Microbial fermentation

# Improve scale-up consistency in *E. coli* plasmid DNA production with a chemically defined medium and feed platform

## Challenges

Microbial fermentation using *Escherichia coli (E. coli)* remains the dominant platform for producing plasmid DNA (pDNA) that serves as the template for in vitro transcription (IVT) of mRNA used in vaccines, gene therapies, and mRNA-based therapeutics. Unfortunately, production teams routinely face significant challenges when scaling plasmid DNA processes from benchtop to pilot or manufacturing scale. Fermentation conditions that appear well-controlled and reproducible at small scale often are unpredictable at larger volumes, leading to deviations in cell growth, plasmid yield, and plasmid quality. Despite constant attention, countless adjustments, and experienced hands at the controls, teams are often challenged with variability that accompanies scale-up, commonly referred to as scale-up nonlinearity [1,2].

Nonlinear behavior can relate to the lack of a robust process for parameters like dissolved oxygen or temperature. However, it may also relate to another foundation of the process: the media. Traditional hydrolysate-based media are rich in growth-promoting components but less defined in precise, consistent nutritional composition. This nutritional imprecision and variability can lie at the heart of challenges when scaling.

## Solutions

Shifting to chemically defined (CD) and optimized media and feed formulations can help address scale-up challenges by enabling precise control of nutrient delivery. By reducing variability in nutrient composition, CD media support more predictable *E. coli* metabolic behavior, which is particularly important under scale-dependent mixing and mass transfer conditions commonly encountered during scale-up [1,2]. When paired with controlled fed-batch strategies, CD formulations can support reduced acetate accumulation, more uniform oxygen demand, and consistent plasmid replication across scales.

To provide solutions for these challenges, Thermo Fisher Scientific developed the Gibco™ Bacto™ CD Supreme Fermentation Production Medium (FPM) Plus and Bacto™ CD Supreme Feed (2X) platform. Using Phenotype MicroArray™ screening (Biolog Inc.) and principal component analysis, the nutrient requirements across diverse auxotrophic *E. coli* strains were identified. These insights supported the design of an optimized medium and feed platform built for production consistency and reliable scalability. For production teams, this signifies a shift where confidence and consistency can replace uncertainty and variability with scaling.

## DH10B scalability study

The platform was evaluated using a commonly used *E. coli* strain DH10B producing a plasmid for *in vitro* mRNA transcription (pIVT). After developing a feeding strategy in 250 mL mini bioreactors, consistency with scaling was evaluated from 3 L benchtop scale to 30 L pilot scale in a single-use fermentation system. The results demonstrated that predictable and scalable high-performance cell growth and plasmid production are achievable with the right platform medium and feed system.

## Feeding strategy development

In high–cell density *E. coli* processes, feeding strategy selection plays a central role in controlling growth rate, carbon flux, and formation of by-products such as acetate. Constant and exponential fed-batch strategies are commonly explored during process development, as they offer different levels of control over substrate availability and metabolic stress, particularly during scale-up [3,4]. The DH10B feeding strategy developed using Bacto CD Supreme Feed (2X) with Bacto CD Supreme FPM Plus was based on maximizing cell density and pIVT productivity in 250 mL mini bioreactors. Constant feeding rates of 1.08 or 4.86 mL $h^{-1}$ and exponential feeding rates of 0.11 or 0.14 $h^{-1}$ were tested. Further assay details are shown in the materials and methods section (Table 1).

These evaluations showed that the lower 0.11 $h^{-1}$ exponential feeding strategy supported a comparable or higher average peak cell density (Figure 1), and between 11% to 72% higher average pIVT productivity (Figure 2), compared to the other feed strategies. Low glycerol and acetate accumulation at the optimized exponential feed rate indicated reduced overflow metabolism, a key contributor to scale-up nonlinearity (Figure 3). Overall, the results supported the use of the 0.11 $h^{-1}$ exponential rate for the 3 L and 30 L scaling studies, utilizing the feeding strategy with the second-lowest total volume of the feeding rates evaluated.

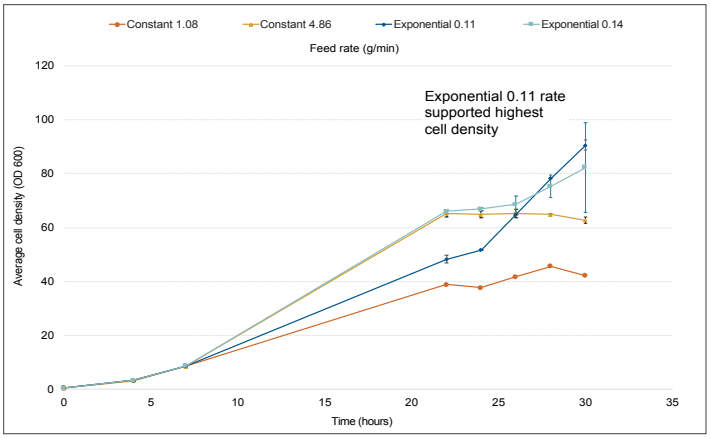



**Figure 1. Cell density in feeding strategy development.** Using Bacto CD Supreme FPM Plus with Bacto CD Supreme Feed at the lower 0.11 $h^{-1}$ exponential feeding rate demonstrated a comparable or higher average peak cell density relative to those shown with the other feeding strategies (n = 2 vessels per condition, except n = 1 for the 1.08 constant feed rate).

