



Process economy and production capacity using single-use versus stainless steel fermentation equipment

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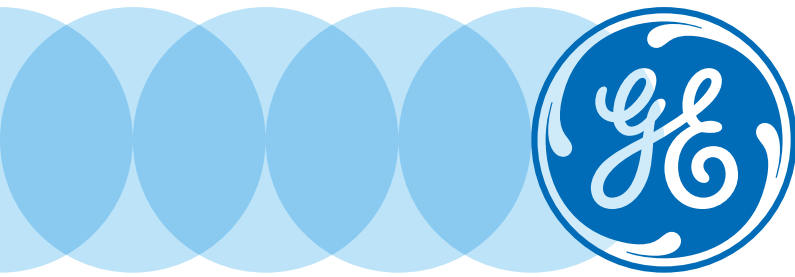
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Process economy and production capacity using single-use versus stainless steel fermentation equipment



Process economic comparison between fermentation in single-use versus stainless steel equipment

In this white paper, we compare production capacity and process economy between stainless steel and single-use equipment in microbial processes. Economy simulations were based on an *E. coli* Dab process. Production scenarios in both single- and multi-product facilities were considered.

In comparison with a stainless steel strategy, this study shows that the annual production capacity can be increased with up to 100% with a single-use strategy due to a faster batch changeover procedure. The increased production capacity with single-use equipment means that a defined amount of batches can be produced in shorter time, for example, in a manufacturing campaign or during process development. The increased batch throughput also generates a greater profit opportunity, which benefits can outnumber the higher production cost per batch associated with the single-use alternative. With the decreased financial risk with single-use equipment, the business case becomes more agile in comparison with stainless steel equipment associated with higher fixed costs.

Introduction

Microbial fermentation is used for manufacturing of a wide variety of products in the biopharmaceutical industry, including small non-glycosylated proteins such as human growth hormone; peptides such as insulin; organic molecules such as antibiotics; and vaccine against pneumonia and cholera. Recently, biosimilars, biobetters, and antibody fragments were added to the list of products produced in fermentation processes.

The main advantages of microbial fermentation include straight-forward cloning procedures, simple culture conditions, and fast culture growth. In comparison with higher-developed cells, however, microorganisms are less complex, with limitations, for example, in terms of their ability of post-translational modifications. Extensive research has been conducted to develop capabilities to enhance microbial expression systems, for example, glyco-engineering to enable correct protein glycosylation. Dominating organisms in today's biomanufacturing are *Escherichia coli* and *Pseudomonas fluorescens* bacteria, *Saccharomyces cerevisiae* and *Pichia pastoris* yeasts, and *Aspergillus* filamentous fungi. Historically, bioreactors and fermentors were constructed from stainless steel or glass. At the end of the 90s, however, plastics entered the scene, and with this, the possibility of using disposables and single-use equipment in culturing

processes. The adoption of the early single-use rocking WAVE Bioreactor™ system showed that it was possible to save both time and money by using this novel disposable approach. The Xcellerex™ stirred-tank bioreactors, with a bottom magnetic drive, pioneered the single-use field, enabling the use of disposables over the technology platform, from small-scale process development to 2000 L manufacturing scale. Although both the WAVE Bioreactor systems and the Xcellerex bioreactors were designed for mammalian cell culture, they found use also in some microbial processes with lower OD requirements.

The benefits of single-use bioreactors, including increased process flexibility; reduced cross contamination risk; and a higher batch throughput, are also of interest for microbial biomanufacturers. However, the engineering requirements are more challenging for fermentors used in microbial processes than for bioreactors used in animal cell culture processes. Sufficient mass transfer of oxygen and the removal of excess metabolic heat are some of the specific requirements of a microbial process. The Xcellerex XDR-50 MO system was the first single-use stirred-tank fermentor that was purpose-designed for microbial cultivation. This 50 L fermentor system was introduced in 2007 and is currently used in both process development and GMP production of recombinant proteins and vaccines. XDR-50

MO has been shown to exhibit performance comparable with stainless steel systems, with an OD as high as 375 achieved in a monoclonal antibody producing *P. fluorescens* culture (1). The success with the 50 L fermentor was followed up with the announcement of the larger 500 L XDR-500 MO fermentor in 2015.

What remains to be understood are the process economic implications from using a disposable strategy for microbial biomanufacturing, that is, to identify scenarios for which single-use solutions can be more favorable than traditional stainless steel equipment. In this white paper, these questions will be discussed based on a model setup for an *E. coli* domain antibody (Dab) production process run at 50 L scale (2). Data and assumptions were validated, that is, prices and costs were verified to generate a non-biased and realistic outcome that may facilitate decision-making related to microbial production scenarios.

