

## UNDERSTANDING THE IMPACT OF HIGH GAS ENTRANCE VELOCITY ON CHO CELL CULTURE PROCESSES TO IMPROVE PROCESS SCALE UP

Robin Luo, Boehringer Ingelheim Fremont Inc., Fremont, CA 94555  
robin.luo@Boehringer-Ingelheim.com

Garima Chaudhary, Immutics, Inc., Menlo Park, CA 94025

Meena George, Boehringer Ingelheim Fremont Inc., Fremont, CA 94555

Lia Tescione, Sanofi, Framingham, MA 01701

Anurag Khetan, Bristol-Myers Squibb, Princeton, NJ 08534

Henry Lin, Sanofi, Framingham, MA 01701

**Key Words:** bioreactor scale-up, bioreactor sparger, gas entrance velocity, CHO cell culture, scale down model

During the transfer and scale-up of three CHO processes from 2L and 100L in process development to 2,000L or 12,000L manufacturing scale bioreactors, significant differences in cell culture performance were experienced. While each process had its own set of contributing factors to the decreased performance in manufacturing scale, one major common root cause related to scale difference to all three processes is the significant higher gas entrance velocity (GEV) in the 2,000L and 12,000L bioreactors compared to the 2L and 100L bioreactors used in process development. This is due to the relatively small sparger area (sparger hole sizes and numbers) in the 2,000L and 12,000L bioreactors and the resulting high GEV caused higher shear stress to cells around the sparge zone. This in turn contributed to lower viability and productivity. Troubleshooting experiments were performed in 2L and 100L scales using spargers with much smaller gas entrance area to model the high GEV in manufacturing bioreactors. Results from these experiments confirmed that high GEV is a major contributing factor for poor cell culture performance in manufacturing scales. Further insight into the cell culture by flow cytometry revealed increased apoptosis due to high GEV. The outcomes from these investigations have driven the implementation of new spargers in manufacturing. The redesigned spargers in 2,000L and 12,000L bioreactors have larger gas entrance area (larger hole diameter and more holes), which lowered GEV to levels close to the 100L pilot scale bioreactor. The implementation of these new spargers has helped improve large scale cell culture performance in all three processes, closing the scale-up gap. These case studies illustrate sparger gas entrance velocity, in addition to other commonly used scale-up/scale-down parameters, is also an important parameter to consider in order to have a successful transfer to manufacturing.