Tutorial Title: **Compare Single-Use and glass/steel fermenter**
Subtitle: **CerCell present the BactoVessel configurable Single-Use-Fermenter**
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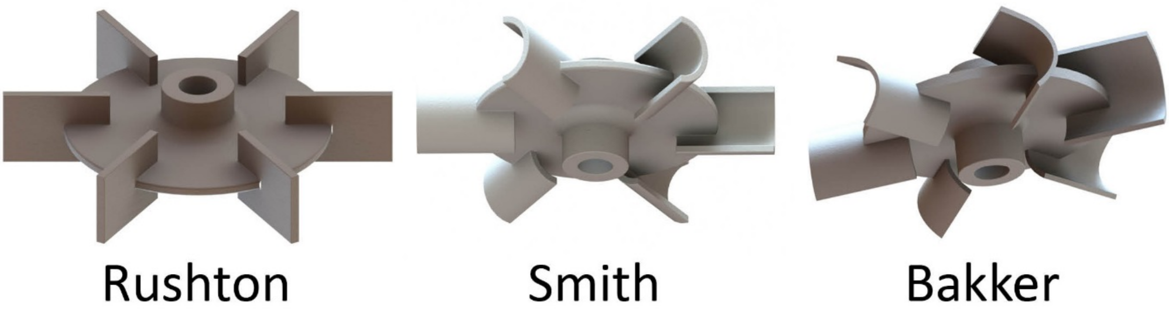
The decision to use single-use systems for manufacturing pharmaceuticals depend on many factors, but the major driving force is a desire to increase throughput. Single-Use-Fermenter´s (SUF) eliminate cross contamination, reduce water consumption, and eliminate time consuming sterilization of conventional glass/steel fermenter (STR).

One glass/steel STR and one BactoVessel SUF both liquid cooled and connected to the twin channel Biostat PCS with liquid thermal control. SUF installed in a Re-Usable-Jacket for efficient heat transfer. Two independent servo motor drives in order to reach the 2000 RPM target. Photo by Per Stobbe.

This tutorial compare process parameters between conventional STR and CerCell latest generation SUF and examine the growth data for a wild-type *E. coli* bacterium. The transfer coefficient KLa value for different sizes bioreactor with 3 different turbine design and 4 different agitation speed. Two high speed optical sensors, Presens OpTrode and Hamilton VisiFerm were used. KLa values are compared relative to the different sizes of bioreactors, turbines, stirring speed and the sensors. Finally, the KLa values of conventional STR are compared with KLa values for SUF.



Fermenting process

Fermentation is commonly used in production of many foods, beverages and pharmaceuticals. Fermentation processes include chemical reactions such as oxidations, reductions, hydrolysis and bio-synthesis. Some processes may require the presence of air (aerobic), other´s without air (anaerobic). The rate of the fermentation depends on the concentration of micro-organisms, cells, cellular components, and enzymes, as well as temperature, pH and oxygen level. For the tests performed the STR and SUF temperature was kept constant as controlled by Biostat PCS at 27.5 °C. The 3 litre BactoVessel SUF is ID 124 mm and the 5.7 litre SUF ID is 144 mm.

KLa

The volumetric oxygen mass transfer coefficient, KLa is the most important parameter in the biochemical art and is defined as the reciprocal of time for the transfer of oxygen from gas to liquid phase. KLa describes the efficiency with which oxygen can be supplied to a fermentation for a given set of operating conditions.

Dissolved Oxygen (DO) is often the limiting substrate in the fermentation. For bacteria and yeast cultures oxygen concentration is usually 10% - 50% of air saturation. For optimum growth, it is important to maintain the DO level above this critical value by aeration (sparging) the bio processes with air or pure oxygen. Oxygen mass transfer rate to the liquid fermentation liquid should be equal to or exceed the rate at which growing cells take up oxygen.

In aerobic fermentation oxygen molecules pass several mass transfer steps engaged in the transport of oxygen from the interior of the gas bubbles before facilitating the intra-cellular reactions.

