Process economy of using Cytodex Gamma microcarriers

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Process economy of using Cytodex™ Gamma microcarriers

Microcarriers are commonly used in bioreactor cultures to provide a large growth surface for anchorage-dependent cells, and are typically provided either untreated or gamma-irradiated and ready for use. The process economy comparison performed in this work was made for the preparation step of Cytodex 1 versus Cytodex 1 Gamma microcarriers. Two scales were included: 100 L pilot and 500 L production scales.

Introduction

Microcarriers are used in bioreactor cultures to provide a growth surface for anchorage-dependent cells, such as the Vero cells traditionally used in virus vaccine manufacturing. In comparison with cell culture roller bottles or flask systems, microcarriers provide a much larger surface-to-volume ratio due to the 3D distribution of the microcarriers inside the bioreactor. In bioreactor cultures, the available surface area can easily be changed by altering the microcarrier concentration. Bioreactor cultures also provide a more sophisticated control of the culture process.

Bioreactor culturing can be conducted in stainless steel or in single-use equipment. Whereas stainless steel bioreactors require extensive cleaning and cleaning validation between batches or campaigns, single-use bioreactors reduce the need for these time-consuming activities. With single-use bioreactors, all process components that have been in contact with the process material can be conveniently disposed after use.

Cytodex Gamma microcarriers are provided gamma-irradiated in containers with flexible connection options for various cell culture vessels. For aseptic transfer, the container can be connected to the bioreactor through a welding connection, eliminating the need for open handling of the microcarriers. Compared with standard microcarriers, Cytodex Gamma microcarriers also require less preparation and handling. This work compares process economy between preparation of untreated Cytodex 1 microcarriers versus gamma-irradiated Cytodex 1 Gamma microcarriers at two different process scales.

Materials and methods

Process economy simulations

The process economy simulation is based on GE Healthcare's list prices as well as general information from the BioSolve™ process economic simulation tool (BioPharm Services) and compares the microcarrier preparation step using either Cytodex 1 or Cytodex 1 Gamma microcarriers at 100 L or 500 L scale. Input data is summarized in Tables 1 and 2. Outputs were generated through in-house Excel® modeling.

Table 1. Input data for process economy calculations at 100 L scale

Process steps	Cytodex 1		Cytodex 1 Gamma	
	Process time (h)	Volume (L)	Process time (h)	Volume (L)
Weighing and filling	2	N/A	N/A	N/A
Hydration and washing	10	N/A	N/A	N/A
Sterilization	12	N/A	N/A	N/A
Settling, rinsing, and preparation for cell culture	7	N/A	5.5*	N/A
Mixing of PBS	2.5	72	N/A	N/A
Mixing of cell culture medium	5.3	200	2.7	100

* For Cytodex 1 Gamma, the process time refers to dispensing and transfer to bioreactor.
N/A = not applicable.

Table 2. Input data for process economy calculations at 500 L scale

Process steps	Cytodex 1		Cytodex 1 Gamma	
	Process time (h)	Volume (L)	Process time (h)	Volume (L)
Weighing and filling	2	N/A	N/A	N/A
Hydration and washing	12	N/A	N/A	N/A
Sterilization	18	N/A	N/A	N/A
Settling, rinsing, and preparation for cell culture	14	N/A	5.8*	N/A
Mixing of PBS	2.7	401	N/A	N/A
Mixing of cell culture medium	3.7	1000	2.8	500

* For Cytodex 1 Gamma, the process time refers to dispensing and transfer to bioreactor.
N/A = not applicable.

Cost categories

The following costs were included in the cost comparison:

- Capital expenditure (CAPEX) for hardware and facility footprint (for microcarrier preparation suite as well as for required facility media as identified).
- Installation and operation qualifications (IQ/OQ), performance qualification (PQ), and annual maintenance/requalification of equipment.
- Microcarrier preparation procedures (labor for the calculated process hours), such as weighing, hydration, sterilization (Cytodex 1 only), and mixing of solutions.
- Cleaning-in-place (CIP) procedure for stainless steel tank (Cytodex 1 only).
- Disposables, chemicals, microcarriers, and facility media (water for injection, steam, gases etc.).
- Quality control (QC) testing for CIP of stainless steel tank.
- Cost for solid and liquid waste.
- Other conditions and media as identified.

