

# Characterization of Novel Pneumatic Mixing for Single-Use Bioreactor Application

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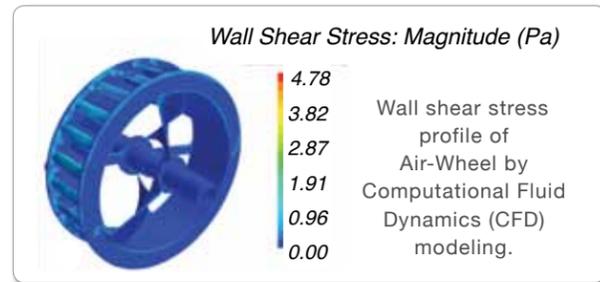
## Abstract

The novel bioreactor system from PBS Biotech<sup>®</sup>, which incorporates the patented Air-Wheel<sup>™</sup> technology, promotes uniform and homogeneous mixing and offers a low shear environment for cell culture, as evidenced by mixing time tests and computational fluid dynamics (CFD) modeling performed over a wide range of working volumes. Recent biological tests performed at various beta test sites have also shown scalable growth performance for a high cell density process in 3L and 15L PBS systems, successful baculovirus expansion in insect cells in the 3L PBS system, and comparable growth and product performance in the 250L PBS system against a 300L stirred-tank system using an Fc-fusion protein-producing process.

## Air-Wheel<sup>™</sup> Mixing Technology

PBS bioreactor systems are the first with a pneumatic mixing device powered solely by gas buoyancy, eliminating the need for an external mechanical agitator.

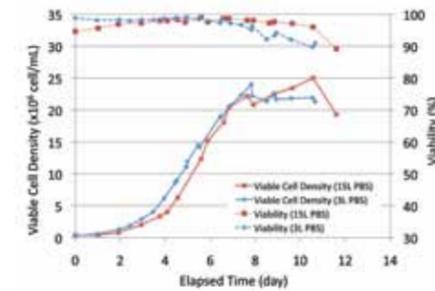
The patented design of the Air-Wheel mixing device promotes not only high tangential fluid flow around the wheel but also efficient radial and axial flow. The leverage effect of the Air-Wheel mixing device allows lower gassing requirement (v/v) with increasing working volume.



## High Density CHO Process at KGI

Lactate-adapted CHO DG44 cells were cultured in fed-batch mode in 3L and 15L PBS systems using CD OptiCHO<sup>™</sup> medium from Invitrogen<sup>™</sup>.

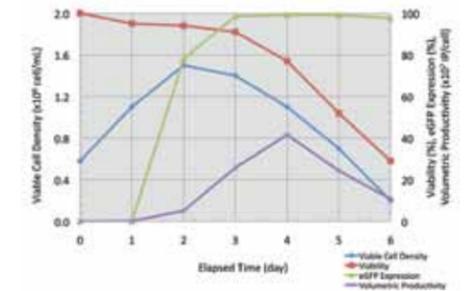
Comparable peak viable cell density of  $25 \times 10^6$  cell/mL was attained in both systems, and >90% viability was maintained through 12 days of culture.



## Baculovirus Expansion in Insect Cells at IBET

Sf9 insect cells were infected with baculovirus expressing enhanced green fluorescent protein (eGFP) in a 3L PBS system. Infection was performed at MOI of 0.8 on day 1 of the run.

Successful expansion of baculovirus in Sf9 cells was demonstrated by a high level of eGFP expression and infected particles after day 1.



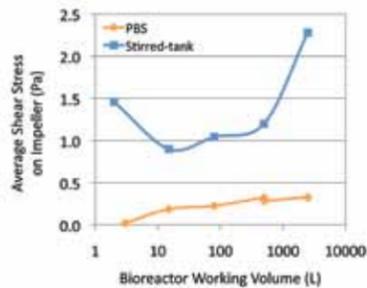
## Scalable Bioreactor System

### Short Mixing Time

	2L	10L	50L	250L	1,000L	5,000L
Gas Flow Rate (VVM)	0.07	0.06	0.05	0.04	0.03	0.02
Mixing Time (sec)	20	25	30	38	53	62

95% mixing time was calculated in PBS systems ranging from 2L to 5,000L working volume by measuring the change in conductivity readings from bolus additions of concentrated salt solution.

