Development of Single-Use Pneumatic Bioreactor System™

Yas Hashimura[1], Daniel Giroux[1], Brian Lee[1], Yoo-Chang Park[2], Yong-Jae Kim[2], Sang-Hoon Paik[2], Jean-Man Kim[2], Jae-Choon Kim[3], Duk-Jae Oh[3], Baron Lumani[4], Dieter Eibl[4]

[1] PBS Biotech Inc., Camarillo, California, U.S.A., [2] Green Cross Corp. Central Research Center, YongIn-Si, KyungGi-Do, Korea, [3] SeJong University, BioScience and Biotechnology Dept., Seoul, Korea,
[4] University of Zurich, Department of Applied Life Sciences, Wadenswil, Switzerland

Abstract

Stainless steel bioreactors have traditionally been used in upstream bioprocesses for culturing eukaryotic cells for recombinant protein expression. Recently the benefits of single-use bioreactors have become more evident, resulting in their rapid adoption over the stainless steel systems. However limited scalability, cumbersome operation, and large footprint are all areas where current single-use bioreactors can improve upon. We have developed a novel Pneumatic Bioreactor System that addresses many of these existing challenges – being comparable in bioprocessing performance, yet scalable, easy-to-use, and more compact than bioreactors currently available in the market.

Why Single-Use Bioreactor?

- Reduces capital investment and construction lead time compared to stainless-steel bioreactors which require steam, cleaning, and hard piping
- Lowers equipment fabrication, installation, commissioning and validation costs
- Minimizes footprint and improves safety of operation (no steam/ pressure)
- Saves operational cost & time for cleaning (CIP/ SIP) between batches
- Reduces risk of cross-contamination from biological and product change-over
- Maximizes flexibility of development time & capacity for multi-product pipelines
- Simplifies process characterization & tech transfer from R&D to manufacturing
- Shortens the product development time & reduces the overall operation cost

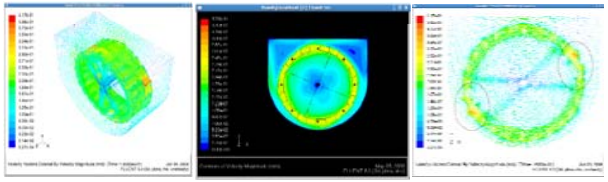
PBS Mixing Technology

- First bioreactor with the pneumatic mixing mechanism inside the bag chamber driven solely by gas buoyancy, which eliminates the need for an external mechanical mixing device
- Readily scalable from 2L to 5000L working volume, allowing for a reliable scale-down model which can be used for consistent and efficient process development, characterization and tech transfer
- With our patented Air-Wheel™ mixing technology, the gassing requirement decreases with scale on a working-volume basis due to the higher leverage effect of gas buoyancy as wheel diameters increase

Key Considerations for Scalable Bioreactor Systems

1) Rapid, homogeneous mixing:	To distribute medium nutrients, heat, metabolites quickly and uniformly
2) High mass transfer rate (k_La):	To provide sufficient oxygen to and remove carbon dioxide from cell culture
3) Low shear stress:	To allow comparable cell growth and productivity when scaling up process

Computational Fluid Dynamics (CFD) Modeling



Fluid velocity vectors and turbulent kinetic energy values were estimated using FLUENT ™ software (v 6.2). Homogeneous, three-dimensional flow patterns and comparable power input (W/m^3) to stirred-tank bioreactors was achieved at 20 RPM in the 10 L PBS bioreactor.

Short Fluid Mixing Time

	2L PBS	10L PBS	50L PBS	250L PBS	1,000L PBS	5,000L PBS
Gas Flow Rate (VVM)	0.07	0.06	0.05	0.04	0.03	0.02
Wheel Speed (RPM)	20	18	16	14	10	8
Mixing Time (sec)	10	17	25	30	36	42

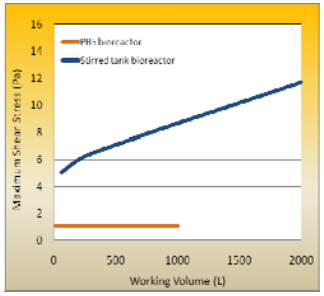
Fluid mixing time in PBS bioreactors was quantified using visual observation upon dye addition, pH changes with acid/base addition, and CFD modeling at various wheel rotation speeds (RPM) for different bioreactor scales. Significantly shorter mixing times were observed in PBS systems with 2L to 5,000L working volumes, compared to those from conventional stirred tanks.

