Efficacy of the Epiphyte3 LEX-10 Bioreactor for Cell Growth and Protein Expression

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The Large-scale Expression (LEX)-10 bioreactor by Epiphyte3 was developed to facilitate high-throughput cell growth and recombinant protein expression with considerations for ease of use and efficient use of space. This technical note compares the LEX-10 with traditional stacked shaking incubators typically used for microbial outgrowth and protein expression to determine which is easiest to operate and most effective for high-throughput protein expression.

Practicality: The LEX-10 employs a stacked setup to save lab floor space: the lowest module is a diaphragmatic pump for draining which doubles as shelving, followed by the circulating water bath that can be cooled or heated between 4 and 45 °C, with extra vertical headroom for the air spargers and reverse airflow module on top. The total width of the bioreactor is 37 inches (**Fig 1A**), compared to the 53.1-inch width of the shaking incubator our lab used as a comparison, making it far easier to fit in a typical lab space. However, it should be noted that because the LEX-10 uses sparged air to provide oxygen to proliferating cells (rather than shaking), it also requires a source of air pumped in from an in-house gas line, slightly increasing its footprint.

The ability of the LEX-10 to use capped glass bottles rather than baffled/non-baffled Erlenmeyer flasks greatly increases the high-throughput capability of the bioreactor compared to the shaking incubator, because Erlenmeyer flasks may only be filled to half of the entire flask volume (or less) to achieve adequate levels of airflow within the culture. The completely submerged air spargers of the LEX-10 do not require significant headroom in the bottle. The LEX-10 can fit up to a maximum of 9 liters (5 1.8-liter cultures) of culture in the water bath. A comparable setup in our shaking incubator would require at least 10 individual 1-liter cultures (in 2-liter flasks). Using fewer culture vessels with the LEX-10 makes media preparation, inoculation, induction, and sample cleanup steps far smoother.

Cell Growth and Protein Expression: We tested the speed at which the LEX-10 could grow two types of organisms (EBY100 yeast and BL21(DE3) *E. coli*) in typical growth media, and its output of soluble protein following a typical recombinant protein expression protocol, compared to our traditional shaking incubator. The results of these experiments are shown in **Fig. 1**. For yeast growth (**Fig. 1B**), the LEX-10 cultures began to significantly outpace the shaking incubator cultures after 8-9 hours in terms of yeast cells per mL and appeared to plateau/reach saturation approximately 3 hours before the shaking incubator cultures. For *E. coli* growth (**Fig. 1C**), the LEX-10 cultures outpaced the shaker cultures after approximately 2 hours during the logarithmic phase of growth, after which the LEX-10 culture growth slowed to be comparable with the shaker culture; however, after 24 hours the optical density of the culture at 600 nm (proportional to the number of cells) of the LEX-10 cultures was nearly double that of the shaker cultures due to the acceleration of growth during the logarithmic growth phase.

Our protein expression comparison between the LEX-10 and shaking incubator followed a typical protocol [1], with T7-mediated IPTG induction taking place in the early/mid-logarithmic phase, followed by protein purification via high-speed centrifugation and immobilized metal affinity chromatography (IMAC). The LEX-10 cultures produced significantly more soluble protein compared to the shaking incubator cultures (**Fig 1D**).

