Evaluation of the BioFlo® 320 Process Capabilities

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Abstract

This poster characterizes the capabilities of the BioFlo 320 in terms of key process parameters including oxygen transfer rate (OTR), volumetric mass transfer coefficient (k_La), tip speed, mixing time, and water consumption. These data enable quantitative analyses of bioprocess engineering parameters, thus allowing potential users to gain insight into the system's cell culture and fermentation capabilities.

Introduction

Historically, stirred-tank fermentors and bioreactors have been the trusted design for cultivating all types of submerged cultures including suspension and anchorage-dependent mammalian, insect, yeast, plant, and microbial cell cultures. The tried-and-true design offers scalability and proven reproducibility which is pivotal for cost-saving process development and productivity. However, the glass or stainless steel autoclavable systems and the single-use equipment have usually remained separate.

In order to meet the demand of improved process flexibility and cost savings, Eppendorf recently developed a new bioprocess controller, the BioFlo 320, seen in Figure 1. It combines the capabilities of autoclavable and single-use systems into one advanced bioprocess control station.

Figure 1: The BioFlo 320 benchtop system with water-jacketed (left) and stainless steel dish-bottom (right) vessels

Design Specifications

As the newest offering from the Eppendorf bioprocess portfolio, the BioFlo 320 blends design and utility into one state of the art package. An industrial design, autoclavable and single-use vessels, intelligent sensors, Ethernet connectivity, and an enhanced software package are only a few of the features that set it apart from the competition. The BioFlo 320 is also available with various types of impellers including the packed-bed and cell lift impeller designs exclusively from Eppendorf.

Figure 2 illustrates the dimensions of the BioFlo 320 stainless steel dish-bottom vessel. Table 1 provides dimension specifications for both stainless steel dish-bottom and water-jacketed vessels. Table 2 provides dimension specifications for the Rushton (6-blade) and pitched blade impellers (3-blade).

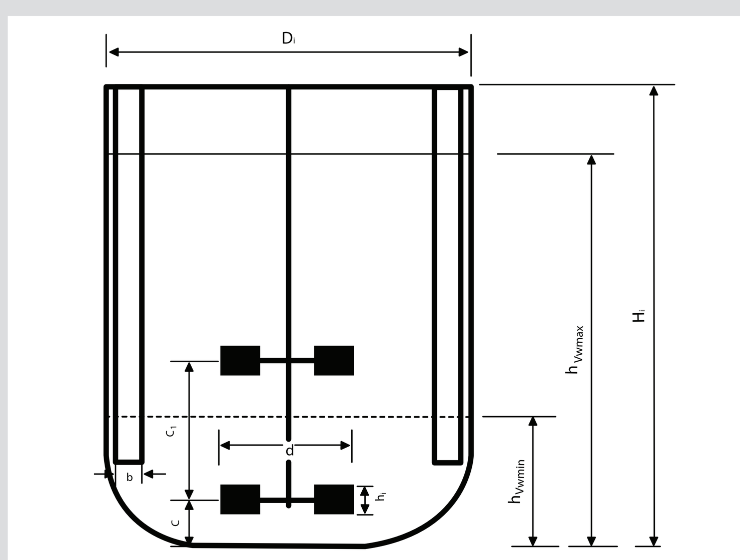

Figure 2: Dimensional drawing of the BioFlo 320 stainless steel dish-bottom vessel

Vessel type		Stainless steel dish-bottom (single wall) or water-jacketed (double wall)			
Vessel size		**1 L**	**3 L**	**5 L**	**10 L**
Total volume (V_{total})	L	2.5	5.0	7.5	14.0
Max working volume (V_{max})	L	1.9	3.8	5.6	10.5
Min working volume (V_{min})	L	0.6	1.3	1.9	3.5
Vessel height (V_{total})	cm	23.9	29.5	32.6	42.0
	in	9.4	11.6	12.8	16.5
Vessel height (V_{max})	cm	17.4	22.5	24.9	32.3
	in	6.9	8.9	9.8	12.7
Vessel inner diameter (ID)	cm	12.0	15.0	17.6	21.1
	in	4.7	5.9	6.9	8.3
Height (V_{total}) : Vessel ID	[-]	2.0	2.0	1.9	2.0
Height (V_{max}) : Vessel ID	[-]	1.5	1.5	1.4	1.5

Table 1: BioFlo 320 vessel dimensions

Impeller type		Rushton (6-blade)			
Vessel size		**1 L**	**3 L**	**5 L**	**10 L**
Material	[-]	316L	316L	316L	316L
Impeller quantity	[-]	2	2	2	2
Impeller outer diameter (OD)	cm	4.80	6.00	7.04	8.44
	in	1.89	2.36	2.77	3.32
Impeller height	cm	1.20	1.50	1.76	2.11
	in	0.47	0.59	0.69	0.83
Impeller spacing	[-]	1 x OD	1 x OD	1 x OD	1 x OD
Vessel (ID) : Impeller (OD)	[-]	2.50	2.50	2.50	2.50

