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Novel compact cell settlers for continuous perfusion bioreactor cultures of microbial (and mammalian) cells

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NOVEL COMPACT CELL SETTLERS FOR PERFUSION BIOREACTOR CULTURES OF MICROBIAL (AND MAMMALIAN) CELLS

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Summary of Abstract

As **secretory expression systems** have become well developed for some larger **microbial cells**, such as yeast *Saccharomyces cerevisiae* and *Pichia pastoris* cells, it is now possible to develop **high cell density continuous perfusion cultures** of ***microbial yeast cells.***

Keywords: Compact cell settlers, Cell retention device, perfusion bioreactor, microbial yeast cells, selective removal of dead cells & cell debris, viable cell recycle.

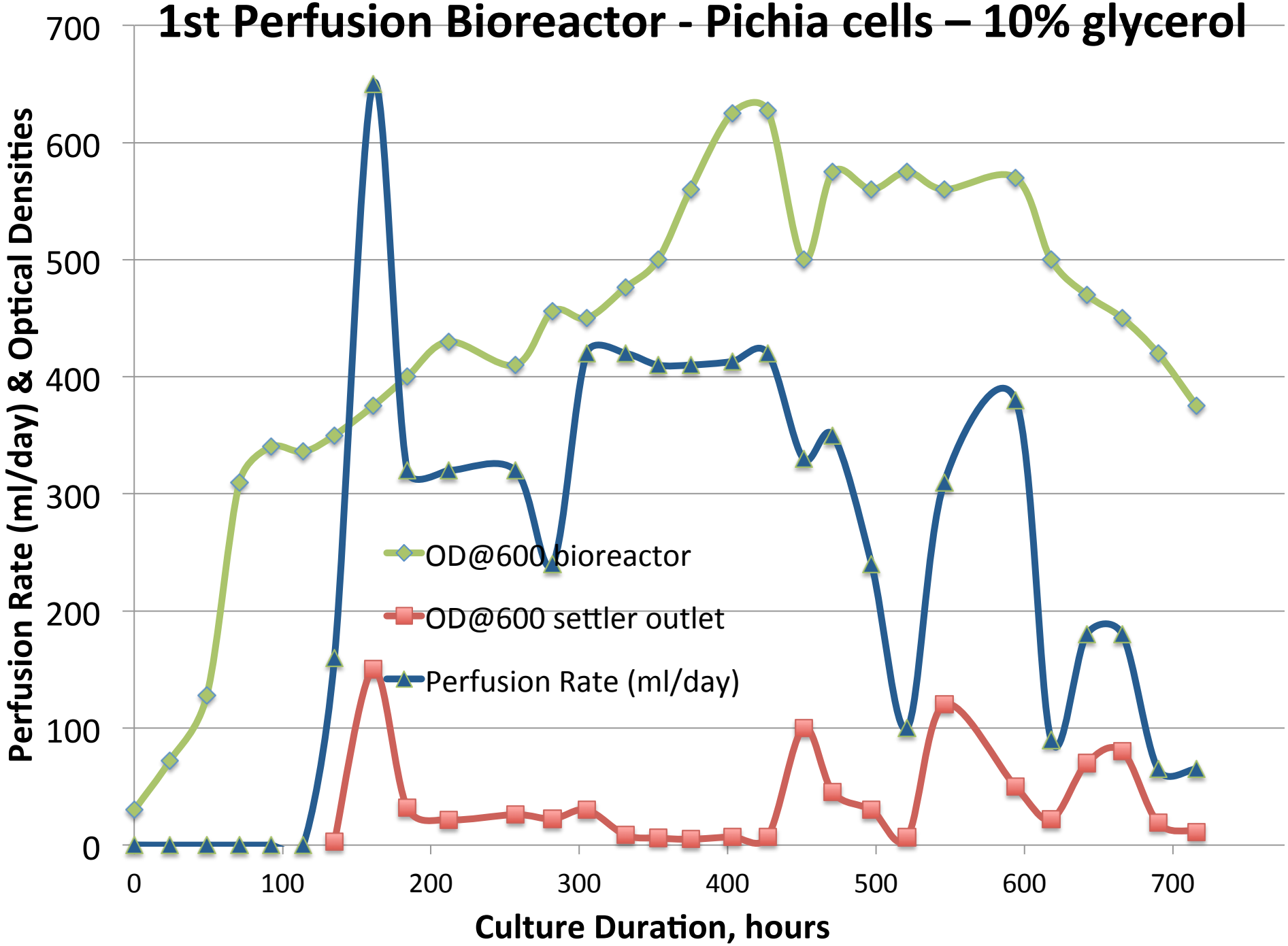
Inclined Settler – a superior cell retention device

