

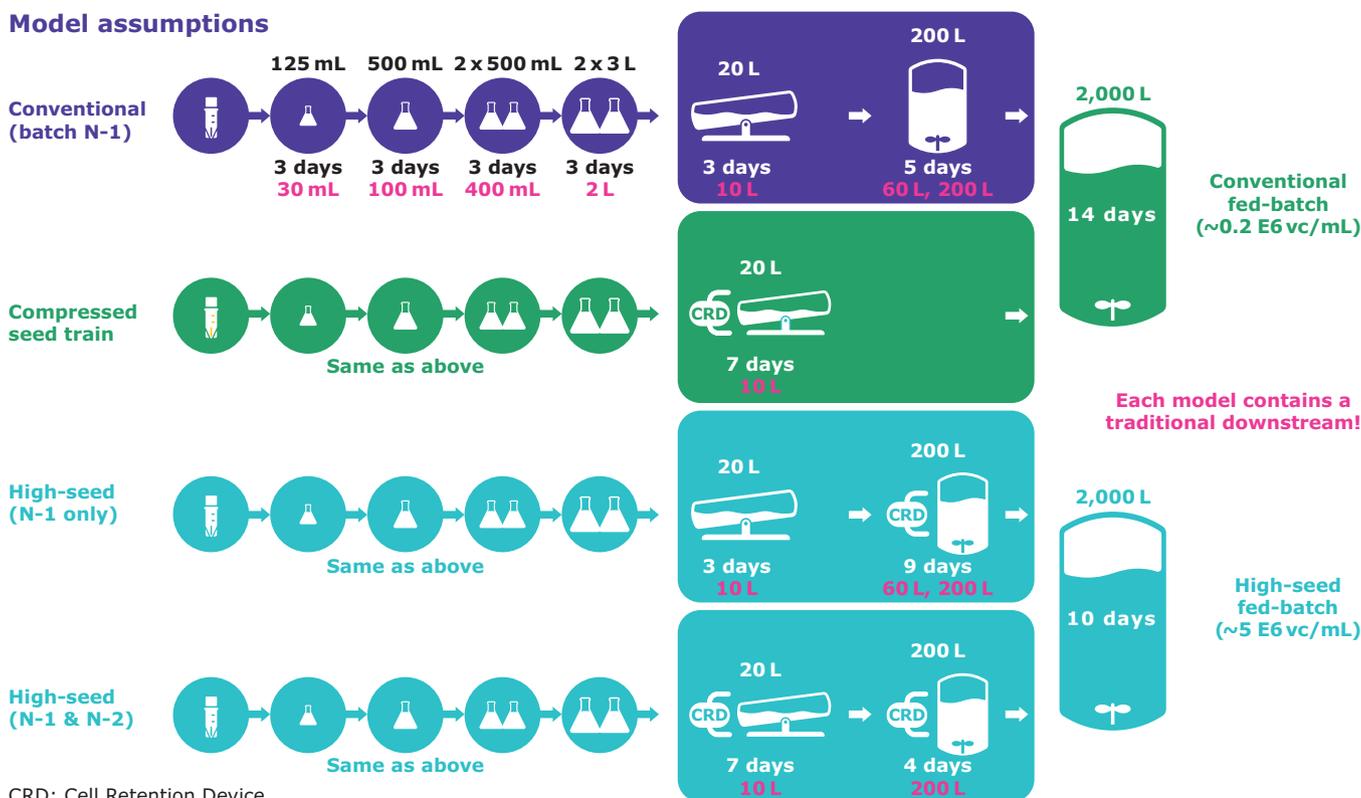
# A Cost Analysis and Evaluation of Perfused Seed Train Scenarios Through Process Modeling

Jeffrey Barna, Research Scientist, Process Solutions – BioContinuum™ Platform  
 Habib Horry, Ph.D, Associate Director Marketing – BioContinuum™ Platform  
 Douglas Rank, R&D Senior Manager – BioContinuum™ Platform

Among the strategies available for upstream process intensification is use of perfusion in the N-1 step to increase cell density prior to the production bioreactor. N-1 perfusion can also be used to eliminate the need for an intermediate-scale bioreactor prior to the production bioreactor. Use of perfusion and continuous protein harvest from the production bioreactor is also a strategy for intensification but is a relatively complex process compared to use of perfusion in the seed train.