Impeller type		Pitched blade (3-blade, 45°)			
Vessel size		**1 L**	**3 L**	**5 L**	**10 L**
Material	[-]	316L	316L	316L	316L
Impeller quantity	[-]	1	1	1	1
Impeller outer diameter (OD)	cm	6.05	7.62	8.89	10.49
	in	2.38	3.00	3.50	4.13
Impeller height	cm	4.01	5.23	6.28	7.19
	in	1.58	2.06	2.47	2.83
Vessel (ID) : Impeller (OD)	[-]	1.98	1.97	1.98	2.01

Table 2: BioFlo 320 vessel and impeller dimensions

Mixing Time

Mixing Time
Mixing time is one of the criteria used to describe the quality and mixing efficiency of a vessel [1]. Proper mixing improves oxygen transfer, nutrient delivery, as well as pH and temperature homogeneity, thus providing an optimized culture environment throughout the entire vessel. Mixing time studies were conducted using the pH disturbance and recovery method as previously described [2] and results can be found in Table 3. To maintain scalability between various vessel sizes, all mixing times were obtained at the same tip speed of 0.6 m/s.

Tip speed (m/s)	1 L vessel mixing time (sec)	3 L vessel mixing time (sec)	5 L vessel mixing time (sec)	10 L vessel mixing time (sec)
0.6	8.33	11.66	15	20

Table 3: Mixing time of BioFlo 320 water-jacketed vessels equipped with a single pitched blade impeller

Oxygen Transfer – Stainless Steel Dish-Bottom Vessels

The OTR is the rate at which oxygen is transferred from air to liquid in a vessel. OTR is of critical importance for the selection, design, and scale-up of bioprocess systems [3]. Since oxygen is often the limiting factor during aerobic fermentation, the OTR is commonly used as a reference for a vessel's fermentation capabilities. Therefore, the OTR is most often obtained using the sodium sulfite chemical method under maximum agitation speed, maximum vessel working volume, and high air flows, such as 1 Vessel Volume per Minute (VVM). Figure 3 illustrates the OTR obtained under these conditions for all four fermentation vessel sizes. However, in reality, high density fermentation may demand supplementation of additional oxygen and much higher total gas flow than 1 VVM. The superior high flow TMFC of the BioFlo 320 is capable of delivering precision air flow of up to 20 Standard Liters per Minute (SLPM), allowing high OTRs to be achieved without any additional oxygen. All experiments were conducted in stainless steel dish-bottom vessels equipped with dual Rushton impellers and baffle assemblies.

In addition to the OTR, oxygen transfer can also be represented in the form of k_La, the volumetric mass transfer coefficient. The k_La is important to establish aeration efficiency and to quantify the effects of the operating variables on the provision of dissolved oxygen [3]. Figure 4 shows the k_La values of BioFlo 320 fermentation vessels under the same conditions used to determine the OTR. The k_La was calculated using the following equation as described by Truesdale, Downing and Lowden [4]:

$$OTR = k_L a . \Delta c (\Delta c = 1.13)$$

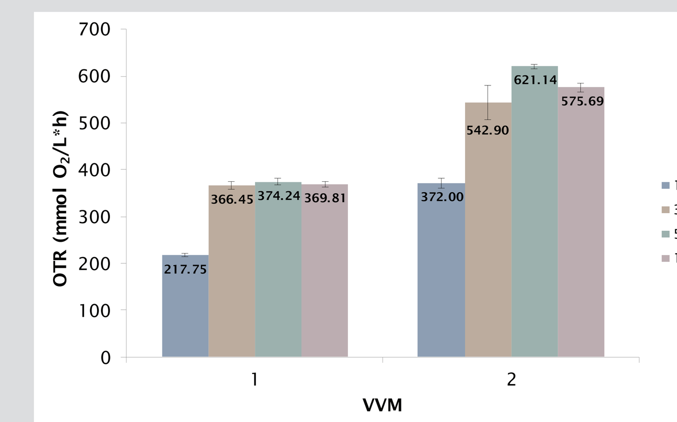

Figure 3: BioFlo 320 fermentation vessel OTR values

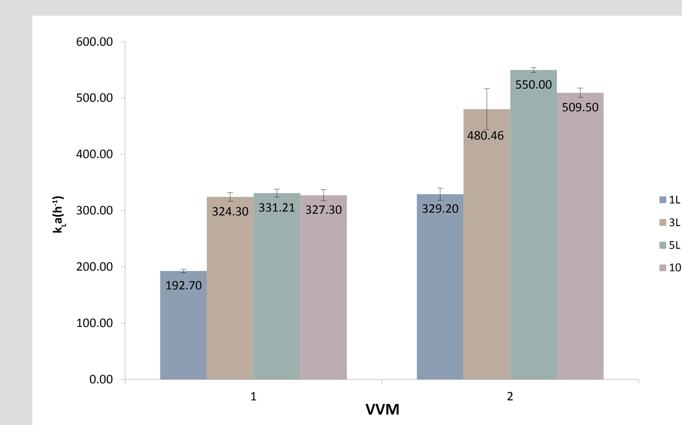

Figure 4: BioFlo 320 fermentation vessel k_La values

Oxygen Transfer – Water-Jacketed Vessels

Typical cell culture conditions do not allow high agitation and thus, cannot fully utilize the full OTR potential of a vessel. Oxygen transfer under cell culture conditions are typically represented only via the k_La. BioFlo 320 cell culture vessel k_La values were experimentally determined under a fixed tip speed of 0.6 m/s and a full vessel working volume of DI water. The k_La was calculated first using aeration with a ring macrosparger and second with a microsparger, seen in Figure 5.

