The orbital shaken bioreactor SB10-X for bacteriophage production

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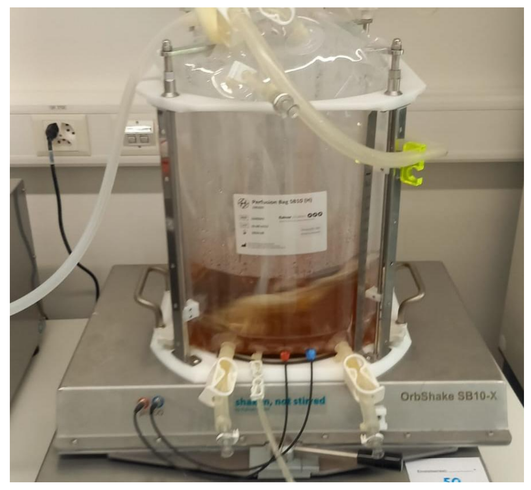
Background

As demand for phages in therapeutic, biocontrol, and diagnostic applications grows, easy-to-scale and reproducible production methods become essential. Bioreactors offer advantages over shake flasks in process control, yield, and scalability. This note presents the first orbital shaken bioreactor (OSB) application of an SB10-X for bacteriophage production.



Scale-up from shake flask to SB10-X

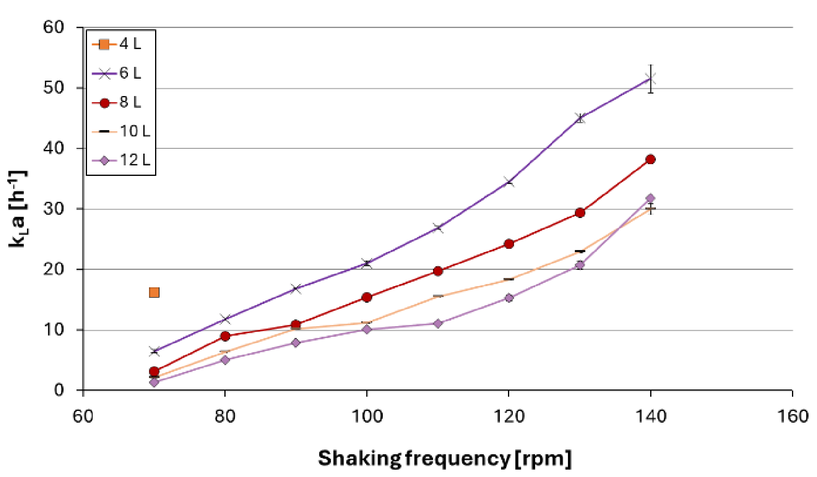


Figure 1: Volumetric mass transfer coefficient k_La at different filling volumes and shaking frequencies in the SB10-X. For a 5 L filling volume, the required shaking frequency was extrapolated from the data.

Orbital shaken bioreactors (OSBs) provide a low-shear hydrodynamic environment, as mixing is driven by orbital motion rather than impeller-induced turbulence, and oxygen transfer occurs via surface aeration instead of bubble sparging. Functionally, OSBs behave as large, controlled shake flasks, maintaining comparable flow regimes across scales. This makes them well suited for shear-sensitive mammalian cells. However, the design limits achievable k_La compared to stirred tank bioreactors, reducing suitability for oxygen-intensive microbial processes such as high-density *E. coli* cultures. To ensure comparable physiological conditions and avoid oxygen limitation during bacteriophage production scale-up, k_La was selected as the key scaling parameter. Based on the empirical correlation for shake flasks (Klöckner & Büchs, 2012) and the conditions in Table 1, a k_La of 11.5 h^{-1} was estimated. This value was matched in the SB10-X by setting the shaking frequency to 72 rpm, as derived from Figure 1.

Table 1: Production conditions for shake flask and SB10-X orbital shaken bioreactor.

Scale	Nominal Volume [L]	Working Volume [L]	Shaking Diameter [mm]	Shaking Frequency [rpm]
Shake Flask	1	0.250	20	120
SB10-X	12	5	50	72

A strain of *E. coli* was cultured overnight (O/N) in brain heart infusion (BHI) shaking at 120 rpm at 37 °C. A 100-fold dilution of the O/N culture was started in either the shake flasks or the SB10-X using a sterile 50 mL syringe. Phage inoculation was performed by injecting a 50 mL suspension of ~2.0×10^{12} PFU/mL of phage to obtain an MOI of 1.

Increase in phage titers after 3 hours

Phage forming units (PFUs) were compared between scales at 3 and 24 hours post-infection. At 3 hours, SB10-X cultures yielded higher average titers (~1.0×10^{9} PFU/mL) than flask cultures (~2.0×10^{8} PFU/mL), indicating faster early amplification. At 24 hours, both systems showed substantial increases, reaching ~1.0×10^{10} PFU/mL, with SB10-X maintaining a slight advantage. Greater variability was observed in the 3-hour shake flask samples (Figure 2). These results suggest that SB10-X cultivation enhances early phage amplification and offers comparable or improved yields at later time points, with greater process consistency.