This white paper describes process modeling and comparison of process economics for a conventional upstream process and three perfused seed train alternatives in combination with either a conventional fed-batch bioreactor or high-seed fed-batch bioreactor (Figure 1).

## Model assumptions



**Figure 1.** Model assumptions for a conventional upstream process and three perfused seed train alternatives.

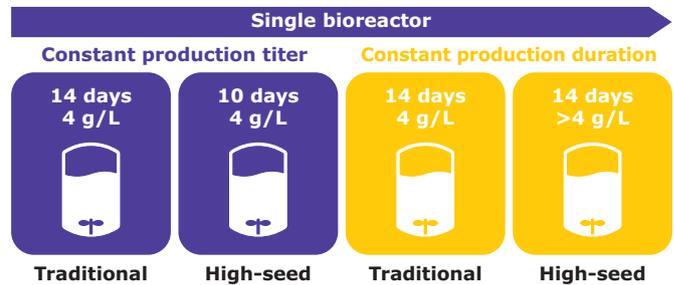
For all four models, the first five steps were identical with differences residing in the N-1 or N-2 steps. For the conventional model, which was used as the baseline, N-2 was a 20 L rocker bag and N-1 was a 200 L bioreactor operating for five days with a working volume of 200 L. In the compressed seed train model, the N-2 and N-1 steps were replaced with a single 20 L rocker bag operating in perfusion mode. Both the compressed seed train model and the conventional model were used to feed traditional, fed-batch bioreactors, operating at 14 days.

Two high-seed options were also modeled. In the first, N-1 was a 200 L bioreactor operating in perfusion mode. The second high-seed model used perfusion for both N-1 and N-2 steps. Both models fed into a high-seed fed-batch bioreactor, seeded at a higher cell density, allowing the target titer to be reached in a shorter duration (10 days).

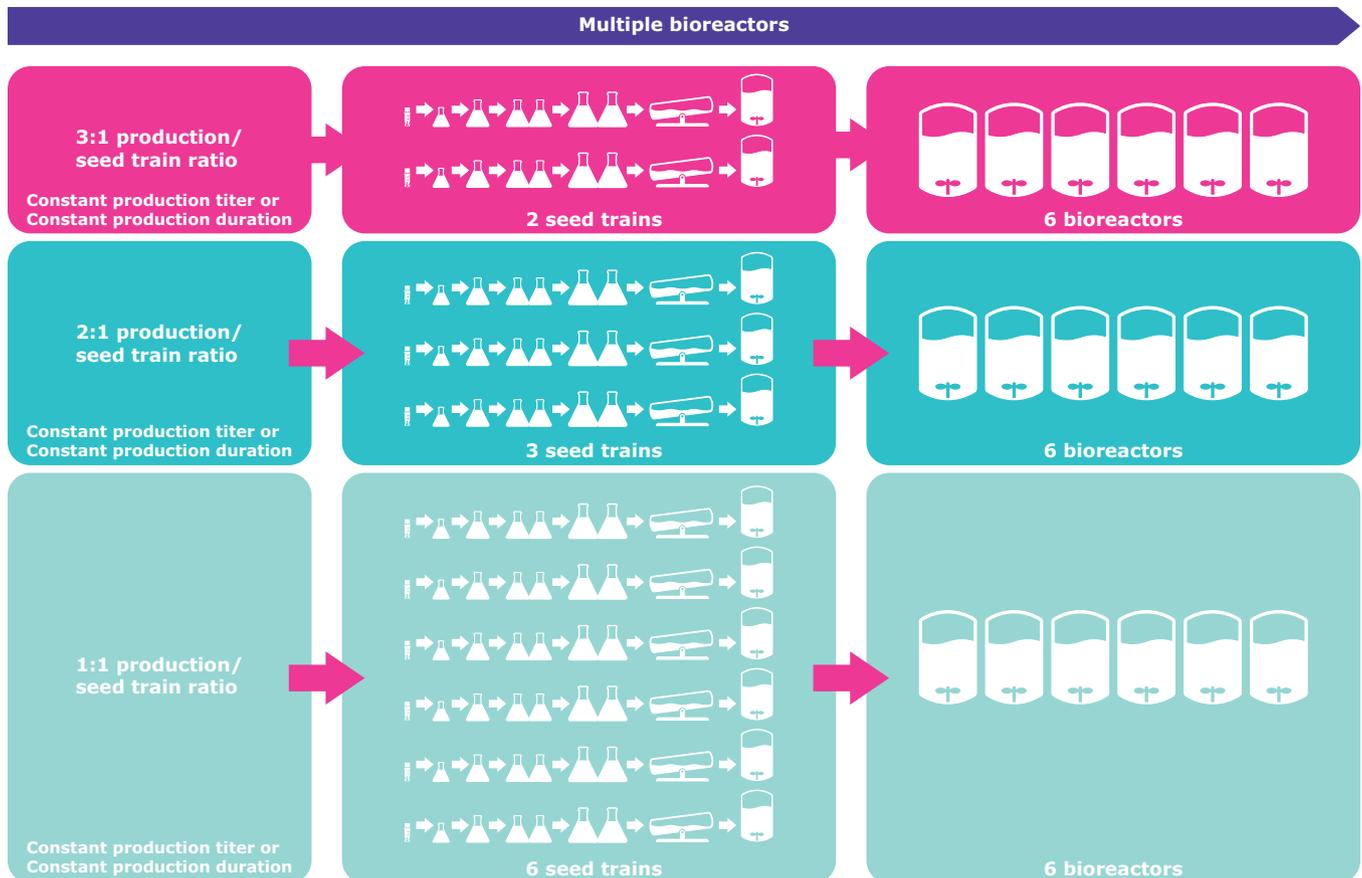
Process modeling was completed using BioSolve® software (Biopharm). Industry averages for equipment, consumables and labor costs, along with user-specified information can be used to model the economics of an entire process or a specific unit operation. The software also includes a database of traditional, standard unit operations as well as next-generation, intensified unit operations.