General cost assumptions

The following assumptions were made:

- Hardware costs were based on BioSolve information, existing official prices, and cost estimations.
- Facility cost was based on estimated required foot print and price for foot print from BioSolve.
- Costs for IQ/OQ/PQ and annual maintenance/ requalification of equipment were based on estimated number of work hours for each unit operation based on in-house experience as well as input from external sources.
- The microcarriers were prepared for one batch at a time.
- Costs were calculated per prepared batch.
- Assumed cost per full-time equivalent (FTE) was set to 30 and 170 USD/h.
- Costs of disposables and raw materials are based on external sources (e.g., official websites) and BioSolve information.

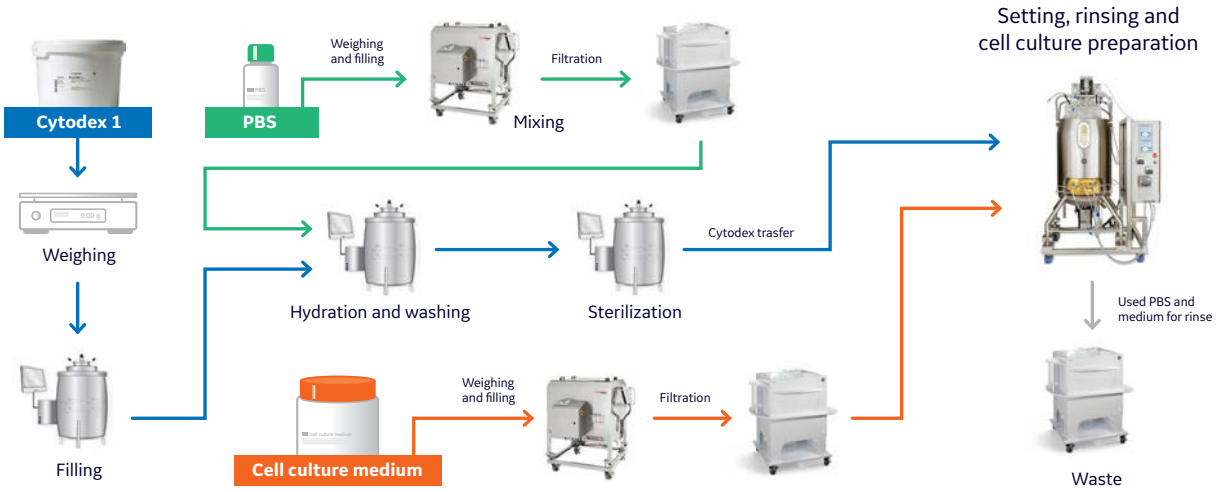

Fig 1. Assumed preparation procedure for Cytodex 1 microcarriers at 100 L scale.

Fig 2. Assumed preparation procedure for Cytodex 1 Gamma microcarriers at 100 L scale.

- Cost of cell culture medium was set to 8 USD/L.
- Cost of PBS was based on BioSolve information.
- Waste costs were based on BioSolve information.
- Failure rate was not considered.
- Microcarrier costs were based on the 5 kg pack size for Cytodex 1 and the 3 kg pack size for Cytodex 1 Gamma.

The following costs were not included in the comparison:

- Minor hardware.
- Environmental monitoring.
- Labor not directly involved in the preparation of the microcarriers.
- Electricity.
- Interest rate and depreciation time for the capital investments.
- Minor disposables such as vials, syringe filters, tubing connectors, etc.

Assumed process outlines

For the 100 L scale, Cytodex 1 is weighed, washed, and sterilized followed by transfer to the Xcellerex™ XDR-200 bioreactor system. PBS and cell culture medium are prepared separately in the Xcellerex XDUO Mixing System and filtered into hold bags. PBS is used for the swelling and washing of Cytodex 1. The process is outlined in Figure 1. The corresponding preparation procedure for Cytodex 1 Gamma, shown Figure 2, requires no PBS. Hence, for Cytodex 1 Gamma, only cell culture medium is prepared separately as above. For 500 L scale, Cytodex 1 and Cytodex 1 Gamma are prepared as above, but instead transferred to the Xcellerex XDR-500 bioreactor system. The processes are outlined in Figures 3 and 4.