Process economy model

The model *E. coli* Dab process was used to assess process economy in four hypothetical production scenarios, including both single-use and stainless steel equipment in a single-product as well as a multi-product facility:

1. Single-product facility with stainless steel equipment
2. Single-product facility with single-use equipment
3. Multi-product facility with stainless steel equipment
4. Multi-product facility with single-use equipment

The scope of the process economic simulation was limited to the upstream fermentation process and the Dab production phase. Other unit operations, such as the downstream purification process, have been omitted for the sake of simplicity. The single-use fermentor selected for this investigation was the Xcellerex XDR-50 MO system. The stainless steel Biostat™ D-DCU 50 L fermentor (Sartorius Stedim Biotech) was used as reference.

The specific objectives for the investigation were the following:

- Investigation of effects of the equipment choice on the production capacity.
- Estimation of batch production cost for processes in which either stainless steel or single-use equipment was used.
- Understanding of how the equipment strategy affects the total annual cost at different batch throughputs.
- Assessment of the profit opportunity for the different equipment strategies.

General assumptions

The following general assumptions were made:

- Available for fermentation are 300 days per year. The remaining time is dedicated for annual maintenance. Capital investments (including 10% interest) and qualification costs are spread over the number of batches that can be produced during the depreciation period (10 years) of the equipment.
- A cost of 100 USD per man-hour.
- The fermentation is assumed to run over night with two operators present to monitor the process (same for all scenarios). Labor is performed in two shifts: one from 6 am to 2 pm and one from 2 pm to 10 pm.
- The batch failure rate was not considered.

The aim of this study was to make an objective comparison between single-use and stainless steel fermentors to provide a representative assessment of the two alternatives and to understand their respective strengths and weaknesses. Hence, all assumptions and costs were verified with data or information from existing processes whenever possible.

The following specific assumptions were made:

- For single-product facilities (scenarios 1 and 2), it was assumed only one product was produced and the production capacity of 300 days was utilized to 100%.
- For multi-product facilities (scenarios 3 and 4), it was assumed the facility could produce different products and the production capacity of 300 days was utilized to 100%. Each product was assumed to be produced in campaigns of five batches.

Cost categories

The following cost categories were included in the model:

1. Capital investments (Table 1)
2. Installation and operation qualifications (IQ/OQ), performance qualification (PQ), and cleaning validation (Table 2)
3. Annual requalification and maintenance (Table 3)
4. Production-related costs (Table 4):
 - a. System preparations prior to fermentation
 - b. Fermentation process in the production facility
5. Disposables, chemicals, water for injection (WFI), steam, and similar (Table 5)

The costs of the various qualifications, cleaning validation, annual requalification and maintenance, as well as production-related costs have been estimated by evaluating the amount of labor (man-hours) required for each respective unit operation.

The following elements were omitted from the model, as the needs and procedures are identical in the stainless steel and single-use scenarios or have minimal differential cost impact:

- Identical needs
 - Seed culture generation procedure in shaker flasks
 - Type and amount of culture medium components
 - Minor hardware such as scales and tube welders
 - Minor disposables such as C-Flex® tubing, pump tubing, syringe filters, vials, and similar
 - Number of autoclave cycles for sterilization of tubing (cost for steam and electricity)
- Nitrogen for calibration of dissolved oxygen (DO) sensors
- Facility requirements
- Minimal impact on overall cost
 - Energy consumption per batch
 - Air and oxygen demands (slightly higher for single-use equipment due to lower maximum stirring speed)

The cost of goods sold (COGS) per amount of final product was excluded from the calculations because of the small production volumes.

Table 1. Capital investment costs

Stainless steel system	Single-use system
Biostat D-DCU 50 L single O ₂ enrichment bioreactor with control unit	Xcellerex XDR-50 MO fermentor system
Bioreactor vessel load cells	Exhaust condenser for XDR-50 MO
System cleaning-in-place (CIP)/steaming-in-place (SIP) operations	Temperature control unit (TCU) for XDR-50 MO
Extended documentation	Xcellerex XDM Quad single-use mixing system
Substrate pump	
Pressure hold test	

Table 2. Unit operations for qualification and cleaning validation

Unit operation	Stainless steel system	Single-use system
IQ/OQ of fermentor and control unit (preparation of protocols)	Included	Included
IQ/OQ of fermentor and control unit (factory acceptance test [FAT] and site acceptance test [SAT])	Included	Included
IQ/OQ of fermentor and control unit (reporting)	Included	Included
OQ temperature mapping of SIP procedure	Included	N/A
PQ of fermentor (using bioindicators for stainless steel system) and control unit	Included	Included
Cleaning validation	Included	N/A
Sterile medium hold test	Included	N/A
IQ/OQ of mixing unit (preparation of protocols)	N/A	Included
IQ/OQ of mixing unit (FAT and SAT)	N/A	Included
IQ/OQ of mixing unit (reporting)	N/A	Included
Analytics for cleaning validation and sterile medium hold test	Included	N/A

N/A = not applicable

Table 3. Unit operations for annual requalification and maintenance

Unit operation	Stainless steel system	Single-use system
OQ temperature mapping of SIP procedure (annual retesting)	Included	N/A
Cleaning validation (annual recovery study)	Included	N/A
Annual maintenance of fermentor system	Included	Included