High Oxygen Gas-Liquid Mass Transfer Rate (k_La)

	10L PBS			
Gas Flow Rate (VVM)	0.125	0.135	0.145	0.155
k_La (hr^{-1})	1.60	12.00	17.20	19.12

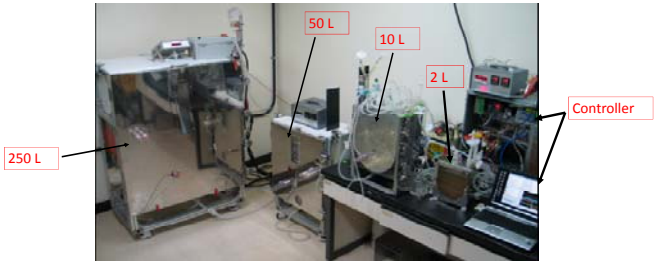
High gas-liquid mass transfer rate (up to 20 hr^{-1}) was achieved in the 10L PBS system using a dual s parger system: 1.25 SLPM gas flow rate through the open-tube sparger and 0.30 SLPM flow rate th rough the microsparger.

Low Shear Stress

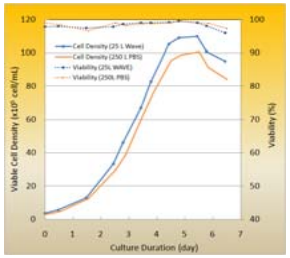


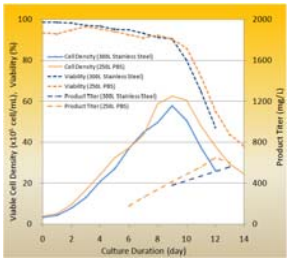
- Shear stress was calculated from CFD modeling on FLUENT ™ software (v. 6.2)
- Lower maximum shear stress was demonstrated in the PBS system compared to a conventional, stirred-tank bioreactor operated at typical impeller speed
- Consistent maximum shear stress in PBS system from 10L to 1,000L working volumes confirmed its broad range of scalability

Biological Test in PBS Bioreactors



A series of PBS bioreactor prototypes was employed for the 250 L cell culture evaluation: 2L, 10L, and 50L PBS for seed expansion and 250L PBS for production stage.





- Cell culture performance of 250L PBS bioreactor was compared with those of 300L stainless steel and 25L WAVE ™ bioreactors using CHO cell lines
- ***Graph on Left***: Comparable peak cell density (1.0x10^7 cell/mL) and viability profile (>95%) were achieved in both the 250L PBS bioreactor and the 25L WAVE ™ bioreactor in chemically-defined medium (*CD-CHO AGT™, Invitrogen™*)
- ***Graph on Right***: Comparable final product titer (600 mg/L), cell growth rate (20-hr D.T.), peak viable cell density (1.0x10^7 cell/mL), and viability profile were achieved in the 250L PBS bioreactor and the 300L stainless steel bioreactor

Conclusions and Future Directions

- A simple, user-friendly, single-use Pneumatic Bioreactor System (PBS) with novel, pneumatic Air-Wheel ™ mixing technology has been developed
- Pneumatic mixing provides sufficient power for homogeneous fluid mixing, high gas-liquid mass transfer, and low shear environment needed for optimal cell culture process, at gas flow rate input comparable to traditional stirred-tank systems
- Pneumatic Bioreactor System has been successfully scaled up from 2L to 250L in biological tests, and proof of concept has been demonstrated at 5,000L working volume
- The broad scalability of the Pneumatic Mixing System will allow PBS Biotech to offer a product line with a wide range of working volumes for upstream bioprocessing needs

PBS Biotech thanks Life Technologies™ for generous contribution of culture media