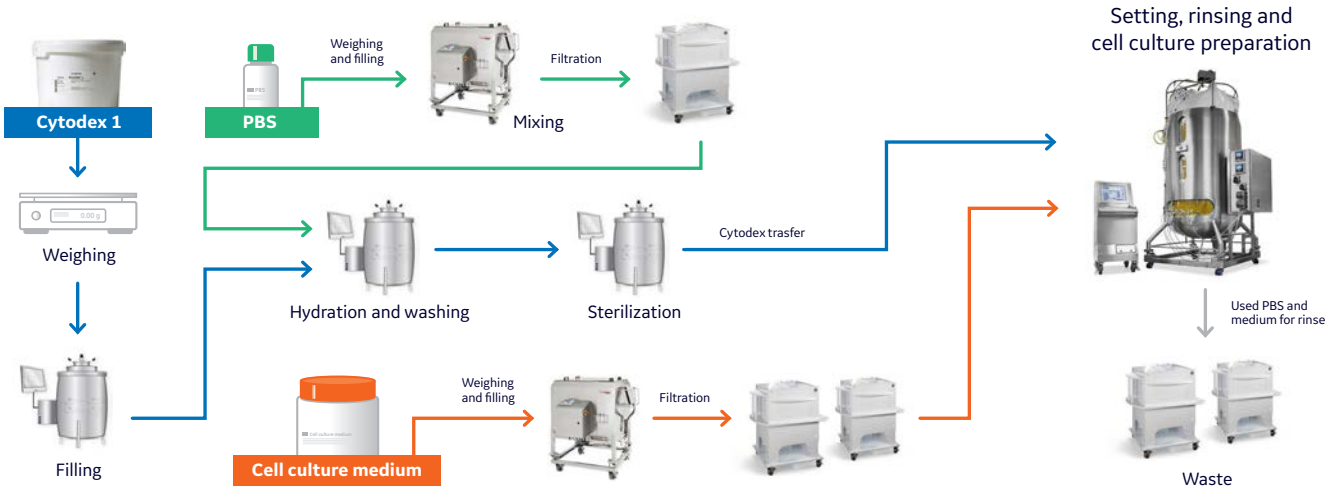

Fig 3. Assumed preparation procedure for Cytodex 1 microcarriers at 500 L scale.

Fig 4. Assumed preparation procedure for Cytodex 1 Gamma microcarriers at 500 L scale.

Results

Capital investments

As shown in Figure 5A, CAPEX for Cytodex 1 Gamma at 100 L scale is 1.11 MUSD less than for Cytodex 1. At 500 L scale, CAPEX is 1.13 MUSD less for Cytodex 1 Gamma compared with Cytodex 1 (Fig 5B).

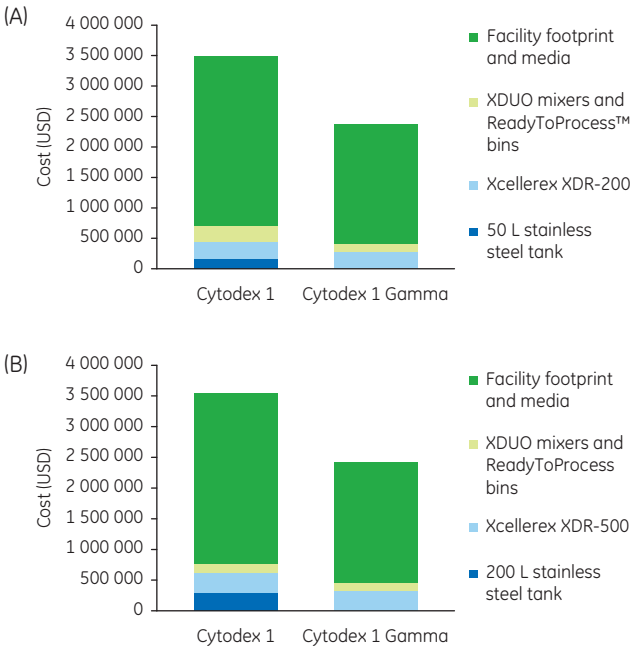

Fig 5. CAPEX for Cytodex 1 versus Cytodex 1 Gamma at (A) 100 L and (B) 500 L scales.

