A novel Chemically-Defined (CD) and Animal Origin-Free (AOF) basal and feed media combination was developed for use in CHO cell fed-batch protein expression culture. Parallel experiments testing media performance in shake flasks and the ambr®15 microbioreactor system demonstrated reduced viability and productivity in the controlled environment of the ambr®15 as compared to shake flasks. Harvest titers in shake flasks for this clone were greater than 3 g/L while the ambr®15 conditions resulted in half the productivity at 1.5 g/L. Reduced productivity and viability were accompanied by significant increases in lactate production which resulted in an increase in base addition and osmolarity. Several design of experiment (DoE) studies testing stir speed, gassing, and pH control set points were designed and performed in the ambr®15 to determine the source of the lactate accumulation. Gassing and pH strategies were found to be statistically impacting lactate accumulation with pH control range being the most meaningful. Several DoE optimized ambr®15 conditions matched or exceeded the productivity and growth levels of the uncontrolled shake flask conditions. However, ambr®15 bioreactors without any pH control enabled failed to sustain viable cultures. The pH, gassing, and stir speed optimized strategies determined in the ambr®15 microbioreactors were then scaled up to and confirmed in a fed-batch study using 5L single-use stirred-tank benchtop bioreactors.