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Pitched-Blade vs. Spin Filter vs. Packed-bed Basket: CHO Cell Culture Comparison

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Abstract

In the following application note, the pitched-blade impeller, the spin filter impeller and the packed-bed basket impeller are discussed, highlighting the uses and advantages for each type. Then examples of actual CHO cell cultures are given for each impeller type; showing the perfusion capability when using the spin filter or packed-bed basket impeller and the resulting higher cell densities over the pitched-blade impeller.

Introduction

In the world of bioprocess, there are many tools and methods that can be used to culture mammalian cells, each with their own strengths, weaknesses and purposes. One of the most critical decisions that is made before a bioprocess system purchase is which impeller type is ideal for a particular cell culture. In this application note, three impeller types were compared using CHO cell culture: The pitched-blade impeller, the spin filter with marine-blade impeller and the packed-bed basket impeller. All experiments were performed using a New Brunswick™ CelliGen® 310 benchtop bioreactor.

The pitched-blade impeller has three flat blades set at approximately a 45 ° angle which produces both axial and radial flow. Right handed or left handed blades are options that can be considered depending on which direction you would like your axial flow. Pitched-blade impellers are low shear impellers, designed to gently mix both suspension cells and cells attached to a microcarrier. Typically, these impellers are used for mammalian, insect or other shear-sensitive cell lines, but have also been used in highly viscous fermentation cultures with bacteria and fungi, as well as some biofuel processes. When using a pitched-blade impeller, a culture is typically grown in a batch-style run (no media is added or removed) or fed-batch-style run (a culture is started at a lower working volume and more media is added later during the run). A perfusion-style run (fresh media is continuously added and old media is removed) is possible, however, unless a filtering device is attached with this system to prevent the cells from being removed, cells will be depleted with the harvested ("waste") media.

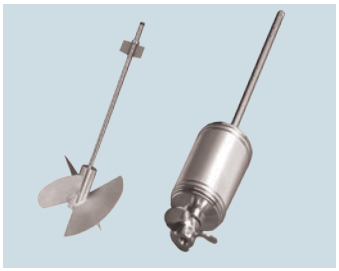


Figure 1: Pitched-blade impeller (left) and spin filter with marine blade (right)

A spin filter is a cylinder-shaped cage that spins with the impeller shaft and is covered with a screen designed to prevent cells from being collected with the waste media. Typically, underneath the spin filter, a marine blade is attached to the impeller shaft. When attached to the vessel, media is added so it covers the spin filter almost to its top, with a specially designed harvest tube that can reach the media inside the spin filter. When used, this device can keep cells in the vessel while old media is perfused out from inside of the spin filter. The spin filter is offered with two screen sizes, 10 µm openings for suspension cultures and 75 µm openings for microcarrier cultures. The marine-blade impeller attached underneath the spin filter provides gentle mixing but, due to its unidirectional flow, has a lower KLa than the pitched-blade. The spin filter is perfect for cultures that secrete proteins or compounds of interest since the desired product can be collected with the media while the cells are left to continue to produce. This also helps with downstream processing as cells will not have to be removed

with centrifugation or filtration. It should be noted that at very high density cultures the spin filter may eventually get clogged with cell debris and require cleaning, which can limit run time.

Figure 2: Packed-bed basket with Fibra-Cel disks

The packed-bed basket impeller, combined with Fibra-Cel® disks, is a system perfect for manufacturing high-yield secreted products from both attachment and suspension cultures with perfusion. Fibra-Cel is a solid supported fiber-mesh matrix microcarrier used predominantly for secreted products with perfusion. Fibra-Cel allows for long-term, high-density cultures without the risk of clogging. Fibra-Cel can be used for both anchorage-dependent cultures and suspension cultures due to its electrostatically-treated material and woven nature that traps the cells in a single step within 15 - 60 minutes (no need to stop agitation). The basket consists of two horizontally positioned, perforated metal screens that isolate a section in the interior of the vessel that is filled with Fibra-Cel. The impeller consists of a hollow tube (draft tube) with three smaller discharge tubes radiating from the top. When media is filled over the three tubes at the top of the impeller and it is spun, the centrifugal force exerted on the media forces out the liquid, causing a gentle suction at the bottom of the impeller, which brings media from the bottom of the vessel to the top. The media then gently flows through the Fibra-Cel packed-bed from the top to the bottom. Gases are sparged into the vessel through the central draft tube; this method oxygenates the media but prevents bubbles from interacting with the cells growing inside the Fibra-Cel packed-bed, thus, preventing bubble shear.