Qualification costs

Qualification costs for Cytodex 1 versus Cytodex 1 Gamma at FTE costs of 30 UDS/h and 170 USD/h for the 100 L and 500 L scales are shown in Figures 6 and 7, respectively. The results show cost-savings with Cytodex 1 Gamma, even at the lower labor cost. The main part of the qualifications cost for Cytodex 1 can be attributed to the stainless steel tank for sterilization of the microcarriers. In addition, an extensive cost for annual retesting and maintenance can also be related to the stainless steel tank. The much lower qualification cost for Cytodex 1 Gamma can be correlated to the less equipment used, and hence, fewer qualification activities.

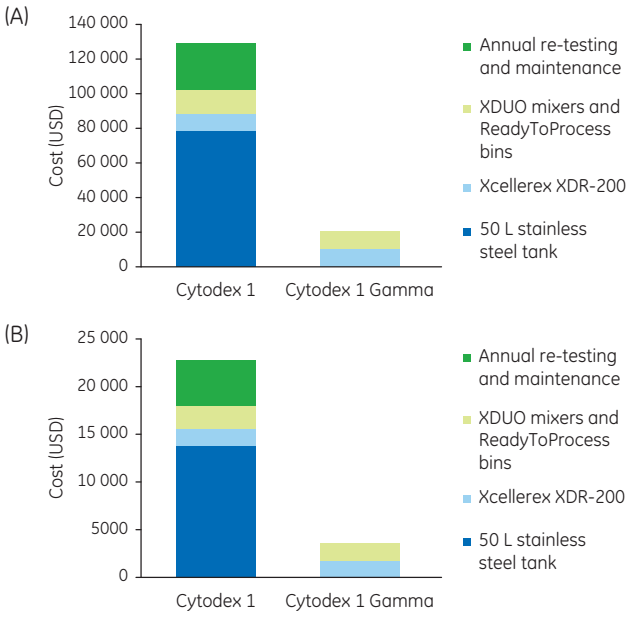

Fig 6. Qualification costs for Cytodex 1 versus Cytodex 1 Gamma at 100 L scale at FTE costs of (A) 170 USD/h and (B) 30 USD/h.

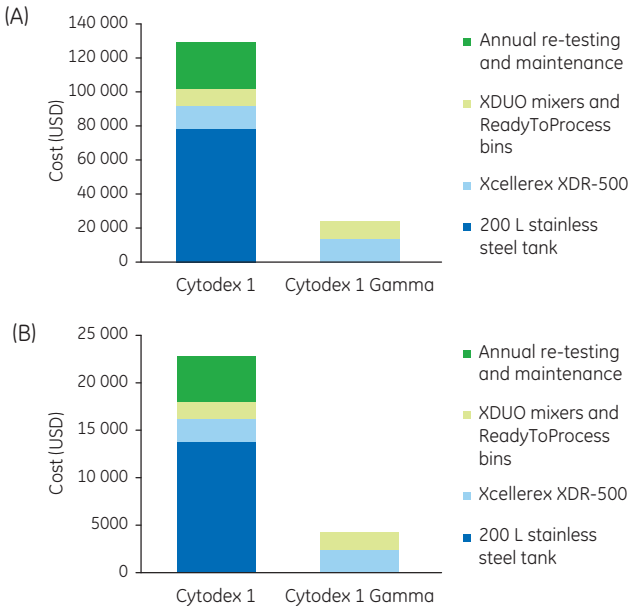

Fig 7. Qualification costs for Cytodex 1 versus Cytodex 1 Gamma at 500 L scale at FTE costs of (A) 170 USD/h and (B) 30 USD/h.