N/A = not applicable

Table 4. Unit operations for production activities

Unit operation	Stainless steel system	Single-use system
Handling of disposables and chemicals	Included	Included
Weighing of medium components and additives	Included	Included
Mixing of medium and additives	Included	Included
Autoclave/filter additives	Included	Included
Assemble fermentor tubing	Included	Included
Autoclave fermentor tubing	Included	Included
Connect tubing to fermentor	Included	Included
Connect medium and additives to fermentor tubing	Included	Included
Preparations in fermentation room	Included	Included
Calibration of fermentor sensors	Included	Included
Sterilization of sensors	N/A	Included
Installation of single-use fermentor bag and sensors	N/A	Included
CIP of fermentor	Included	N/A
Pressure hold test of fermentor	Included	N/A
Transfer medium concentrate (including autoclavable additives) and WFI to stainless steel system	Included	N/A
SIP of fermentor including sensors	Included	N/A
Filtration of additives (non-autoclavable) into stainless steel system	Included	N/A
Transfer medium to single-use fermentor bag via filtration	N/A	Included
Transfer additives to single-use fermentor bag	N/A	Included
Inoculation with seed culture	Included	Included
Fermentation	Included	Included
Heat treatment prior to harvest	Included	Included
CIP of fermentor	Included	N/A
SIP of fermentor	Included	N/A
Waste disposal	Included	Included

N/A = not applicable

Table 5. Disposables, facilities, and chemicals included in the study

Unit operation	Stainless steel system	Single-use system
XDA single-use fermentor bag	N/A	Included
Plus Quad MBA single-use mixing bag for medium preparation	N/A	Included
Probe sheath	N/A	Included
Tap water for CIP (NaOH/acid solutions)	Included	N/A
WFI for CIP (final rinse)	Included	N/A
NaOH solution for CIP	Included	N/A
Acid solution for CIP	Included	N/A
Plant steam for heating of CIP solutions	Included	N/A
Steam for SIP	Included	N/A
Plant steam for system heating during fermentation	Included	N/A
Air vent filter inlet (Sartofluor™ Junior, Sartorius Stedim Biotech GmbH)	Included	N/A
Air vent filter outlet (Sartofluor mini, Sartorius Stedim Biotech GmbH)	Included	N/A
ULTA™ HC, 6" filter for medium transfer to system	N/A	Included

N/A = not applicable

Results

Production capacity

Production schedules for stainless steel and single-use fermentation scenarios in the single-product facility were developed. Stainless steel fermentation batches can be harvested every third day, which means that a maximum of 100 batches can be produced per year at 100% utilization, given the assumption that 300 days are available for fermentation. Under the same conditions, single-use fermentation batches can be harvested every second day, meaning that a maximum of 150 batches can be produced per year. For the production scenario outlined in Figure 1, a batch produced with single-use fermentation equipment will take 33% less time to complete compared with when using stainless steel equipment.

The production schedules for the stainless steel and single-use scenarios in the multi-product facility are outlined in Figure 2. As can be seen, the stainless steel equipment supports production of 67 batches per year, which translates to about 13 full production campaigns annually. The corresponding number for the single-use equipment is 135 batches per year, corresponding to 27 full campaigns. For the described multi-product facility, the production capacity can be doubled with single-use equipment compared with stainless steel equipment.

