

Dramatic Improvement in Recombinant Protein Yields using a 96-well High Throughput High-Speed Shaking Format and the INFORS HT 3mm Orbit Multitron Pro

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Abstract

A growing demand for recombinant proteins has driven the Biotechnology Industry to develop high throughput production and screening methods. Mammalian cell lines are preferable but have been difficult to adapt to 96-well culture due to their high oxygen and CO₂ demands. Recently, INFORS HT released the Multitron Pro incubator shaker with a 3mm orbital diameter. By using this instrument at 800–1000 min⁻¹, we have been able to increase recombinant protein yields significantly and improve well-to-well consistency compared to cultures using a 25mm orbit. These gains can be attributed to improved cell growth and viability because of optimal circulation. Additionally, we investigated different sealing systems and a peg tray that will enable us to stack up to 40 96-deepwell plates per incubator. This will allow for the development of an automatable high-throughput protein expression system that will shave months off of our current development time.

Methods

Cell Culture and Transfection

The 293E cell line, a suspension adapted HEK293 line licensed from the Biotechnology Research Institute, Canada was used in all experiments. Cells were cultivated as a seed stock in spinner flasks under the following conditions: 37°C, 5% CO₂, and 150 min⁻¹ agitation speeds. A 1:1 mixture of F-17 (Life Technologies) and SFM4 Transfix (GE Healthcare Life Sciences) was used as both the seed and production medium. Transfections were performed using a 25-kDa linear PEI (Polysciences) and purified plasmid DNA (Qiagen).

Experimental Design

All experiments were conducted on 96-deep well deep well plates (Greiner Bio-One Masterblock), and tested two agitation speeds (800 and 1000 min⁻¹) and two working volumes (500 and 1000uL). Transfections were performed in bulk at a 50mL working volume an aliquoted into the final working volume in 96 well deep well blocks. At the indicated time of harvest cultures were removed from the incubators, clarified by centrifugation, and the supernatant samples were analyzed by HPLC for protein expression.

Cell Count and Protein Concentration Measurement

Viable cell density and cell viability were measured using a TC 20 Automated Cell Counter (Bio-Rad Laboratories). Cell culture samples from 96-deep well plates were collected on culture days 0–7 for analysis. For protein concentration determination, supernatant samples assayed in replicate using affinity chromatography (Agilent Technologies).

Evaporation Rate Determination

The evaporation rate with three sealing systems were compared: Duetz-System (Kunher Technology), gas permeable membrane sealers (Axygen Scientific), and a Microplate box (INFORS HT). Evaporation was determined by measuring the difference in mass of each plate over 5 days calculated via linear regression and divided by 96 to generate a mg/well*day value. Plates were filled with 1000mL per well and agitated at 1000 min⁻¹ at a 3mm orbital diameter in an 75% humidity incubator.

Conclusion

By using the INFORS HT Multitron Pro incubator shaker with a 3mm orbital diameter, we have been able to increase recombinant protein yields significantly and improve well-to-well consistency compared to cultures using a 25mm orbit. These gains can be attributed to improved cell growth and viability because of optimal circulation. Additionally, we tested two agitation speeds, two working volumes, and three plate sealing formats. The optimal parameters were identified to be a fill volume of 1000ul and an agitation rate of 1000 min⁻¹ using a 3mm orbital diameter. By using a membrane-based sealer with the peg tray we were able to maximize throughput in our process. Although other sealing options could have the added benefit of reduced evaporation. Together these findings allow for a high throughput platform for recombinant protein expression.

Figure 1: Recombinant protein yield improvements using the INFORS HT Multitron Pro incubator shaker with 3mm orbital compared to a 25mm orbital shaker.

