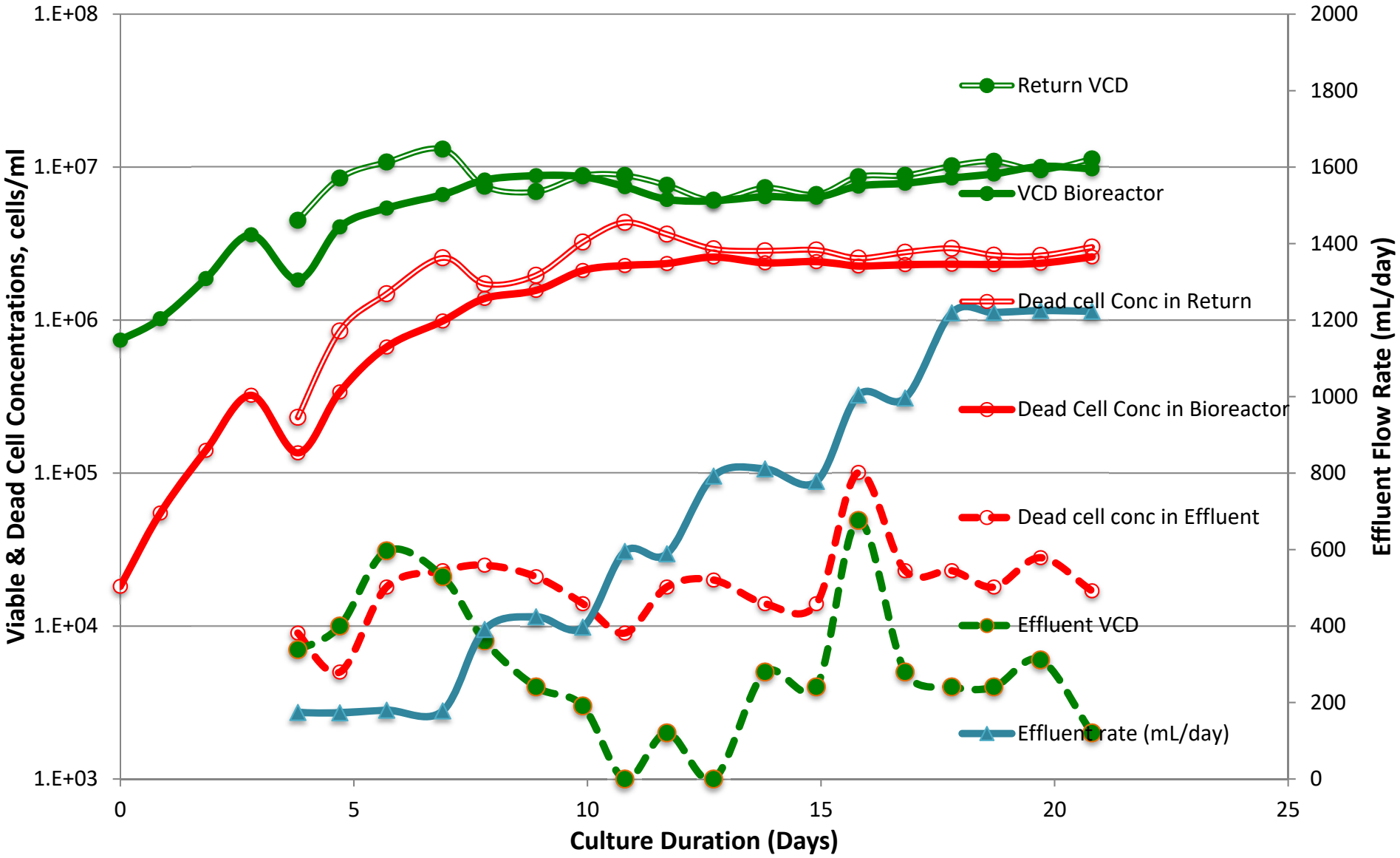


Single Use Disposable BioSettler 150 developed with NIIMBL funding

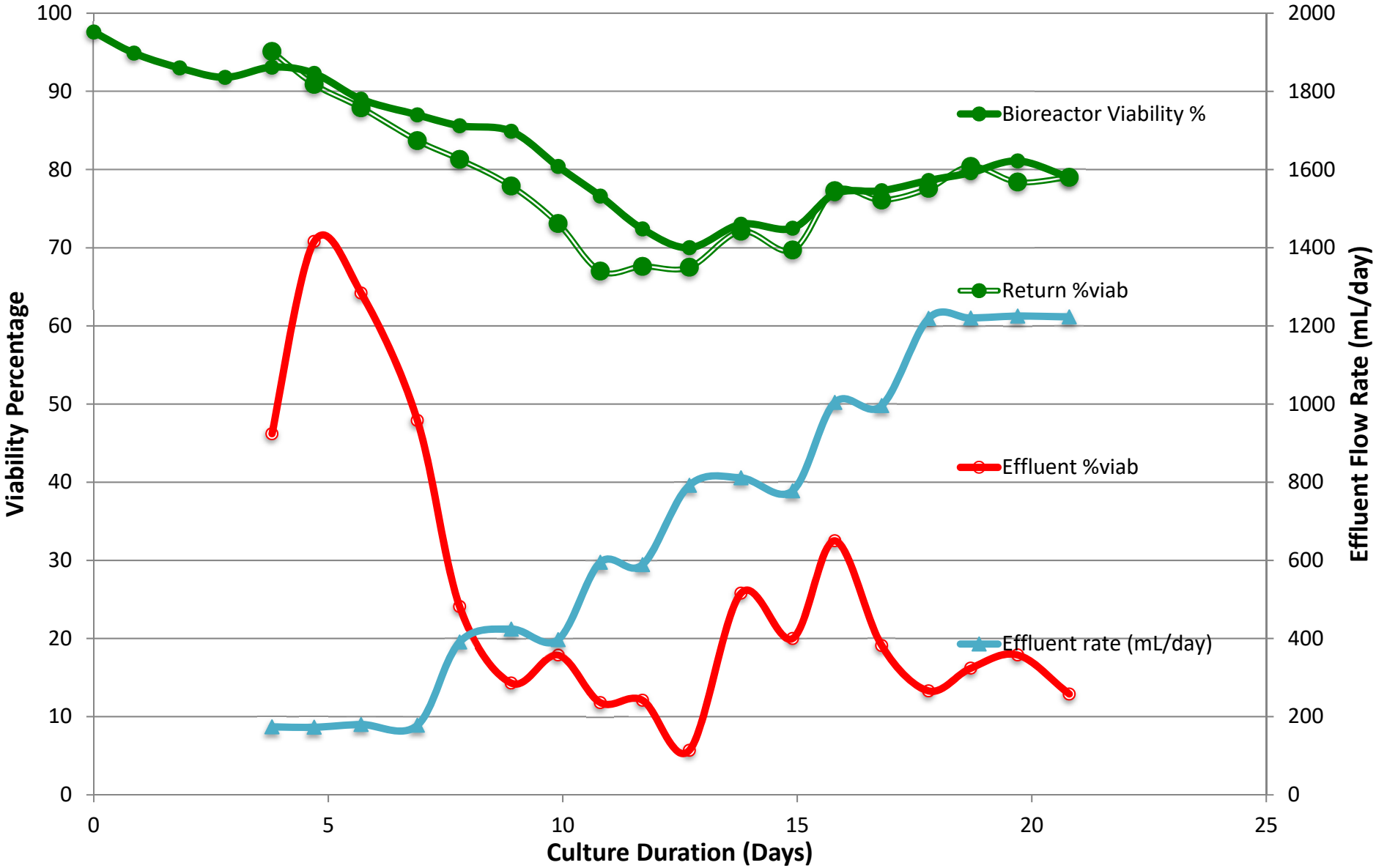


BioSettler for 1. CHO Perfusion Bioreactor

CHO Cell Density profiles from our first perfusion bioreactor expt w BioSettler150