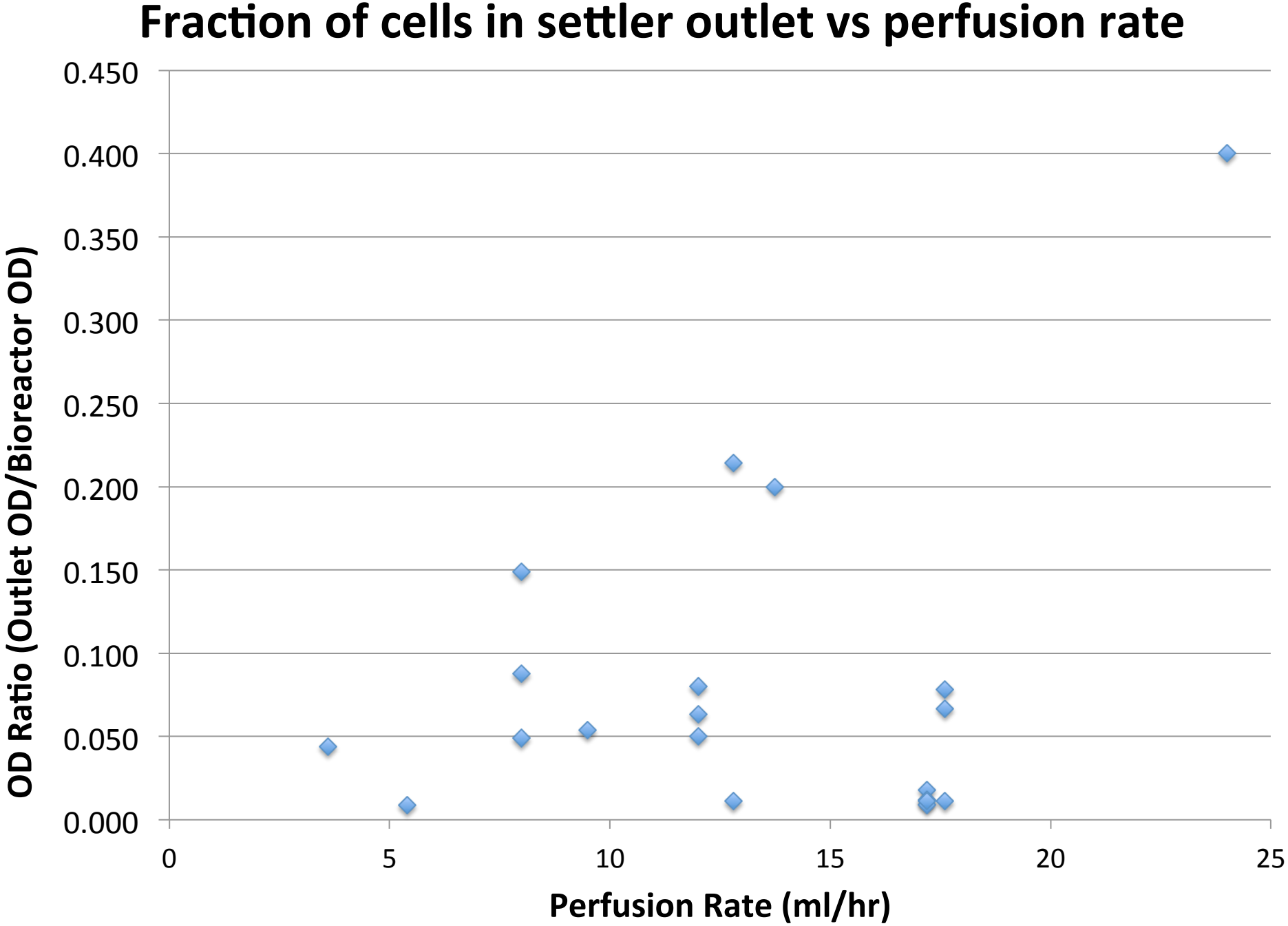
- Simple, passive, robust & powerful technology
- Uses no membrane barriers that can clog!
- Uses no moving parts that can break down!
- Proven at large scale for mammalian cell cultures
- Selective removal of dead cells and cell debris
- Complete recycle of live cells to bioreactor
- High *viable* cell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