Eppendorf also offers other impellers for various bioprocess needs. Some impellers offered but not explored in this application note include the Rushton-type impellers; which are ideal for fermentation cultures with bacteria, yeast and fungi that require higher dissolved oxygen level (oxygen transfer rate) but are not sensitive to mechanical shearing damage; and the Cell-Lift impeller; which is an ultra-low-shear impeller that provides uniform circulation for microcarrier cultures and a bubble free environment for the cells.

Materials and methods

Table 1: Materials, media and cells

Material	Supplier	Catalog no.
CelliGen® 310 Control Station	Eppendorf	See ordering information, page 6
4 TMFC (0 - 1 SLPM)	Eppendorf	
2.5 L water jacketed vessel (with motor)	Eppendorf	
2.5 L pH/DO Sensor Kit (with cables)	Eppendorf	
2.5 L Pitched-Blade Impeller Kit	Eppendorf	
2.5 L Spin Filter Impeller Kit (10 µm)	Eppendorf	
2.5 L Basket Impeller Kit	Eppendorf	
YSI 2700 Select™ analyzer	YSI® Life Science	2700D
Vi-CELL® XR	Beckman Coulter®	731050
Media and cells		
Fibra-Cel® Disks	Eppendorf	M1292-9988
Freestyle® CHO-S	Life Technologies®	R800-70
CD CHO media	Gibco®	10743
L-glutamine	JRH Biosciences®	90114
Penicillin/streptomycin 100x	Gibco®	15140-122
D-(+)-Glucose	Sigma-Aldrich®	G5146
Sodium Bicarbonate	Thermo Fisher Scientific® Chemical	S631-3

Bioreactor conditions

Figure 3: CelliGen 310 with packed-bed basket impeller

During all three of the following CHO bioprocess examples, a CelliGen 310 Bioreactor with four 0-1 Standard Liters Per Minute (SLPM) Thermal Mass Flow Controllers (TMFC) were used. A TMFC is a device that monitors specific gas flow and is used by the cabinet to automatically control the gases flowing into the vessel. The vessel was a 2.5 L glass, water-jacketed vessel with a magnetic drive motor. The water jacket provides uniform temperature distribution with gentle heating and cooling for the culture while the magnetic drive motor provides a sterile vessel environment. All three culture types utilized 3 gas mixing (Air, O_2 and CO_2) for DO and pH control with a base addition (Pump 2, 0.3 M sodium bicarbonate solution). Table 2 shows all of the settings for each loop used during all three runs. Both the DO and pH were controlled using the cascade parameters seen in Tables 3 and 4.

Table 2: Loop settings

Loop	Setpoint
Agitation	See each example
Temperature	37 °C
pH-1	7.20 (Deadband 0.05)
pH-2	Off
DO-1	50
DO-2	Off
Air	Auto
O_2	Auto
Gs3Flo	Off
CO_2	Auto

Table 3: DO-1 cascade

	Start setpoint	@ DO start output %	End setpoint	@ DO end output %
Air	0.0	0.0	0.5	60
O_2	0.0	10	1	100

Table 4: pH-1 cascade

	Start setpoint	@ pH start output %	End setpoint	@ pH end output %
Pump 2	0.0	0.0	100	100
CO_2	0.0	0.0	0.3	-50

Cells were grown in CD CHO media supplemented with 8 mM of L-glutamine and 1 % penicillin/streptomycin and kept at a total working volume of ~1.6 L. Each vessel was inoculated at identical densities of 0.3 x 10^6 cells/mL. Glucose was added to the perfusion media as needed. Cell counts were performed on the pitched-blade and spin filter reactors using a Vi-CELL®. A YSI® 2700 Biochemical Analyzer was used to determine glucose and lactate concentrations for all three reactors.