Cell Viability profiles during our first perfusion bioreactor expt w plastic settler



2. Clarification of Cell Culture Broth in the BioSettler150 at Sudhin Biopharma

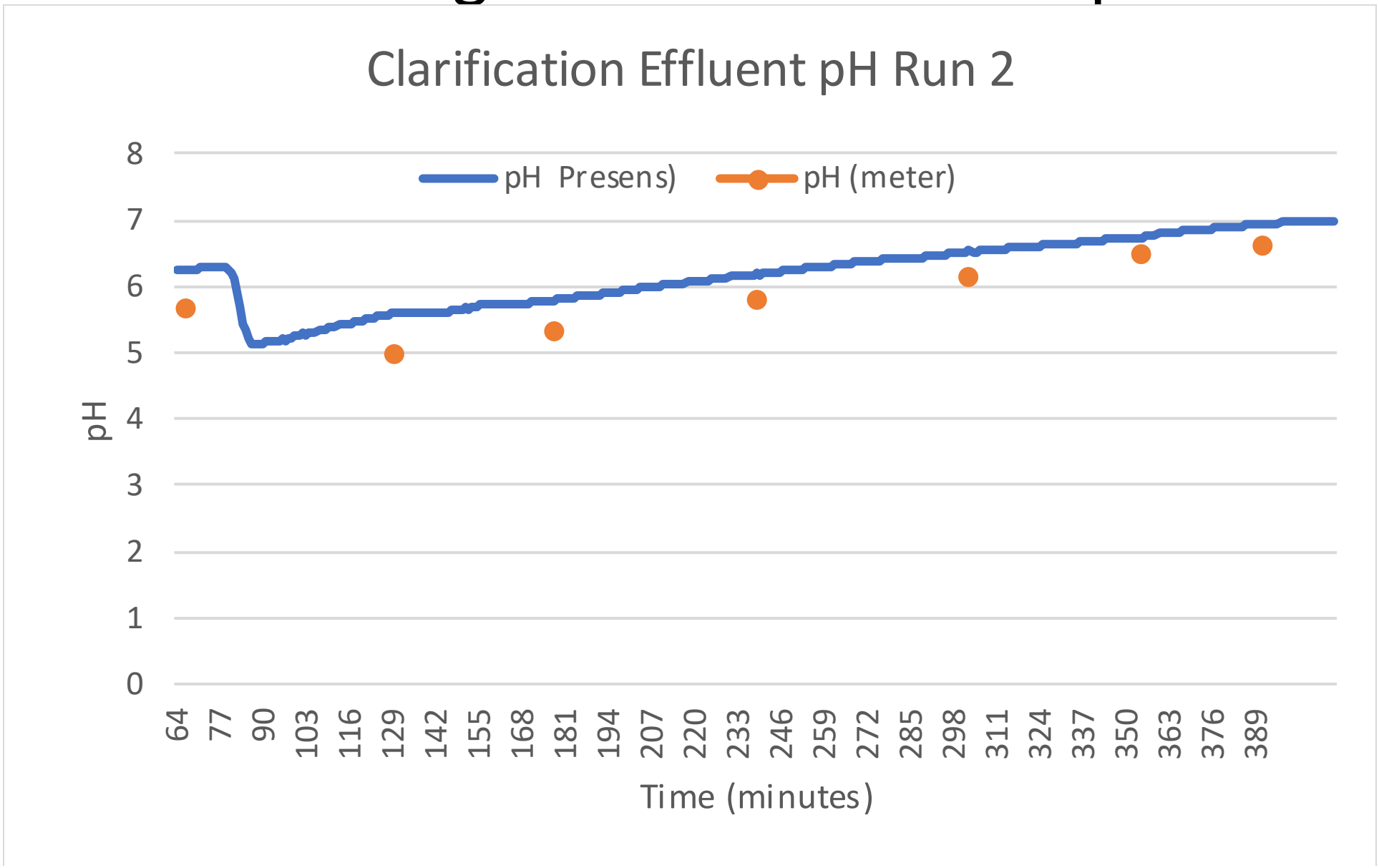
Reducing culture broth's pH from 7 to 5 or lower by adding a small amount of acetic or citric acid causes cell clumping and rapid settling of clumps.