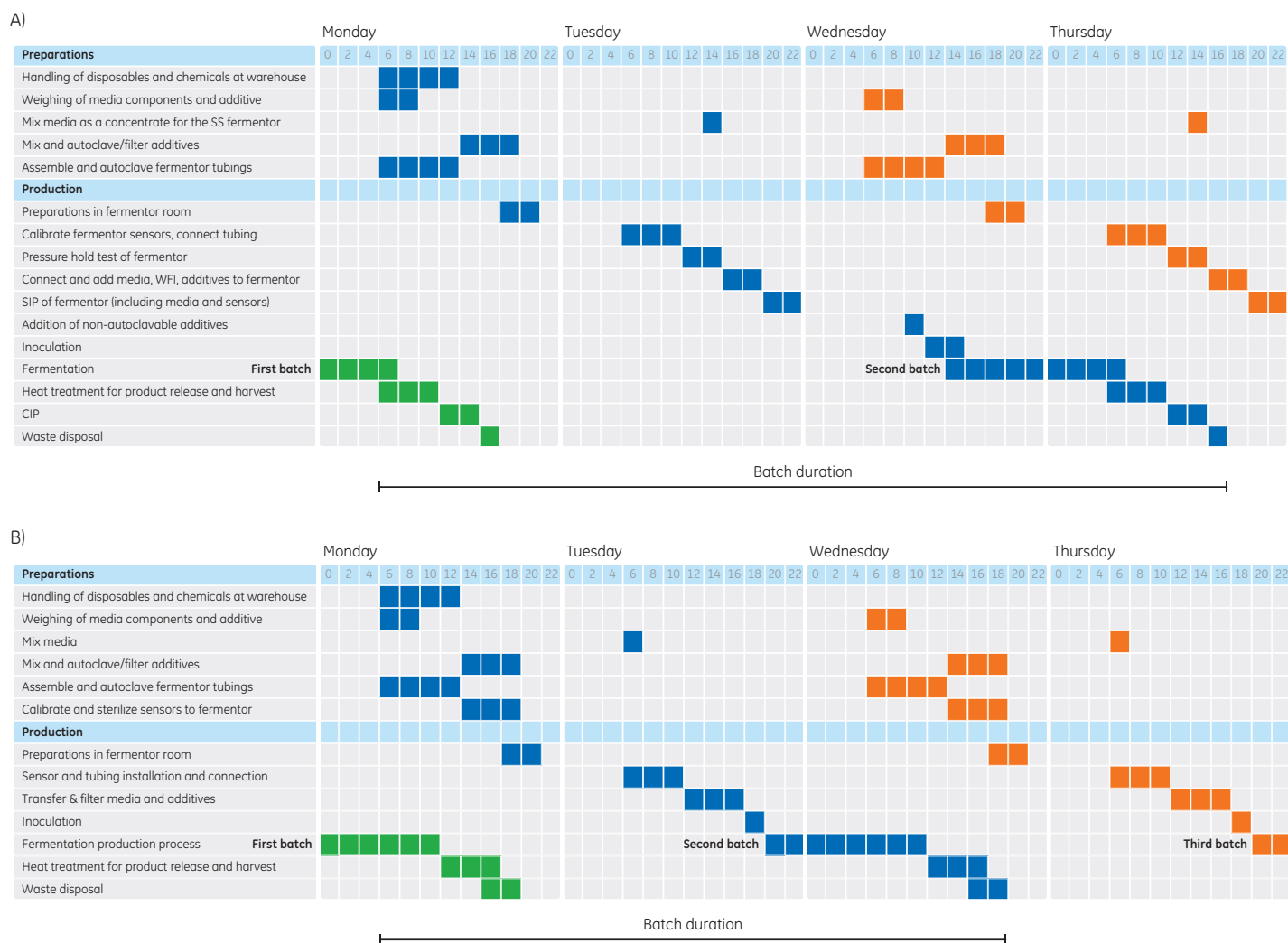


Fig 1. Production schedules for a single-product facility using either (A) stainless steel equipment or (B) single-use equipment. In the stainless steel alternative, one complete culture, including equipment preparation, takes four working days to complete. With single-use equipment, the culture can be harvested already after three days, saving one working day.

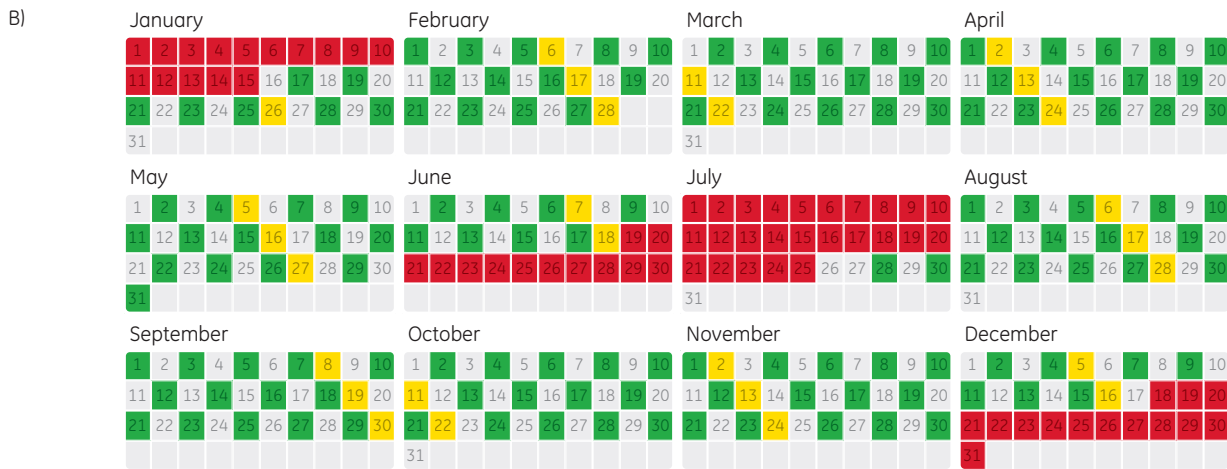
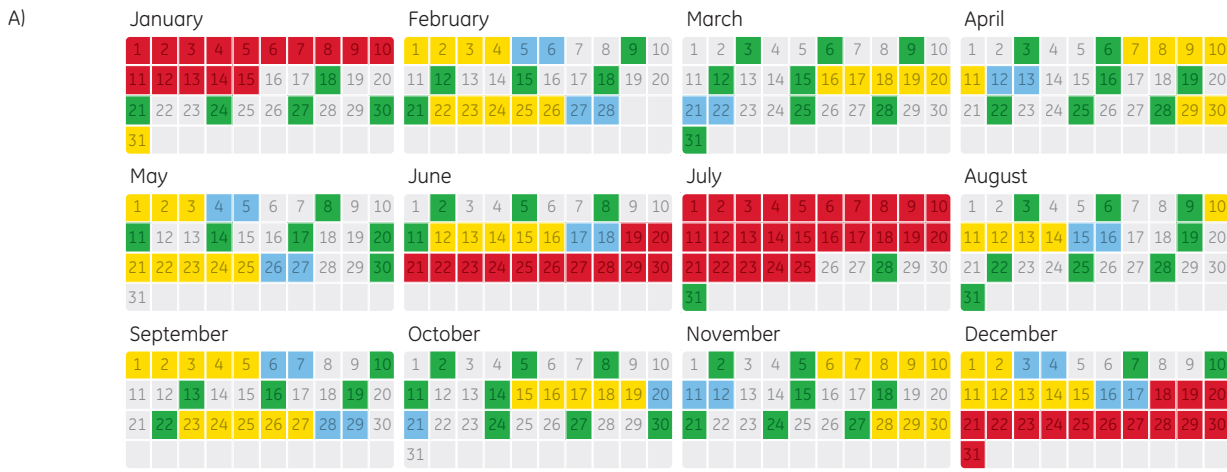


