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Bioprocessing Tutorial

Microbial Secretion via ESETEC® Technology

System Was Developed to Create a Cost-Efficient Alternative to Mammalian Cell Culture

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The success of biopharmaceuticals started about 30 years ago with the first production of recombinant human insulin (Humulin®) in *Escherichia coli*, followed by the first production of human tissue plasminogen activator (tPA) in mammalian host cells some years later.

Due to the lack of glycosylation in *E. coli*, which is required for the biological activity of most monoclonal antibodies, the use of Chinese hamster ovary (CHO) cell lines soon became the industrial gold standard for the production of biopharmaceuticals.

CHO cells possess the machinery for post-translational modifications and, in contrast to conventional *E. coli* systems, make it possible to purify correctly folded and secreted proteins directly from the culture broth. Consequently, the booming demand for antibodies led to the success of CHO cells in biomanufacturing.

Nevertheless, CHO-based systems still suffer from slow cell growth and thus low productivity. Moreover, process development using mammalian cells is time-consuming, due to tedious clone screening and selection, which can take up to five months.

The demand for fast, safe, and cost-efficient manufacturing solutions is triggered by increasing pressure on clinical development timelines and public healthcare systems. Personalized medicine and biosimilars are just two examples underlining the need for innovative expression platforms that combine high productivity, protein secretion, and rapid process development.

ESETEC®

E. coli is a well-studied and quick-replicating host with a genetic system that is easily manipulated. The fast-growing nature of *E. coli* accelerates process development, with less time spent on clonal screening, cell line development, cultivation, and testing.

Common disadvantages are the laborious purification from the periplasm and refolding from inclusion bodies. Such constraints, however, have been conquered by the proprietary *E. coli* expression system ESETEC® (*E. coli* secretion technology) developed by **Wacker Biotech**.

Safe *E. coli* K12 strains have been engineered to secrete correctly folded

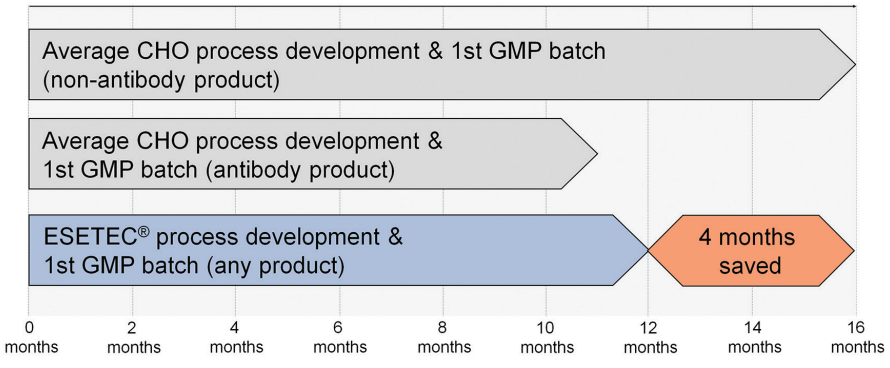

Figure 1. From gene to first GMP batch—timeline comparison ESETEC® versus CHO. Duration of cell-line/strain selection, process development, scale-up, and GMP manufacturing is shown. Data for CHO cells is based on data of two relevant market players. The typical development and production timeline of ESETEC® saves up to four months compared to mammalian cell culture. Advantages of ESETEC® are faster strain development and the lack of development of viral depletion steps (not necessary for microbial systems).

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proteins directly into the culture medium. The unique ability to export the proteins enables purification of the product without cell disruption and results in higher yields and quality. The secretion of the target protein reduces process-related impurities, like host cell DNA and endotoxins, which need to be removed by more extensive purification in conventional *E. coli* procedures. Recent improvements of the ESETEC® technology have allowed high-level expression and secretion of proteins that are difficult to express. The broad range of secreted products with molecular weights of 5 to 150 kDa renders ESETEC® a versatile and cost-efficient alternative for any nonglycosylated biopharmaceutical.

Time Is Critical

Biopharmaceutical drug development requires several rounds of clinical testing with an increasing amount of drug substance needed. Regrettably, the failure rate of early clinical candidates is more than 90%, fueling the demand for rapid and reliable production systems to cope with the increasing number of clinical studies.