Preparation costs

The main part of the microcarrier preparation cost for Cytodex 1 can be related to weighing, transfer to tanks/bioreactors, hydration, and wash. A significant part can also be associated with handling of the stainless steel tank, including CIP, waste handling, and similar. The buffer/medium preparation cost is less for the Cytodex 1 Gamma process, as no PBS and less cell culture medium are required. In general, the cost of microcarrier preparation is much lower for Cytodex 1 Gamma, at both 100 and 500 L scale, at both high and low FTE cost. The results are shown in Figures 8 and 9.

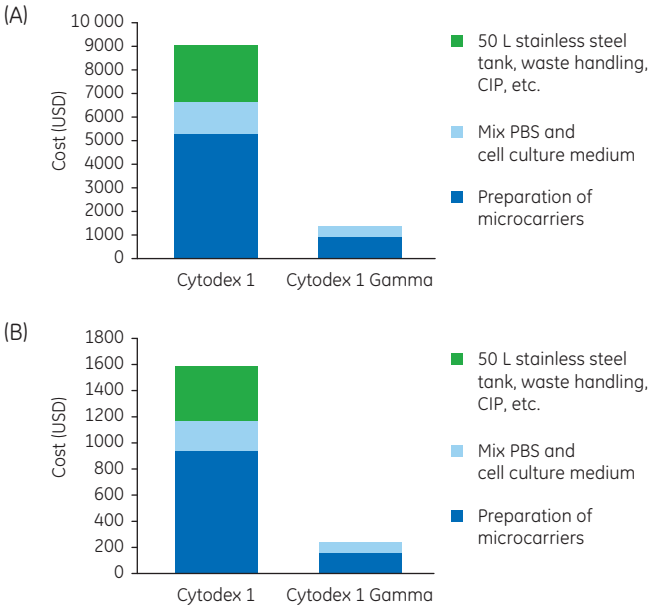

Fig 8. Microcarrier preparation costs for Cytodex 1 versus Cytodex 1 Gamma at 100 L scale at FTE costs of (A) 170 USD/h and (B) 30 USD/h.

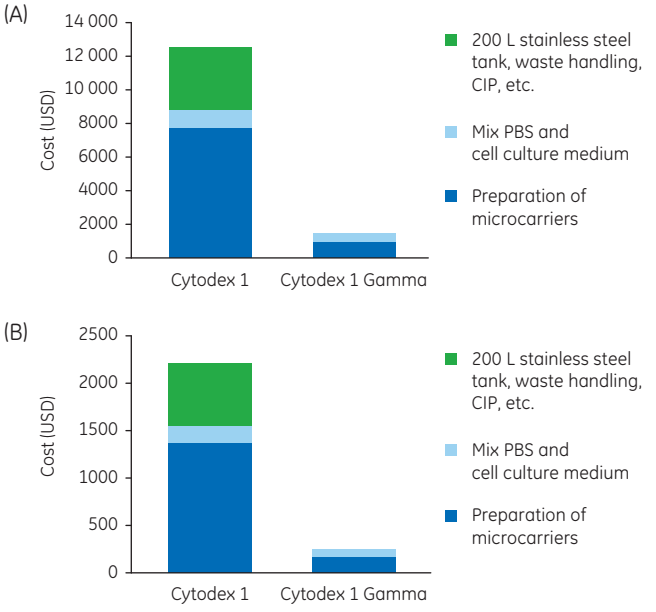

Fig 9. Microcarrier preparation costs for Cytodex 1 versus Cytodex 1 Gamma at 500 L scale at FTE costs of (A) 170 USD/h and (B) 30 USD/h.

Preparation time

Microcarrier preparation time, summarized in Figure 10, shows that much more work hours are required for the Cytodex 1 process due to the more extensive handling of the Cytodex 1 microcarriers. As handling of the stainless steel tank also requires a significant amount of work hours, the time spent on sterilization of the Cytodex 1 microcarriers was added to show the true process time spent on preparing these microcarriers.