## Description of Scenarios

A number of scenarios were defined to explore the potential benefits of a perfused seed train (compressed, high-seed perfused N-1 and high-seed perfused N-2 and N-1) as compared to the conventional approach (Figure 2 and 3).



**Figure 2.** Each of the four process models were incorporated into a single-bioreactor scenario. The conventional and compressed seed train models fed into a traditional bioreactor at 14 days with a titer of 4 g/L; the high-seed options fed into a high-seed bioreactor operating at 10 days, also at 4 g/L. A scenario in which the high-seed bioreactor was operated at 14 days was also evaluated; this allowed a determination of whether it was better to have the shorter duration for high-seed options or have the same duration with potentially increased titers.

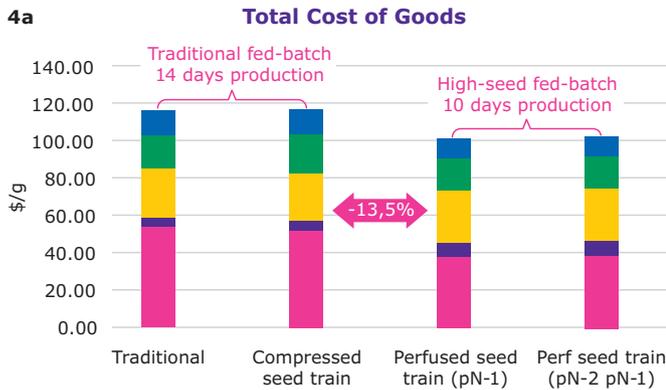


**Figure 3.** The study also considered multiple bioreactor scenarios; constant production titer versus constant duration were evaluated using a model of six bioreactors. In addition, the process modeling included different production to seed train ratios as another variable. For a 3:1 ratio, one seed train was used for every three bioreactors; for the 2:1 ratio, one seed train was used for every two bioreactors. For the 1:1 ratio, every bioreactor had a dedicated seed train.

