Fed-Batch IVT mRNA synthesis using the BioLector XT and ReadyToProcess™ WAVE™ 25 bioreactors system

Darius Menezes[1], Patrick Francis[1] and Gary Pigeau[1]

[1]Cytiva, 400-1055 Vernon Drive, V6A 3P4 Vancouver, Canada

Introduction

RNA-based therapeutics are rapidly transforming modern medicine, offering novel treatment modalities for genetic disorders, infectious diseases, and cancer (1–3). As the field matures, the demand for scalable, cost-effective manufacturing solutions continues to grow. Central to RNA therapeutic production is *in vitro* transcription (IVT), a cell-free, enzymatic process that synthesizes RNA from nucleotide triphosphates (NTPs) using a linear DNA template and polymerase. However, IVT—especially with co-transcriptional capping—remains the most expensive step in RNA manufacturing, posing challenges to the global adoption of RNA therapeutics (4).

Fed-batch IVT—wherein NTPs and magnesium salts are supplemented to maintain their concentrations—is emerging as a promising strategy to improve RNA yield, reagent usage efficiency, and product quality. This study highlights how the Beckman Coulter BioLector XT Microbioreactor and the Cytiva ReadyToProcess™ WAVE™ 25 system provide a powerful platform for process development and scale up of fed-batch IVT.

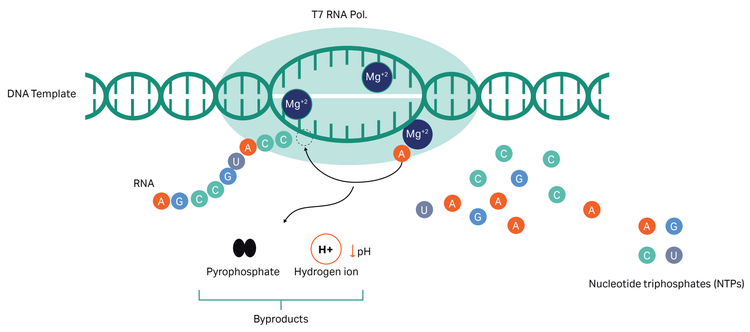


Fig 1. Fed-batch IVT reaction where NTPs are supplemented into the reaction. Magnesium ions facilitate binding of NTPs to the polymerase and stabilize the polymerase-DNA complex. Inorganic pyrophosphatase and hydrogen ions are released with every nucleotide addition.

Experimental methods

Small scale fed-batch IVT process development with the Beckman Coulter BioLector XT Microbioreactor

Small scale (0.8 mL) process development of continuous fed-batch IVT reactions was enabled using the BioLector XT Microbioreactor and microfluidic 48 well plates for high-throughput optimization.

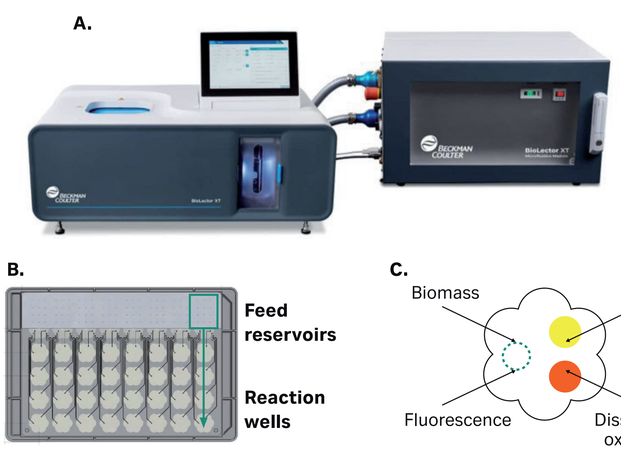


Fig 2. (A) BioLector XT Microbioreactor with valve control unit. Beckman Coulter Life Sciences 2021 **(B)** Microfluidic fed-batch plate. Reservoirs are loaded with NTP and Mg mixture and fed to the four wells directly below. Beckman Coulter Life Sciences 2022 **(C)** Optical sensors embedded in each well for real time process tracking. Beckman Coulter Life Sciences 2022.