Perfusion Bioreactor Culture of Yeast *P. pastoris* Cells using Inclined Settlers

Materials & Methods

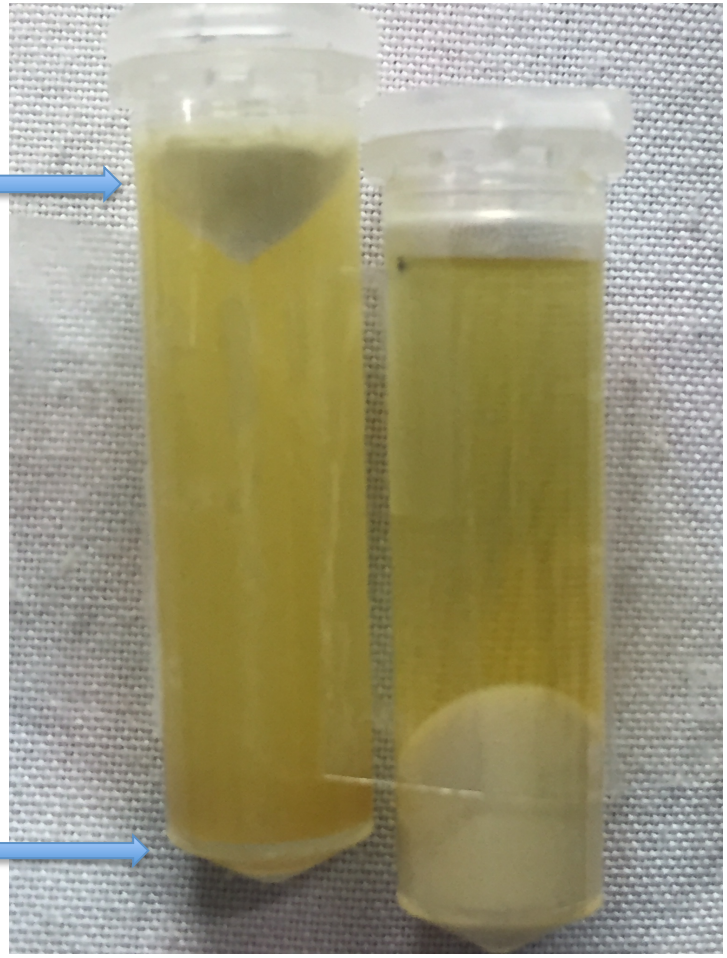
- Cells: *Pichia pastoris (x-33)* size: 3 – 6 microns
- Bioreactor size: 1.5 liters, computer controlled
- Inclined Settler: CS-10 (from Dr. P. Brown, Biotechnology Solutions, Inc.) lamellar settler designed for 2 - 4 liter mammalian bioreactor
- Suggested max perfusion rate of ~8 liters/day for mammalian cell culture, i.e. 333 ml/hr
- Media: Glycerol, plus basal salts medium
- Temp: 28 ^{o}C pH: 5.6 Air flow: ~1 vvm


1st Perfusion Bioreactor - Pichia cells – 10% glycerol
Perfusion Rate (ml/day) & Optical Densities
Culture Duration, hours
0
100
200
300
400
500
600
700
OD@600 bioreactor
OD@600 settler outlet
Perfusion Rate (ml/day)


Fraction of cells in settler outlet vs perfusion rate
OD Ratio (Outlet OD/Bioreactor OD)
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
0
5
10
15
20
25
Perfusion Rate (ml/hr)

