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Application Note

Optimization Strategy and Process Economics of DNA Digestion in Viral Vector Production for Gene Therapy

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Benzonase® endonuclease is employed to reduce the levels of host cell nucleic acids during production of viral vectors used for gene therapies. The use of this enzyme can reduce the levels of DNA by more than 100,000-fold while also decreasing viscosity and protecting downstream equipment from DNA fouling. The enzyme activity is strongly influenced by the matrix of the process intermediate and optimization of its use is often a crucial step in process development.

Benzonase® endonuclease is originated from bacteria *Serratia marcescens* and expressed in *E.coli* K12. It is nonspecific, making it highly active against all kinds of nucleic acids (DNA, RNA, circular, single or double stranded). The recombinant protein has 30 kDa molecular weight of each subunit and exists as a dimer. The isoelectric point (pI) is 6.85 and is effective in wide ranges of pH (6–10) and temperature (0–42 ºC). The presence of Mg^{2+} (1–2 mM) is required for enzyme activity.

In this application note, we highlight an optimization strategy and process economics of DNA digestion in viral vector purification.

Benefits of using Benzonase® endonuclease in a viral vector manufacturing process

- Prevents yield loss due to virus-nucleic acid complexes
- Prevents fouling of downstream equipment
- Reduces viscosity of process intermediates

Regulatory expectations

- Residual DNA considered a contaminant requiring removal[1] (size of residual DNA no more than 100–200 bp, less than 10 ng per dose).
- FDA Bulk Biological Master File (BBMF; FDA Reg. No. BBMF 5403) and Emprove® dossiers available.

Need for Optimization

Benzonase® endonuclease is a high-quality product delivering immense value to viral vector processes. Using it in the most economical way is crucial. The enzyme activity is strongly influenced by the matrix and optimization of its use should be a mandatory step in process development.

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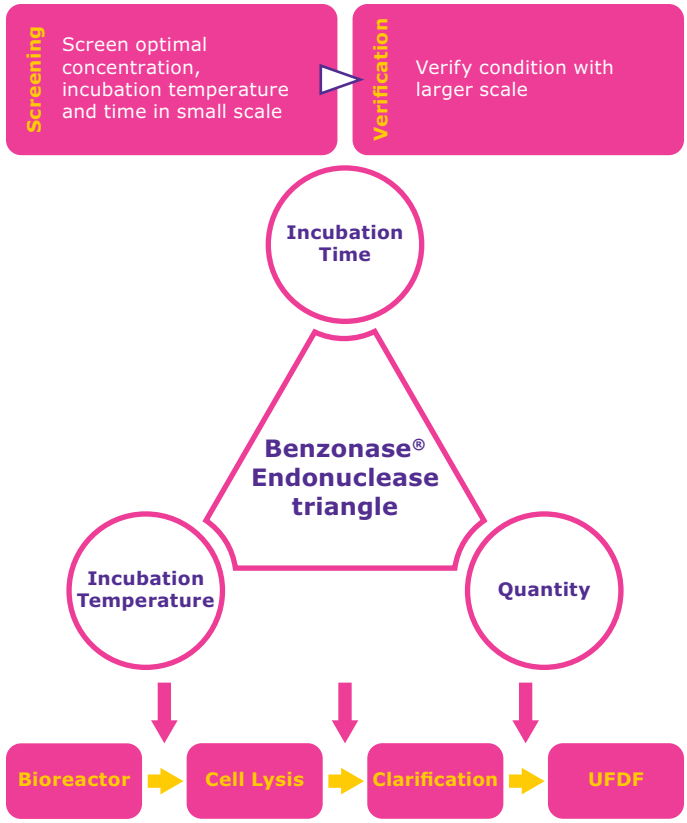
The following steps provide general guidance for an optimization framework:

1. Determine the DNA concentration of the process intermediate.
2. Calculate the theoretical concentration, X, needed of Benzonase® endonuclease:

   X (U/mL) = DNA concentration (ug/mL) / 37
3. Test different concentrations of Benzonase® endonuclease (e.g., 2x; 3x; 10x and 0.5x; 0.25x; 0.1x of the theoretical concentration) combined with different incubation times (e.g., 1, 2, 4, 8, 12, 18 and 24 hr) and different temperatures if applicable (eg., 15 °C, 20 °C, 37 °C or other)
4. Test scaling (e.g., by moving from mL to L scale).
5. Test optimal concentration at different locations in the process.