Scale up of fed-batch IVT using Cytiva ReadyToProcess™ WAVE™ 25 system

The optimized fed-batch process was scaled up to 20 mL and 200 mL initial volume in a ReadyToProcess™ WAVE™ 25 system, using either the standard WAVE™ trays or Cytiva™ enzyme micro reactor accessory. The WAVE™ 25 system is a GMP-ready, closed bioreactor with integrated pumps and real-time pH monitoring.

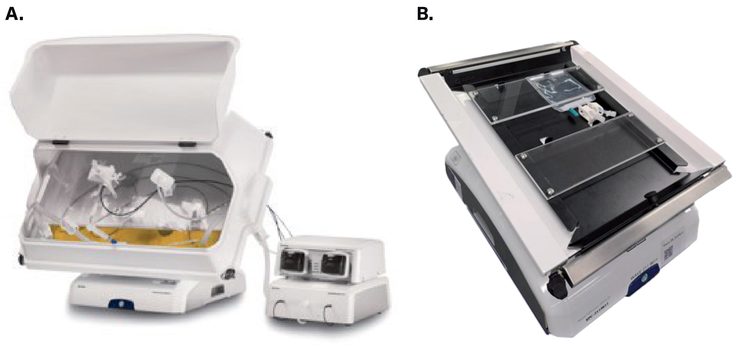


Fig 3. (A) ReadyToProcess™ WAVE™ 25 system capable of supporting IVT volumes from 100 mL to 25 L. Feeding using integrated pumps. **(B)** Enzyme micro reactor accessory capable of IVTs from 20–100 mL. Feed performed using syringe pump (not shown).

High initial NTP concentration inhibits RNA synthesis

- Higher initial concentrations of NTPs lead to a reduced rate of RNA production
- There is an optimal magnesium (Mg) concentration, beyond which the RNA production rate decreases
- Feeding NTPs and Mg gradually over time sustains RNA production while preventing inhibition due to high NTP concentrations leading to greater overall yield

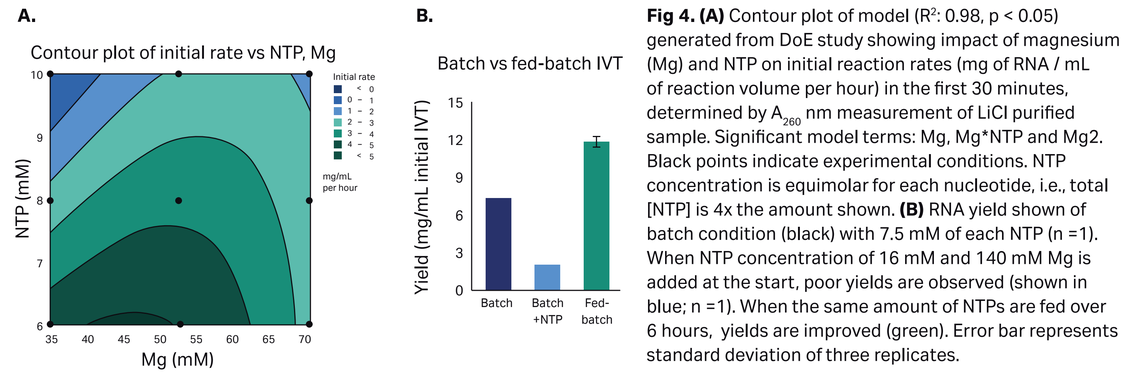
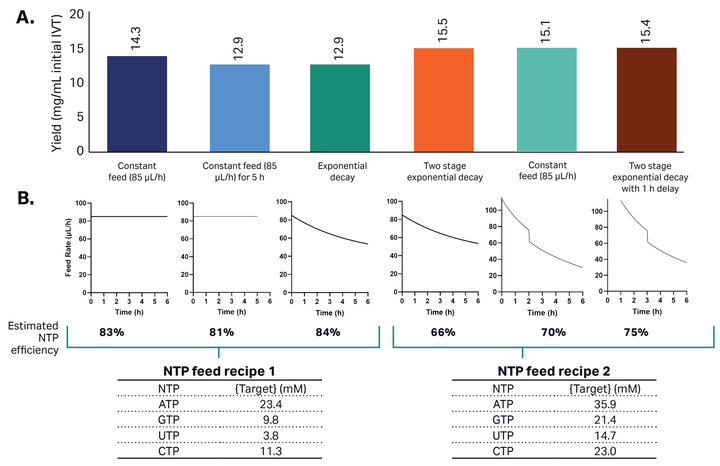


