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A Simple Approach for Characterizing Oxygen Transfer Rate (OTR) within a BIOne Single-Use Fermentor

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BIOPROCESSING

Abstract

Gene therapy is a rapidly advancing technology, where modified genes are introduced into a patient to address specific genetic conditions. These therapies often utilize plasmid DNA as the vector to deliver the modified genes into patient's genome. Most commonly, the plasmid DNA is expressed in E. coli cultures through a process known as microbial fermentation. As the demand for these therapies increases, so does the need high quality plasmid DNA, which is achievable through precise and repeatable fermentation techniques. This includes maintaining optimal dissolved oxygen concentrations within in the bioreactor system. Oxygen Transfer Rate (OTR) characterization for the bioreactor is necessary for process design and scale up to ensure sufficient dissolved oxygen control for the microbial culture.

Traditional OTR measurement methods, such as the static gassing out method for k_La determination, are often inadequate for the dynamic conditions of microbial fermentation. This study demonstrates the use of the sulfite depletion method to accurately determine OTR in the Distek BIOne 2L Single-Use Fermentor (SUF). The sulfite depletion method provides reliable OTR results, with the BIOne 2L SUF showing an OTR of 427.95 mmol O_2/L/hr under typical E. coli processing conditions. This approach offers significant advantages over traditional techniques, supporting optimal fermentation conditions and high-quality plasmid DNA production.

Introduction

Over the last decade, gene therapy has emerged as a revolutionary technology in which modified exogenous genes are introduced into a patient to address diseases caused by defective or abnormal genes. For many of these therapies, plasmid DNA is used as the vector to deliver the modified genetic material to in vitro mammalian, typically HEK293, host cell cultures. In these cultures, the DNA is incorporated into the host cell genome and replicated as a viral vector, which is ultimately administered to a patient to treat a targeted genetic condition. These therapies continue to be an ever-increasing reality, with 36 gene therapies approved by the FDA as of August 2, 2024, and an additional 500 in the pipeline, according to the US Food and Drug Administration and the Segal Group.[1]

The plasmid DNA containing modified genetic material and used to produce viral vectors is first expressed and amplified in E. coli cultures, through a process known as microbial fermentation. Therefore, as the number of approved of gene therapies grows so does the demand for high quality plasmid DNA. This demand has driven a shift in the field of microbial fermentation towards adoption of single-use technologies (SUT), which do not require any extensive cleaning or sterilization validation and allows for more flexibility in the manufacturing process. Additionally, increased effort is being placed on E. coli fermentation process development and specifically bioreactor control strategies to ensure consistent and repeatable processes that yield high quality product.

One of the primary control strategies analyzed in a microbial fermentation process is maintenance of suitable dissolved oxygen (DO) concentration for cell growth. Although E. coli can proliferate under either aerobic or anaerobic conditions, plasmids are more efficiently expressed under aerobic conditions, where molecular oxygen is required for the oxidative phosphorylation pathways.[2,3] DO limitation in E. coli cultures can affect not only biomass accumulation, but also reduce the replication of plasmid and the expression of plasmid gene.[4] Therefore, maintaining a sufficient dissolved oxygen concentration can be necessary for optimal cell culture productivity.

For microbial processes in bioreactors, DO is maintained primarily through the introduction of molecular oxygen directly into the culture medium through the sparging of gases directly into the liquid. The rate by which oxygen is transferred from the gaseous to the aqueous phase within the overall bioreactor system is defined by the Oxygen Transfer Rate (OTR). If the OTR is not sufficient to meet the demands of highly aerobic cultures, also known as the Oxygen Uptake Rate (OUR), the metabolic potential of the culture could be reduced and may lead to by-product accumulation.[5] Hence, a thorough understanding of OTR in the bioreactor system is vital when designing a microbial bioreactor system and developing a microbial fermentation process.

Many factors can influence the OTR within a bioreactor system including physical, chemical, and biological aspects of the overall bioreactor system. Specific factors include impeller design, vessel aspect ratio, number of impellers, system volume, agitation rate, sparged air flow rate, media salt and surfactant concentrations, extracellular protein concentrations, and overall system biomass accumulation.[6] Understanding these factors and their impact on OTR is essential for designing fermenters that can maintain optimal oxygen levels throughout the fermentation process.[7]

The variety of factors that can significantly influence the OTR of a bioreactor system makes characterization of the OTR a considerable challenge. This challenge is further increased due to the dynamic nature of a bioprocess, which cause the OTR to fluctuate throughout the duration of the process. However, limiting OTR characterization to factors that are associated with the bioreactor system itself and not the process allows scientists to determine a best case or maximum OTR achievable with a specific bioreactor design. This maximum OTR can then be used as a target parameter for process development engineers when scaling up to production volumes.[8]

Sulfite Depletion Method for Determination of Oxygen Transfer Rate

The BIOne Single-Use Fermentor (SUF) system is a novel liner-based benchtop disposable bioreactor system, designed and manufactured by Distek, Inc. The SUF has been designed for microbial fermentation processes as a suitable single-use alternative to traditional glass bioreactors. The team at Distek has recognized that SUF can serve as an excellent process development and material generation platform for plasmid DNA to be used in gene therapy applications. However, to demonstrate its suitability, a methodology for oxygen transfer rate characterization of the BIOne SUF system is required.