Centrifuged samples from Settler outlet and Bioreactor

Cell debris



Sample from Bioreactor

Sample from settler outlet

Low fraction of dead cells

~ 25 % packed cell volume
High cell density in Bioreactor

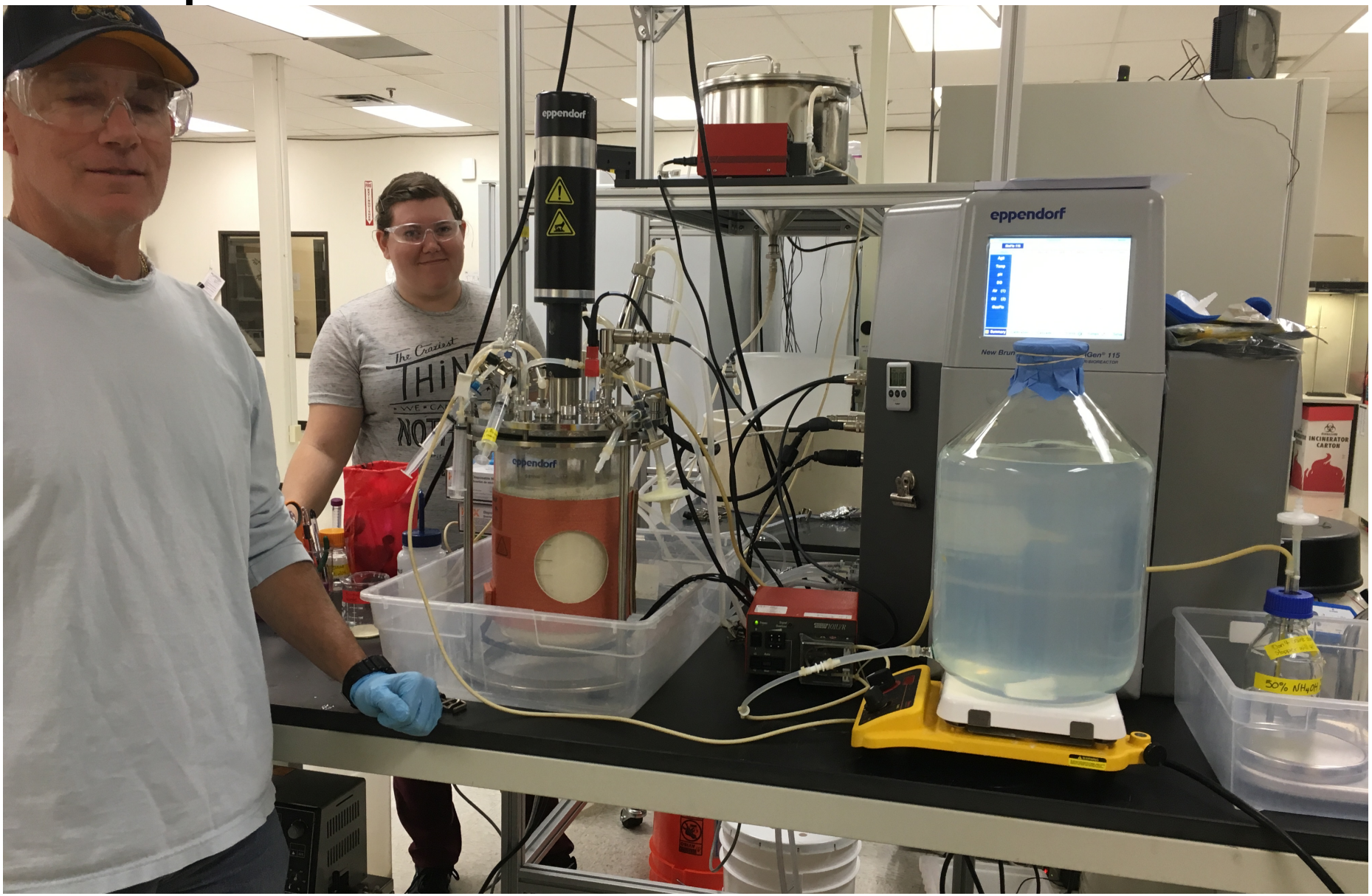
Lessons Learned from Lamellar Settler for the smaller yeast *Pichia* cells