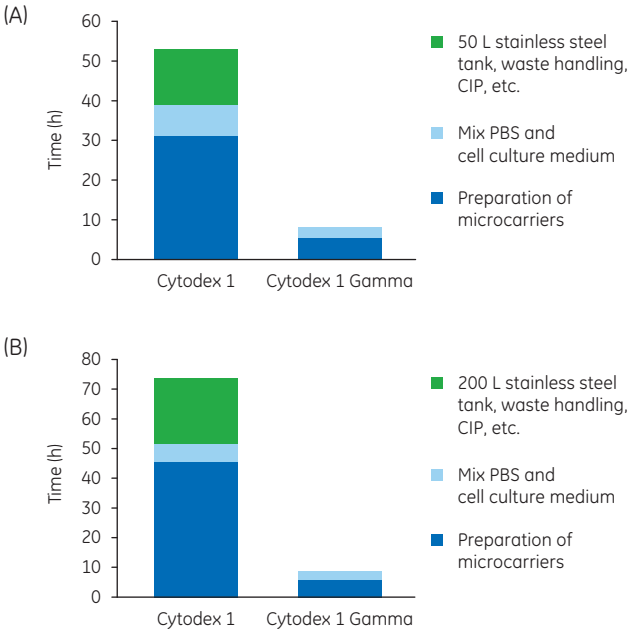

Fig 10. Microcarrier preparation time for (A) the 100 L scale and (B) the 500 L scale.

Buffer and medium volumes

Volumes of PBS and cell culture medium required for the Cytodex 1 and Cytodex 1 Gamma processes are shown in Figure 11. As shown, these volumes are lower for the Cytodex 1 Gamma process, requiring no PBS. The higher volumes of cell culture medium for the Cytodex 1 process is an effect of exchanging the PBS used for hydration of the microcarriers. The exchange of PBS with cell culture medium leads to a double consumption of cell culture medium in the Cytodex 1 process.

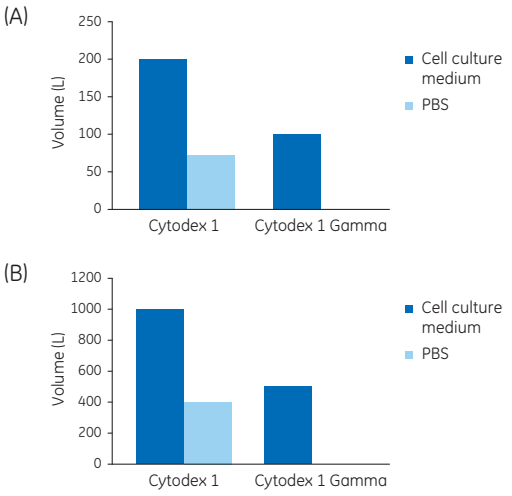

Fig 11. Volume of PBS and cell culture medium required for the Cytodex 1 and Cytodex 1 Gamma processes at (A) 100 L and (B) 500 L scales.

Cost of consumables

As shown in Figure 12, the cost for consumables per batch is higher for Cytodex 1, regardless of scale. For the 100 L scale, the cost of all consumables per batch is about 18% lower for the Cytodex 1 Gamma process. At 500 L scale, the Cytodex 1 Gamma process shows about 11% lower cost than the Cytodex 1 process.

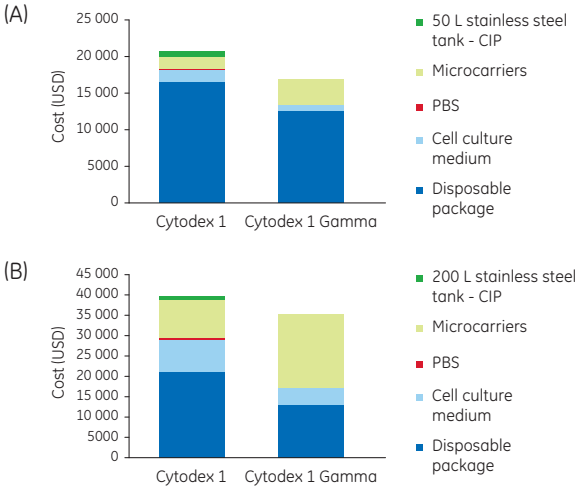

Fig 12. Consumable costs per batch for the Cytodex 1 and Cytodex 1 Gamma processes at (A) 100 L and (B) 500 L scales.