## Single Bioreactor Scenario – Process Economics

- 1 x 2,000 L Bioreactor
- 1 x seed train

### Constant production titer 4 g/L

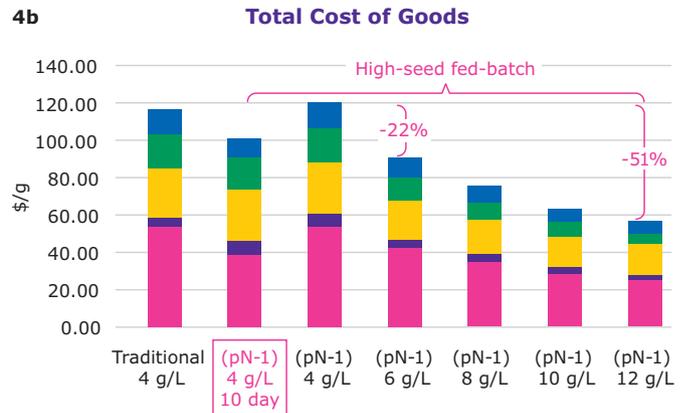


|                           |        |        |        |        |
|---------------------------|--------|--------|--------|--------|
| <b>CoG's (\$/g)</b>       | 116.39 | 116.80 | 101.17 | 101.64 |
| <b>Throughput (kg/yr)</b> | 90.18  | 90.18  | 125.25 | 125.25 |
| <b>Batches/yr</b>         | 18     | 18     | 25     | 25     |

Capital Materials Consumables Labor Other

### Constant production duration 14 days

Control: (pN-1 4 g/L) operated at 10 days



|                           |        |        |        |        |        |        |        |
|---------------------------|--------|--------|--------|--------|--------|--------|--------|
| <b>CoG's (\$/g)</b>       | 116.40 | 101.20 | 120.10 | 90.60  | 75.60  | 64.00  | 57.00  |
| <b>Throughput (kg/yr)</b> | 90.18  | 125.25 | 90.18  | 135.27 | 180.36 | 225.45 | 270.54 |
| <b>Batches/yr</b>         | 18     | 25     | 18     | 18     | 18     | 18     | 18     |

**Figure 4a/4b.** Comparison of modeling scenarios: integration of the 4 seed models into a single bioreactor at constant production 4 g/L (a) or constant production duration 14 days (b).

**At the constant production titer scenario (Figure 4a),** where the high-seed production bioreactor was operating at 10 days and producing 4 g/L, the high-seed fed-batch option increased the number of batches per year from 18 to 25 and throughput from 90 to 125 kilograms per year versus traditional fed-batch with 14 days production. This increase in throughput drives down the capital cost of goods, for a total cost of goods reduction of about 13.5 percent.

**At constant production duration scenario (14 days) with a range of titers (Figure 4b),** experimental data suggest that titer can be increased by two to three times through high seeding of the production bioreactor, especially with use of a robust media platform.

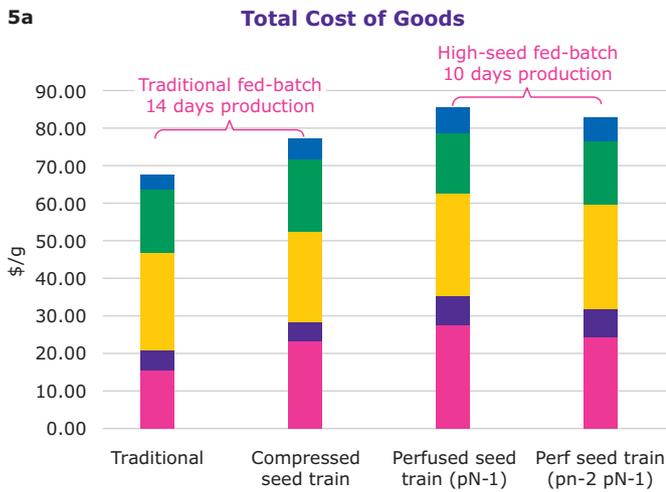
With higher titers, the benefits in terms of cost of goods were greater. With the worst-case scenario (4 g/L), the cost of goods increased slightly, and throughput remained the same. At a titer of 6 g/L, however, cost of goods decreases to below that of the 10-day, perfused N-1 and traditional options and throughput was increased. At a conservative 6 g/L increase, cost of goods was reduced by approximately 22 percent; at 12 g/L, there was a 51 percent decrease.