Fast settling cell clumps may be removed from the bottom port with a peristaltic pump quickly before they get packed or compacted. After draining out the cell clumps, the clarified supernatant can be collected via bottom port.

Alternately, the secreted product in the settling cell clumps may be removed by pumping in a buffer via bottom port to wash out the product from these cell clumps and collecting the diluted and clarified supernatant from the top port.

This photograph shows the collected cell clumps (~70 ml) at the bottom of settler from 3.5 liters of cell culture broth at 2 – 4 million cells/ml plus about 300 ml of acetic acid at pH 4.

pH profile of settler effluent during PBS washing of settled cell clumps

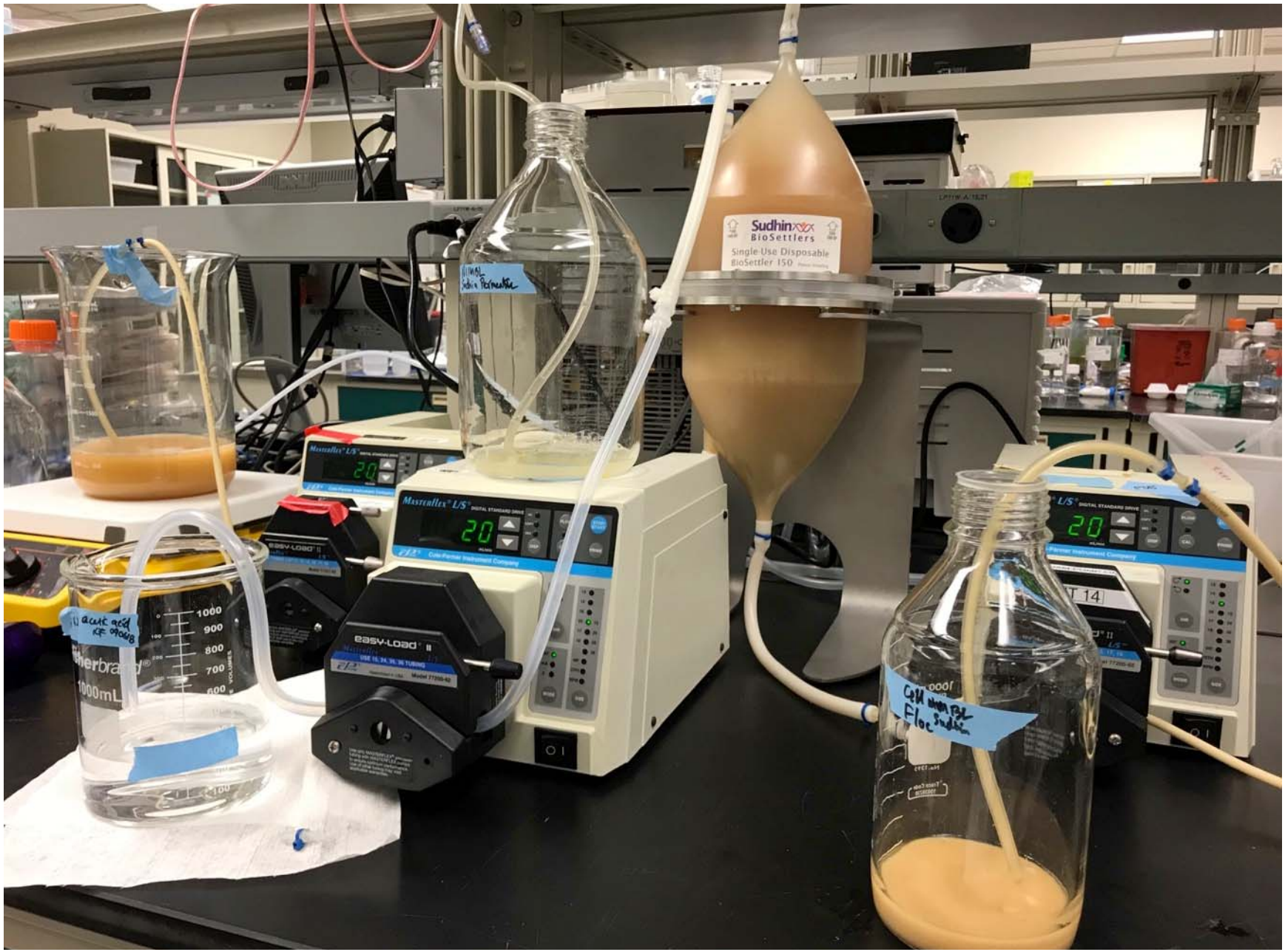


Recovery or Yield of Total Protein & IgG in collected settler effluent pools (2 expts)