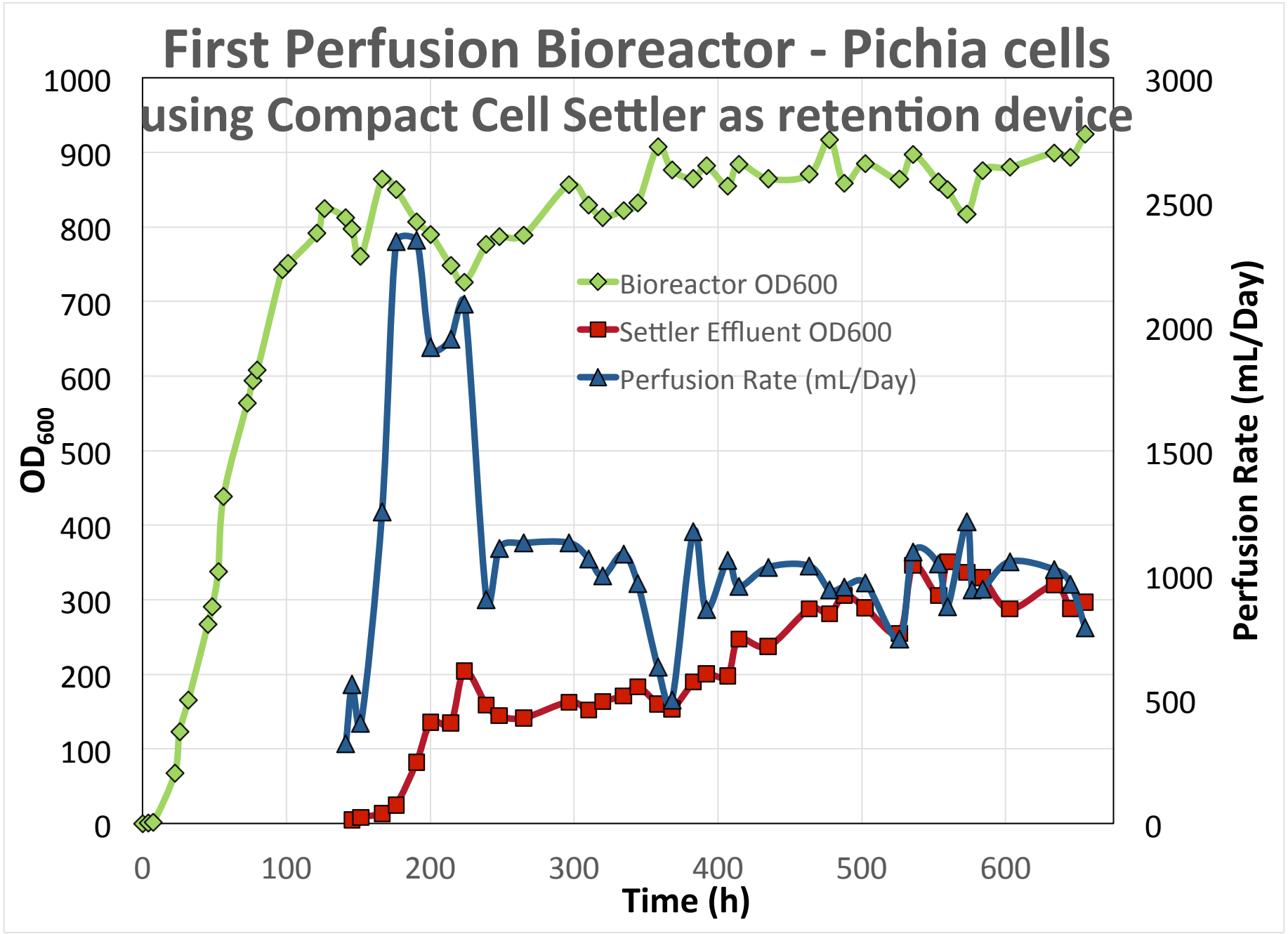
- Inclined settler can separate dead cells and cell debris into the settler top outlet, while the larger live yeast cells are recycled back to the bioreactor.
- Very high cell densities (> 500 OD) are achieved and maintained for several weeks (interrupted by power outages in India shutting off pumps in weekends).
- CS-10 designed for up to 10 liters/day perfusion rate in mammalian cell cultures can be operated up to only 400 ml/day, before losing a significant fraction (>25%) of the smaller yeast cells.