**The benefit was proportional to the increase in titer that can be achieved.**

## Multiple Bioreactor Scenario – Process Economics

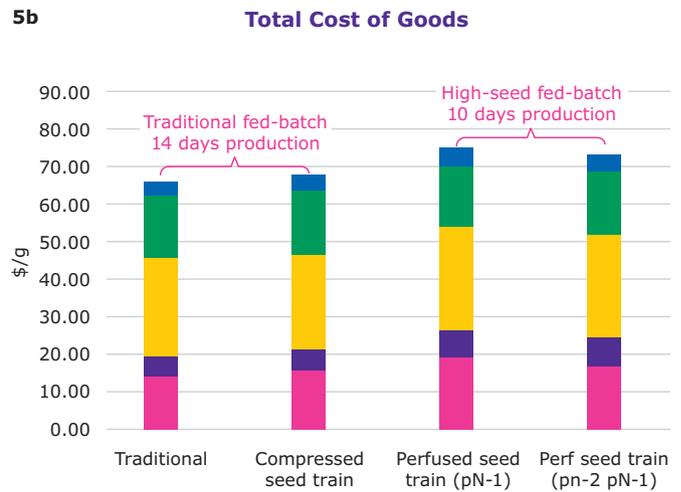
### Multiple bioreactor scenario with different production and seed train ratios at constant production titer 4 g/L

- 6 x 2,000 L Bioreactor
- 3 options of production/seed train ratio



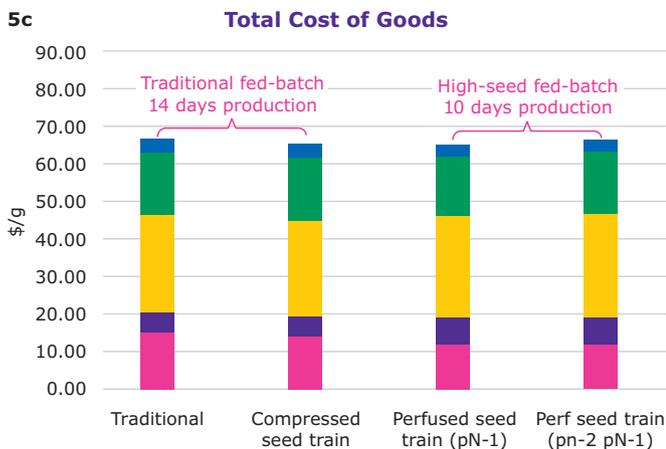
| CoG's (\$/g)                 | 67.38  | 77.25           | 85.46  | 82.80      |
|------------------------------|--------|-----------------|--------|------------|
| Throughput (kg/yr)           | 495.99 | 315.63          | 275.55 | 315.63     |
| Batches/yr                   | 99     | 63              | 55     | 63         |
| Bottleneck unit operation    | N-1    | Perfused Rocker | N-1    | N-2 Rocker |
| Bottleneck time (days/batch) | 2.64   | 4.12            | 4.76   | 4.12       |
| Batches/yr                   | 99     | 63              | 55     | 63         |

**3:1 production/seed train ratio – 2 seed train**



| CoG's (\$/g)                 | 65.88      | 67.58           | 75.03  | 73.08      |
|------------------------------|------------|-----------------|--------|------------|
| Throughput (kg/yr)           | 551.11     | 475.95          | 410.82 | 475.95     |
| Batches/yr                   | 110        | 95              | 82     | 95         |
| Bottleneck unit operation    | Production | Perfused Rocker | N-1    | N-2 Rocker |
| Bottleneck time (days/batch) | 2.39       | 2.74            | 3.17   | 2.74       |
| Batches/yr                   | 110        | 95              | 82     | 95         |

**2:1 production/seed train ratio – 3 seed train**



| CoG's (\$/g)                 | 66.88      | 65.35      | 65.06      | 66.52      |
|------------------------------|------------|------------|------------|------------|
| Throughput (kg/yr)           | 551.11     | 546.10     | 761.53     | 761.53     |
| Batches/yr                   | 110        | 109        | 152        | 152        |
| Bottleneck unit operation    | Production | Production | Production | Production |
| Bottleneck time (days/batch) | 2.39       | 2.40       | 1.73       | 1.73       |
| Batches/yr                   | 110        | 109        | 152        | 152        |

**1:1 production/seed train ratio – 6 seed train**

Capital Materials Consumables Labor Other

**Figure 5a/b/c.** Comparison of modeling scenarios: integration of the 4 seed models into multiple bioreactors at constant titer 4 g/L and variable production/seed train ratio.

went down, from 99 batches per year to 63 or 55. The reason for this decrease is that the bottleneck shifted from production to the N-1 or N-2 steps with the increased duration (Figure 5a).