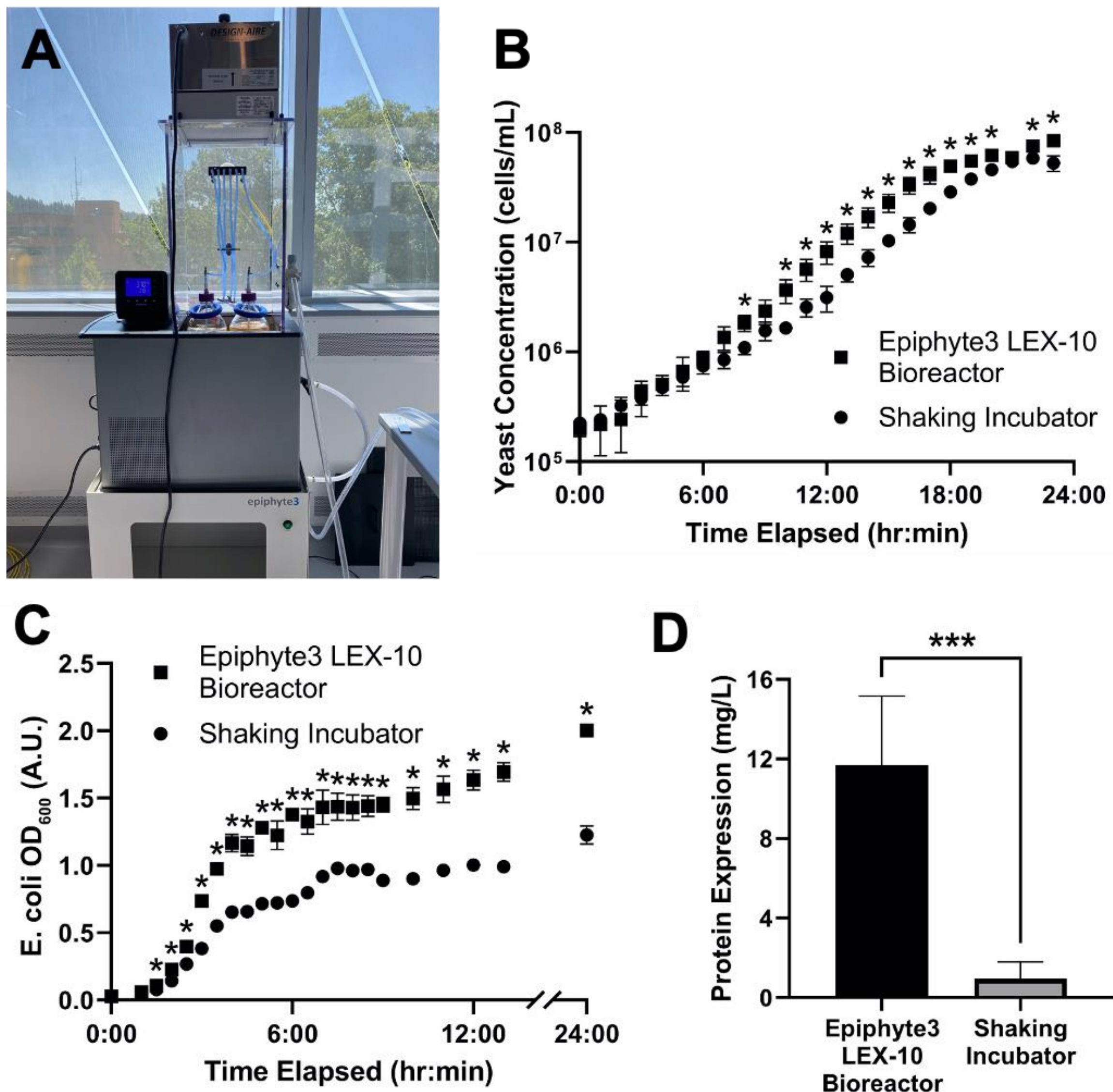

Figure 1. **LEX-10 Bioreactor facilitates improved cell growth and protein expression compared to traditional shaking incubator.** (**A**) Photograph of LEX-10 Bioreactor. (**B**) The concentrations of yeast growing in the bioreactor and shaking incubator were measured at hour intervals. (**C**) Absorption of light at 600 nm by *E. coli* growing in the bioreactor and shaking incubator was measured at half-hour and hour intervals for 13 hours, and again after 24 hours. (**D**) Soluble protein concentration of purified samples from pooled *E. coli* cultures grown in the bioreactor and shaking incubator were measured via light absorption at 280 nm. Data and error bars represent mean ± standard deviation. 1-way or 2-way ANOVA with Bonferroni's post-hoc test. * $p < 0.05$, *** $p < 0.001$.