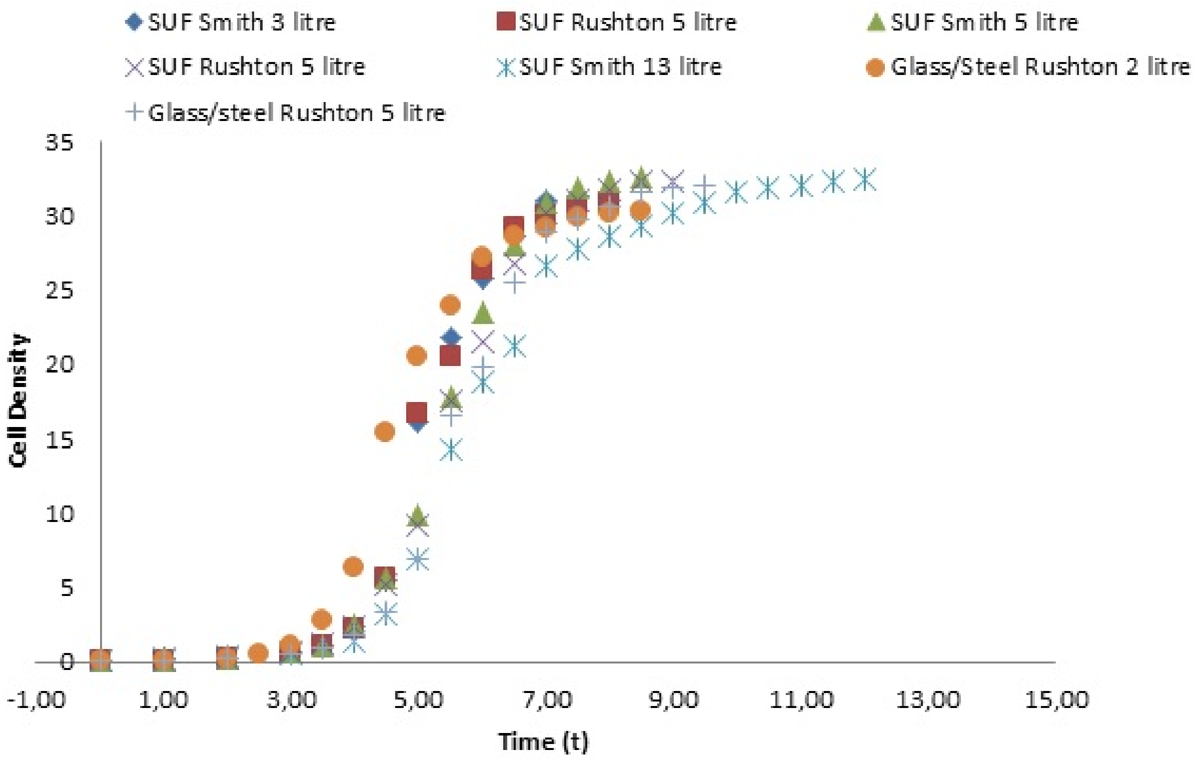
Results

Table 1	SUF	SUF	SUF	SUF	SUF	Glass/steel	Glass/steel
Turbine	Smith	Rushton	Smith	Rushton	Smith	Rushton	Rushton
# turbines	2	2	3	3	4	2	3
Turbine (mm)	60	60	70	70	70	50	60
VV (litre)	3	3	5.7	5.7	13	2	5
WV (litre)	2	2	4	4	12	1.5	4
Aeration (VVM)	1	1	1	1	1	1	1
Aeration (l/min)	2	2	4	4	12	1.5	4

Table 1 explain an overview of the parameters used for the 7 fermentation experiments. Fermenter type and their size, turbine type and diameter at 1,000 RPM, ring-sparger/aeration volume, stirring speed and aeration.

Table 2 - Turbine	Smith	Rushton	Smith	Rushton	Smith	Rushton	Rushton
Fermenter VV, litre	3	3	5.7	5.7	13	2	5
Number of hours	7.5	8	8.5	9	12	8.5	9.5
Cell Density, CD_{max}	31.35	30.85	32.55	32.3	32.4	30.35	32.05
μ_{max}(t^-1)	0.379	0.384	0.367	0.368	0.395	0.370	0.384
DW_{max} (g/l)	0.0273	0.0274	0.0289	0.0268	0.0281	0.0262	0.0283

Table 2 show the 7 different fermentation results of table 1. Number of hours each run and values for CD_{max}, μ_{max} and DW_{max} for each fermentation.