Total Protein		IgG	
Total in samples 2-8 (mg)	8927.2	Total in samples 2-8 (mg)	120.5
Percent Recovery	74.3	Percent Recovery	67.5
Total in samples 2-8 (mg)*	11985.4	Total in samples 2-8 (mg)*	145.6
Percent Recovery	99.7	Percent Recovery	81.5
Total in samples 2-8 (mg)	6680.7	Total in samples 2-8 (mg)	128.2
Percent Recovery	63.2	Percent Recovery	60.7
Total in samples 2-8 (mg)*	8432.7	Total in samples 2-8 (mg)*	156.0
Percent Recovery	79.8	Percent Recovery	73.8
* for estimation purposes, includes			
settler volume			

2a. Washing of Settled Cell Clumps

2b. Draining of Cell Clumps from bottom



2c. Fed-Batch Cell Clump & Clarification

3.4 liters of cell culture broth (pH 7) were pumped with about 400 ml of citric acid (pH 4) in to fill the settler (3.8 liters) over 30 minutes and reduce the pH to below 6.

More cell culture broth (4.6 liters) and citric acid (600 ml) were pumped in at a lower rate over the next 3 hours, as the clarified supernatant is collected at 1.8 liters/hr from the top port.

Total of 8 liters of cell culture broth was pumped in the settler along with 1 liter of citric acid and was clarified in < 4 hours while the cell clumps were retained in the single use disposable biosettler.

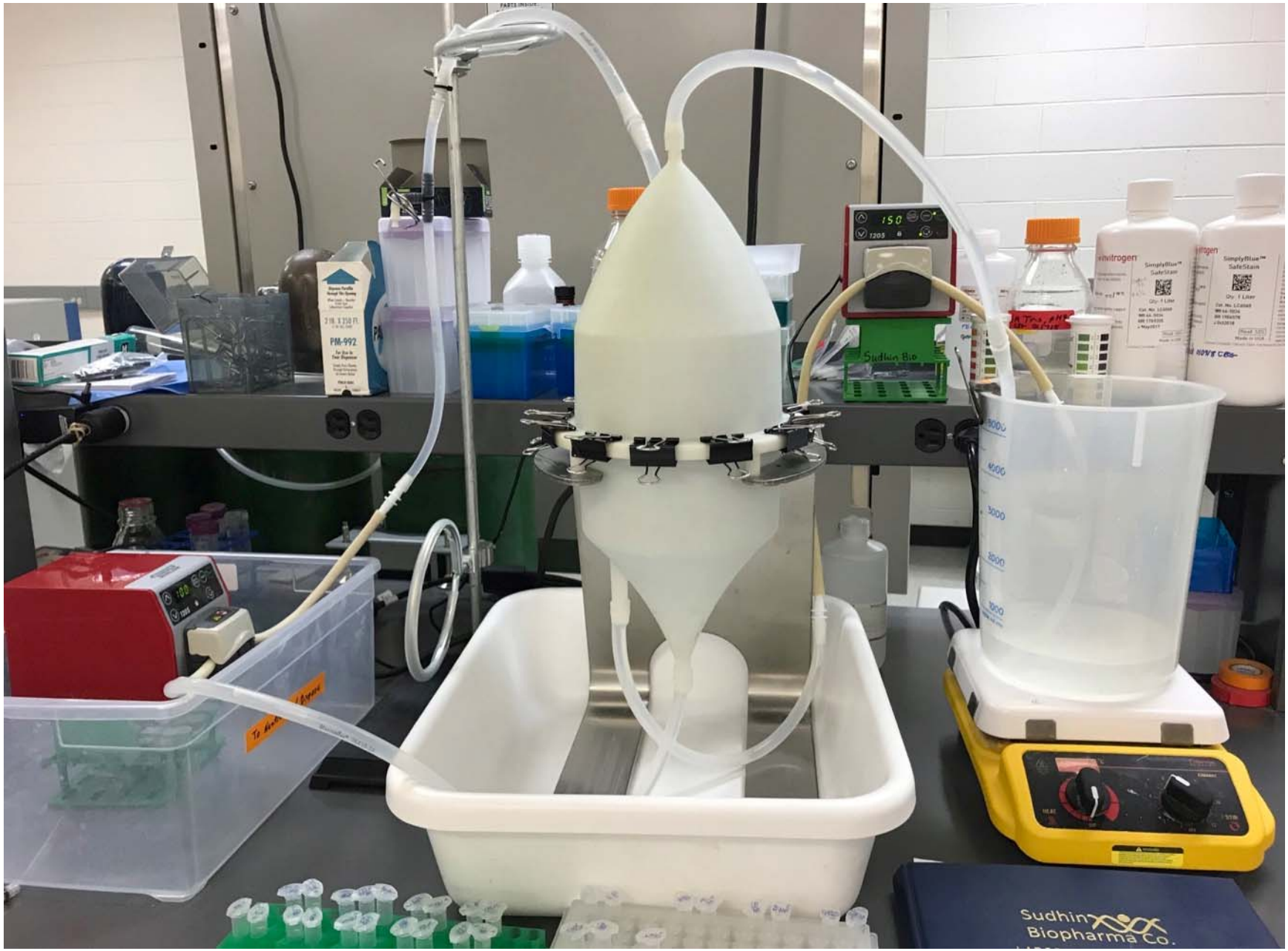
91.4% of secreted antibody product was recovered in the settler clarified supernatant, plus an additional 7.3% of IgG was recovered after centrifuging the cell clumps.

Centrifugation of the clarified supernatant resulted in a packed cell volume (PCV) of 0.5%, whereas the PCV in the starting cell culture broth was 3.0% (reduction of 83.3 %).

