

# Technology Transfer and Scale-Up of a High-Density *E. coli* Fermentation from a 200 L Stainless Steel to a 1000 L Single-Use Fermenter

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## Introduction

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While biopharmaceutical CDMOs (*Contract Development and Manufacturing Organizations*) have widely embraced single-use technologies for mammalian cell culture, their adoption for microbial fermentation applications has been slow. In contrast to single-use bioreactors that are available in volumes up to 6000 L, single-use fermenters are mostly restricted to volumes up to 300 L, with the ABEC single-use fermenter being the only one available up to 1000 L. The challenges associated with the development of production-scale single-use fermenters are related to the high demands of cooling capacity and mass transfer due to the higher oxygen consumption and metabolic heat production of microbial applications compared to mammalian cells. Not only does the single-use fermenter design need to be capable of achieving the required mass and heat transfer demands, but the single-use components also must be robust to cope with the mechanical stress of the higher agitation and gas flow rates.

ABEC's Custom Single Run (CSR®) single-use technology has overcome these challenges. Leveraging 50 years of fermenter design experience, ABEC engineered its CSR® bioreactors and fermenters to provide the same performance and scalability as stainless-steel systems. As a result, ABEC offers CSR® fermenters up to 1000 L whose designs are similar to conventional stainless-steel vessels. In addition, key design attributes such as agitators, spargers, and instrumentation can be customized for specific processes.

BIOVECTRA has extensive experience in microbial fermentation producing commercial quantities of drug substance for its clients within a GMP environment. As a CDMO, flexibility is a key consideration for BIOVECTRA when choosing new equipment for manufacturing, making single-use technologies more suitable than their stainless-steel counterparts for use in clinical scale manufacturing. This was demonstrated by a requirement to scale up a fermentation process established in a stainless-steel 200 L fermenter when BIOVECTRA selected ABEC's single-use 1000 L CSR® fermenter for achieving its need for a large-scale microbial process, combining the flexibility of single-use equipment with the high heat and mass transfer capabilities characteristic from ABEC's CSR® fermenters.

This paper demonstrates the successful scale-up of a high-cell density *Escherichia coli* process from an established 200 L stainless-steel fermenter to a 1000 L CSR® single-use fermenter. Typical fermenter characterization and scale-up techniques were used to ensure similar mass transfer and process control performance between the two systems. Key process control parameters were measured and compared, and similar *E. coli* growth was achieved seamlessly in the first microbial culture performed in the 1000 L CSR® fermenter.

## Methodology

### Scale-up Approach

Oxygen is a key substrate for microbial growth, maintenance, and other metabolic routes, including product biosynthesis. However, due to its low solubility, oxygen must be continuously fed into the system through aeration. The oxygen transfer rate (*OTR*, mmol/(L s)) from the gas to the liquid broth in a fermenter or bioreactor vessel is calculated by the product between the mass transfer coefficient of the liquid side, ( $k_L$ , m/s), the specific surface area for the mass transfer ( $a$ , m<sup>2</sup>/m<sup>3</sup>), and the gradient of oxygen concentration between the interface with the aeration gas ( $C^*$ , mmol/L) and the liquid broth ( $C$ , mmol/L):

$$(1) \quad OTR = k_L a (C^* - C)$$

Along with heat transfer (cooling), *OTR* stands as a limiting factor for high-cell density microbial cultures. Experimentally, it is not possible to determine individually the parameters  $k_L$  and  $a$ , thus they are lumped together as one single parameter called volumetric oxygen mass transfer coefficient ( $k_L a$ , s<sup>-1</sup>). The most widely used empirical equation to correlate  $k_L a$  to the operation parameters of a fermenter was proposed by Van't Riet (1979) and relates the  $k_L a$  to the mixing power input in the gassed broth ( $P$ , W), per volume of fluid ( $V$ , m<sup>3</sup>), and the superficial gas velocity ( $v_S$ , m/s):

$$(2) \quad k_L a = k \left( \frac{P}{V} \right)^\alpha v_S^\beta$$

For non-viscous systems, the exponents  $\alpha$  equal to 0.4 and  $\beta$  equal to 0.5 are the most frequently used to describe the  $k_L a$  behavior with  $P/V$  and  $v_S$  (Garcia-Ochoa and Gomez, 2009). The parameter  $k$  depends on the geometry of the vessel and culture temperature and is used as 0.55 in this work.