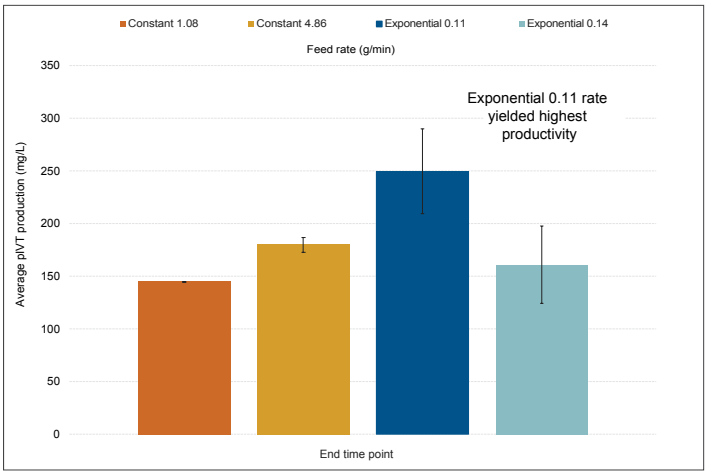
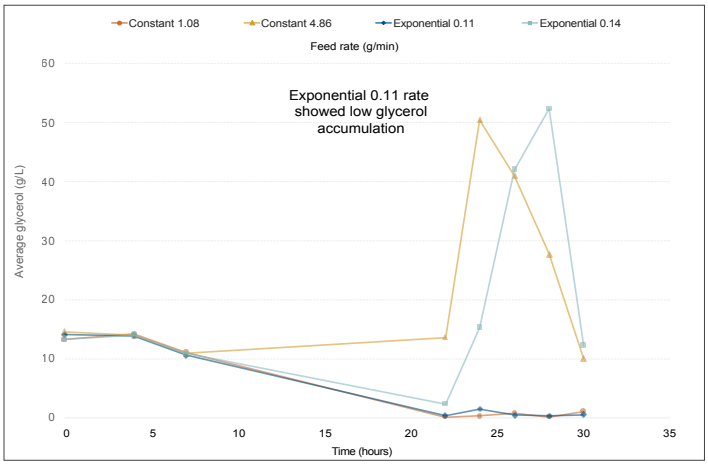



**Figure 2. Productivity results in feeding strategy development.** The lower 0.11 $h^{-1}$ exponential feeding rate with Bacto CD Supreme Feed (2X) yielded 11%–72% higher average pIVT production than the other feeding rates.

**A**

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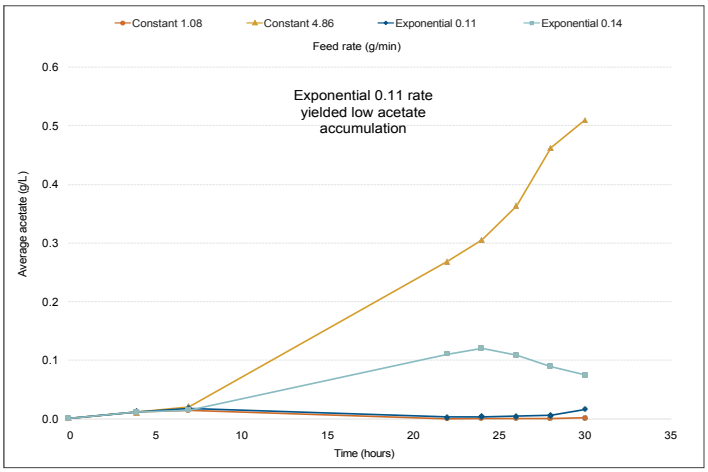


**Figure 3. Glycerol and acetate in feeding strategy development.** Bacto CD Supreme FPM Plus, with Bacto CD Supreme Feed (2X) at the lower 0.11 $h^{-1}$ exponential feeding rate, supported **(A)** low average glycerol accumulation and **(B)** low average acetate accumulation.