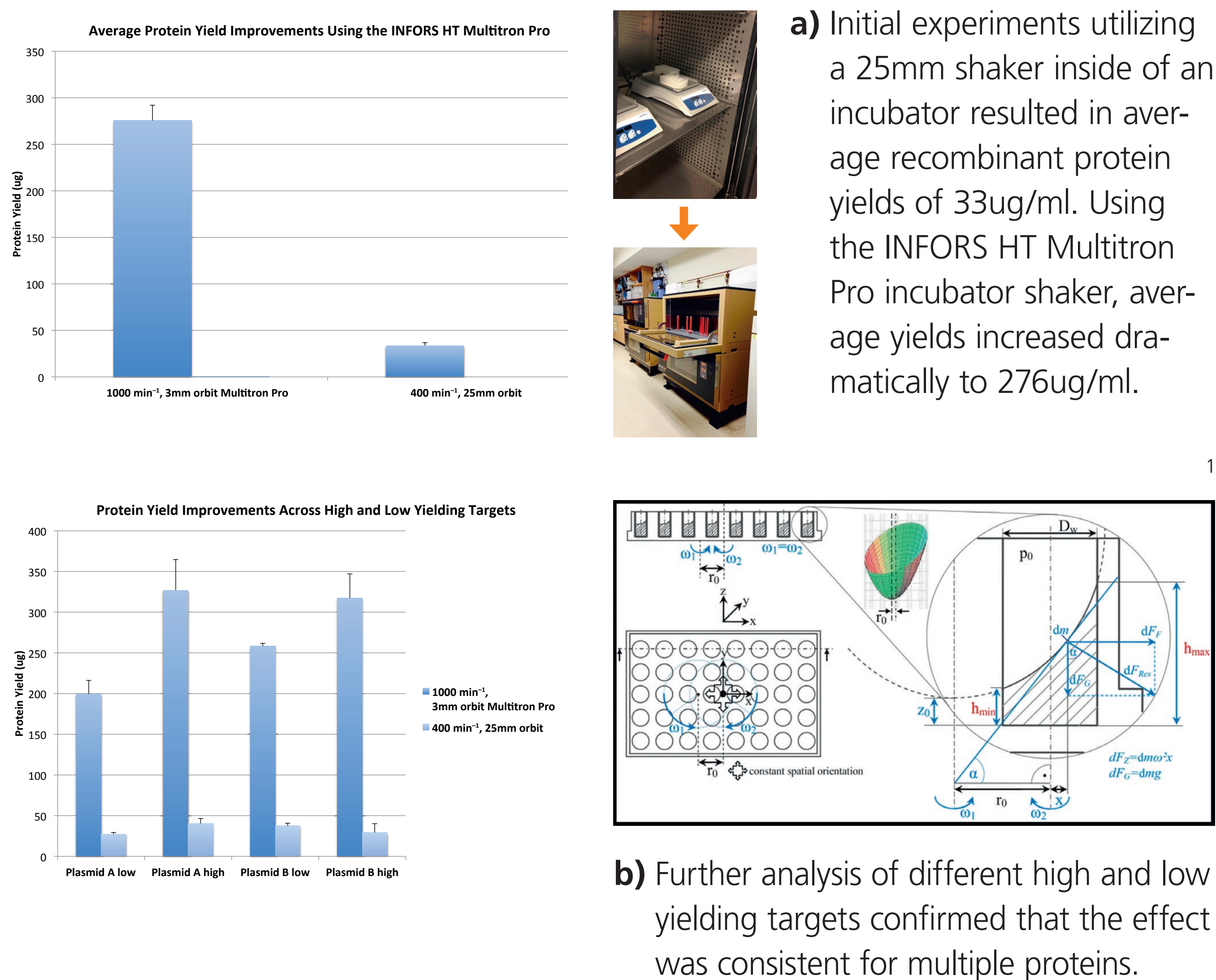


Figure 2: Increased oxygen transfer enhances cell growth

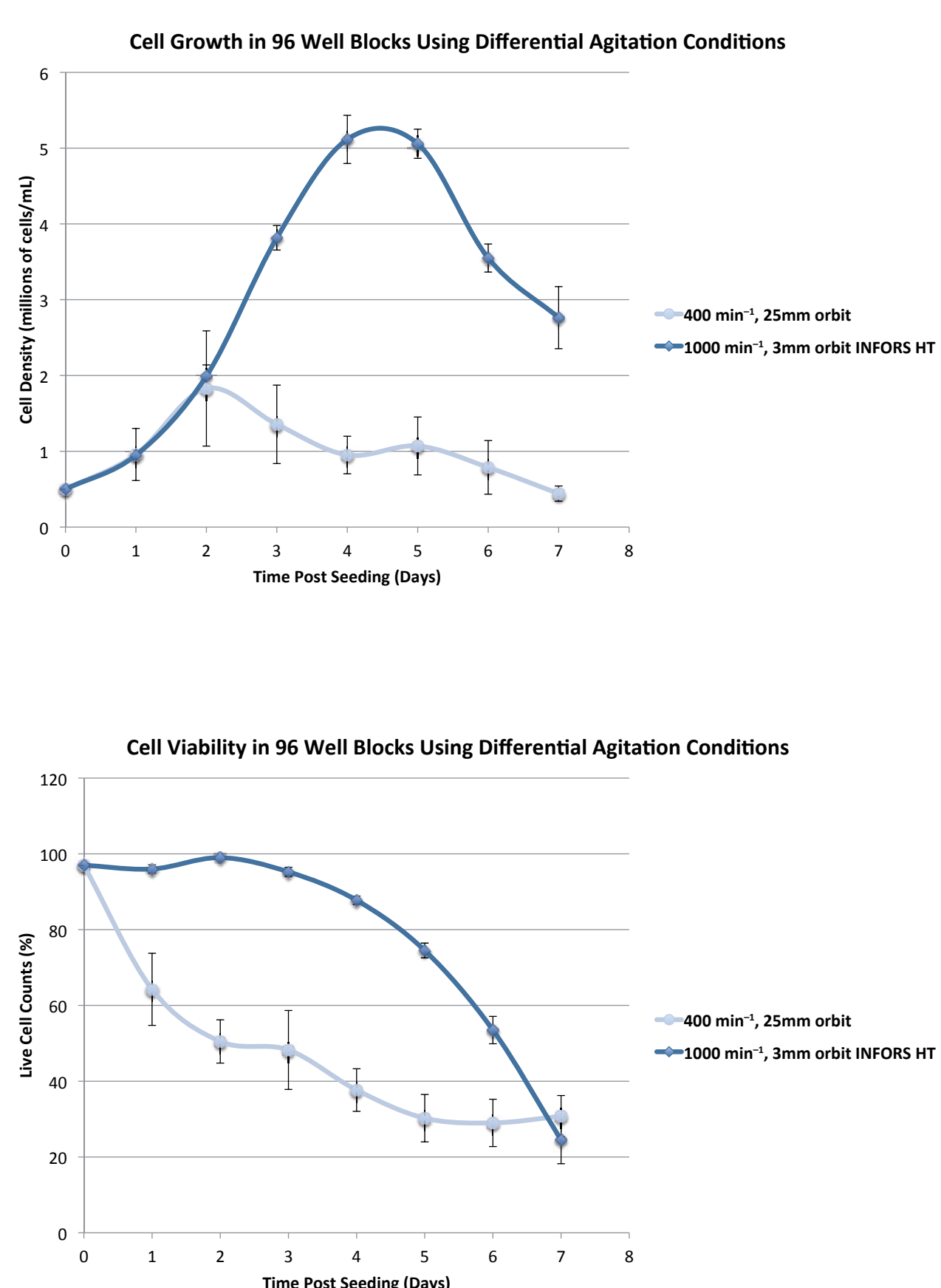


Figure 3: Differences in protein yield resulting from sealing method, fill volume and agitation speed