Overview of a generic AAV manufacturing process

1	2	3	4	5	6	7	8	9	10	11	12	13
N-2 Seed Train	**N-1 Seed Train**	**Production Suspension**	**Cell Lysis**	**Clari-fication**	**DNA Digestion**	**TFF**	**1st Chromato-graphy**	**Dilution**	**2nd Chromato-graphy**	**TFF**	**Sterile Filtration**	**Fill Finish**
Batch Seed Culture (SU)	Batch Seed Culture (SU)	Fed Batch Production Culture (SU)	Detergent Mixing	Filtration	Mixing	UFDF (Particu-late reduction)	Capture (Bind & Elute)	Mixing	Polishing (Bind & Elute)	UFDF (Concen-tration & Buffer Exchange)	Filtration	Fill & Storage

Process Economics and Cost Modeling Scenarios

In this study, we took a closer look at a comparison between 50 L and 2000 L AAV suspension process using HEK293 cells and applied the cost modeling analysis on a generic set of midstream and downstream unit operations using BioSolve software. The rationale for choosing 50 L is to represent small dose indications like ocular diseases, while 2000 L is commonly used for muscular indications and is also the max bioreactor size used in the gene therapy industry.

The total cost per batch was calculated approximately as $1.5 M for every 50 L batch and $3.3 M for every 2000 L batch. The number of batches per year for were 2 and 37, respectively, based on the target indications with typical yields in a single product greenfield facility using single-use manufacturing technologies. Other key assumptions include transient triple-transfection with plasmids, 25 U/mL of Benzonase® endonuclease for DNA Digestion, two-column chromatography (affinity capture and ion-exchange polishing) towards production of purified AAV drug substance.

There are some remarkable trends comparing the two volumes, going from 50 to 2000 L. The percent cost of materials goes up from 3.9% to over 74%, and Benzonase® endonuclease falls under the materials category in this case. Consumable costs go up as well, about five times from 3.8% to near 20% while capital expenses go down from 64% to under 5%.