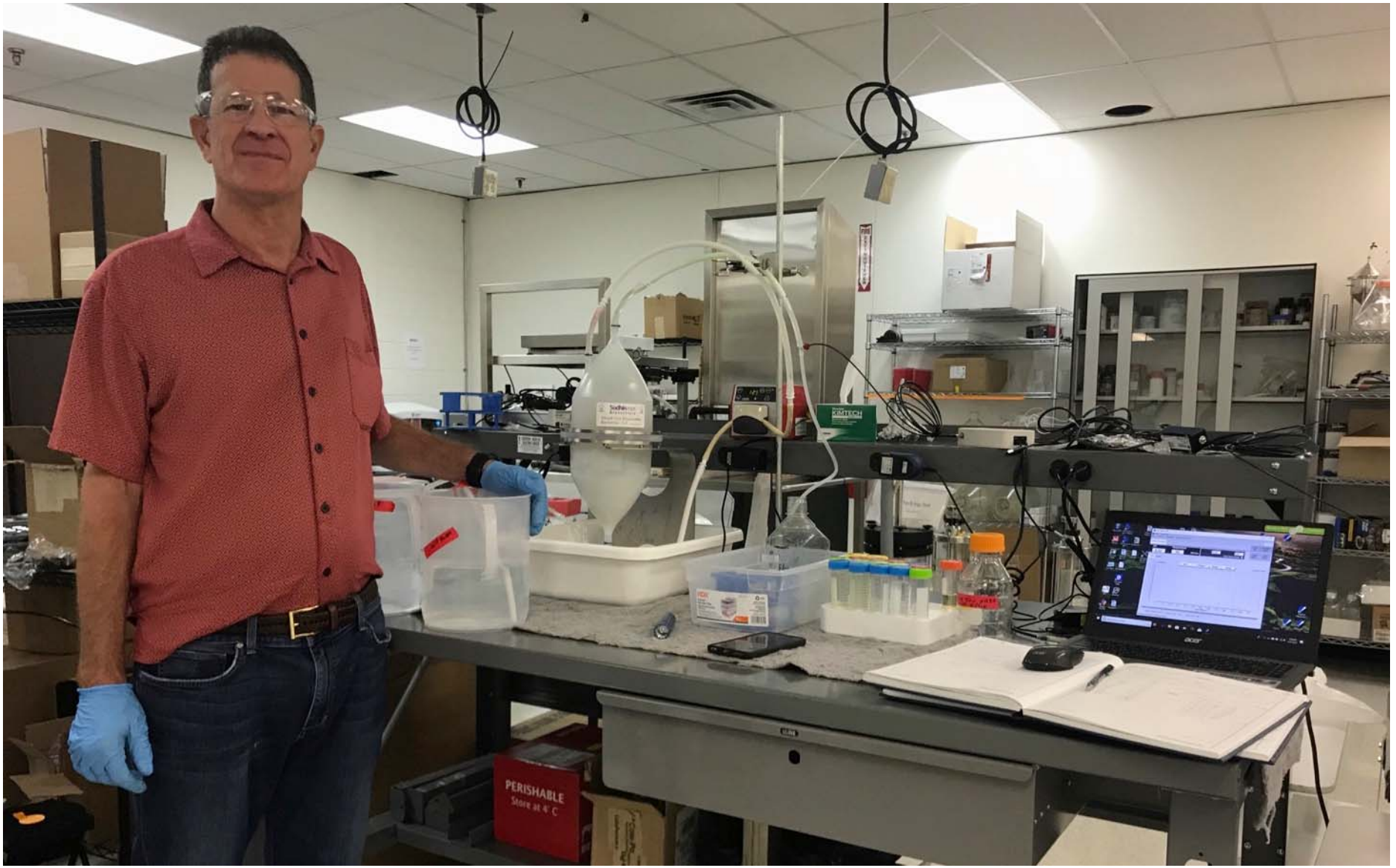
3. Affinity Capture of Secreted Antibodies on Protein A beads suspended in settler