The mixing power input is directly correlated to the impeller diameter ( $D_i$ , m) and rotation ( $N$ , s<sup>-1</sup>) by:

$$(3) \quad P = K \cdot N_p N^3 D_i^5 \rho$$

Where  $N_p$  is the impeller power number,  $\rho$  is the fluid density (kg/m<sup>3</sup>) and  $K$  is the gassing factor, which depends on the impeller shape and Froude number ( $N_{Fr}$ ), and on the aeration (or gas flow) number ( $N_A$ ) (Paul et al, 2004):

$$(4) \quad N_A = \frac{Q}{ND_i^3} \quad (5) \quad N_{Fr} = \frac{ND^2}{g}$$

Where  $Q$  is the volumetric gas flow rate ( $Q$ , m<sup>3</sup>/s) and  $g$  is gravity (9.81 m/s<sup>2</sup>).

The superficial gas velocity is given by the ratio between the volumetric gas flow rate ( $Q$ , m<sup>3</sup>/s) and the cross-sectional area of the vessel:

$$(6) \quad v_S = \frac{4Q}{\pi D_T^2}$$

Where  $D_T$  is the vessel diameter (m).

Considering the importance of keeping similar oxygen transfer across different scales, a traditional scale-up approach maintaining constant gassed  $P/V$  to scale agitation rate ( $N$ ), and constant superficial gas velocity to scale the gas flow rate ( $Q$ ) was used in this study.

Using equations (3) and (6), the gas flow rate and the agitation rate were scaled up from the established process parameters in the 200 L stainless steel fermenter to the 1000 L CSR® fermenter. Current and scaled up process parameters are shown in Table 1.

The process was operated at approximately 7.5 psig of head pressure in the 200 L stainless steel fermenter; for scale up to the 1000 L CSR single-use fermenter, the head pressure was limited to approximately 0.3 psig. The decrease in head pressure results in a decrease in the driving force for the oxygen concentration gradient from equation (1); therefore an increase in the oxygen ratio supplied through the gas flow rate  $Q$  was required to maintain the necessary *OTR*.

	SSF 200 L	CSR® 1000 L
Working volume, $V$	190 L	800 L
Maximum agitation rate, $N$	7.1 s <sup>-1</sup> (423 rpm)	3.83 s <sup>-1</sup> (230 rpm)
Gas flow rate, $Q$	0.00472 m <sup>3</sup> /s (283 slpm)	0.010 m <sup>3</sup> /s (600 slpm)
Aeration number, $N_A$	0.057	0.050
Froude number, $N_{Fr}$	1.01	0.55
Power per volume, $P/V$	4.18 kW/m <sup>3</sup>	3.93 kW/m <sup>3</sup>
Superficial gas velocity, $v_S$	0.0165 m/s	0.0163 m/s
Head Pressure, $P$	7.5 psig	0.3 psig
Oxygen % in Gas flow rate	30%	50%
Volumetric oxygen mass transfer coefficient, $k_L a$	0.125 s <sup>-1</sup> (451 h <sup>-1</sup> )	0.124 s <sup>-1</sup> (447 h <sup>-1</sup> )

**Table 1:**  
Scale-up parameters from the 200-L stainless steel fermenter (SS 200 L) to the 1000-L Custom Single Run fermenter (CSR® 1000 L)

## Fed-batch cultivation in the 1000 L CSR® fermenter

The fed-batch high-cell density process was carried out in an ABEC CSR® fermenter that has a maximum working volume of 1000 L (Figure 1). The single-use fermenter was designed with three six-flat-blade impellers (Rushton) with a  $D_i:D_r$  ratio of 0.43. The fermenter controller has the capabilities of pH control by acid/base addition, and dissolved oxygen control by a cascade of agitation rate, gas flow rate and oxygen enrichment of the inlet gas. Extra ports for feed addition are also available. Feed can be added according to a pre-defined profile or changed manually throughout the process. Temperature is maintained at the target setpoint by flowing chiller fluid through the fermenter jacket.

A proprietary *E. coli* strain was grown in shake flasks that were used to inoculate the fermenter. The process started as batch culture with 632 L of culture medium. The feed (54% glycerol) started at 3 h fermentation time and was increased stepwise hourly until a final volume of 800 L. Temperature was controlled at 32°C through the use of chiller fluid (-8°C) in the fermenter jacket, and pH was controlled at  $7.0 \pm 0.1$  through the addition of ammonium hydroxide.

Dissolved oxygen was controlled at the setpoint of 13% of air saturation by first increasing the agitation rate to the maximum speed of 230 rpm ( $P/V \cong 4 \text{ kW/m}^3$ ), followed by the enrichment of the gas flow with pure oxygen. Gas flow rate was kept constant at 600 L/min ( $v_s = 0.0163 \text{ m/s}$ ). The dissolved oxygen setpoint was increased from 10% in the 200 L stainless steel fermenter to account for the reduction in the saturation solubility of oxygen in the broth due to the lower head pressure in the 1000 L CSR.

*OTR* was estimated during the culture through material balance between the oxygen concentration in the inlet and exhaust gas.