## Scaling evaluation

DH10B cell density and pIVT plasmid production were tested to evaluate the consistency of scaling using Bacto CD Supreme FPM Plus with Bacto CD Supreme Feed (2X) at the 0.11 $h^{-1}$ exponential feeding rate in 3 L stirred glass bioreactors and at 30 L in a Thermo Scientific™ HyPerforma™ Enhanced Single-Use Fermentor (eS.U.F.) System. Further test details are shown in the materials and methods section (Table 1).

The results showed that the platform medium and feed supported similar growth profiles and peak cell densities of $OD_{600}$ between ~90 and 120 across scaling to 30 L (Figure 4). Comparable plasmid yields of 250–306 mg/L were supported (Figure 5), from the 250 mL development scale to the 3 L benchtop and 30 L pilot scale in the HyPerforma eS.U.F. system. Equivalent performance across scaling suggests the process is robust to scale-dependent mass transfer and mixing differences. The use of the HyPerforma eS.U.F. further supports robust scale-up by providing improved cooling capacity and oxygen mass transfer, which are critical for maintaining temperature and dissolved oxygen control during high–cell density *E. coli* fermentations.

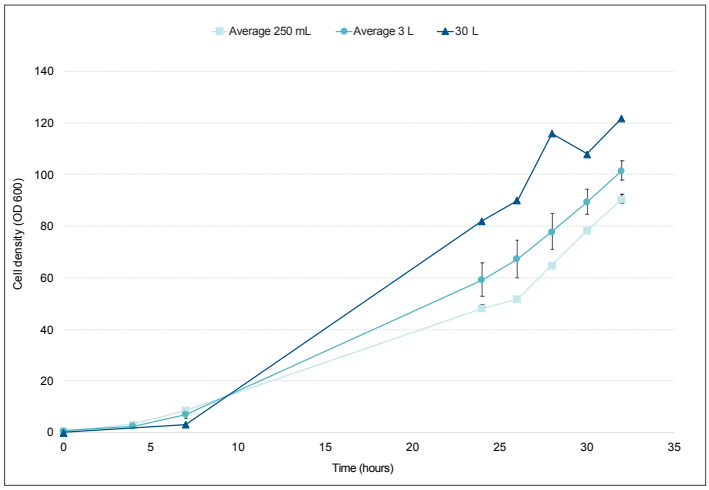


**Figure 4. Cell density with scaling.** Across scaling to 30 L, the Bacto CD Supreme FPM Plus and CD Feed platform supported similar growth profiles and peak cell densities of $OD_{600}$ ~90 to 120 (n = 2 or 3 mini bioreactors and 3 L vessels per condition, and n = 1 vessel at 30 L).

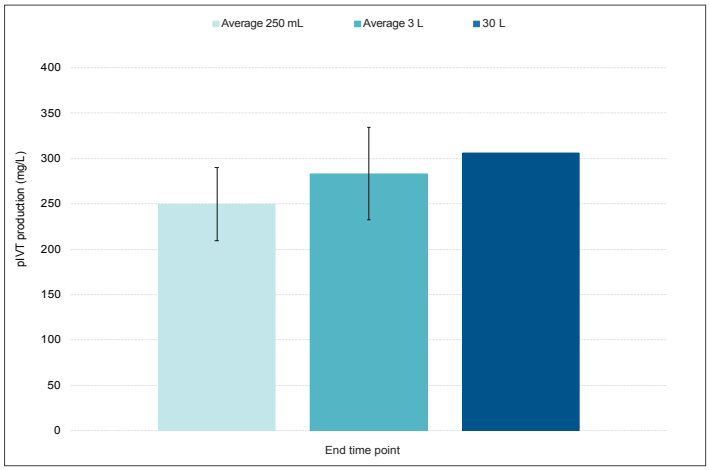


**Figure 5. Productivity with scaling.** When scaling to 30 L using Bacto CD Supreme FPM Plus with Bacto CD Supreme Feed (2X) at the 0.11 $h^{-1}$ rate, comparable pIVT yields of 250–306 mg/L were shown.

## Conclusions and discussion

Previous work demonstrated that the Bacto CD Supreme FPM Plus with Bacto CD Supreme Feed (2X) platform supports enhanced growth and plasmid DNA (pDNA) production in an NEB™ Stable auxotrophic *E. coli* strain, compared to traditional hydrolysate-based and lab-prepared chemically defined (CD) media and feeds (see related application note).

In this study, DH10B *E. coli*—another commonly used auxotrophic strain for high-quality plasmid production—showed similarly strong performance. The results demonstrate the platform's robustness across multiple auxotrophic strains, supporting consistent cell growth and plasmid yields from development scale up to a 30 L pilot scale, using a low exponential feeding rate of 0.11 $h^{-1}$.