- In the 3:1 production/seed train ratio, there was no benefit of incorporating a perfused seed train; the cost of goods went up and the throughput

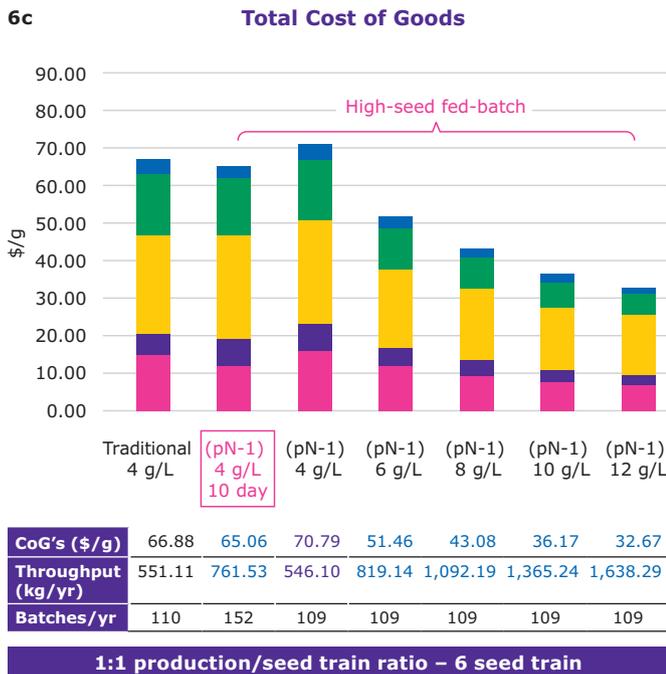
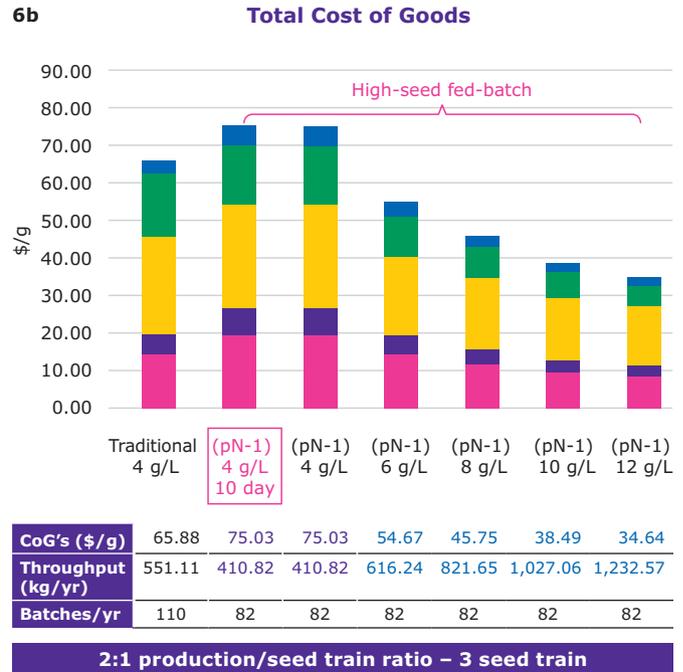
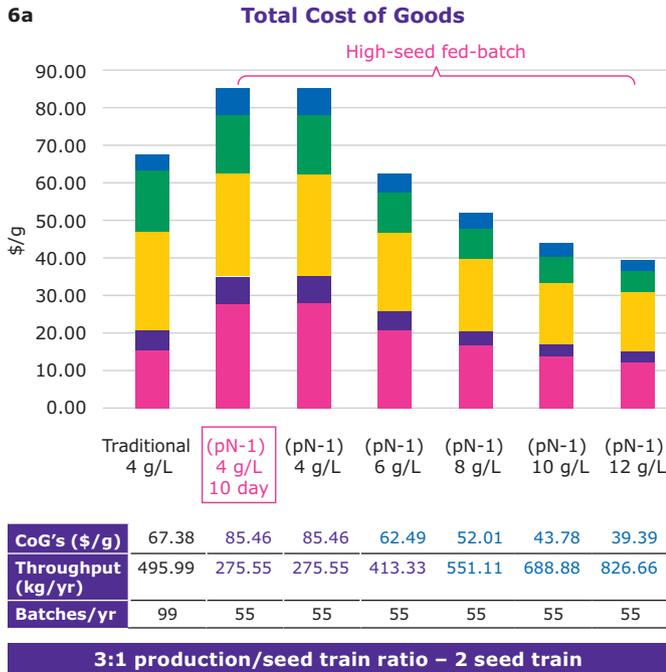
- For the 2:1 production/seed train ratio (Figure 5b), similar to the 3:1 ratio, there wasn't a significant impact on cost of goods or throughput. While the additional seed train dampened the effect on the number of batches per year, the total was still reduced. The bottleneck did, however, shift back to production for the traditional scenario but remained at the N-1 step for the remainder of the scenarios.

