Recognizing the Potential Impact of Agitation Directionality On Oxygen Mass Transfer Through a k_La Evaluation Within the BIOne Single-Use Bioreactor

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Agitation: A Necessary Element Within Upstream Mammalian Cell Culture Bioprocesses

Defining a suitable agitation strategy is essential for the successful development of upstream mammalian bioprocesses. Such a strategy can drive oxygen mass transfer within the bioreactor system to effectively support the aerobic needs of the expanding culture.[1] An inadequate agitation strategy can result in the emergence of chemical gradients within the system. The existence of such nonuniformity within a bioreactor can result in extracellular conditions which may be unfavorable to the overall growth of the cell culture.

Hypoxic conditions in a bioreactor are defined by low dissolved oxygen concentrations. The formation of such conditions can occur when an agitation strategy is insufficient for an individual bioreactor system.[2] Hypoxic conditions have been demonstrated to be potentially detrimental to the health and productivity of mammalian cell cultures.[3] Cellular responses to hypoxic conditions include upregulation of inefficient aerobic glycolysis metabolic pathways and downregulation of pathways necessary for DNA and RNA synthesis.[4] Additionally, hypoxic conditions may adversely affect product quality, as they can potentially increase variability across glycosylation patterns of manufactured proteins.[5]

Using k_La as a Criterion to Define Suitable Agitation Operational Parameters

To help mitigate the risk of engineering agitation operational parameters which produce hypoxic conditions, parameters can be strategically defined based upon the aerobic demands of the culture. An understanding of the relationship between agitation and the oxygen transfer rate (OTR) within the bioreactor system can be an essential part of such a strategy.

The relationship between agitation and the bioreactor OTR may be understood through the characterization of the volumetric oxygen mass transfer coefficient (k_La) of the system. For a given bioreactor system, the OTR can be defined as the product of both the bioreactor system k_La and the dissolved oxygen concentration gradient. Therefore, the system k_La can serve as a suitable criterion by which to define agitation operational parameters. Such a strategy can reduce the risk of hypoxic conditions developing within the bioreactor.

During bioreactor k_La characterization, the agitator rotational rate is typically considered as the sole agitation process input. However, the agitation flow directionality may also be worth consideration when performing system characterization work. Including this additional parameter may help improve system understanding and facilitate the engineering of optimal agitation parameter definitions.

Directionality of Axial Flows within Mammalian Bioreactor Systems

Pitch blade impellers are commonly used during mammalian cell culture bioprocesses. Such impellers have been demonstrated to reduce the generation of hydrodynamic shear forces in comparison to other impeller types.[6] Hydrodynamic shear forces can be potentially detrimental to mammalian cell culture health.[7] Therefore, reducing the presence of such forces can be beneficial to both the growth and productivity of the culture.

Pitch-blade driven agitation processes result in either upward or downward axial flows. The directionality of the flow will be determined by both the orientation of the impeller and the rotation directionality of the agitator shaft. The impeller may be right-handed (RH) or left-handed (LH), while the shaft may be rotated either clockwise (CW) or counterclockwise (CCW). The combination of these two parameters will determine the ultimate directionality of the axial flow within the system. The details of this relationship are described within **Figure 1**.