Conclusion

In conclusion, we found the LEX-10 bioreactor to improve yeast, bacteria, and overall soluble protein yield compared to traditional stacked shaking incubators, in addition to providing better easy-of-use and space efficiency.

Experimental Methods

Yeast outgrowth – EBY100 *Saccharomyces cerevisiae* yeast were seeded at an initial concentration of 10^4 cells/mL in SD-CAA yeast growth media (16.8 g/L sodium citrate dihydrate, 3.9 g/L citric acid, 20.0 g/L dextrose, 6.7 g/L yeast nitrogen base, 5.0 g/L casamino acids) with 1 µg/mL ciprofloxacin, 100 µg/mL ampicillin, and 500 µL of Antifoam 204. Three bioreactor cultures (0.9 L each) were prepared in 1 L glass bottles and grown with constant air sparging at 30 °C. Three shaker cultures (0.9 L each) were prepared in 2 L baffled Erlenmeyer flasks and grown

with constant shaking at 250 rpm at 30 °C. Yeast concentration was quantified using a Countess II Automated Cell Counter (ThermoFisher Scientific).

E. coli outgrowth – BL21(DE3) *E. coli* cultures were grown overnight in 10 mL lysogeny broth (LB) with 50 μg/mL kanamycin. The following day, three 0.9 L bioreactor cultures in 1 L glass bottles and three 0.9 L shaker cultures in 2 L baffled Erlenmeyer flasks were prepared with Terrific Broth (TB) and 500 μL of Antifoam 204, inoculated with overnight cultures, and grown at 37 °C with constant air sparging or shaking at 250 rpm. *E. coli* growth was quantified by measuring culture optical density at 600 nm (OD_{600}) in a 1 cm pathlength cuvette using a NP80 spectrophotometer (Implen).

E. coli protein expression – Overnight BL21(DE3) *E. coli* cultures containing a pET-28b(+) expression vector for a novel, soluble protein with a hexahistidine (H_6) purification tag were prepared as described previously. Liquid culture was transferred to 1.8 L of Terrific Broth (TB) containing 50 μg/mL kanamycin, 0.4% glycerol, and 500 μL of Antifoam 204. TB cultures were grown at 37 °C with constant air sparging or shaking until $OD_{600} \geq 1.25$, at which point Isopropyl ß-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM to induce protein expression. Growth temperature was reduced to 18 °C and cultures were grown for 18 hr. TB cultures were centrifuged at 6,000 rpm at 4 °C for 20 min and cell pellets were frozen at -20 °C for 24 hr. Cell pellets were resuspended in 45 mL binding buffer (50 mM Tris pH 7.5, 500 mM sodium chloride, 5 mM imidazole) with 150 mg tris(2-carboxyethyl) phosphine (TCEP) and lysed via sonication at 55% amplitude for 7.5 min at 10 s intervals. Sonicated cell lysates were centrifuged at 13,000 rpm at 4 °C for 40 min, and the soluble fractions were incubated with 7.2 mL of cobalt-nitrilotriacetic acid (Co-NTA) agarose beads for 20 min at 4 °C. The bead-lysate solutions were poured into a glass chromatography column and the beads were washed with 10 × 10 mL of wash buffer (50 mM Tris pH 7.5, 500 mM NaCl, 30 mM imidazole). Following the washes, bead-bound proteins were eluted using elution buffer containing a high concentration of imidazole (50 mM Tris pH 7.5, 500 mM NaCl, 250 mM imidazole). Proteins were exchanged into 30 mM Tris pH 8 buffer, and protein concentration was measured using a NP80 spectrophotometer.

References

[1] Sivashanmugam, A., Murray, V., Cui, C., Zhang, Y., Wang, J., & Li, Q. (2009). Practical protocols for production of very high yields of recombinant proteins using Escherichia coli. *Protein science*, *18*(5), 936–948. https://doi.org/10.1002/pro.102.