Evaluation of PBS 15 Air-Wheel® Single Use Bioreactor for a Fed-Batch Monoclonal Antibody Process

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ABSTRACT

The Upstream Process Development team at MassBiologics has demonstrated successful cell culture process development utilizing MassBiologics's chemically defined media and feed platform in addition to optimized DASGIP bioreactor conditions to boost mAb expression and growth. In contrast to stirred tank bioreactors, the Air-Wheel® Single Use Bioreactor system utilizes rising gas bubbles and converts them into rotational mixing power, lowering the amount of shear stress on the cells. In this work we evaluated the PBS 15 Air-Wheel® using MassBiologics's optimized media and feed platform, pH control, dissolved oxygen control, temperature conditions, and a stable CHO cell line expressing an IgG1 mAb product. We compared its titer and growth patterns to concurrent DASGIP 1L bioreactors running under the same optimized conditions. All three 14 day fed-batch runs were conducted with one PBS 15 Air-Wheel®, two duplicate 1L DASGIP bioreactors with 15µm microspargers, and 100mL shake flask controls inoculated from each bioreactor system. However, the first experiment showed that evaporation for the Air-Wheel® culture was 43% higher than the DASGIP culture. As a result, the second experiment used diluted feed in order to compensate for the evaporating water calculated from the first experiment. Using the diluted feed reduced the evaporation rate to 27% when compared to DASGIP cultures without diluted feed. This led to the use of a lower Air-Wheel® agitation rate for the third run, which reduced the amount of gassing by 60%. Lower gassing reduced the evaporation rate further to 12%. All three experiments show that viable cell density, viability, titer, specific growth and death rates, productivity, and metabolism are comparable between the two bioreactor systems, and that the Air-Wheel® system provides better CO_2 stripping.

MATERIALS & METHODS

Materials:

- Stable CHO cell line producing IgG1 monoclonal antibody
- Chemically defined medium and feed platform
- All three runs include the use of:
  - 2 DASGIPs (900mL initial working volume (w.v.))
  - 1 PBS 15 Air-Wheel® system (9L initial w.v.)
  - 250 mL shake flasks (100 mL initial w.v.)

Methods:

- Cells were seeded at 0.4x10^6 cells/mL
- All bioreactors were run for 14 days in fed-batch mode
  - DASGIP gassing via 10 µm microsparger, 0.1 – 1.2 L/hr
    - Agitation initially set to 120 rpm, and increased daily based on the increasing volume
  - Air-Wheel gassing via macrosparger (air) and microsparger (oxygen)
    - Runs 1 and 2 used 150L/hr (38 rpm)
    - Run 3 used 60L/hr Day 0-7, then lowered gradually to 48 L/hr (29-26 rpm)
  - Bioreactor cultures were kept in 36.5ºC and 40% DO
  - CO_2 was sparged in order to maintain pH at a maximum of pH 7.3 (with a lower bound of pH 6.8)
  - Glucose was supplemented daily to 3.9 g/L (bolus)
  - Continuous feed
- Shakers were incubated at 140 rpm, 36.5ºC and 5% CO_2
  - Glucose was supplemented daily to 3.9 g/L (bolus)
  - Bolus feed
- Cell viability and density was determined by Cedex HiRes Cell Counter (Roche)
- Metabolites, pH, and pCO_2 and osmolality determined by Nova BioProfile 400 Analyzer (Nova Biomedical)
- Additional samples were centrifuged to remove cell debris. The supernatant was analyzed by Octet Qk (FortéBIO) for mAb quantitation
- Specific productivity was evaluated using IVCC

RESULTS

Part 1: Findings from Air-Wheel® 15 vs. DASGIP Run 1 and Run 2

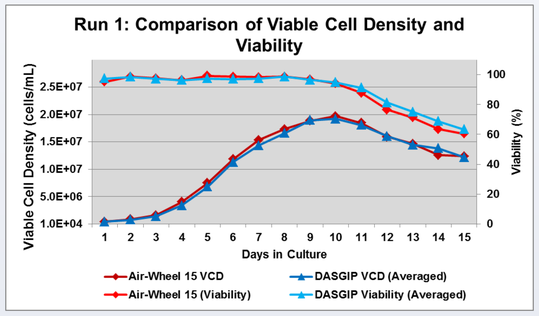

Figure 1: Comparison of viable cell density and viability between the Air-Wheel and DASGIP bioreactor systems. As shown in the results from the first run, the daily viable cell density and viability is very comparable between the two bioreactor systems. These trends were also seen in the subsequent two runs.

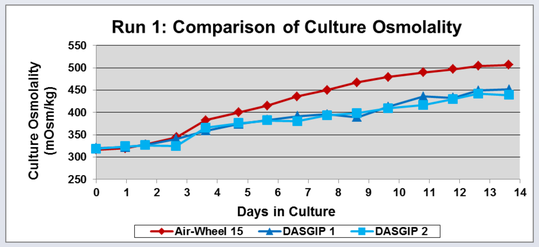

Figure 2: Comparison of culture osmolality between the Air-Wheel and DASGIP bioreactor systems. The osmolality of the Air-Wheel culture increased at a higher rate than the DASGIP culture, despite the nutrient addition into the systems being equal. This was the first indication that there was excessive evaporation in the Air-Wheel bioreactor system when run at 38 rpm (this agitation translates to about 150L/hr of gassing). The actual final and initial volumes were compared to the theoretical, and it was determined that the Air-Wheel culture had 43% more evaporation than the DASGIP cultures.

In order to compensate for the evaporating water, the feed was diluted 15% with dH_2O for the second run (based on calculations from the first run). The amount of nutrients being added to the culture on a daily basis was kept the same. However, diluting our feed is not a practical solution. Furthermore, adding back additional water to the culture reduced the amount of evaporation to 27% more than the DASGIP cultures, so there was still a considerable increase in osmolality for the second run.