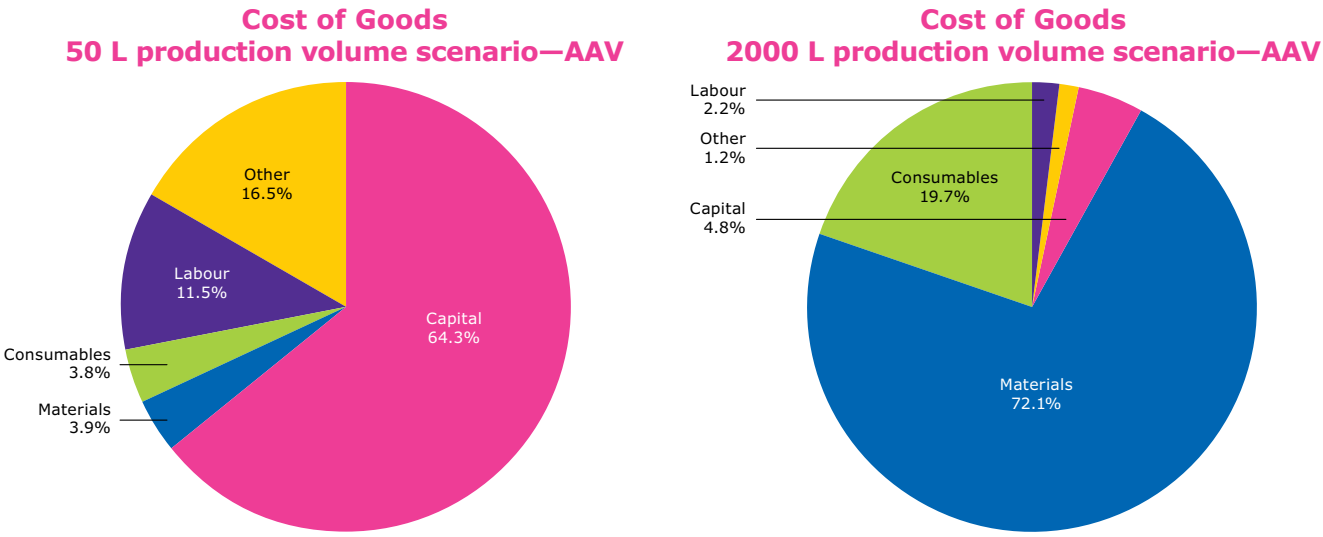


Figure 1a. Comparison of cost of goods for 50 L vs 2000 L production volume. Capital expenses (CAPEX) amount to a major portion of the 50 L batch costs while material costs (includes Benzonase® endonuclease) contributes to a low percentage of the total costs calculated. The scenario is different for 2000 L where material costs is a major factor due to the volumes of raw materials required as detailed further in Figure 1b. Figure 1b. Individual unit operation cost for 50 L vs 2000 L. Holistically, the total cost of the production bioreactor and chromatography steps are typically much higher than Benzonase® digestion.

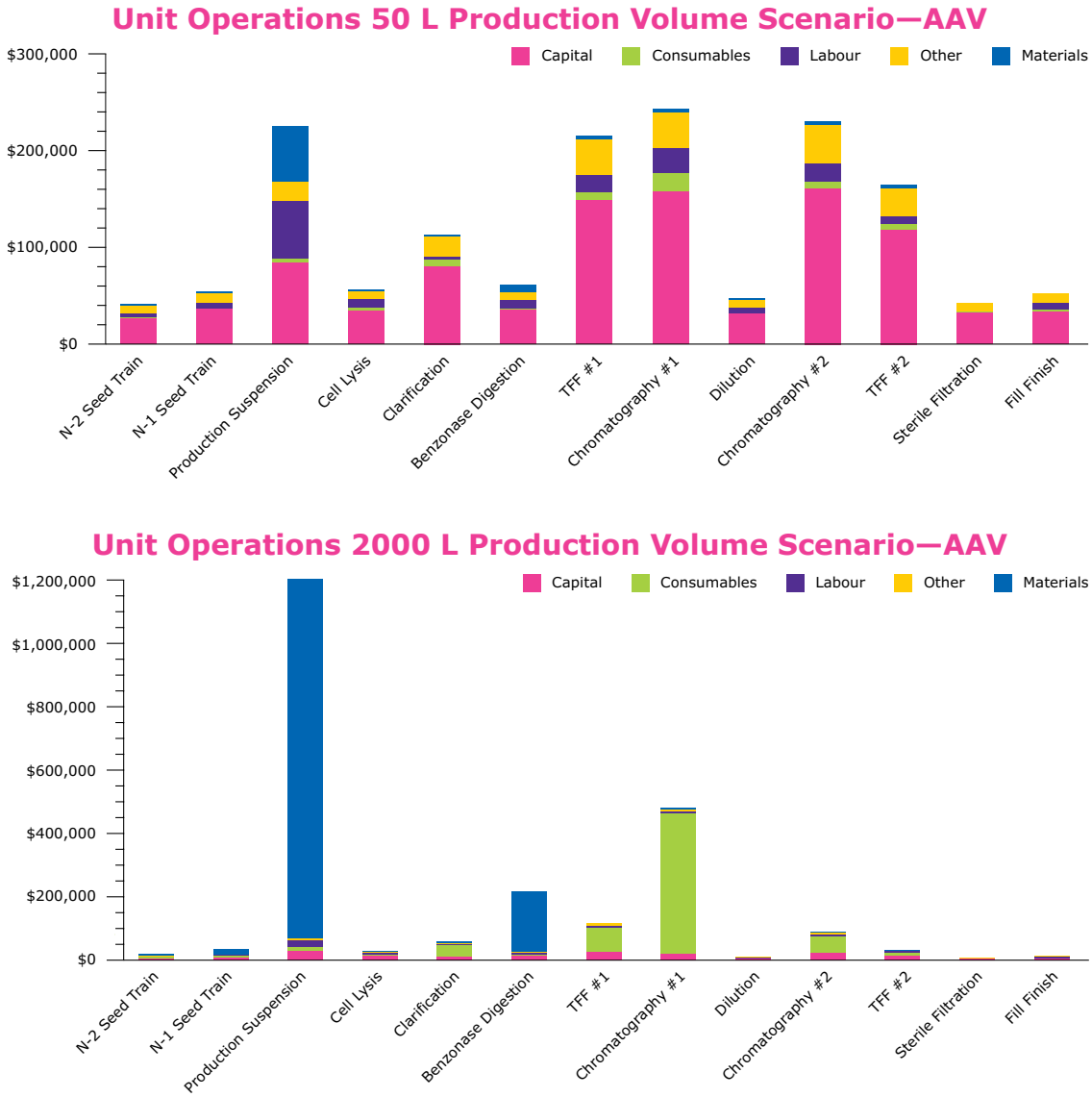
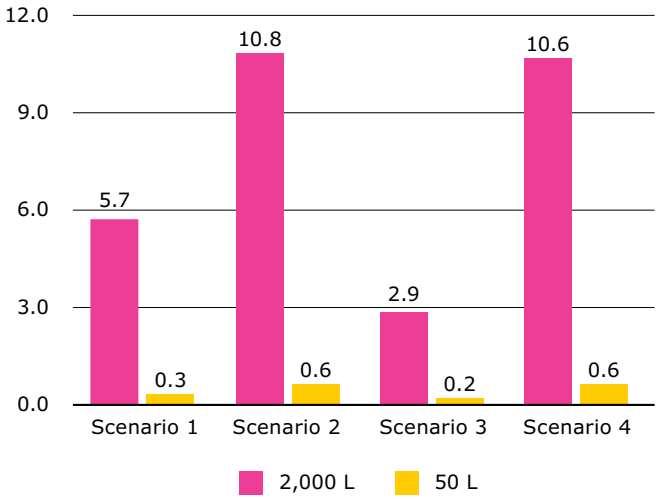