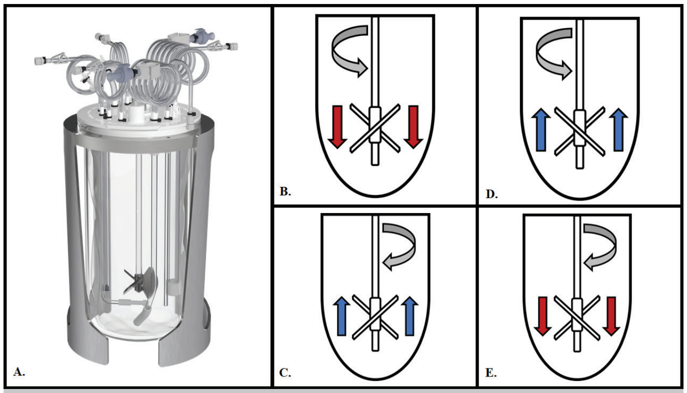

Figure 1. Pitch blade impeller orientation and agitation direction effect axial flows within a bioreactor A. BIOne SUB featuring a righthanded pitch blade impeller B. RH orientation and CCW direction result in Downward Axial Flow C. RH orientation and CW direction result in Upward Axial Flow D. LH orientation and CCW direction result in Upward Axial Flow E. LH orientation and CW direction result in Downward Axial Flow.

Evaluation of the Effects of Axial Flow Direction on k_La within the BIOne Single-Use Bioreactor System

The relationship between axial flow direction and system k_La is likely unique to the physical properties of the specific bioreactor. The BIOne Single-Use Bioreactor (SUB) models utilize right-handed pitch-blade impellers. To improve system characterization, an evaluation was performed to describe the relationship of the axial flow directionality on the k_La within the bioreactor system. The goal of this work was to improve system characterization to support successful upstream bioprocess development for end users of the BIOne SUB.

The mass transfer evaluation was performed in a 2000 mL bioreactor at a working volume of 1450 mL. Throughout the evaluation, air was introduced through a drilled hole sparger at a rate of 82.5 sccm. During the testing, agitation was increased stepwise from 200 rpm to 300 rpm through 25 rpm increments. At each set of parameters, k_La values for both upward and downward axial flow processes were evaluated using the static gassing out method. The method was performed as described in de Ory, Romero, and Cantero, 1999.[8] A model medium was used during the evaluation to increase the relevance of the characterization data across numerous cell culture processes. The model medium was prepared as described in Matsunaga et al., 2009.[9]

Upon completion of the testing, the mean k_La values for upward and downward axial flows were compared across conditions through multiple t-tests. Statistical significance for comparisons was determined using the Holm-Sidak method, with alpha = 0.05. A total of n = 2 trials were completed for all conditions during this evaluation.

The results of the evaluation are presented in **Figure 2**. The k_La values for both upward and downward axial flow processes were comparable at the lower agitation rates. However, the upward axial flow process produced significantly higher k_La values at agitation rates greater than 250 rpm. This observation may be due to the emergence of a secondary circulation loop at higher agitation rates for the upward axial flow process.

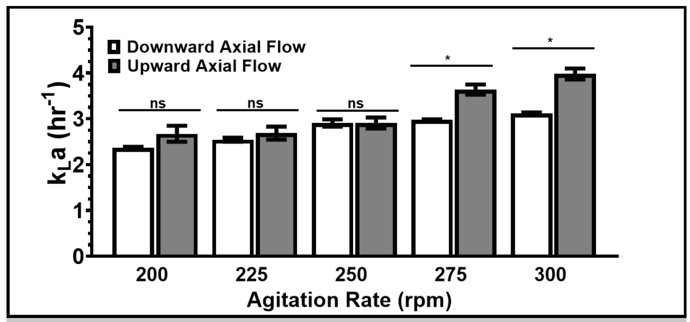

Figure 2. Demonstration of potential for improved oxygen mass transfer with Upward Directional Flow Axial Agitation within the BIOne SUB. As agitation rate was increased over 250 rpm, improved mass transfer of oxygen was observed for upward axial flow agitation. Data are mean values of n = 2 trials. Bars represent standard error for each condition. Key: ns indicates no significant difference between mean values. *indicates statistically significant difference between values.

Conclusion

Detailed understanding of the effects of agitation on oxygen mass transfer within a bioreactor system can help facilitate successful upstream bioprocess development and characterization. As shown in this work, the directionality of the axial flow may influence the overall k_La of a system. Process scientists, engineers, and researchers may find value in considering directionality of the axial flow as a process parameter during their own upstream bioprocess characterization and development work.

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