Figure 3: The Air-Wheel® system utilizes rising gas bubbles and converts them into rotational mixing power, lowering the amount of shear stress on the cells. Lowering the agitation of the system significantly reduces the amount of total air gassing. Photo courtesy of PBS Biotech.

RESULTS

Part 2: Reducing the Overall Gassing to Prevent Excessive Evaporation of the Air-Wheel® 15 Culture

In the third run, a reduced gassing and agitation method was used to compensate for the evaporating water in the Air-Wheel system. The gassing tied to the vertical agitation of the wheel was reduced from 150L/hr to 60L/hr (38 rpm to 29 rpm). As the culture approached it's maximum cell density, it was further reduced to 48 L/hr (26 rpm). This was determined to be a low enough agitation to prevent excessive evaporation, while still allowing for sufficient mixing and CO_2 stripping.

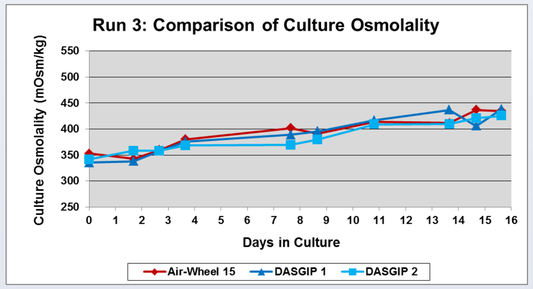

Figure 4: Comparison of culture osmolality between the Air-Wheel and DASGIP bioreactor systems. The osmolality of the Air-Wheel culture is very comparable to the osmolality of the DASGIP culture throughout this 14-day run. This indicates that running the Air-Wheel bioreactor system at 48-60 L/hr gassing allows for sufficient culture agitation and gassing without causing excessive evaporation.

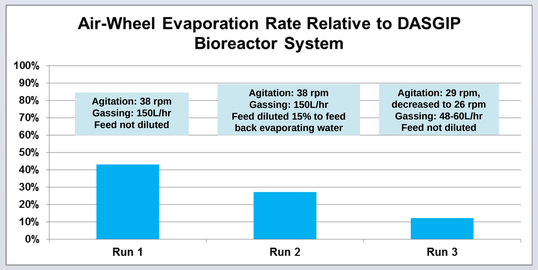

Figure 5: The Air-Wheel culture's evaporation rate as compared to the control DASGIP cultures for all three experiments. Diluting the feed by 15% reduced the relative evaporation to 27%. Lowering the amount of gassing by 60% reduced the relative evaporation rate to 12%.

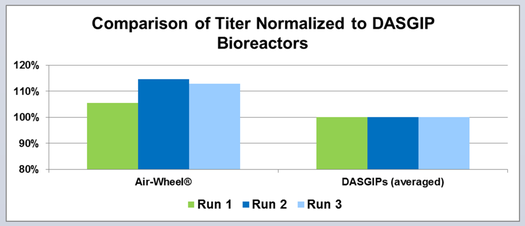

Figure 6: Comparison of titer normalized to DASGIP bioreactors. All data values were calculated with the evaporation in the Air-Wheel taken into account in order to show a fair comparison. There is no significant difference in titer between the Air-Wheel system and the DASGIP system.

RESULTS

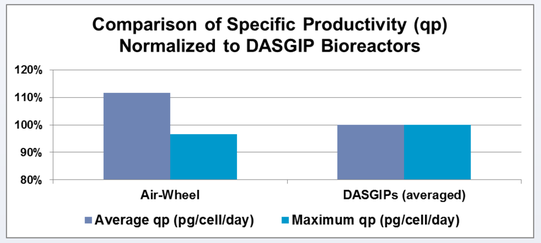

Figure 7: Average specific productivity. There is no significant difference between the specific productivity (amount of antibody produced per cell per day) between the Air-Wheel system and the DASGIP system.

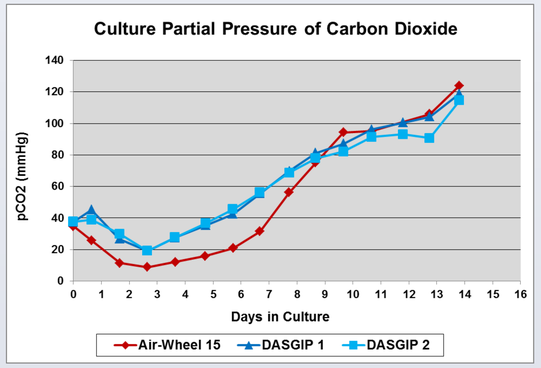

Figure 8: Partial pressure of carbon dioxide in culture. The Air-Wheel system has better CO_2 stripping during the first seven days of growth. This trend was seen in all three runs.

CONCLUSIONS

- The daily viable cell density and viability is very comparable between the two bioreactor systems.
- There is no significant difference in titer between the Air-Wheel system and the DASGIP system.
- There is no significant difference between the specific productivity (amount of antibody produced per cell per day) between the two bioreactor systems.
- The Air-Wheel system has better CO_2 stripping during the first seven days of growth.
- Cells grown in the Air-Wheel bioreactor display similar metabolism to cells grown in concurrent DASGIP bioreactors (data no shown).
- Lowering the amount of gassing by 60% reduced the evaporation rate (as compared to DASGIPs) to 12%.
- For processes that require a higher Air-Wheel agitation and gassing rate, the authors advise adding a chiller to prevent excessive evaporation.

ACKNOWLEDGEMENTS

The authors would like to thank Daniel Giroux, Joe Petrosky (PBS Biotech), Bernie Creswick, Kristina Papa (MassBiologics) for their assistance with this project.