Figure 1b. Details of the cost-split between different unit operations in the process.

Four scenarios of optimization and impact to cost

Assumptions	Scenario
Baseline (25 U/mL); post-clarification	1
Increase quantity to 2x (25 to 50 U/mL)	2
Decrease quantity added to 0.5x (25 to 12.5 U/mL)	3
Change location to pre-clarification, increase quantity (vol) to 2x and decrease clarification surface area to 0.5x	4

% Cost of Benzonase® endonuclease per batch



In **Scenario 1**, which is the baseline, a process utilizes 25 U/mL Benzonase® endonuclease in a step post-clarification as a standalone unit operation. This process assumes that the residual DNA at the drug substance level meets the release specs.

Scenario 2 shows the process with double the quantity of Benzonase® endonuclease from 25 to 50 U/mL and incorporates a safety factor to ensure robustness of DNA digestion upon scale up which is relatively common.

Scenario 3 shows a decrease in Benzonase® endonuclease quantity from 25 to 12.5 U/mL upon process characterization.

Finally, **Scenario 4** shows changing the location of the addition to the bioreactor which results in better temperature control, elimination of the need for a separate unit operation, better clarification flux and reduction of surface area.

% Change in total batch cost

Increased quantity to 2× (25 to 50 U/mL)	Decreased quantity to 0.5× (25 to 12.5 U/mL)	Change location to pre-clarification, increased quantity (vol) to 2x and decreased clarification surface area by 2×
Incorporation of 2× safety factor **LOWER RISK**	Amount reduced after process character-ization experience **MEDIUM RISK**	Better clarification performance expected **MEDIUM RISK**

2,000 L 50 L

Figure 2. There are only minor changes in cost savings upon Benzonase® endonuclease optimization from the four scenarios analyzed.

Conclusion

- Benzonase® endonuclease is a critical raw material widely used in purification of AAV and lentiviral vectors.
- Optimization of enzyme use is most critical at larger scales, where material costs become much more important as described in the cost modelling analyses.
- Data obtained from the application of various cost modeling scenarios to a viral vector process underscore the importance of incorporating an optimization strategy for DNA digestion. This can be accomplished within reasonable overall cost expectations while at the same time ensuring robust residual nucleic acid clearance.

References

[1] Bauer SR, Pilaro AM, Weiss KD. Testing of adenoviral vector gene transfer products: FDA Expectations. In Adenoviral Vectors for Gene Therapy. Curiel DT, Douglas JT, Eds.; Academic Press: New York, 2002; pp 615-654.

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More information

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