Results

Pitched-blade culture

The pitched-blade reactor was run at an agitation speed of 80 rpm. It was cultured as a batch-style reactor so no media was added or removed throughout the process run. As you can see from Figure 4, viable cell concentration continued to rise until all of the glucose was consumed from the media at which point the cell viability began to drop. Lactate levels increased until the drop in glucose concentrations caused a shift in cellular metabolism which caused the cells to consume lactate.

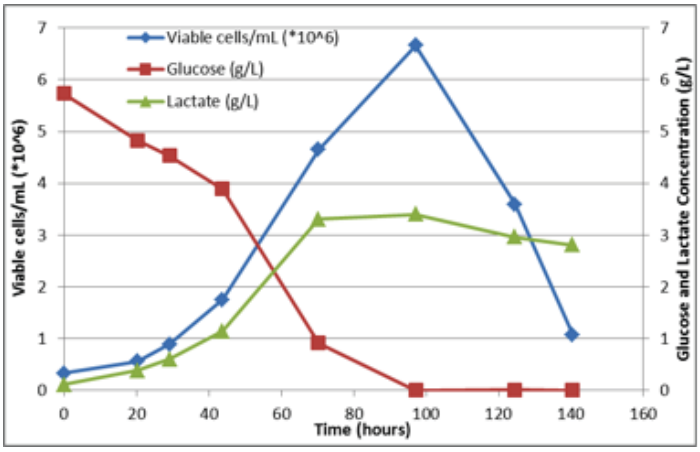


Figure 4: The pitched-blade viable cell concentration and glucose and lactate concentrations. Viable cell concentration begins to decrease when all the glucose is consumed in the vessel due to it being a batch-style run.

Spin filter culture

The spin filter reactor was run at an agitation speed of 100 rpm with a 10 µm filter screen. With the spin filter, the culture was run using continuous perfusion. One of the CelliGen 310 cabinet pumps was calibrated and run at varying rates of input as needed to maintain a glucose level above 1 g/L and to keep waste metabolites low. Another pump was cascaded to a level sensor so media was automatically removed from the vessel anytime it reached a volume over 1.6 L. Since the media being removed was from inside the spin filter, the cells were retained outside of the 10 µm spin filter cage. Figure 5 shows that the cells achieved a high density and viability with perfusion using the spin filter. Although the spin filter can achieve 3X, the run ended due to the high cell concentration eventually clogging the spin filter.

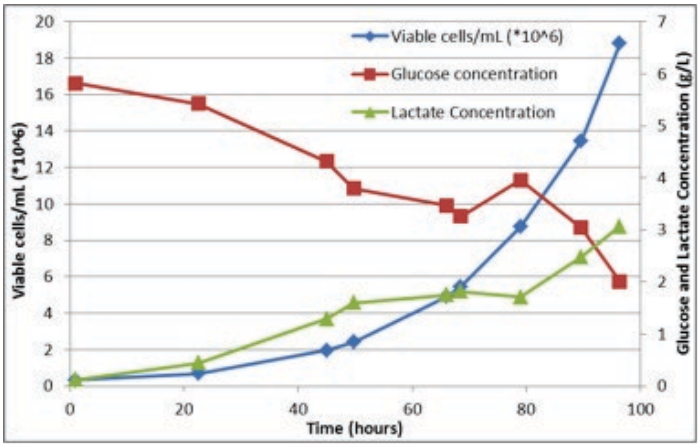
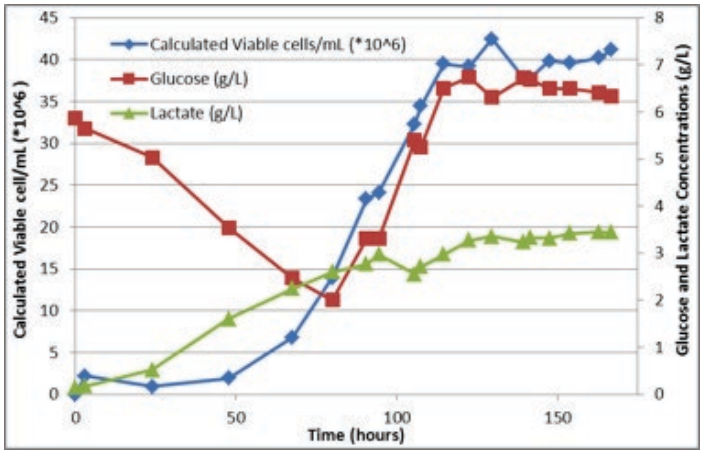