Graph 1 show cell density measurements of 7 different SUF/STR relative to agitation speed of 1,000 rpm and time in hours according to table 2.

Table 3 – sensor comparison	KLa values (t^{-1})	KLa values (t^{-1})
Fast acting DO sensor type (sec)	Presens Optrode - 6	Hamilton VisiFerm - 30
Rushton 5.7 VV litre 1,000 rpm	142	141
Rushton 5.7 VV litre 1,500 rpm	173	169
Rushton 2 VV litre 1,000 rpm	83	79
Rushton 2 VV litre 1,500 rpm	110	98

Table 3 explain obtained KLa precision comparing results from the two in parallel operating fast acting DO sensors. DO sensor response time is 6 sec. for the Optrode and 30 sec for VisiFerm.

Table 4 – servo motor power consumption in Watt							Glass/steel STR	
Turbine	**Smith**	**Rushton**	**Smith**	**Rushton**	**Bakker**	**Smith**	**Rushton**	**Rushton**
VV litre	**3**	**3**	**5.7**	**5.7**	**5.7**	**13**	**2**	**5**
0 RPM	19.7	19.6	19.8	19.5	19.6	19.8	20	20.1
500 RPM	30.2	32.6	36.8	37.6	40.2	66.2	37.1	51.2
1,000 RPM	39.5	38.6	55.9	56.4	126.7	167.9	54.5	82.4
1,500 RPM	41.8	42.1	111.7	113.4	308.1		79.1	137.5
2,000 RPM	43.8	44.6	203.6	204.5			93.4	225.1

Table 4 show power consumption in Watt relative to fermenter VV size, RPM and turbine design.

Table 5 - BactoVessel 5.7 litre SUF	Presens	Hamilton
Dual turbine setup and RPM	**KLa værdier (t^{-1})**	**KLa værdier (t^{-1})**
Rushton 1,000 rpm	144	142
Rushton 1,500 rpm	182	180
Rushton 2,000 rpm	176	175
Smith 1,000 rpm	146	144

Smith 1,500 rpm	187	186
Smith 2,000 rpm	182	180
Bakker 1,000 rpm	184	182
Bakker 1,500 rpm	212	210

Table 5 show the interesting KLa values of the test though not describing the gradients created.

Discussion

The KLa value increases with increasing agitation speed. The best KLa value is obtained in the 5.7 litre SUF with Bakker turbine at 1,500 rpm and 212 h^{-1}. At very high agitation speed (2,000 rpm) the KLa value drops of.

Growth data showed no significant difference for conventional STR and BactoVessel SUF. SUF can deliver the same results as conventional STR fermenters. Data for the growth of bacteria E. coli showed an OD max about 30, a specific growth rate of 0.3 h^{-1} and a maximum of about 0.028 DW g / L for all bioreactor types.

KLa values for a 5 litre STR with Rushton turbine measured to 142 h^{-1} at 1,000 rpm and the KLa value of 5.7 litre SUF with Rushton turbine is 144 h^{-1}.

Photo 1 illustrate visual inspection the mixing gradients, differences. Flat blade Rushton left being the worst, Smith middle significant better and Bakker right even slightly better. Photo by André Bakker.

Conclusion

Tray turbines has good KLa values, Bakker at 212 h^{-1}, Smith turbine at 187 h^{-1}, and Rushton at 182 t^{-1}. Bakker tray turbine has highest gas collection level relative to Smith tray and Rushton flat blade. Further the oxygen mass transfer rate seems to be linked to power take up / RPM – more power gives higher KLa. Visual inspection of mixing gradients show flat blade Rushton being the worst in gradient free mixing and Smith significant better and Bakker slitghly better.

Abbreviations:

G/S = Glass/steel fermenter = STR = Stirred-Tank-Reactor
SUF= Single-Use-Fermenter = BactoVessel® from www.cercell.com
VV = Vessel Volume in litre
WV = Working Volume (VV minus WV = head space)
CD = Cell Density, mio cell/ml measured with capacitance system
μ_{max} (t^-1) = exponential regression = specific growth speed ($x = x_0 e^{\mu t}$)
DW_{max} (g/l) = dead weight, dry stuff

Full Application Note available as a PDF file in Danish on http://cercell.com/products/bactovessel-configurable-single-use-fermenter/application-notes/
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