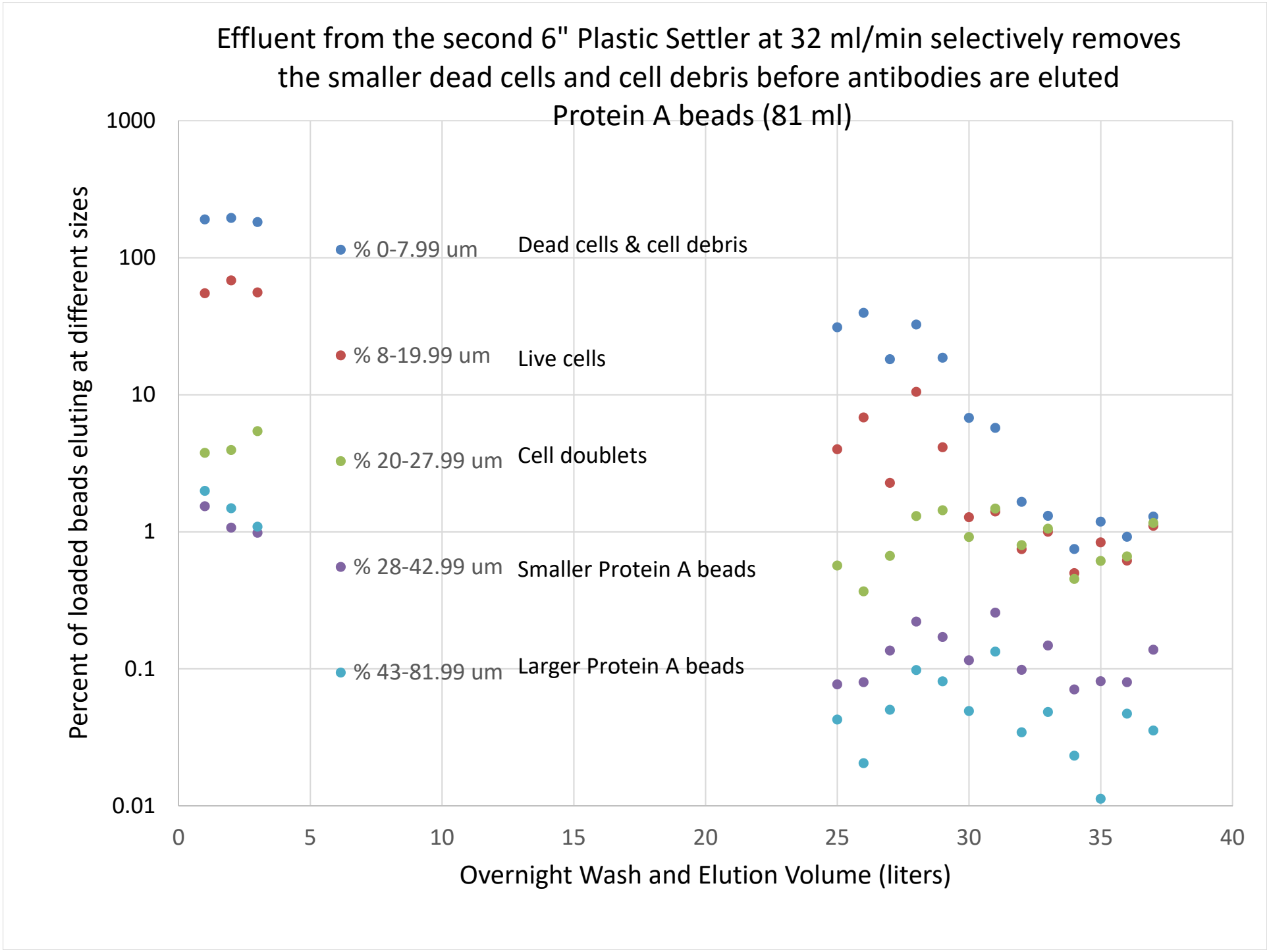
- Protein A beads (GE MabSelect SuRe LX) have a size range of 28 – 82 microns(μ), with a median of 43 μ.
- Size range of live CHO cells is 10-20 μ, dead cells: 8 – 10μ, and cell debris: < 8μ.
- Cell culture broth from end of Fed-batch bioreactor can be directly loaded into our settler, along with Protein A beads.
- After secreted antibodies are bound to the larger beads suspended in the settler, all smaller cells, debris and unbound host cell proteins can be washed out the top.
- Elution and regeneration steps can also be carried out sequentially with the beads suspended in the settler.