Fig 2. Production schedules for a multi-product facility using either (A) stainless steel equipment or (B) single-use equipment. The time dedicated to maintenance (red) is equal in both scenarios, whereas the time dedicated to cleaning and associated analyses (yellow) is less with single-use equipment. The time needed for carry-over calculations, reporting, and quality assurance (QA) approval (blue), included in the stainless steel scenario, is omitted in the single-use scenario. If five batches are harvested (green) each campaigns, 67 batches can be harvested annually with stainless steel equipment versus 135 annual batches with single-use equipment.

The production capacities for all four scenarios are summarized in Figure 3. As shown, the throughput is higher in the single-product facility than in the multi-product facility. In both single- and multi-product facilities, single-use equipment enables a higher throughput than stainless steel equipment. However, the difference between single-use and stainless steel equipment is most prominent in the multi-product facility scenario.

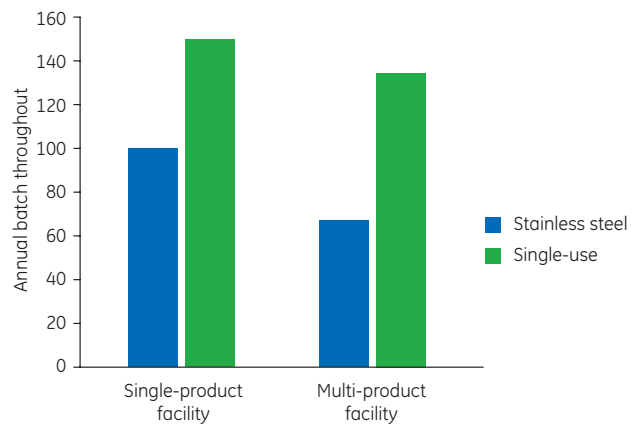


Fig 3. Production capacity for the four scenarios included in the study.

Cost analyses and profit opportunities

Cost per batch

The total cost per batch was calculated for all four scenarios, and a detailed analysis was performed by assessing the costs within six main categories:

- Capital investments
- Qualification
- Annual maintenance and requalification
- Production preparations
- Production (fermentation)
- Consumables (disposables, facility media, and chemicals)

The costs for a batch production in the stainless steel scenarios were used as a reference. This cost was normalized for all categories and set to 1. The results are summarized in Figure 4. As can be seen, the total cost and the individual cost profiles are very similar for the two facility scenarios. Relative to the stainless steel processes, the total cost per batch is higher for the single-use processes: 29% higher in the single-product facility and 25% higher in the multi-product facility. The higher batch cost with single-use equipment is due to the increased cost for consumables. However, the capital investments, qualification costs, and annual maintenance costs are higher for stainless steel, which can be expected as a stainless steel facility includes a larger amount of fixed infrastructure in comparison with a single-use facility. The production-related costs are comparable in all scenarios.