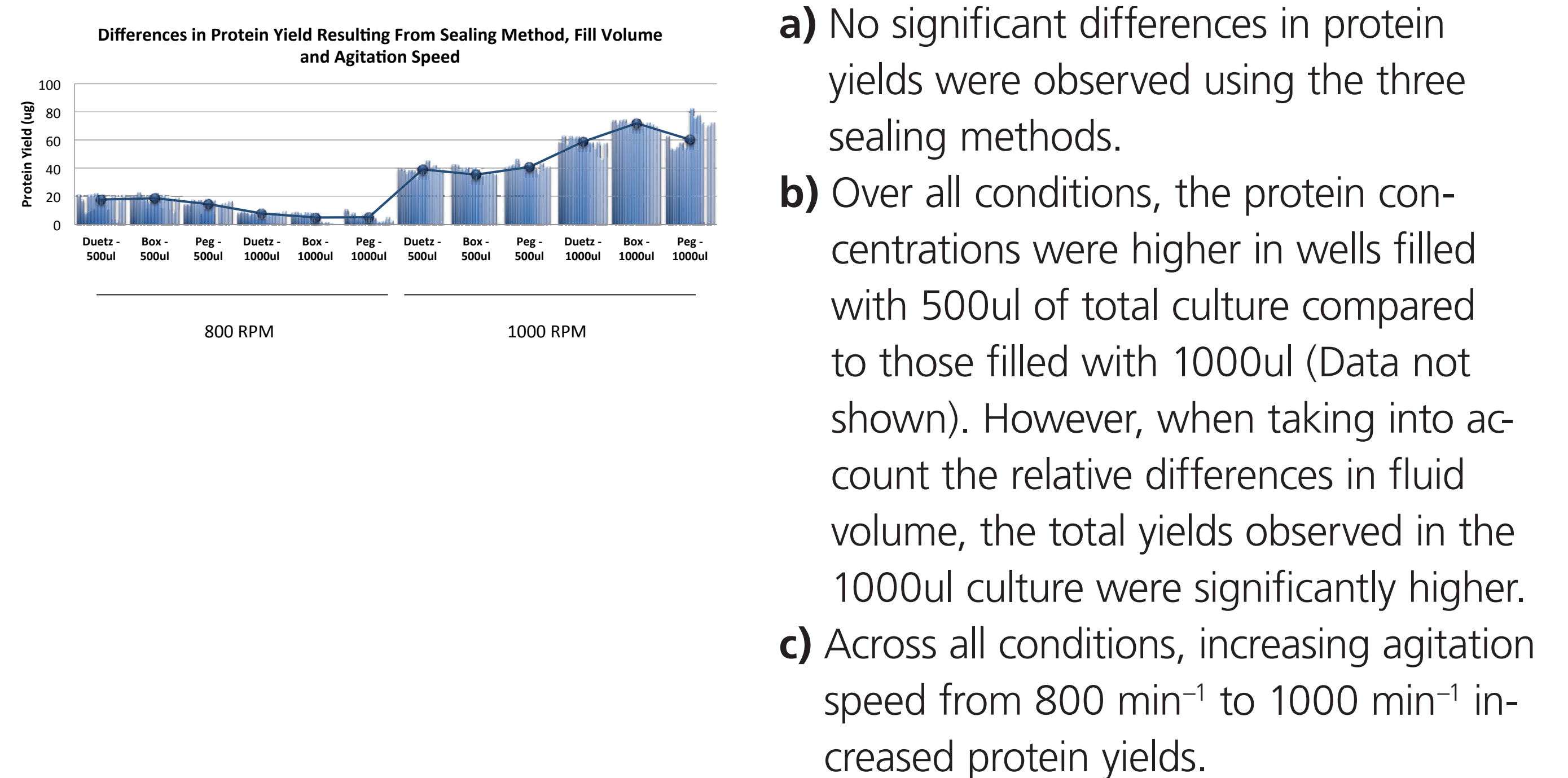
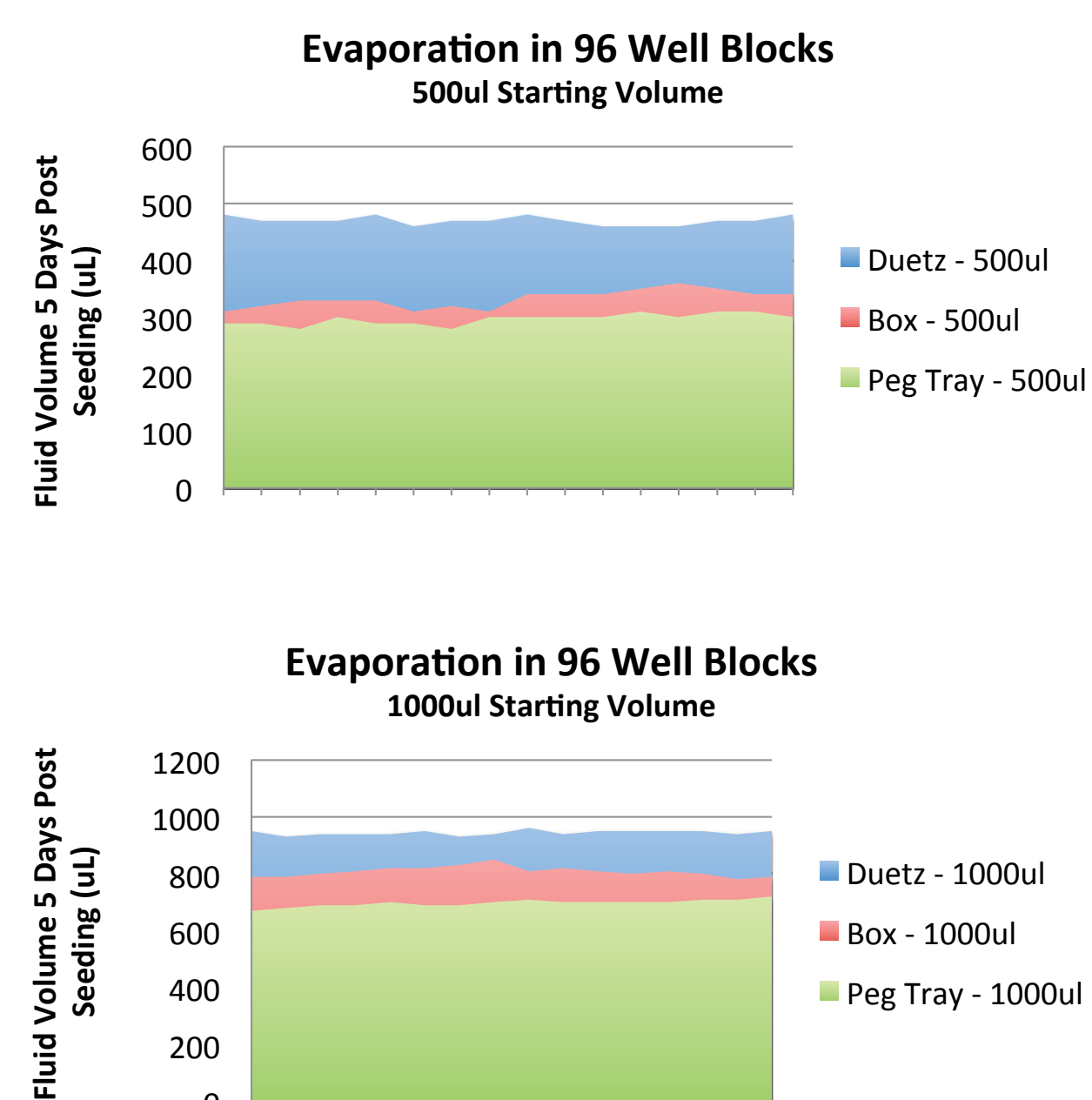


Figure 4: Evaporative loss using different sealing formats



A comparison of three commercially available plate-sealing formats resulted in significant differences in the residual fluid volume following five days of culture in the INFORS HT Multitron Pro. The least evaporative loss was seen using the Duetz-System followed by the Microplate Box (INFORS HT). Membrane based sealers resulted in the highest level of evaporative loss which was roughly 30–40% depending on the various culture formats.

Reference

- 1 www.thermoshaker.com/?tag=orbital-shaker
- 2 Bos, A.B., Luan, P., Duque, J.N., Reilly, D., Harms, P.D., Wong, A.W. (2015) Biotechnology and Engineering. 112 (9) 1832-1842.



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