Affinity Capture Experimental Set-up

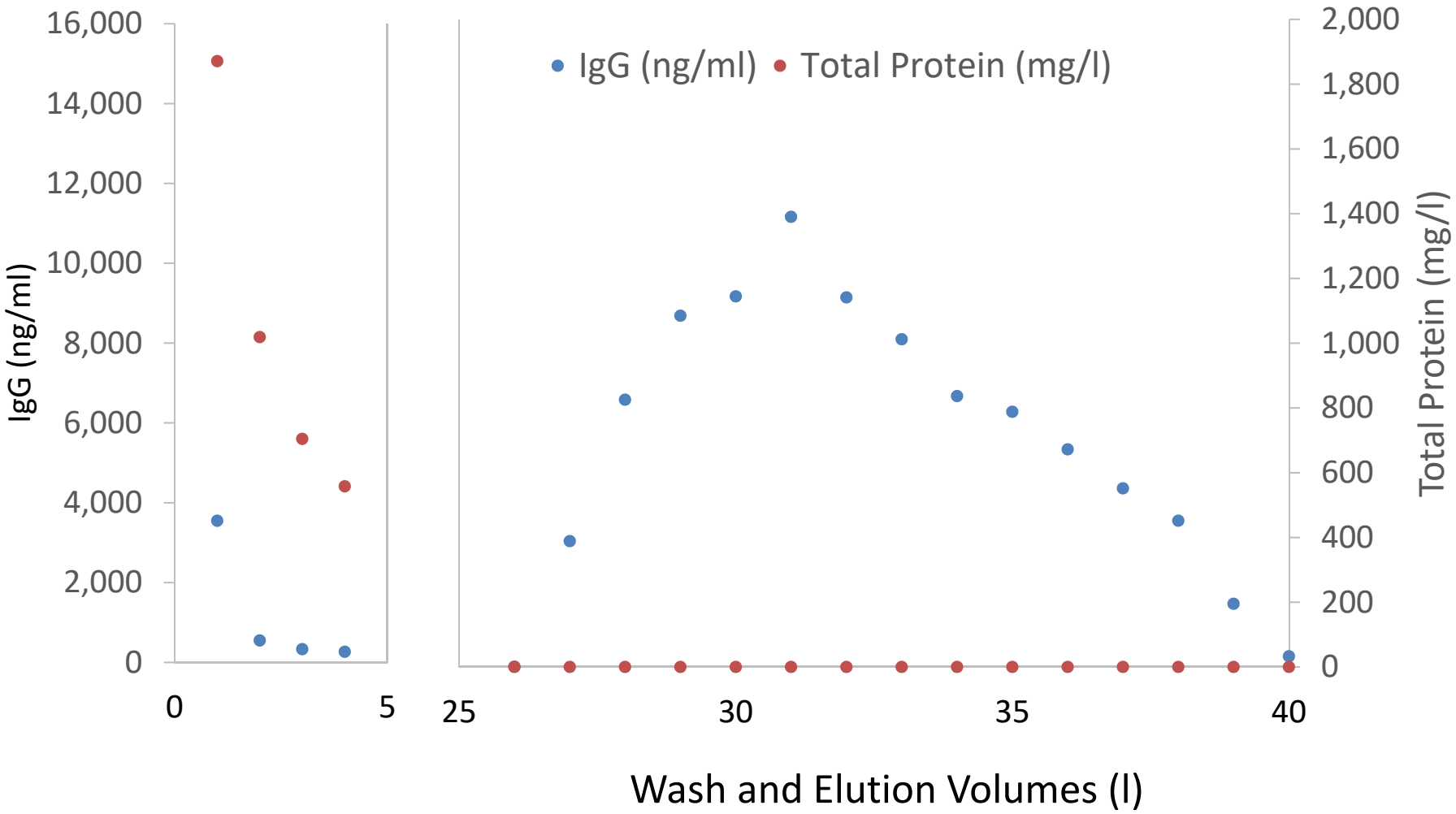


3. Affinity Capture Experimental Setup

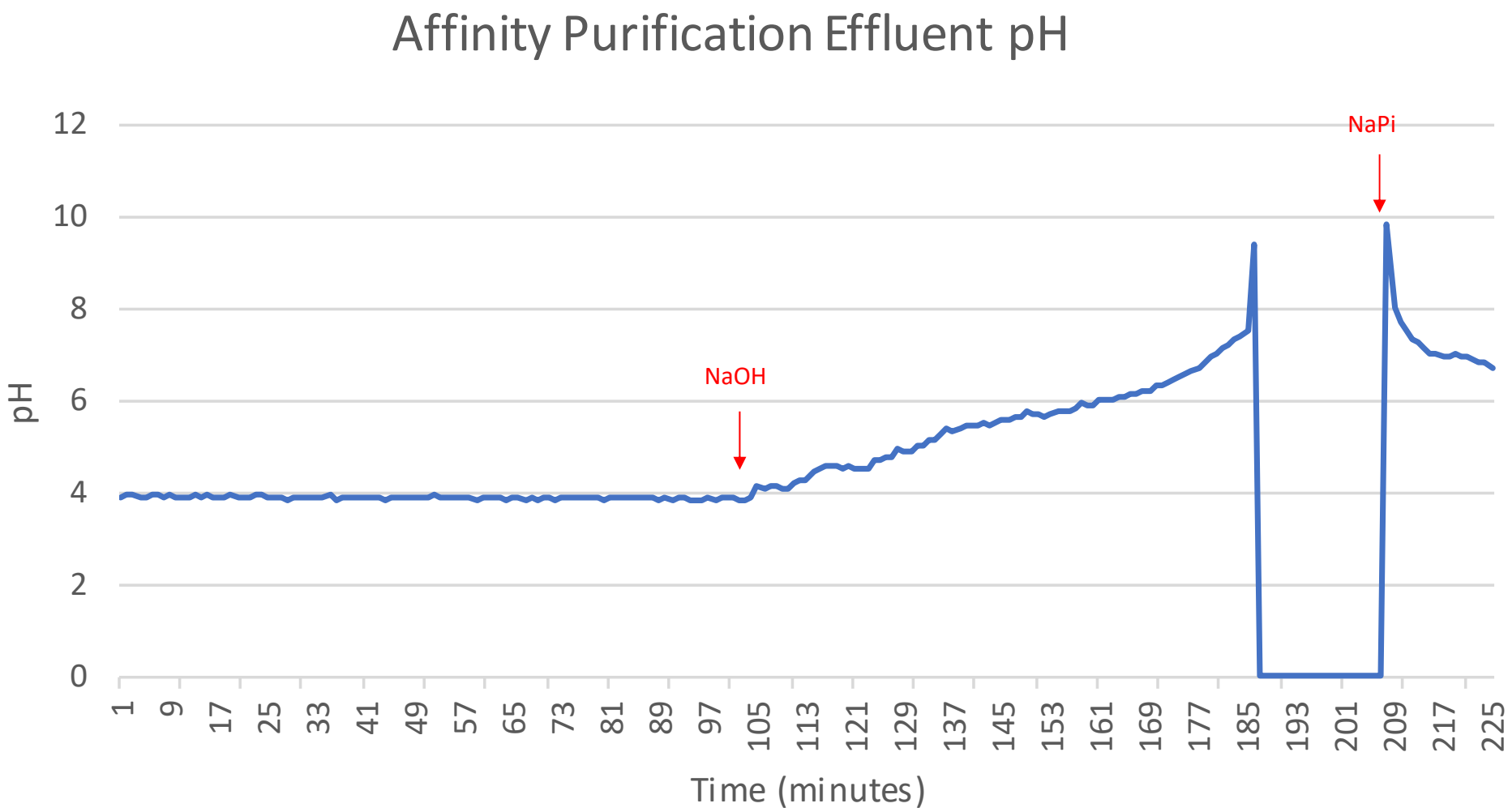



Effluent from the second 6" Plastic Settler at 32 ml/min selectively removes the smaller dead cells and cell debris before antibodies are eluted
Protein A beads (81 ml)
Percent of loaded beads eluting at different sizes
1000
100
10
1
0.1
0.01
0
5
10
15
20
25
30
35
40
Overnight Wash and Elution Volume (liters)
% 0-7.99 um
Dead cells & cell debris
% 8-19.99 um
Live cells
% 20-27.99 um
Cell doublets
% 28-42.99 um
Smaller Protein A beads
% 43-81.99 um
Larger Protein A beads