The long history of CHO cells for the standardized production of antibodies has helped to improve development timelines. Ideally, generic purification approaches counterbalance time-consuming cell-line development, therefore reducing the advertised time from gene to GMP-grade antibody material to 11 months.

Process development and GMP manufacturing of nonantibody products, however, require at least 16 months, mostly due to higher efforts and longer process development timelines (*Figure 1*). ESETEC® takes advantage of a fast-growing host strain and protein secretion, which speeds up process development and production. In total, only 12 months are required from gene to the first GMP batch, even for nonplatform products (*Figure 1*).

By leveraging the ESETEC® advantage in GMP manufacturing, the typical time-in-facility of a batch is just one third of that of mammalian cell cultures. Due to shorter fermentation times and based on a simulated process, batch production with ESETEC® is completed after seven days, while CHO cells require approximately 20 days.

Comprehensive Cost Analysis

A comprehensive cost analysis for different expression systems is difficult, as each process varies, depending on the protein of interest. To make matters even worse, manufacturers have their own preferred procedures, expression hosts, media, and purification strategies. For an unbiased head-to-head comparison of both technologies, we employed cutting-edge process simulation software to calculate the cost of goods based on ESETEC® and a CHO cell culture.

The analysis for a hypothetical nonglycosylated protein, assuming identical secretion titers of 2.2 g/L, was performed with BioSolve Process 7 (**Biopharm Services**). As far as possible, identical input cost data were used for CHO and ESETEC®. Since both technologies secrete the active protein into the culture broth, primary downstream processing (pDSP) and downstream processing (DSP) are widely comparable (*Figure 2A*). Therefore, the simulation is based on a similar pDSP/DSP sequence with the exception of the mammalian-specific viral inactivation/filtration steps.

The purification setup contains three chromatography columns with identical yields and life cycles. The calculations are based on three working shifts and stainless steel fermenters. The estimated overall costs for facility investments, capital costs and labor were identical. Initially, total manufacturing costs of a single batch with 1,200 L working volume (w/V), corresponding to 1,500 L total fermenter volume, were analyzed.

Processing of a mammalian batch

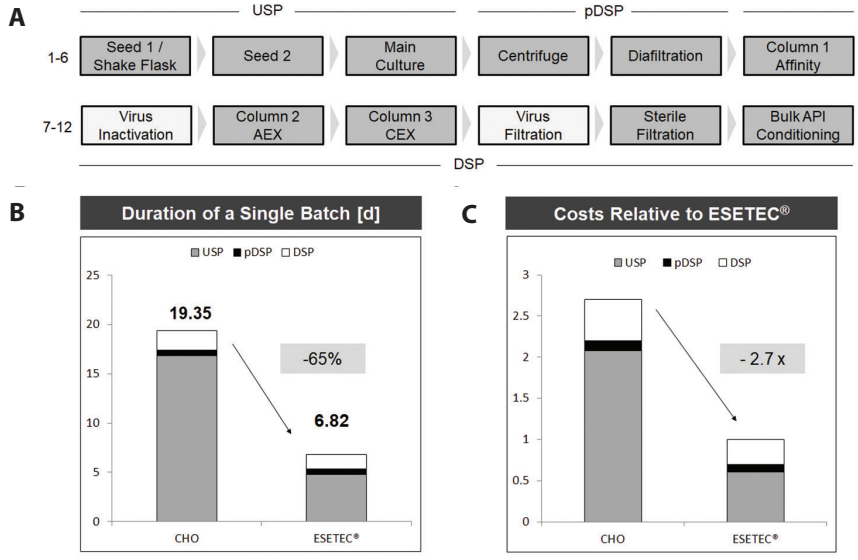

Figure 2. Process simulation results for fermentations with ESETEC® and CHO cells in 1,200 L (w/V).
(A) Simulated manufacturing process for ESETEC® and CHO cell culture using BioSolve Process 7 (Biopharm Services). Steps displayed in light grey only apply for CHO cell. (B) Total process times of a single ESETEC® and CHO GMP batch based on a three-shift model. Due to short fermentation times, ESETEC® reduces the process duration by up to 65%. (C) Total costs of single GMP batches relative to ESETEC®. The cost of goods is reduced up to 2.7-fold due to shorter process times, reduced media costs, and viral depletion steps.