When combined with an optimized feeding strategy, the platform supports a stable culture environment, reduces acetate accumulation, and enables consistent, easily scalable plasmid manufacturing. Together, these results demonstrate that the Bacto CD Supreme FPM Plus and Feed (2X) platform supports *E. coli* plasmid DNA processes that are less sensitive to scale-dependent variability, reducing the need for production teams to micromanage or re-optimize a process with scale-up.

**Additional benefits:**

- Supports regulatory submissions with chemically defined (CD), animal origin–free (AOF) formulations and supporting documentation
- Flexible liquid and dry powder media (DPM) formats and packaging options
- Operational flexibility with autoclave or filter sterilization options for DPM
- Manufacturing versatility—supports a range of microbial strains, making it suitable for pDNA and protein bioproduction
- Supports reliable supply with global, redundant manufacturing facilities
- Process development and scale-up support with experienced field and R&D support
- Added confidence in consistency and scalability with highly automated HyPerforma eS.U.F. systems (see related application note)

## Materials and methods

Table 1. Details of each study.

| Assay description | Feeding strategy evaluation | 3 L scale | 30 L scale |
|---|---|---|---|
| Culture vessel and maximum volume | DASbox™ Mini Bioreactor System (Eppendorf), 250 mL | BioFlo™ 320 (Eppendorf) Glass Vessel, Rushton Impeller, 3 L | Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.) System, 30 L |
| Vessel fill volumes | 160 mL starting and 180 mL maximum | 1.6 L starting and 2.5 L maximum | 21.6 L starting and 30 L maximum |
| Basal medium, feed, sterilization method, and supplements | Bacto CD Supreme FPM Plus, Bacto CD Supreme Feed (2X) with glycerol, sterile filtered, and kanamycin sulfate (following the user guide) | | |
| Feeding strategy and feed rate | Constant feed rates of 1.08 or 4.86 (mL/hr ) and exponential fed-batch* with C1 = 0.13 g/min and C2 = 0.11 or 0.14 $hr^{-1}$ | Exponential fed-batch* with C1 = 0.4 g/min and C2 = 0.11 $hr^{-1}$ | |
| Inoculum | $OD_{600}$ = 0.5, 20 mL volume | $OD_{600}$ = 0.5, 200 mL volume | $OD_{600}$ = 0.058, 200 mL volume |
| pH | 7.0 ± 0.1 | | |
| Temperature | 35°C | | |
| Agitation | 300–1,800 rpm | 200–1,200 rpm | 300–600 rpm |
| Dissolved oxygen (DO) and air sparging rates | DO 30% and air 1.8–24 sL/hr | DO 30% and air 0.3–4 sL/min | DO 30% and air 12–50 sL/min |
| Antifoam | 250 µL | Diluted to 30% and added as needed at 5 mL/min | 50 mL |
| Cell density quantification | Cedex™ Bio Analyzer (Roche), $OD_{600}$ | | |
| Plasmid extraction and isolation | Thermo Scientific™ GeneJET™ Plasmid Miniprep Kit | | |
| Plasmid quantification | Thermo Scientific™ NanoDrop™ Spectrophotometer | | |

*The exponential feeding profile started when a dissolved oxygen (DO) spike occurred or there was a drop in the stir speed, typically 10–14 hours after fermentation start. The feed rate (pump rate) is programmed to increase exponentially based on the equation $F = C1 \times e^{C2t}$, where F = flow rate (g/min), C1 = feed rate constant = 0.13 g/min, C2 = growth rate constant ($hr^{-1}$) (adjusted per strain growth rate), and t = EFT (hr). The C1 coefficient is adjusted according to vessel working volume, and C2 is based on experimental strain growth rate testing and carbon requirements.

## References


1. Enfors, S.O., et al. Physiological responses to mixing in large-scale bioreactors. *Trends Biotechnol*, 19(9), 2001, 379–384.
2. Lara, A.R., et al. Living with heterogeneities in bioreactors: understanding the effects of environmental gradients on cells. *Mol Biotechnol*, 34, 2006, 355–381.
3. Shiloach, J., Fass, R. Growing *E. coli* to high cell density—A historical perspective on fed-batch fermentation. *Biotechnol Adv*, 23, 2005, 345–357.
4. Riesenberg, D., Guthke, R. High-cell-density cultivation of microorganisms. Appl *Microbiol Biotechnol*, 51, 1999, 422–430.


| Cat. No. | Product name | Format | Packaging size |
|---|---|---|---|
| A9378401 | Bacto CD Supreme Fermentation Production Medium Plus | Liquid | 1,000 mL bottle |
| A9378201 | Bacto CD Supreme Feed (2X) | | 500 mL bottle |
| A9378501 | Bacto CD Supreme Fermentation Production Medium Plus | DPM | 500 g |
| A9378502 | | | 10 kg |
| A9378301 | Bacto CD Supreme Feed (2X) | | 250 g |
| A9378302 | | | 5 kg |

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