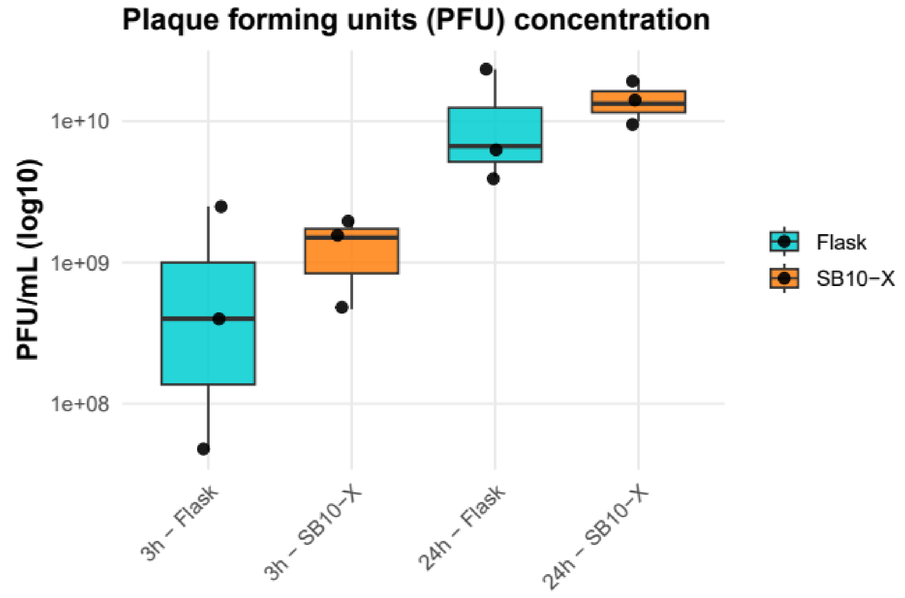


Figure 2: Average phage concentration (PFU/mL) in shake flask and SB10-X cultures at 3 and 24 hours post-infection. SB10-X cultures showed higher titers at both time points, with the most pronounced difference at 3 hours. Error bars represent standard deviation from biological replicates.

Increase of total phage production

Total phage output was also assessed at both time points. At 3 hours, SB10-X cultures produced ~1.0×10^{13} PFU on average, markedly higher than flask cultures (~1.0×10^{11} PFU) with high variation. By 24 hours, flask cultures reached ~1.0×10^{12} PFU while SB10-X cultures achieved ~1.0×10^{14} PFU, confirming a significant benefit in both early amplification and total output (Figure 3).

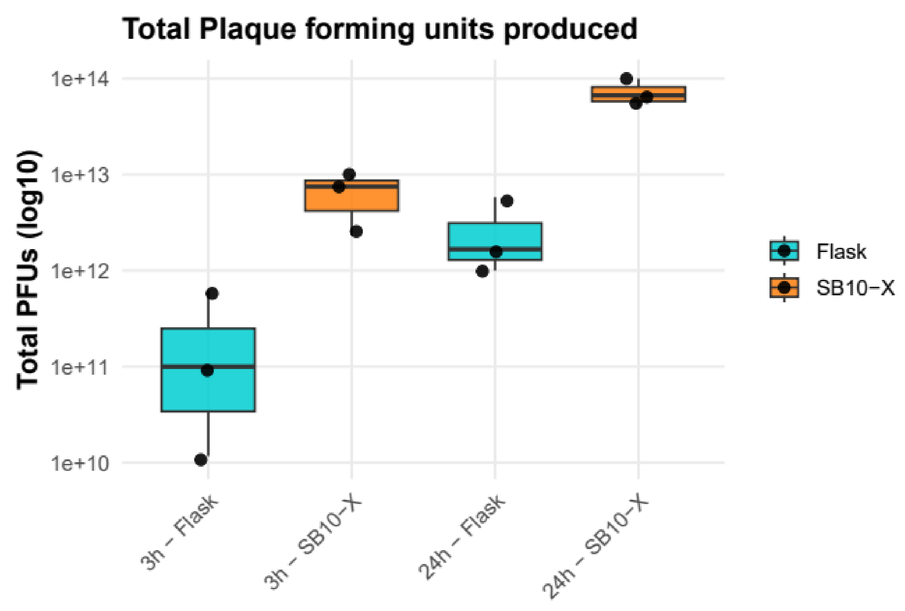


Figure 3: Total phage yield (PFUs) in shake flask and SB10-X cultures at 3 and 24 hours post-infection.

Conclusion

The SB10-X outperformed shake flasks in both phage concentration and total yield, particularly at the 3-hour time point. After 24 hours, both systems achieved high titers, but the OSB maintained a consistent lead in productivity and replicate robustness. These findings highlight the benefits of bioreactor-based cultivation – enhanced process control, improved oxygen transfer, and superior scalability – while the OSB's shake-flask-like hydrodynamics facilitate straightforward scale-up. Integration with USP and DSP systems further supports full process monitoring, making the transition to OSBs a compelling step toward consistent, efficient phage production for therapeutic development.

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Klöckner, W., & Büchs, J. (2012). Advances in shaking technologies. *Trends Biotechnol*, 30(6), 307–314.

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