Elution Profiles of IgG and Total Protein

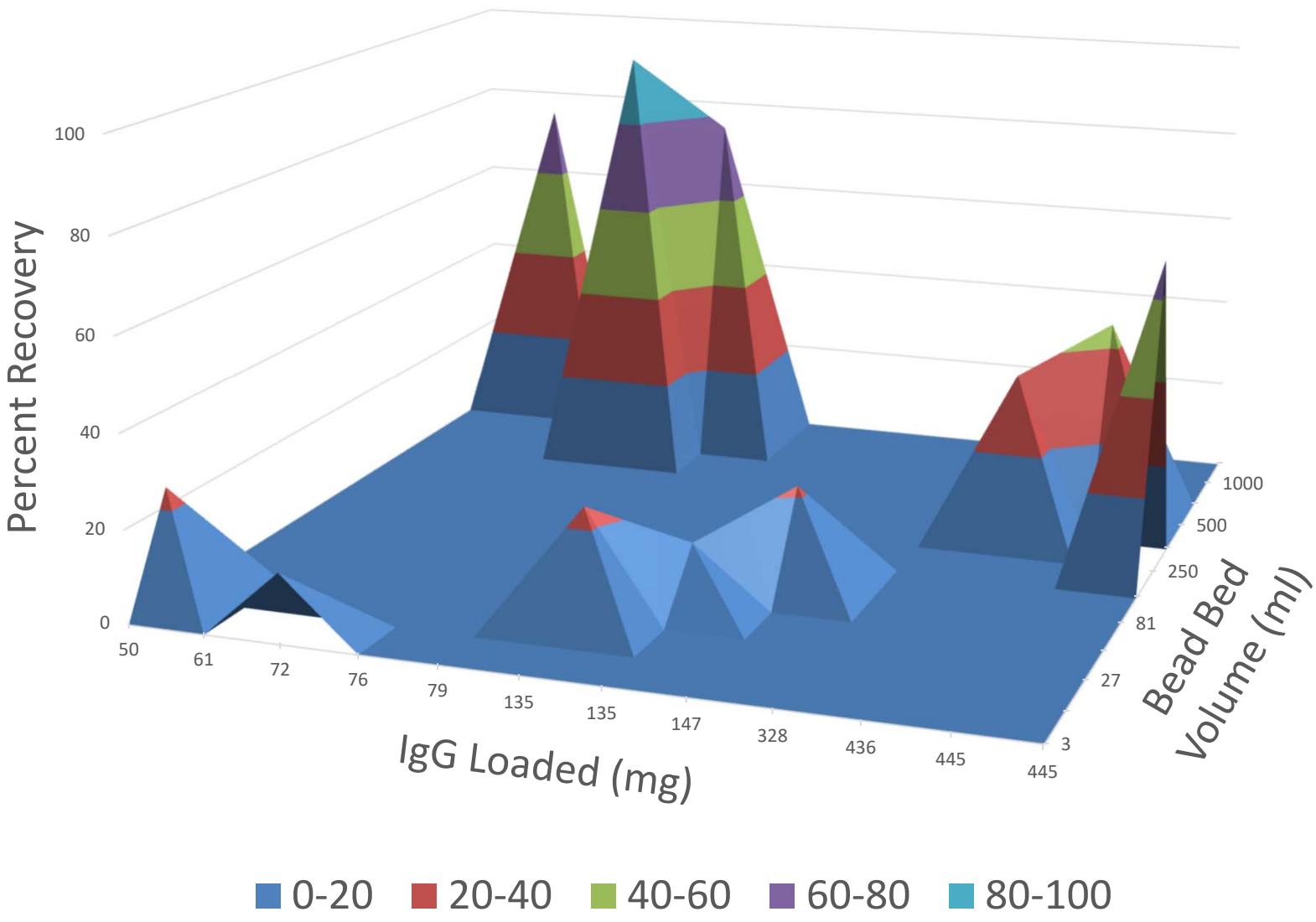


Effluent pH during Antibody Elution, Protein A bead Clean-in-place and Regeneration steps



pH is measured with an online PreSens fluorescent dye probe, which is calibrated between 6 – 8

Summary of Results obtained so far



Summary & Conclusions

Single use disposable BioSettlers can be useful in many novel bioprocessing applications:

1. We have demonstrated selective recycle of live and productive cells, while removing dead cells and cell debris continuously from the perfusion bioreactor.
2. We are developing novel clarification methods for harvesting secreted product while cells are settled inside BioSettler in batch, fed-batch or continuous operation.
3. We are optimizing the affinity capture of secreted antibodies on protein A beads suspended inside BioSettler from perfusion effluent and culture broth directly, again in batch and continuous modes.

Key References

- Batt, B.C., R.H. Davis, and D.S. Kompala, Biotechnology Progress 6: 458-464, 1990
- Searles, J.A., P.W. Todd, and D.S. Kompala, Biotechnology Progress 10: 198-206, 1994.
- Freeman, C.A., P.S.D. Samuel and D.S. Kompala, Biotechnology Progress 33: 913- 922, 2017

Acknowledgements

- National Science Foundation, Presidential Young Investigator award to DSK, $500,000; 1988-1993
- National Science Foundation, Small Business Innovation Research Phase I grant $180,000; 2015
- National Science Foundation, Small Business Innovation Research Phase I grant $225,000; 2016
- National Institute for Innovation in Manufacturing (NIIMBL) Biopharmaceuticals, QSP 1.18, $1.5 M, 2017-2019