- With the 1:1 production/seed train ratio, each of the six bioreactors had a dedicated seed train. As shown in Figure 5c, the difference in cost of goods across all scenarios is negligible. The throughput is increased significantly, however, for the high-seed options. The reason for this is that the production step once again becomes the bottleneck. The four-day reduction in the high-seed options becomes more important again and leads to the benefit in throughput and number of batches per year, from about 110 to 150, for the perfused-seed options.

## Multiple bioreactor scenario with different production and seed train ratios at constant production time duration 14 days

Control: (pN-1 4 g/L) operated at 10 days

- 6 x 2,000 L Bioreactor
- 3 options of production/seed train ratio



Capital Materials Consumables Labor Other

**Figure 6a/6b/6c.** Comparison of modeling scenarios: integration of the 4 seed models into multiple bioreactors at constant production duration 14 days.

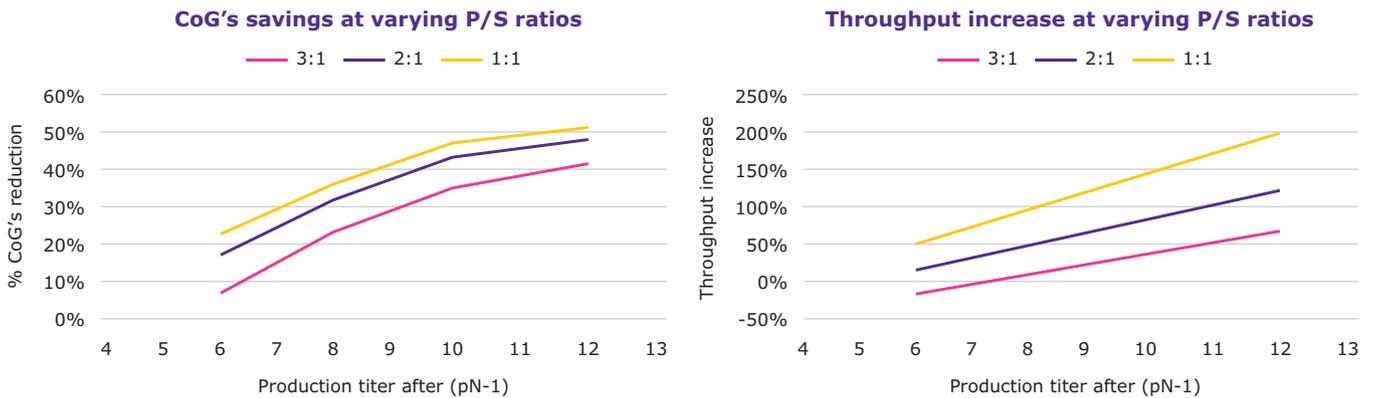
produced per year reduced from 99 to 55. However, if a titer of 6 g/L can be achieved, benefits in the cost of goods were realized, although throughput might suffer slightly. Benefits increase at higher titers with a noticeable increase in throughput and the need to produce about half as many batches per year. Higher throughput and titer drove down the cost of goods.

- The 3:1 production/seed train ratio (Figure 6a) results show that the process bottleneck remained; when comparing a high-seed option to traditional fed-batch, the number of batches that can be

- With a 2:1 ratio, the benefits were increased and more pronounced, including at the level of 6 g/L (Figure 6b). The bottleneck effect was diminished, which allowed for increased throughput from 55 to 82 batches per year versus the 3:1 ratio. At 10 g/L, throughput was nearly doubled versus traditional fed-batch.
- Similarly, for the 1:1 ratio of bioreactors and seed trains, production became the bottleneck and the batches per year were roughly equivalent (Figure 6c). However, the benefits in terms of reduction in cost of goods and increased throughput were optimized.