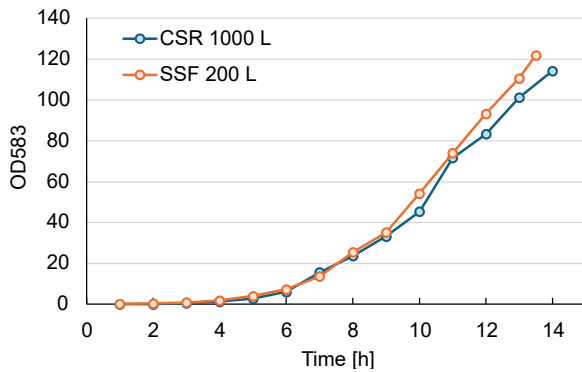
Figure 1: Single-use 1000 L fermenter holder and control unit.



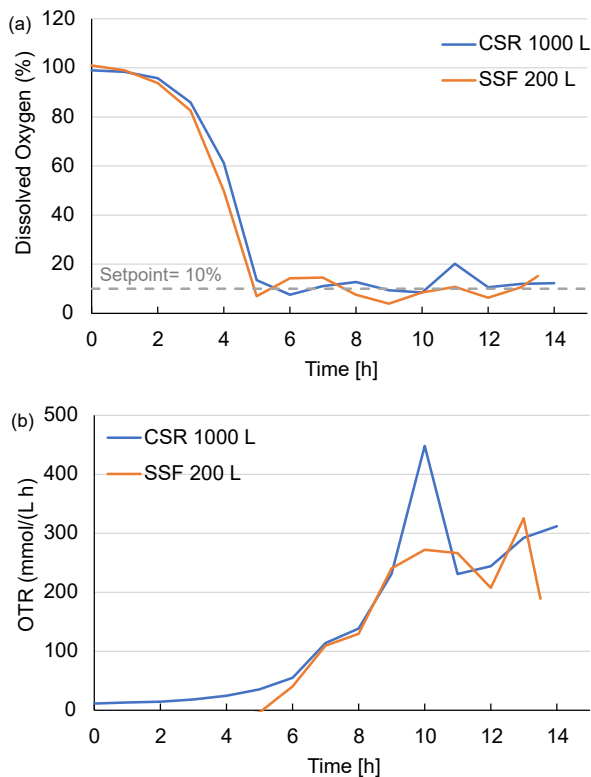
Figure 2: Single-use Rushton impeller for CSR® fermenters.

## Results

**Figure 3** compares the *E. coli* biomass yield in the 200 L stainless steel and the 1000 L CSR® fermenters measured as optical density at 583 nm (OD583). Both fermenters showed very similar growth curves, demonstrating that the scale-up of microbial processes in single-use fermenters can be accomplished by using traditional transfer of scale approaches, constant  $P/V$  and  $v_s$  in this work, and that the 1000 L CSR® can achieve similar heat and mass transfer to its stainless-steel counterpart.



**Figure 3 :** Biomass measured as optical density (OD) in the 200 L stainless-steel fermenter (SSF 200 L) and ABEC's 1000 L single-use CSR fermenter (CSR 1000 L).



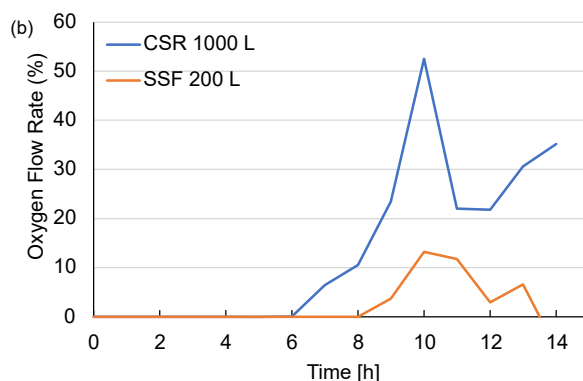
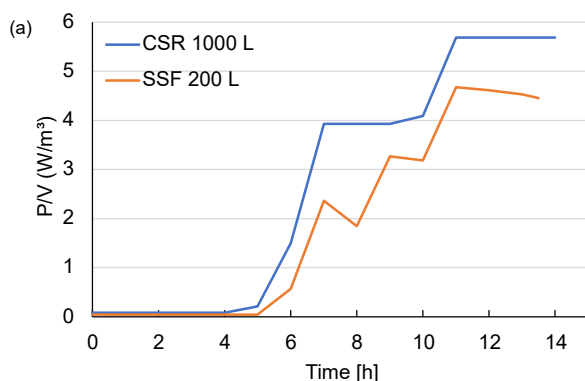
**Figure 4 :** Dissolved oxygen (a) and oxygen transfer rate (OTR, b) comparison between the 200 L stainless-steel fermenter (SSF 200 L) and ABEC's 1000 L single-use CSR fermenter (CSR 1000 L).