Waste costs

Solid waste is more expensive to dispose than liquid waste. The waste costs for the Cytodex 1 and Cytodex 1 Gamma processes are presented in Figure 13. As the Cytodex 1 process requires more disposables, the waste cost for this process is also higher. The cost of liquid waste is small in comparison, and is non-existent for the Cytodex 1 Gamma process.

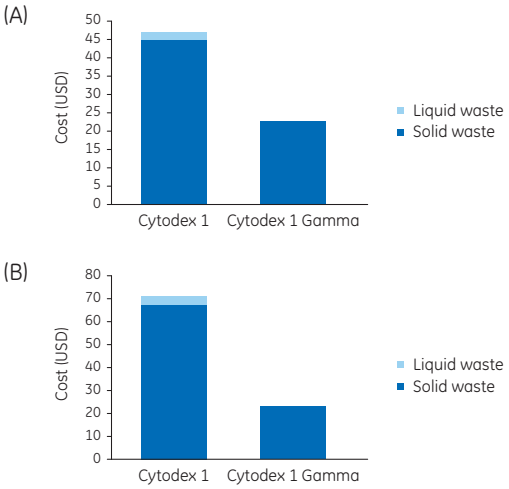

Fig 13. Waste costs per batch for the Cytodex 1 and Cytodex 1 Gamma processes at (A) 100 L and (B) 500 L scales.

Waste amounts

Figure 14 shows the amounts of waste generated for the Cytodex 1 and Cytodex 1 Gamma processes. The Cytodex 1 process generates a lot of liquid waste that requires treatment in an inactivation tank (kill tank). Regardless of if the process liquids require an inactivation tank, waste in this comparison is considered contaminated and in the need of a kill tank, which will add to the facility footprint. Although the weight of the solid waste is much lower compared with the liquid waste, the solid waste has a higher price tag. Here, the weight of the XDA bioreactor bag is also included as this is required for process preparation and setup.

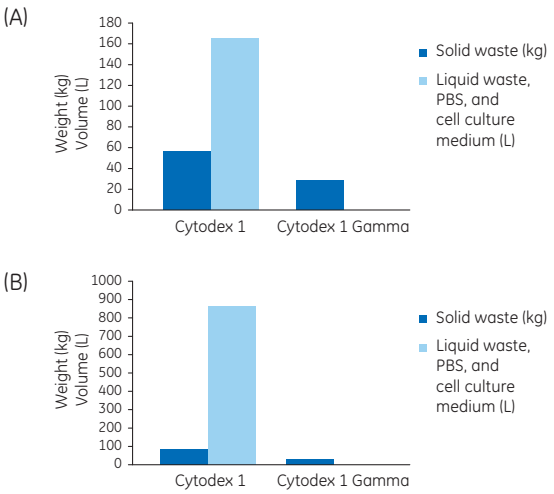

Fig 14. Waste amounts per batch for the Cytodex 1 and Cytodex 1 Gamma processes at (A) 100 L and (B) 500 L scales.

Total cost per batch

Total cost per batch is obtained when combining costs for preparation, consumables, and waste. As shown in Figures 15 and 16, consumables account for the largest part of the total batch cost, even at high labor cost. Cost of waste is the smallest contribution to the total batch costs (see Section Waste costs).

Table 3 summarizes the total batch costs for Cytodex 1 and Cytodex 1 Gamma at 100 and 500 L scales at FTE costs of 170 and 30 USD/h. At 100 L scale and an FTE cost of 170 USD/h, using Cytodex 1 Gamma can lower the total batch cost by about 38%. For the 500 L scale, even at the lower labor cost (30 USD/h), the total batch cost is still about 16% lower for Cytodex 1 Gamma.