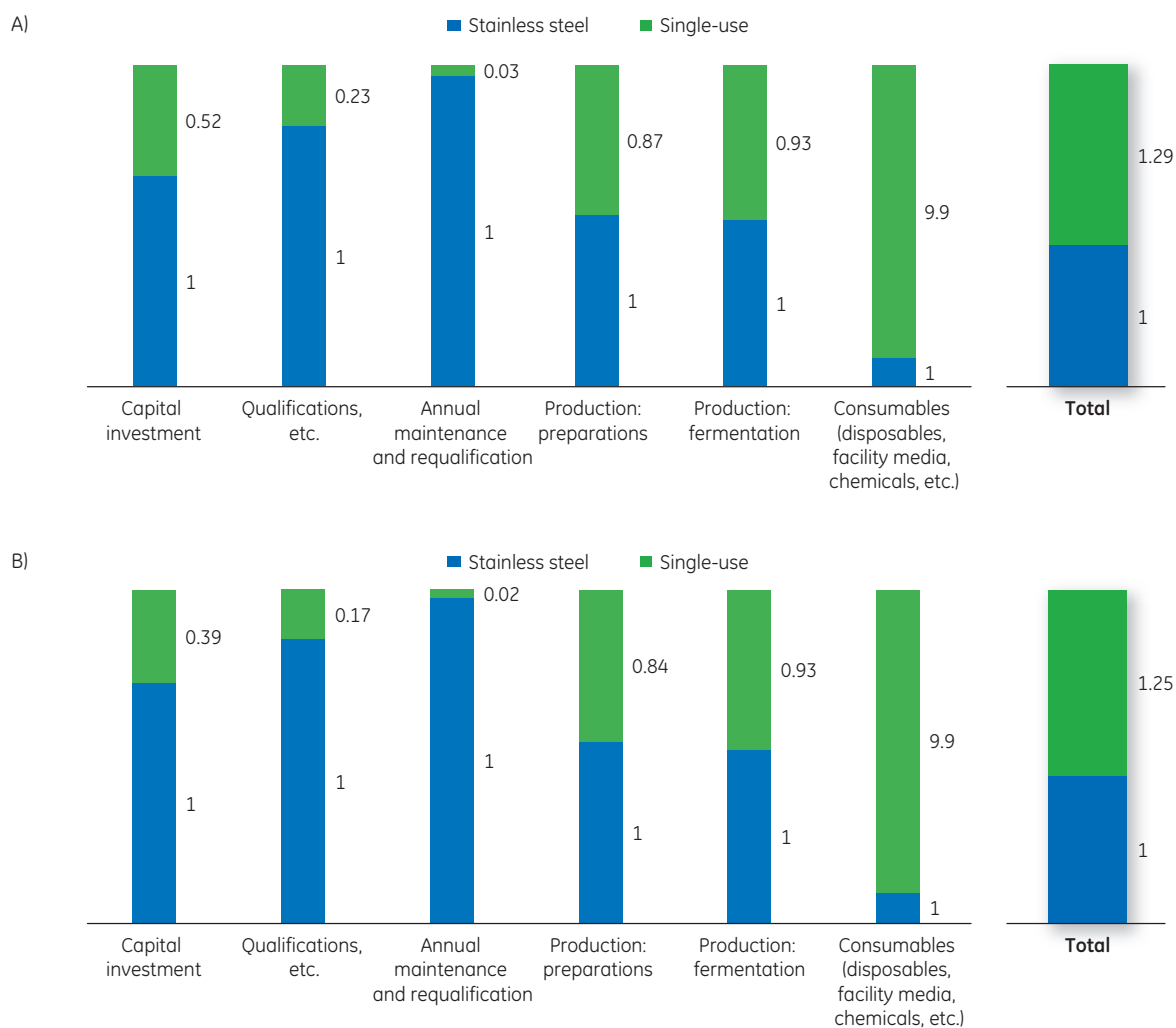


Fig 4. Relative cost per batch in the (A) single-product and (B) multi-product facilities using either stainless steel (SS) or single-use (SUB) equipment. The cost for a batch production using stainless steel equipment was used as reference and set to 1 for all categories.

Costs during varying facility utilization rate

The batch cost analysis was performed based on the assumption that the production capacity of the facility is fully utilized. In reality, however, many facilities are run at a lower utilization rate, which changes the dynamics in the cost calculation model. In certain cases, such as during a manufacturing start-up scenario, the utilization rate might be very low. For example, if only four batches are required for toxicology studies during the first year and an additional 15 batches are required for phase I studies during the second year, the company would still need to invest in the equipment. The equipment qualification costs and the costs for annual maintenance and requalification would also need to be considered.

To investigate this further, 4, 15, 30, 50, 100, and 150 annual batches were used as input data for the model, and the annual costs were calculated for the stainless steel and single-use scenarios, respectively (Fig 5A).

At low utilization rates, the data show that a single-use strategy is more beneficial from an annual cost perspective. For 4 and 15 batches annually, a single-use strategy will be associated with approximately 27% and 10% less cost, respectively, compared with a stainless steel strategy. The main reason for the lower cost for single-use equipment

is less time spent on equipment qualification. For stainless steel equipment, more than three times as much time is spent on equipment qualification. When this time was translated into cost, the annual cost for maintenance of stainless steel equipment was shown to be 21 times higher than the corresponding cost for single-use equipment, as maintenance cost remains constant regardless of equipment utilization rate. As the utilization rate increases, however, the difference between the stainless steel and the single-use strategies is levelled out. At 30 batches annually, the annual costs are more or less equal between strategies. As the number of batches increases, the stainless steel strategy becomes a feasible alternative up to 100 batches annually when the production capacity becomes limiting for the stainless steel scenario. For capacity needs above 100 annual batches, the single-use strategy would be the alternative of choice. In the extreme case where a facility is not used at all during a whole year, our model shows that the annual costs for capital investment (assuming a 10 years depreciation cycle and an interest rate of 10%) and for qualification, annual maintenance, and requalification are 122% higher for a stainless steel facility compared with a single-use facility. In summary, single-use equipment offer flexibility and benefits at both low and high capacity needs.