Maintaining an adequate oxygen supply during a fermentation process is a key factor for a successful

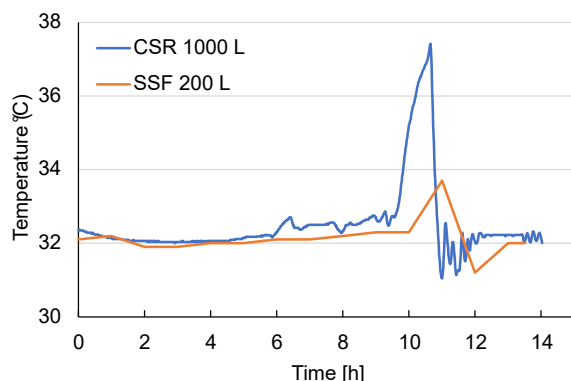
fermentation. **Figure 4a** compares the dissolved oxygen (DO) profile recorded during the process in both fermenters, and **Figure 4b** shows the calculated oxygen transfer rate. Results show an overlap between the DO profiles of the two fermenter scales, that decreased from 100% of air saturation reaching the setpoint of 10% to 13% (a.s.) around 5 h of elapsed fermentation time. After reaching the setpoint, both fermenters were able to keep a steady dissolved oxygen concentration over the remainder of the process. The two fermenters also presented high similarity between their *OTR* profiles. The *OTR* peak observed in the 1000 L CSR® fermenter is likely to be the response to an issue with the chiller used for temperature control, which allowed the temperature to briefly reach 37°C at around 10 h of elapsed fermentation time (**Figure 6**), increasing the cell metabolism and the rate of oxygen consumption by the culture as a consequence.

The *OTR* in a fermenter is a function of the mixing power per volume,  $P/V$ , that directly influences the volumetric mass transfer coefficient,  $k_L a$  ( $h^{-1}$ ), and of the difference between oxygen concentration in the gas and liquid phases. In the featured fermentation process, the dissolved oxygen is controlled both by increasing  $P/V$  (agitation rate) and by the enrichment of oxygen in the gas phase. **Figure 5** compares the evolution of  $P/V$  and gas composition over the course of the fermentation process.  $P/V$  starts to ramp up around 4 h of elapsed fermentation time in both processes. In the 200 L SSF and the 1000 L CSR®,  $P/V$  increases until it reaches its scale-up target ( $4 \text{ kW/m}^3$ ), then DO control switches to oxygen enrichment in the inlet gas. The oxygen usage was higher in the 1000 L CSR® as shown in **Figure 5b**; this was expected due to reduction in head pressure at the 1000 L scale. However, in the face of the higher oxygen consumption in the 1000 L CSR® at approximately 10 h of elapsed fermentation time, the  $P/V$  was allowed to increase to  $5.7 \text{ kW/m}^3$  to lower the amount of pure oxygen in the inlet gas and meet the higher demand of oxygen by the culture due to the higher temperature. The period of high oxygen consumption noted above would not be expected during normal chiller operation. The higher percentage of oxygen usage in the 1000 L CSR compared to the 200 L SSF aligned with the prediction from Table 1.

The similar biomass yield and consistent DO levels throughout the process in both systems emphasize that the oxygen demand of the cells is being met, ensuring optimal conditions for cell growth and productivity. This demonstrates the ability to effectively reproduce results obtained in stainless steel fermenters in large scale single-use units. The success can be attributed to a suitable scale-up strategy combined with the high performance of the 1000 L CSR® fermenter. The resulting product from both the 200 L SSF and the 1000 L CSR® met all quality attributes as tested at the end of the fermentation.



**Figure 5:** Dissolved oxygen control cascade comparison between the 200 L stainless-steel fermenter (SSF 200 L) and ABEC's 1000 L single-use CSR fermenter (CSR 1000 L): (a) mixing power per volume of in the gassed fermenter; and (b) oxygen flow rate calculated as the percentage of pure oxygen added in the gas flow.



**Figure 6:** Temperature comparison between the 200 L stainless-steel fermenter (SSF 200 L) and ABEC's 1000 L single-use CSR fermenter (CSR 1000 L)

## Conclusion

The successful transfer and 5-fold scale-up of a high-cell density microbial process between stainless steel and single-use systems demonstrates the suitability of single-use fermenters for large-scale production of microbial drug substance. The single-use technology brings the capability of quickly switching between different processes in the

same vessel, flexibility that is key for CDMOs producing a variety of biomolecules for different customers. In addition, the single-use CSR® units are highly customizable; thus, a single holder can accommodate several custom CSR® configurations, designed to meet the demands of each individual process application.

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