Figure 7 summarizes the high-seed benefits that can be observed at various titer scenarios. The x-axis represents the titer that can be achieved in the high-seed production bioreactor, following perfusion

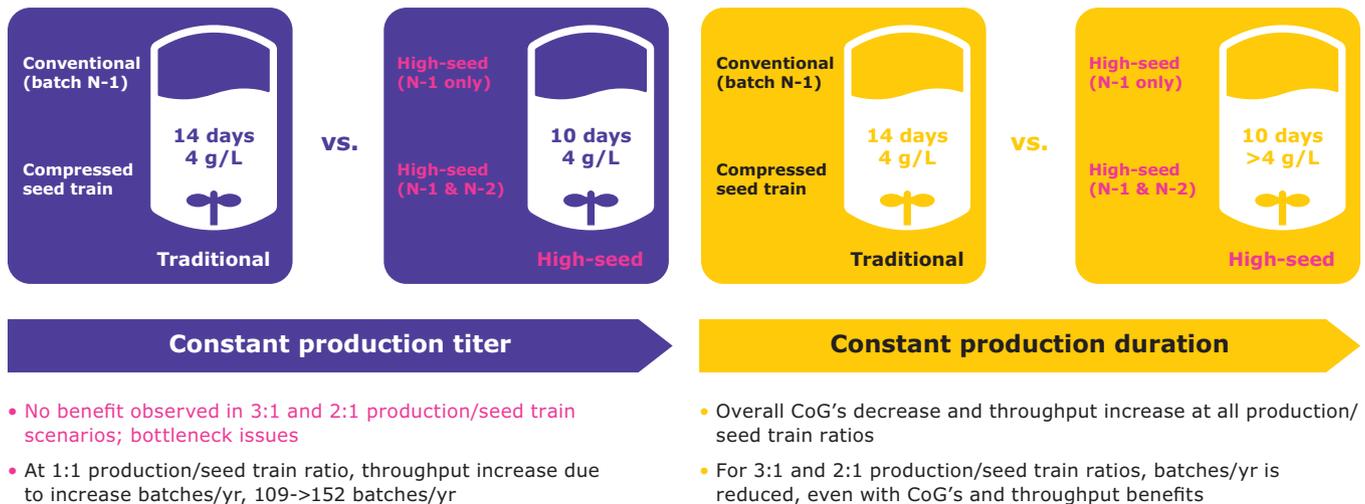
N-1. The y-axis represents the percent cost of goods reduction or the percent throughput increase, calculated by comparing the perfusion N-1 process to a traditional process.



**Figure 7.** Improvements in cost of goods and throughput at different production to seed train ratios.

When considering the potential for increased titer through high seeding of a fed-batch bioreactor, virtually all the scenarios offer benefits, with the 1:1 production/seed train ratio offering the

strongest benefits in terms of both cost of goods and throughput. With a 1:1 ratio, the cost of goods decreased 23 to 51 percent and throughput increased 65 to 200 percent.



**Figure 8.** Summary of constant production titer and constant production duration scenarios and benefits, for multiple bioreactors scenario.

## Conclusion

With higher titers, adding more seed trains and production bioreactors at a 1:1 ratio delivers the most benefit. Benefits can also be achieved at the 3:1 and 2:1 ratio, while requiring fewer batches per year to realize that cost-of-goods and throughput benefit.

Even with no increase in titers, the single-bioreactor scenarios still experience cost-of-goods reduction and throughput increases at shorter, high-seed fed-batch durations. Adding seed trains and production bioreactors at a 1:1 ratio delivers an increase in throughput.

Implementing perfused seed trains can reduce the costs of manufacturing and increase product throughput, all while maintaining the production bioreactor in a more simple-to-operate fed-batch mode. In addition, perfused seed trains allow for high seeding of the production bioreactor, which enables an increase in titer, especially when incorporating a robust media platform.

## What is the Biosolve® software?

A software that allows you:

- Gain access to impartial and industry-wide data to rationalize and de-risk your business decision-making.
- Access up-to-date information from across the biopharmaceutical sector to deliver impartial knowledge and advantage.

## More information

<https://biopharmservices.com/software>

**BioContinuum™ Platform**  
Converge, Intensify and Evolve. Together.



[EMDMillipore.com/BioContinuum](https://EMDMillipore.com/BioContinuum)

**MilliporeSigma**  
400 Summit Drive  
Burlington, MA 01803

For additional information, please visit [EMDMillipore.com](https://EMDMillipore.com)  
To place an order or receive technical assistance, please visit [EMDMillipore.com/contactPS](https://EMDMillipore.com/contactPS)