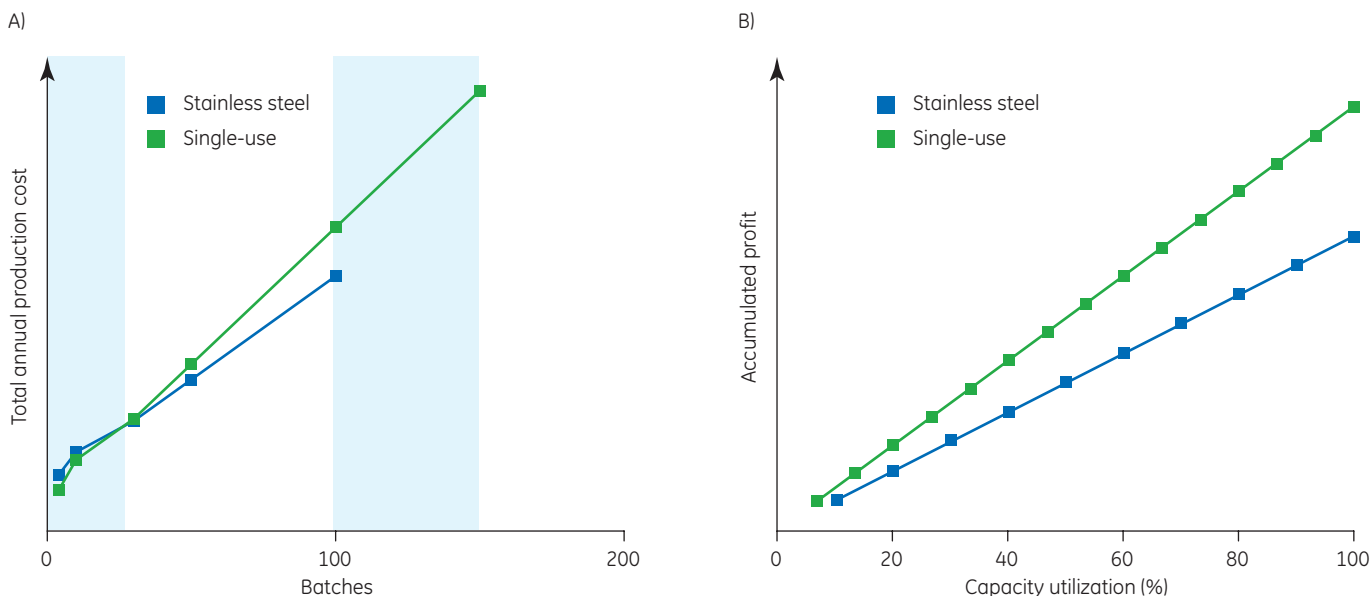


Fig 5. The annual total production cost to number of batches produced and annual accumulated profit to total capacity utilization with the single-use versus the stainless steel alternative were analyzed. (A) Total production cost to number of batches produced annually in single-use and stainless steel scenarios. After 100 batches, the maximum annual production capacity is reached using stainless steel equipment. With single-use equipment, 150 batches can be produced annually. (B) Total accumulated profit plotted against total annual capacity utilization. For the stainless steel scenario, 100% capacity represents 100 batches. For the single-use scenario, 100% capacity represents 150 batches.

Profit

This study is performed based on fermentors at a 50 L scale. In reality, few, if any, manufacturing processes are run at this scale. More appropriate applications at 50 L include process development, pilot-scale production of clinical material and seed preparations for larger-sized fermentors.

Still, we wished to get an understanding of the profit dynamics of a single-use strategy versus a stainless steel strategy. Hence, a profit calculation was performed. Revenue of 1 MUSD was assumed for each batch and the production cost was subtracted to obtain the gross profit. This calculation was performed for both the stainless steel and the single-use scenarios in a single-product facility. The result plotted against total capacity utilization for the two scenarios is displayed in Figure 5B. As shown, the profit opportunity is higher for the single-use alternative. The main reason for this outcome is the increased batch throughput, which benefits essentially outnumber the slightly higher production cost per batch for the single-use scenario.

Discussion

A model was set up to understand the cost and capacity implications for use of single-use equipment in microbial fermentation processes in comparison with reference scenarios based on stainless steel equipment. The main conclusion from this study is that a substantial amount of time can be saved by using single-use equipment instead of stainless steel equipment. For a single-product facility based on single-use equipment, batches can be harvested every second day. With stainless steel equipment, harvest takes place every third day. Thus, single-use equipment enables a higher throughput of the facility compared with a stainless steel strategy. For a single-product facility, 50% more batches (150 batches) can be produced annually using single-use equipment compared with stainless steel equipment (100 batches).