Fig 4. (A) Contour plot of model (R^2: 0.98, $p < 0.05$) generated from DoE study showing impact of magnesium (Mg) and NTP on initial reaction rates (mg of RNA / mL of reaction volume per hour) in the first 30 minutes, determined by A_{260} nm measurement of LiCl purified sample. Significant model terms: Mg, Mg*NTP and Mg2. Black points indicate experimental conditions. NTP concentration is equimolar for each nucleotide, i.e., total [NTP] is 4x the amount shown. **(B)** RNA yield shown of batch condition (black) with 7.5 mM of each NTP (n =1). When NTP concentration of 16 mM and 140 mM Mg is added at the start, poor yields are observed (shown in blue; n =1). When the same amount of NTPs are fed over 6 hours, yields are improved (green). Error bar represents standard deviation of three replicates.

Feed strategy optimization using BioLector XT Microbioreactor can improve process efficiency

Improve NTP incorporation efficiency

- Fed-batch with a constant feed rate and equimolar feed stock is highly inefficient (approximately 55% NTP incorporation efficiency) due to slowing down in RNA production rate
- Different feeding strategies and feed stock recipes can be used to improve incorporation efficiency



NTP feed recipe 1

NTP	(Target) (mM)
ATP	23.4
GTP	9.8
UTP	3.8
CTP	11.3

NTP feed recipe 2

NTP	(Target) (mM)
ATP	35.9
GTP	21.4
UTP	14.7
CTP	23.0

Fig 5. (A) Optimisation of NTP incorporation efficiency (n = 1 replicate for each condition) estimated using RNA mass (A_{260} nm measurement of LiCl purified sample) and RNA sequence composition. All IVTs started with 6 mM of each NTP. **(B)** NTP Feed 1 is derived from the nucleotide sequence and was chosen to achieve a predicted efficiency of 85% with constant feed of 85 µL/h over 6 h. NTP Feed 2 matches the exact frequency of the nucleotide in the RNA sequence. Line graphs illustrate feed rate profiles over time for each condition.

Reduce DNA template usage

- Choosing appropriate feeding strategies and template concentrations can improve the RNA yield per input DNA amount
- Conditions with DNA concentration of 50 µg/mL produced yield greater than 200 fold (highlighted in yellow) with three out of four feeding strategies tested

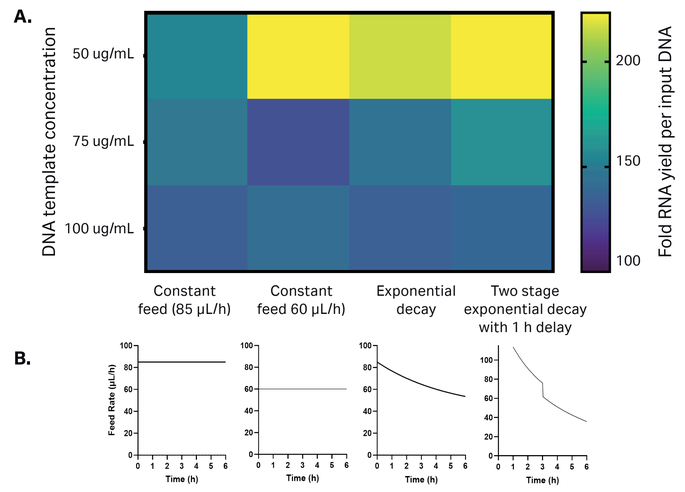


Fig 6. (A) Optimisation of DNA template usage (n = 1 replicate for each condition) by monitoring the amount of RNA produced per amount of input DNA. Three DNA template concentrations were tested (50, 100, and 150 µg/mL) with four different feed strategies. Gradient shows the ratio of RNA mass produced to input DNA mass. **(B)** Line graphs illustrate feed rate profiles over time for each condition.