Traditional methods of determining system oxygen transfer rate, such as the static off-gassing method for k_La determination, rely on DO sensor response when saturating media with oxygen.[9] For microbial fermentation processes, the high agitation and sparge gassing rates cause media to saturate extremely quickly, and so much so that timing DO probe signal recovery would lead to highly variable and inaccurate results. Therefore, an industry standard chemical reaction process known as the sulfite depletion method is a widely recognized technique for determining the oxygen transfer rate (OTR). This method is particularly valued for its ability to provide accurate measurements irrespective of the dissolved oxygen (DO) probe response time.[10]

The sulfite depletion method uses a rapid and well-characterized oxidation reaction of sodium sulfite in the presence of a copper catalyst to produce sodium sulfate as a byproduct, shown in **Equation 1**. Oxygen is used as the oxidizing agent, where it's concentration in the bioreactor is dictated by the remaining sodium sulfite in solution. Once sodium sulfite has been depleted, oxygen is no longer consumed and its concentration within the bioreactor recovers, demonstrated in **Figure 1**. The speed of this reaction ensures that oxygen depletion and recovery are almost instantaneous, thereby circumventing the potential variability associated with DO probe response time.

$$2Na_2SO_3 + O_2 \xrightarrow{Cu^{2+}} 2Na_2SO_4$$

(Eq. 1)

The sulfite depletion method's robustness and reliability make it particularly suitable for microbial fermentation applications, offering a significant advantage over other techniques that might be more susceptible to measurement inaccuracies due to probe lag or other factors. In this application note, we demonstrate OTR characterization within the Distek BIOne 2L SUF using the sulfite depletion method.

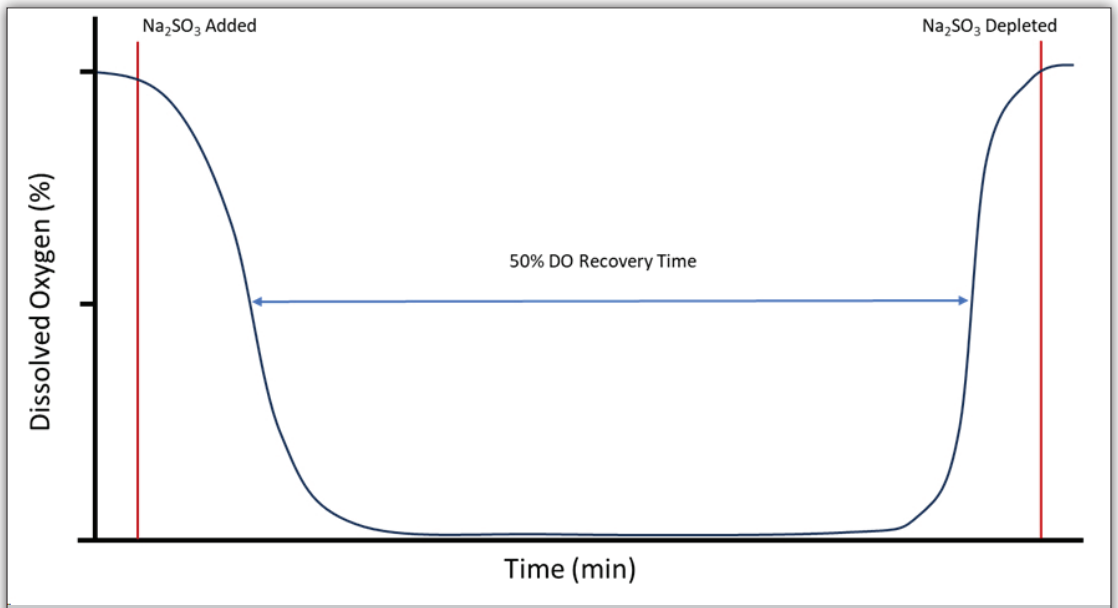

Figure 1. Time course dissolved oxygen profile during the sulfite oxidation reaction. Time used to calculate OTR is the duration between DO dropping below 50% to the time it takes to recover to 50%.

Methods and Materials

A BIOne Single-Use Fermentor (SUF) manufactured by Distek, Inc was utilized for this study. The specific BIOne SUF evaluated was a 2L system, model number 2022-1078. This model features dual six paddle Rushton impellers spaced 82 mm apart and four internal baffles. Gas transfer into the system is facilitated with the use of a drilled hole flute sparger, an element which includes seven 1.5 mm holes.

To measure the dissolved oxygen (DO) concentration of the system, a 225mm Hamilton OxyFerm FDA analog DO sensor was installed in the non-invasive sleeve on the SUF headplate. A Distek BIOne 1250 Dual Controller was used to control temperature, agitation, and bottom air sparge rate into the SUF. Operating conditions within the system were chosen as representative values for a typical E. coli process. The SUF was filled to 1.5L with deionized (DI) water with agitation fixed at 1250 rpm, temperature at 30°C, and air sparge at a constant 1 VVM (1500 sccm).