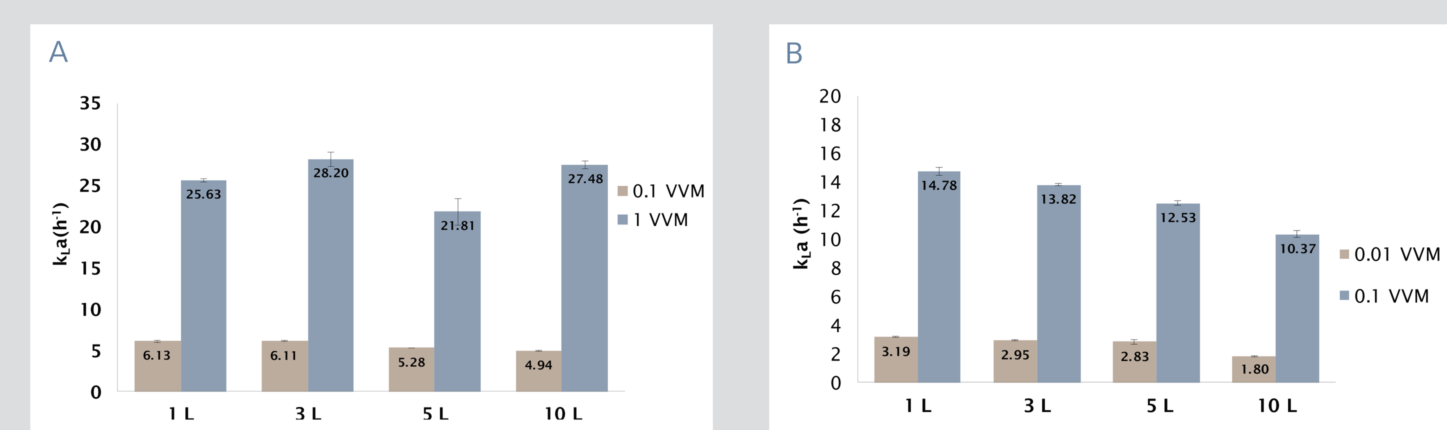

Figure 5:
A: The k_La values obtained using BioFlo 320 cell culture vessels with aeration by macrosparger (ring sparger)
B: The k_La values in the same vessels with a microsparger

Water Consumption Rate

Definition:
The amount of water used to maintain internal vessel temperature of 30 °C with 50 W/L heat input, and cooling liquid maintained at 5 °C. For this test, 1 L and 10 L stainless steel dish-bottom vessels were chosen as they are the most typical choice for exothermic fermentations requiring rapid temperature transfer for cooling.

Results:
1 L stainless steel dish-bottom vessel = 1 L/min
10 L stainless steel dish-bottom vessel = 1.5 L/min

Vessel Heating and Cooling Capacity

Cooling Capacity: Maintain internal vessel temperature of 37 °C with a controlled heat input (W/L) and cooling liquid maintained at 5 °C.
Max Temperature: Maximum achievable controlled temperature with maximum agitation rpm and no gas flow (0.0 VVM).
Vessel Heating Rate: Rate at which the vessel inner temperature increases at 100 % heat output between 30 °C and 60 °C under maximum agitation rpm and without gas flow.
Vessel Cooling Rate: Rate at which the vessel inner temperature decreases at 100 % cool output between 37 °C and 20 °C under maximum agitation rpm, without gas flow, and with cooling liquid maintained at 5 °C.

Vessel type		Stainless steel dish-bottom (single wall)			
Vessel size		**1 L**	**3 L**	**5 L**	**10 L**
Cooling capacity @ 37 °C	W/L	> 80	> 80	> 80	> 80
Max temperature	°C	90	90	90	85
Vessel heating rate	°C/min	3.45	2.02	1.53	0.97
Vessel cooling rate	°C/min	1.44	0.96	0.67	0.5

Vessel type		Water-jacketed (double wall)			
Vessel size		**1 L**	**3 L**	**5 L**	**10 L**
Cooling capacity @ 37 °C	W/L	> 80	> 80	> 80	> 80
Max temperature	°C	80	80	80	80
Vessel heating rate	°C/min	2.44	1.35	0.9	0.6
Vessel cooling rate	°C/min	1.51	0.72	0.6	0.49

Table 4: BioFlo 320 heating and cooling parameters

Conclusion

The BioFlo 320 characterization revealed excellent bioprocess engineering parameters, including:
> Proportional design and superior scalability between various vessel sizes
> High oxygen transfer under both fermentation and cell culture conditions
> Fast mixing time across all vessels sizes
> Ample heating and cooling capacity for bioprocess applications

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