Figure 5: The spin filter viable cell concentration and glucose and lactate concentrations. Perfusion prevented glucose from being totally consumed from the vessel and lactate levels from getting too high.

Packed-bed basket culture

The packed-bed basket impeller was run at an agitation speed of 100 rpm and the basket was filled with 70 g of Fibra-Cel disks. This culture, like the spin filter, was run using continuous perfusion using the same methods as described above, except that media was removed from a normal harvest tube, not from inside of the basket. Since all of the cells were trapped in the Fibra-Cel disks and could not be counted using standard methods, the cell number was determined using the amount of glucose consumption. Due to glucose levels being too high during the run, the cells transitioned from a log phase to stationary phase resulting in a plateau in cell growth, as seen in Figure 6. Higher cell numbers were expected.

Figure 6: Packed-Bed Basket results showing calculated viable cells as well as glucose and lactate concentrations. Perfusion prevented



glucose from being totally consumed from the vessel and lactate levels from getting too high.

Discussion

Each impeller and cell culture method results in a different growth pattern and it is necessary to determine what is best for the desired process. When comparing the viable cell growth curves for each of the impellers (Figure 7), it can be seen that each results in a different cell concentration and rate of growth. More importantly, as discussed earlier, some of the impellers/methods allow for perfusion (Packed-bed Basket and Spin filter) resulting in higher and possibly continually sustainable cultures.

The pitched-blade impeller provided a simple way to grow a low-density culture, but it is not possible to grow the culture to a higher density without extra cell separation

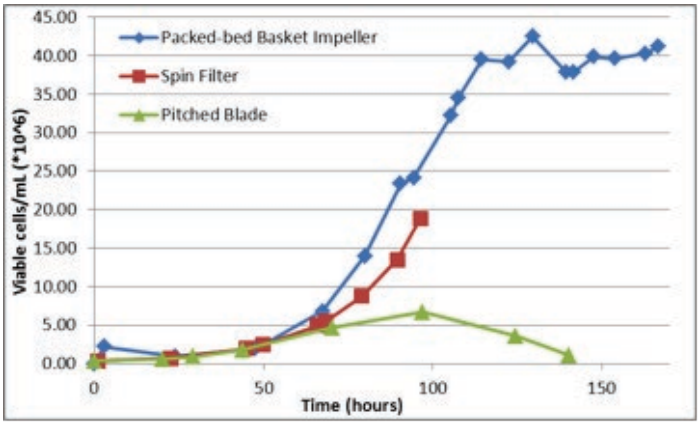


Figure 7: A comparison of viable CHO cell concentration for all three impeller experiments. The packed-bed basket impeller provided long term, high-density cell growth. The spin filter also provided high density cell growth compared to the pitched-blade impeller. Since the pitched-blade impeller was run as a batch-style reactor, a lower viable cell density was reached which eventually drops due to all the glucose being consumed in the vessel.