For a multi-product facility, the capacity difference is even more pronounced, with a doubled annual throughput using single-use equipment (135 batches) compared with stainless steel equipment (67 batches). The higher productivity of a single-use facility is related to the omitted need for equipment cleaning and cleaning validation procedures after a campaign.

In a stainless steel facility, the final equipment CIP procedure at the end of each campaign is followed by cleaning validation. For example, equipment swab samples are commonly analyzed for total organic carbon (TOC) and the final rinse water is analyzed for both TOC and endotoxins. The analytical results will typically be available five days after sampling. The time for the carry-over calculations, reporting, and QA approval of the report is estimated to be an additional two days. This cleaning validation procedure, totaling seven days, is significantly reduced or eliminated when producing in a single-use fermentor, as all materials that have been in contact with the product are disposed after use. Thus, during the downtime of the stainless steel fermentor, the single-use fermentor can be up and running producing additional batches.

In a multi-product facility, not only the equipment, but also the production suite needs to be cleaned before starting a new campaign for a different product. Facility cleaning procedures include emptying the production suite followed by cleaning of walls, ceiling, and floor. Cleaning verification is conducted through environmental monitoring performed by quality control (QC). However, the environmental risk from the production suite can be assessed from previous analytical results. The final QC results and the QA approval of the environmental monitoring report are therefore typically not required before starting a new campaign. The critical activity is instead the equipment cleaning and cleaning validation, which becomes the limiting factors for facilities using stainless steel equipment due to the risk of product carry-over. With single-use equipment, however, the risk for product carry-over from the production vessel is non-existent. A risk-based strategy may be used for valuable time savings and a new campaign can be started already the day after sampling for environmental monitoring.

The increased productivity with single-use equipment can also have implications beyond increasing the total capacity of a production facility. In the product development stage, for example, the shorter process time with single-use equipment can contribute to significant time savings and an overall decreased development time. A compressed time for product development can, in turn, have positive financial impact and improve overall market access. Alternatively, more batches can be produced over a set product development time. Consequently, more experiments can be conducted, generating more regulatory support data to aid in the development of a strong chemistry, manufacturing, and control (CMC) package, as well as allowing poor therapeutic candidates to be eliminated more quickly.

When studying the batch cost, our model shows that the total cost per batch at a 100% equipment utilization rate is 25% to 29% higher for a single-use scenario compared with a stainless steel scenario. The category that drives the majority of the cost for the single-use scenarios is the cost for consumables including the disposable fermentor bag and the mixing and sterile filtration consumables. However, when looking at the fixed costs, including capital investment, annual maintenance, and qualification costs, the cost burden is higher for stainless steel equipment. The fixed costs will remain whether the facility is in use or not, whereas the variable consumable costs only occur when the facility is in use for production of profit-generating biologics.

The implications from having a larger portion of fixed costs versus having a larger portion of variable operational cost become apparent when studying production scenarios at a low facility utilization rate, for example, in a start-up scenario. At a low facility utilization rate of less than 30 batches per year, the annual cost for the facility is lower for single-use equipment. As the number of batches increases, stainless steel equipment becomes the least costly alternative until the point where the stainless steel production capacity becomes a limiting factor. In our model, this point is at 100 batches per year. If a higher production capacity is required, single-use equipment is the preferred option.

The vast majority of microbial GMP manufacturing processes are performed at much larger scales than 50 L. However, we were still interested in an initial assessment of the profit dynamics for single-use and stainless steel scenarios. Our results clearly show that the profit opportunity is higher for a single-use strategy due to the larger batch throughput possible with such equipment. For a representative image of the process economy at a larger production scale, the model used in this study should be adjusted for the specific scenario. However, the general principles that are applied in this study are expected to be valid for microbial fermentation processes at larger scales.

Conclusions

The following conclusions can be drawn from this study.

A single-use upstream equipment strategy is advantageous in microbial fermentation under the following conditions:

- When a certain number of batches need to be produced in the shortest possible time: single-use equipment offers an agile solution, for example, in the development stage of the target product, in production for clinical trials, in toxicity studies, or in vaccine surge capacity scenarios.
- When high production capacity is needed: in this study, a single-use strategy enabled a 50% increased batch capacity in the single-product scenario and a 100% increased capacity in the multi-product scenario compared with a stainless steel strategy.
- At full facility utilization, the profit opportunity was higher for the single-use alternative, with 150 annual batches compared with 100 batches annually for the stainless steel alternative.
- If the facility utilization rate is low: a lower capital investment, due to a low upfront investment and low costs for annual maintenance and qualification, contributes to the lower fixed cost associated with single-use equipment in comparison with stainless steel equipment.

Disclaimer

The results and conclusions presented in this white paper are valid for this specific study. Other study conditions and assumptions could have significant impact on the outcome. The overall finding in this study is that despite the higher batch cost, single-use fermentation equipment can generate more batches annually, and if all batches would lead to sold product, the single-use alternative would contribute to a higher gross profit.

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