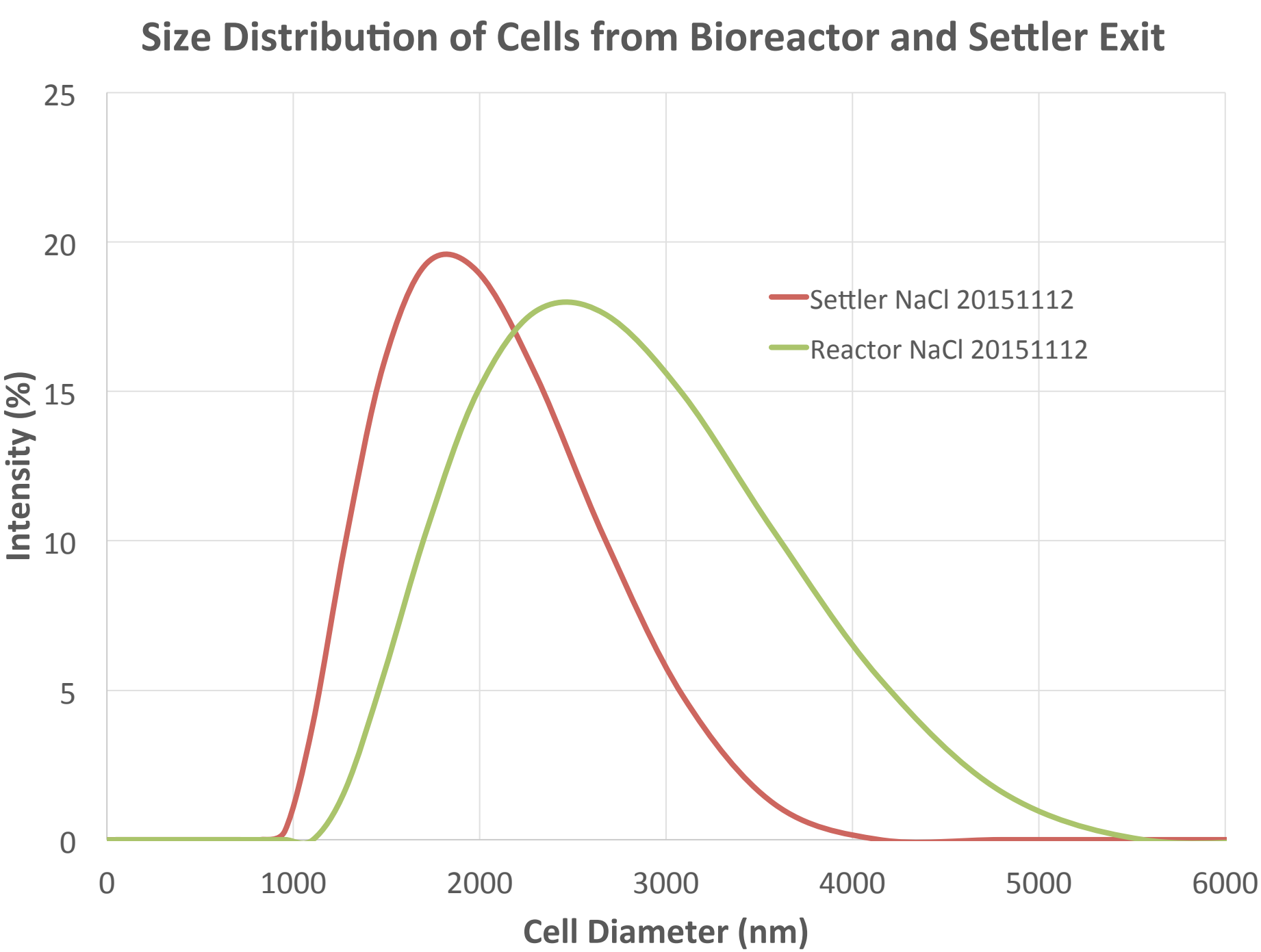
Novel Compact Cell Settlers

- We have now built and patented some more efficiently scaled-up compact cell settlers.
- These novel cell settlers are smaller, need less foot-print, and can scale up in true 3D.
- These novel cell settlers can be used for **microbial** and mammalian cell cultures.