Prior to initiating the chemical reaction, the DO sensor was calibrated to 100% saturation at the operating conditions referenced previously. A 3mL volume of 50 g/L copper (II) sulfate pentahydrate solution was added to the SUF for a final concentration of 0.3 g/L. Immediately following, 16.5g of sodium sulfite anhydrous powder was weighed and added to the SUF at a concentration of 11 g/L. The DO sensor reading was monitored through the process, with the time recorded between the DO dropping to 50% and recovering to 50%. The time of 6.12 minutes was then used to calculate the OTR for the system.

Results and Discussion

Upon completion of the study, the Oxygen Transfer Rate (OTR) for the SUF was calculated using **Equation 2** and **Equation 3** below. In **Equation 2**, W is the weight in grams of sodium sulfite (molecular weight of 126 g/mol) added to the SUF. **Equation 3** takes the calculated mmol of O2 from **Equation 2** along with the working volume of the bioreactor (V) in liters and the 50% DO recovery time (t) in minutes.

$$n_{O_2}(mmol) = \frac{W_{Na_2SO_3}(g)}{126\ (\frac{g}{mol})} * \frac{1000(\frac{mmol}{mol})}{2}$$

(Eq. 2)

$$OTR\left(\frac{mmol_{O_2}}{L * hr}\right) = \frac{n_{O_2}(mmol) * 60\left(\frac{min}{hr}\right)}{V(L) * t(\text{min})}$$

(Eq. 3)

Results of these calculations provided an OTR of 427.95 mmol O_2/L/hr. This study demonstrates the sulfite depletion method as a simple, yet reliable chemical reaction process that can be used to characterize the OTR in a microbial fermentation vessel such as the Distek BIOne 2L SUF.

Conclusion

The sulfite depletion method proves to be an effective and reliable technique for accurately measuring the Oxygen Transfer Rate (OTR) in microbial fermentation systems, as demonstrated with the Distek BIOne 2L Single-Use Fermentor (SUF). By providing precise OTR measurements, this method enhances our ability to optimize fermentation conditions, crucial for the consistent production of high-quality plasmid DNA across bioreactor scales. The successful application of this technique underscores its value in scaling up fermentation processes and meeting the growing demands of the gene therapy industry, ultimately contributing to more efficient and effective therapeutic process development.

References

1. *Approved Cellular and Gene Therapy Products. (2024, August 2). US Food and Drug Administration. Retrieved August 11, 2024, from https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products*
2. *McDaniel, L.E., E. G. Bailey, E.G., Zimmerli, A. (1965) Effect of Oxygen Supply Rates on Growth of Escherichia coli. Applied Microbiology, 13, 109-114. https://doi.org/10.1128/am.13.1.109-114.1965*
3. *Cunningham, D. S., Koepsel, R. R., Ataai, M. M., Domach, M. M. (2009). Factors affecting plasmid production in Escherichia coli from a resource allocation standpoint. Microbial cell factories, 8, 27. https://doi.org/10.1186/1475-2859-8-27*
4. *Li, X., James W Robbins, J.W., Taylor, K.B. (1992). Effect of the levels of dissolved oxygen on the expression of recombinant proteins in four recombinant Escherichia coli strains. Journal of Industrial Microbiology, 9(1), 1–9. https://doi.org/10.1007/BF01576362*
5. *Varma, A., Boesch, B.W., Palsson, B.O. (1993). Stoichiometric interpretation of Escherichia coli glucose catabolism under various oxygenation rates. Appl Environ Microbiol, 59. https://doi.org/10.1128/aem.59.8.2465-2473.1993*
6. *Garcia-Ochoa, F., & Gomez, E. (2009). Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. Biotechnology Advances, 27(2), 153-176. https://doi.org/10.1016/j.biotechadv.2008.10.006*
7. *Garcia-Ochoa, F., & Gomez, E., Merchuk, J.C., Santos, V.E. (2010). Oxygen uptake rate in microbial processes: an overview. Biochemical Engineering Journal, 49(3), 289-307. https://doi.org/10.1016/j.bej.2010.01.011*
8. *Nauha, E. K., Visuri, O., Vermasvuori, R., & Alopaeus, V. (2015) A new simple approach for the scale-up of aerated stirred tanks. Chemical Engineering Research and Design, 95, 150-161. https://doi.org/10.1016/j.cherd.2014.10.015*
9. *Ory, I. D., Romero, L. E., & Cantero, D. (1999) Laboratory scale equipment for the determination of k L a in bio-reactors. Bioprocess Engineering, 20(1), 73-75. https://doi.org/10.1007/pl00009036*
10. *Thaibult, J., Leduym, A., Denis, A. (1990) Chemical Enhancement in the Determination of $k_L a$ by the Sulfite Oxidation Method. The Canadian Journal of Chemical Engineering, 68(2), 324-326. https://doi.org/10.1002/cjce.5450680219*

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