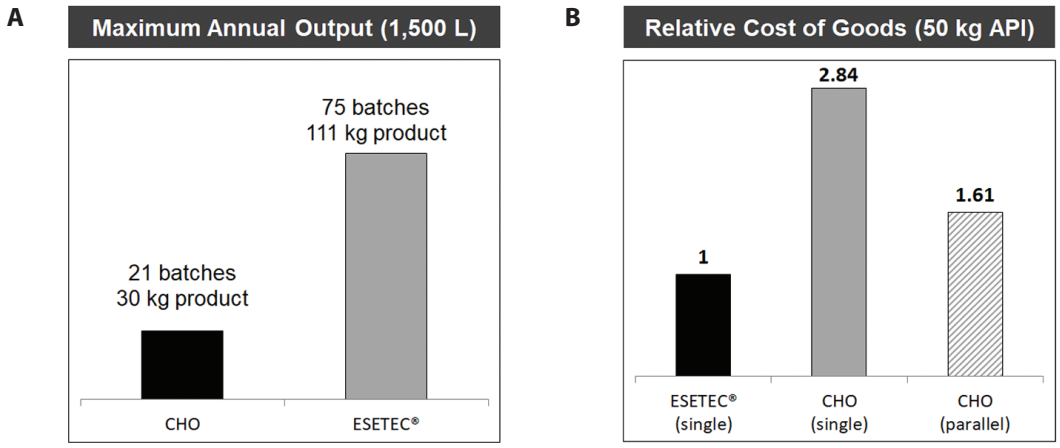

Figure 3. Annual production capacity and relative cost-of-goods based on the simulated ESETEC® and CHO processes. (A) Total number of GMP batches per year based on the simulated batch times (Figure 2) and three working shifts. The corresponding production quantity is calculated for 1,200 L w/V (1,500 L total volume) fermentation with primary titers of 2.2 g/L. (B) Relative costs to manufacture 50 kg bulk API with 4,800 L w/V (6,000 L total volume). A facility with a single CHO fermenter is up to 2.8 times more expensive than ESETEC®. Even four parallel CHO fermenters feeding one DSP unit (fully utilized) result in 1.6-fold higher cost of goods compared to a single ESETEC® fermenter.

takes almost three times longer than ESETEC®, driven by the extensive fermentation times of CHO cells. The fast-growing ESETEC® host is clearly superior and reduces the batch duration by 65% (*Figure 2B*). Cost drivers for GMP manufacturing are capital and labor costs; both are drastically reduced with the faster ESETEC® batch.

Together with viral inactivation/filtration and more cost-efficient media, a single batch of the conventional CHO process is 2.7-times more expensive (*Figure 2C*). The simulated annual output of the aforementioned facility, equipped with a single fermenter, reaches up to 75 batches per year for ESETEC®, compared to just 21 CHO batches (*Figure 3A*). As production of several consecutive batches allows staggering, which is common for commercial manufacturing, we further assessed the relative cost of goods for the production of 50 kg bulk drug substance using a 6,000 L facility with 4,800 L w/V (*Figure 3B*).

The short fermentation of the ESETEC® process reduces the production time 3.3-fold, which equates to savings of ~64% compared to the cost of using CHO cells (*Figure 3B*). To overcome the slow growth of mammalian systems in commercial operations, parallel CHO fermenters are used, which feed one DSP line to achieve 100% utilization.

Even compared to such an optimized CHO plant that is equipped with four identical fermenters, ESETEC® is faster and ~37% cheaper (*Figure 3B*, shaded bar), highlighting its tremendous advantage and high productivity.

Conclusion

Wacker Biotech's microbial secretion technology ESETEC® offers a cost- and time-efficient alternative for the production of any nonglycosylated therapeutic protein. With straightforward strain and process development, ESETEC® combines all benefits of microbial and mammalian systems.

In a process simulation, assuming similar titers/yields, CHO manufacturing on a 1,500 L GMP-scale proved to be 2.7-times more expensive than ESETEC®. The advantage is mainly driven by shorter fermentation times and obsolete viral depletion steps.

The overall superior productivity, shorter development times and lower cost of goods distinguish ESETEC® as a novel, cost-efficient production system, ideally suited for manufacturing nonglycosylated biopharmaceuticals. GEN