equipment to allow for perfusion. The spin filter resulted in almost 4X the number of cells as the pitched-blade impeller due to its ability to run in perfusion mode. The perfusion process usually does not last as long as the Fibra-Cel basket due to the tendency of clogging at very high cell densities. However, the cost of the spin filter is much less than that of the Fibra-Cel basket. It is reusable and it does not rely on consumable Fibra-Cel disks. The packed-bed basket impeller resulted in 8X the number of cells as the pitched-blade impeller and over 2X the spin filter. The packed-bed impeller culture also grew faster than the spin filter culture which was most likely due to the lack of direct physical agitation and bubble shear on the cells while they are trapped in the Fibra-Cel disks. Table 5 shows a general list of the advantages for each impeller type. Every cell line is different and what will work best for each culture and purpose can vary. Table 6 is a general guide for choosing impellers based on some common cell lines. The CHO cell cultures in this paper were not optimized and are just a general example of what can be expected for each impeller type.

Table 5: Advantages for each impeller type

Impeller	Advantages
Pitched-Blade Impeller	> Axial and Radial flow > Simple design > Suspension or Microcarrier attached cultures
Spin Filter Impeller	> Easy to use with perfusion > Capable of higher cell densities
Basket Impeller	> Higher cell densities without the risk of clogging > Gentler environment for cells

Table 6: A general guide to choosing impellers by cell line

Cell line	Rushton, Rushton-Like	Pitched-Blade	Marine Blade	Spin Filter	Cell Lift	Basket
Human						
HEK 293		■	■	■	■	■
HeLa		■	■	■		■
HL60		■	■	■		■
Lncap		■	■	■		■
THP-1		■	■	■		■
UMSCC		■	■	■	■	■
HFF		■	■	■	■	■
KB		■	■	■	■	■
MRC-5		■	■	■	■	■
Hybridoma						
DA4.4		■	■	■		■
123A		■	■	■		■
127A		■	■	■		■
GAMMA		■	■	■		■
67-9-B		■	■	■		■
SP20		■	■	■		■
Primate						
Vero		■	■	■	■	■
COS-7		■	■	■	■	■
Rat Tumor						
GH3		■	■	■		■
9L		■	■	■		■
PC12		■	■	■		■
Mouse						
3T3		■	■	■		■
MC3T3		■	■	■		■
NS0		■	■	■	■	■
Hamster						
CHO		■	■	■	■	■
BHK		■	■	■	■	■
Zebrafish						
ZF4		■	■	■	■	
AB9		■	■	■	■	
Insect						
SF9		■		■		■
Hi-5		■		■		■
Sf21		■		■		
Bacteria						
Streptomyces	■	■				
Bacillus	■					
Escherichia coli	■					
Yeast						
Saccharomyces cereviseae	■					
Baker's yeast	■					
Pichia pastoris	■					
Candida albicans	■	■				
Algae						
Red/Green		■	■			

References

1. Mirro, R, and K. Voll. 2009. *Which Impeller Is Right for Your Cell Line?*. BioProcess Int. 7:52-57.

Ordering Information

Product	Description	International order no.	N. America order no.
Voltage Option	Cabinet voltage	M1287-1020 (200V)	M1287-1010 (120V)
CelliGen® 310 Control Station	Cell culture control cabinet	M1287-2110	M1287-2110
4 TMFC (0 - 1 SLPM)	Gas flow control	M1287-2020	M1287-2020
2.5 L water jacketed vessel (with motor)	Cell culture vessel	M1287-0310	M1287-0310
2.5 L pH/DO Sensor Kit (with cables)	pH and Dissolved oxygen sensors	M1287-0400	M1287-0400
2.5 L Pitched-Blade Impeller Kit	Pitched-blade impeller	M1287-5068	M1287-5068
2.5 L Spin Filter Impeller Kit (10 µm)	Spin Filter Impeller	M1287-1125	M1287-1125
2.5 L Basket Impeller Kit	Basket impeller	M1287-1140	M1287-1140
Fibra-Cel® Disks	Microcarrier	M1292-9988	M1292-9988

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