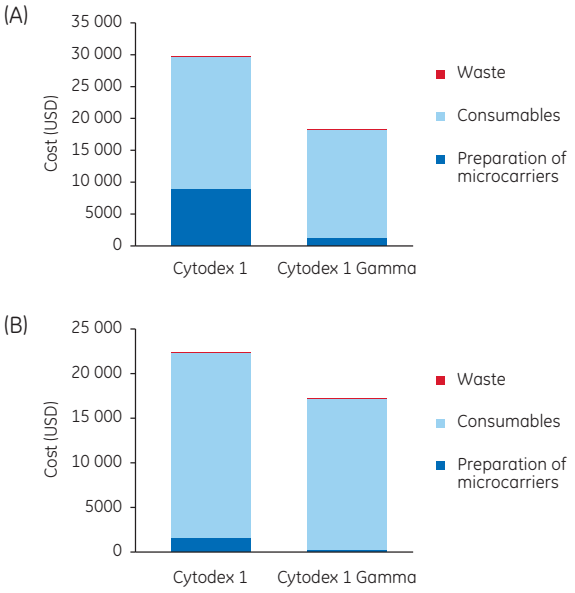

Fig 15. Total cost per batch for Cytodex 1 versus Cytodex 1 Gamma at 100 L scale at FTE costs of (A) 170 USD/h and (B) 30 USD/h.

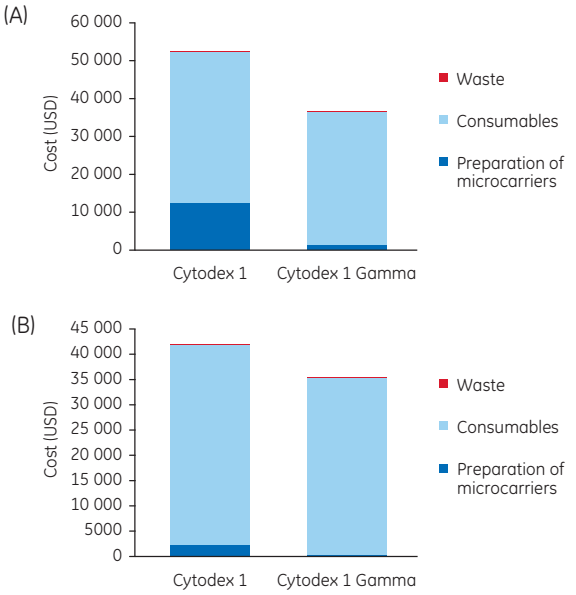

Fig 16. Total cost per batch for Cytodex 1 versus Cytodex 1 Gamma at 500 L scale at FTE costs of (A) 170 USD/h and (B) 30 USD/h.

Table 3. Total batch costs

Process scale	Labor cost (USD/h)	Total batch cost (USD)		Cost savings (%) of using Cytodex 1 Gamma compared with using Cytodex 1
		Cytodex 1	Cytodex 1 Gamma	
100 L	170	29 813	18 342	38
	30	22 388	17 208	23
500 L	170	52 371	36 662	30
	30	42 034	35 460	16

Conclusion

The goal of this work was to compare process economy of using Cytodex 1 versus Cytodex 1 Gamma. The results strongly support the use of Cytodex 1 Gamma due to the opportunity of significant cost-savings. CAPEX as well as qualification and annual maintenance costs are much lower for Cytodex 1 Gamma. The costs per batch, covering microcarrier preparation, liquid preparation (PBS, cell culture medium), use of stainless steel equipment, sterilization, consumables, and generated waste, are also much lower for Cytodex 1 Gamma. In addition to a 1.13 MUSD lower CAPEX and a reduction in total batch cost by up to almost 40% compared with the Cytodex 1 process, the use of Cytodex 1 Gamma also offers a reduced contamination risk due to less manual handling as well as a decreased environmental impact due to less generated waste.

Disclaimer

The results and conclusions presented in this application note 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 compared with Cytodex 1, Cytodex 1 Gamma microcarriers offer:

- Lower CAPEX due to the less equipment and facility support (CIP, steam, etc.) required.
- Lower total facility footprint.
- Lower cost for qualification as well as minimized need for annual maintenance.
- Time-savings due to the lower number of unit operations required during preparation.
- Reduced number of required FTEs due to the less unit operations required.
- Reduced manual handling, which increases the probability of maintaining the sterility of the bioreactors.
- No required swelling in PBS in an external vessel (stainless steel tank), saving costs of both raw materials and hardware.
- Less need for process liquids (PBS and cell culture medium).
- Lower total cost of consumables.
- Less generated waste, reducing cost as well as environmental impact.

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