Seamless scale up into the Cytiva ReadyToProcess™ WAVE™ 25 system

- A fed-batch IVT condition developed using the BioLector XT Microbioreactor was successfully scaled to 20 mL and 200 mL in the WAVE™ 25 system.
- For the 200 mL reaction, real-time pH monitoring was conducted using a pH-enabled 2 L Cellbag™.

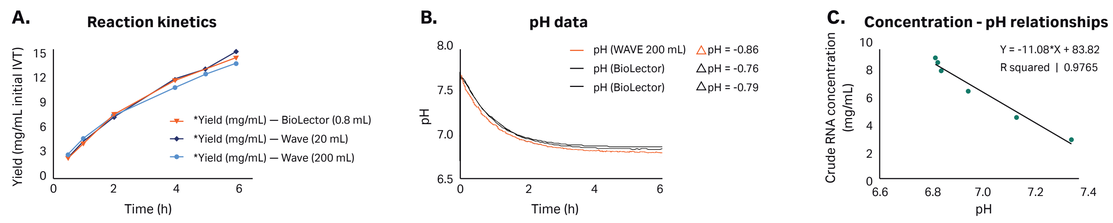


Fig 7. (A) Time course production of RNA at various scales. *RNA yield defined as the mass of RNA divided by the initial reaction volume. Data points generated by A_{260} nm measurement of LiCl purified sample. **(B)** Drop in reaction pH over time as measured on the WAVE™ 25 system and BioLector XT Microbioreactor. **(C)** RNA crude concentration is plotted as a function of pH (measured on WAVE™ 25 system) and a linear fit is applied. Crude concentration refers to the concentration of the RNA in the IVT after dilution from feed stock.

Quality of RNA produced using a fed-batch process

Table 1. Quality attributes of RNA produced in BioLector XT Microbioreactor and WAVE™ 25 systems.

Quality attributes	Batch (0.8 mL)	Fed-batch (0.8 mL)	Fed-Batch (20 mL)	Fed-Batch (200 mL)
Integrity (%)	84%	83%	82%	85%
dsRNA content (%)	≤ 4%	≤ 4%	≤ 4%	≤ 4%
Potentcy (%GFP positivity [1 µg/mL])	92%	91%	95%	88%
Instrument	BioLector	BioLector	WAVE™ 25	WAVE™ 25

Conclusions

- NTP and magnesium fed-batch strategies **enhance IVT productivity** by generating more RNA from the same starting volume as a batch process. This translates to improved DNA template and IVT enzyme utilization, driving better process economics
- RNA generated from fed-batch IVTs show **comparable quality attributes** to traditional batch methods
- Process development is essential to create an efficient and high yielding fed-batch process. The Beckman Coulter BioLector XT Microbioreactor is a powerful tool for this type of work as it allows for **high throughput IVT optimization** at low volumes with pH monitoring and fed-batch capabilities
- The fed-batch processes developed on the BioLector XT Microbioreactor were found to **scale linearly** into GMP-suitable ReadyToProcess™ WAVE™ 25 system from Cytiva including the newly launched enzyme micro reactor accessory
- RNA crude concentration tracks linearly to pH and the latter may be useful as a **real-time measure of reaction progress**

References

1. Daniel S, Kis Z, Kontoravdi C, Shah N. Quality by Design for enabling RNA platform production processes. Trends Biotechnol. 2022; 40(10):1213-1228. doi: 10.1016/j.tibtech.2022.03.012
2. Youssef M, Hitti C, Fulber JPC, Kamen AA. Enabling mRNA therapeutics: current landscape and challenges in manufacturing. Biomolecules. 2023; 13:1497-1524. doi: 10.3390/biom13101497
3. American Society of Cell and Gene Therapy. Gene, Cell, & RNA Therapy Landscape; Q4 2023 Quarterly Report. American Society of Cell and Gene Therapy: Waukesha, WI, USA, 2024. https://asgct.org/publications/landscape-report
4. Kis Z, Kontoravdi C, Shattock R, Shah N. Resources, Production Scales and Time Required for Producing RNA Vaccines for the Global Pandemic Demand. Vaccines. 2021; 9 (1):3. doi:10.3390/vaccines9010003

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