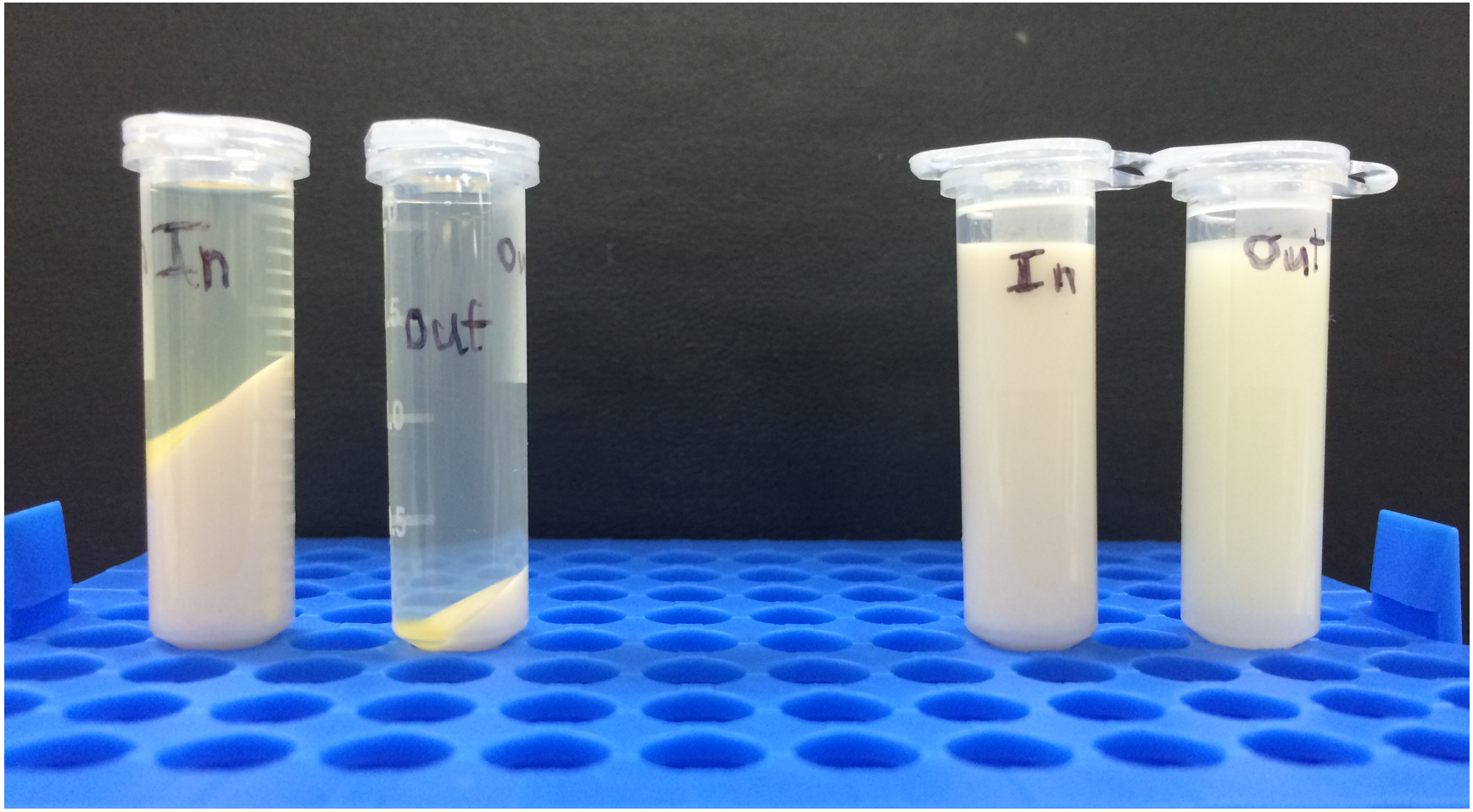
Perfusion Bioreactor of *P. pastoris* cells with Compact Cell Settler as retention device




First Perfusion Bioreactor - Pichia cells
using Compact Cell Settler as retention device
Bioreactor OD600
Settler Effluent OD600
Perfusion Rate (mL/Day)
OD600
Perfusion Rate (mL/Day)
Time (h)
0
100
200
300
400
500
600
700
800
900
1000
1500
2000
2500
3000


Size Distribution of Cells from Bioreactor and Settler Exit
Intensity (%)
25
20
15
10
5
0
0
1000
2000
3000
4000
5000
6000
Cell Diameter (nm)
Settler NaCl 20151112
Reactor NaCl 20151112

Bioreactor & Settler Effluent Samples Centrifuged & Control



Conclusions

- Inclined settler technology can be successfully used to remove smaller dead cells / cell debris and achieve high cell densities for microbial perfusion cultures (as proven before for mammalian cultures).
- Rectilinear scale up of inclined settlers as lamellar settlers is inefficient for microbial cell cultures.
- More efficiently scaled up “compact cell settlers” can be used with microbial yeast cells, which are maintained at very high cell density (> 800 OD) for > 3 weeks so far in continuous perfusion bioreactors.

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.

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