Mixing times were found to be between 20 sec and 62 sec over a wide range of working volumes in the PBS systems, significantly shorter than reported values in conventional stirred tank systems.

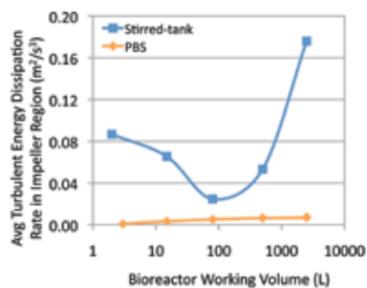


### Low Shear Stress

Shear stress was calculated from CFD modeling on Star CCM software.

Lower average shear stress ( $T_{avg}$ , in Pa) on the impeller was found in the PBS system compared to stirred-tank bioreactor at typical impeller speed.

Comparably low level of shear stress was found in the PBS system ranging from 3L to 2,500L working volume.



### Consistent Turbulent Energy Dissipation Rate

Turbulent kinetic energy dissipation rate ( $\epsilon$ , in  $m^2/s^3$ ) was calculated from CFD modeling on Star CCM software.

Lower average  $\epsilon$  in the impeller region was found in the PBS system compared to stirred-tank bioreactor at typical impeller speed.

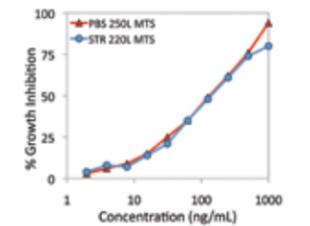
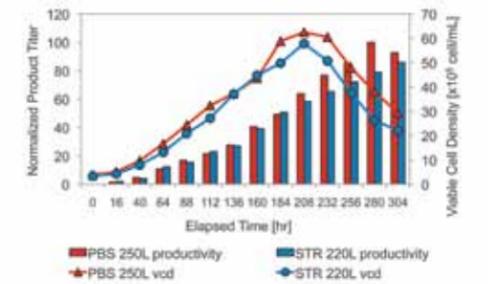
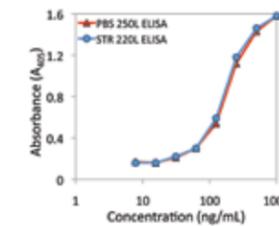
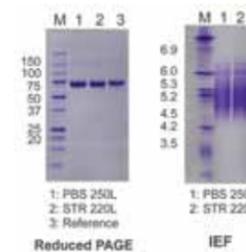
The uniformity of mixing in the PBS system was confirmed with the consistency of  $\epsilon$  from 3L to 2,500L working volume.

## Fc-Fusion Protein Process at Green Cross

A process for producing Fc-fusion protein was run in the 250L PBS system in parallel with the 300L (220L working volume) stirred-tank bioreactor in fed-batch mode over a span of 19 days.

Superior peak viable cell density and product titer were achieved in the PBS system compared to the stirred-tank system.

Protein physical methods (reduced PAGE and IEF), ELISA, and in-vitro bioactivity assay (MTS cell inhibition assay) revealed equivalence of products made in PBS and stirred-tank systems.



## Conclusions

A novel bioreactor system from PBS Biotech utilizes the patented Air-Wheel technology to promote uniform and homogeneous liquid mixing and offers a low shear environment for cell culture. Recent biological tests performed at beta test sites have demonstrated successful performance in three key tests:

- 1) Scalable growth for a high cell density process in 3L and 15L PBS systems,
- 2) Successful baculovirus expansion in insect cells in the 3L PBS system, and
- 3) Comparable growth and product performance in the 250L PBS system against a 220L stirred-tank bioreactor using an Fc-fusion protein-producing process.



## Technical Report of Pneumatic Bioreactor System



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