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Measuring Oxygen Transfer Rate (OTR) of the Eppendorf® Fermentation Vessels

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Abstract

This short protocol describes the procedure to measure the oxygen transfer rate (OTR) of the Eppendorf fermentation vessels, an important parameter of the vessel specifications. It is crucial to measure OTR under industry standard conditions with 1 vessel volume per minute (VVM) of air sparging only without oxygen enrichment, as the increase of VVM or oxygen sparging will significantly inflate OTR values. We used the industrial standard sulfite depletion method for our measurement under maximum agitation and air sparging. In a model experiment, we employed a BioFlo® 320 3 L glass vessel and followed the equation to calculate the mean OTR to be 366.4 mmol O_2/(L×h). This short protocol can serve as a reference for the bioprocess professionals who want to perform OTR measurements of Eppendorf's large collection of fermentation vessels.

Content

Fig. 1: BioFlo 320 bioreactor control system.

1. Introduction

The oxygen transfer rate describes the rate of oxygen delivery from gas into liquid such as an agitated suspension culture in a bioprocess vessel. The typical unit for OTR is mmol O_2/(L×h), i.e., the amount of oxygen transferred into 1 liter of culture per hour. OTR is a very important parameter of fermentation vessels which affects the selection, design, and scale-up of a bioprocess protocol [1].

The OTR of a vessel provided by most manufacturers represents the maximum oxygen transfer capability of this specific vessel under 1 vessel volume per minute (VVM) air sparging. There are many factors that affect the vessel's OTR including the physical properties of the gas and liquid, operating conditions, geometric characteristics of the vessel, impeller size and configurations, to just list a few. The mass transfer of oxygen is usually a limiting factor in aerobic fermentation bioprocess. Under oxygen limitation, microbial cell growth and product formation can be affected [2]. However, the term OTR is not widely used in mammalian cell culture bioprocess since cell culture normally underutilizes a vessel's OTR capability under relatively low agitation and gas flow.

Here we describe in detail how we measure the OTR of an Eppendorf fermentation vessel using the industrial standard sulfite depletion method [3]. Maximum agitation and 1 VVM air flow provide industry standard oxygen transfer to the system. With the addition of sodium sulfite (Na_2SO_3), oxygen is then rapidly absorbed to generate sodium sulfate (Na_2SO_4) in the presence of the copper catalyst. The chemical reaction is shown below:

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

Since the oxygen entering the solution is immediately consumed in the oxidation of sulfite into sulfate, the reaction rate of sulfite oxidation represents the oxygen transfer rate of the vessel. OTR can be calculated by measuring the recovery speed of the dissolved oxygen (DO) in the vessel after sodium sulfite depletion. To further establish this as a proof of principle, we used the BioFlo 320 3 L glass vessel as the experimental device to define the parameters of the OTR.

2. Materials

2.1 Equipment

In this protocol, we used a 3 L autoclavable glass vessel with stainless-steel dished bottom and direct drive for BioFlo 320 (Eppendorf Catalog No. M1379-0301, Figure 1).

> BioFlo 320 Control Station (Eppendorf Catalog No. 1379963011)

> Mettler Toledo® Analog polarographic DO sensor (12mm diameter with 220 mm insertion depth, Eppendorf Catalog No. P0720-6282)

2.2 Chemicals

> Sodium sulfite anhydrous (Na_2SO_3, Fisher Scientific® S430-10)

> Cupric sulfate stock solution at 50 g/L (copper(II) sulfate pentahydrate, $CuSO_4 \cdot 5\ H_2O$, J.T. Baker 1843)

2.3 Other materials

Weigh dish, lab balance, stopwatch, funnel, transfer pipettes, Easypet® 3

Table 1: Process parameters applied in OTR measurement.

Parameter	Configuration
Vessel	Autoclavable glass vessel with stainless-steel dished bottom or BioBLU® f Single-Use vessel
Baffles	With baffles
Working volume	Maximum working volume of the vessel
Agitation	Maximum agitation of the vessel
Gassing	100 % air at 1 VVM
Temperature	30 °C
Impeller	Rushton impellers, factory default set-up
Sparger	Ringsparger for the glass vessel or macrosparger for the BioBLU f Single-Use vessel

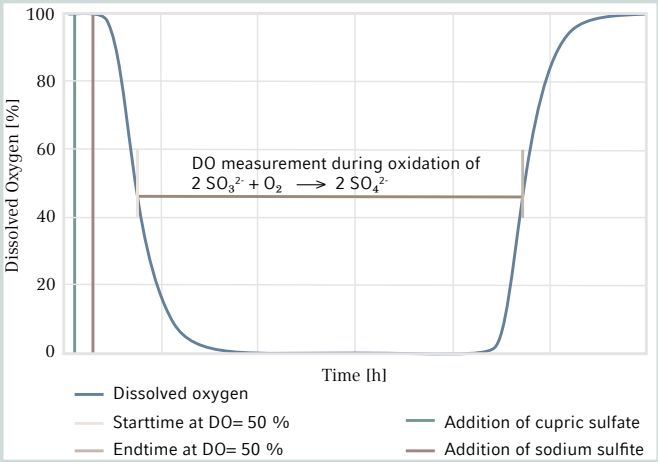

Fig 2. Time tracking according to the DO change during the sulfite oxidation reaction.

3. Experimental procedures

3.1 Vessel assembly

Assemble the glass vessel as per the user manual or take a ready-to-use BioBLU f vessel. Fill the vessel with DI water to its maximum working volume.

3.2 DO sensor calibration

Install the polarized analog DO sensor onto the vessel head plate and calibrate the sensor. Set the temperature at 30 °C and adjust agitation to the maximum rate of the vessel recommended by the user manual. Sparge 100 % nitrogen gas into the vessel at 1 VVM. When the DO reading stabilizes after 10-30 minutes, set zero (0 %). Then change the gas composition to sparge 100 % air into the vessel at 1 VVM, wait for another 10-30 minutes for the DO reading to stabilize to set the span (100 %).

3.3 OTR measurement

Keep the temperature at 30 °C and gas sparging at 1 VVM with 100 % air. The experimental conditions are summarized in Table 1. At the beginning of the experiment, DO stays at 100 %. Open a port on the vessel head plate and transfer 50 g/L cupric sulfate stock solution as the catalyst at a volumetric ratio of 2 mL per 1 L into the vessel. Weigh Na_2SO_3 powder and add them all to the vessel through a funnel as quickly as possible to reach a final Na_2SO_3 concentration of 11 g/L. Track time using a stopwatch from the time point when DO reaches 50 % on the downward trend to the time point when DO recovers to 50 % on the way up. The total DO recovery indicates the exhaustion of the amount of Na_2SO_3 added to the system (Figure 2). DO control is turned off during the entire experiment. Read the present values of DO manually on the control interface of BioFlo 320 or through the trend graph auto-collected by the unit. Carry out the experiment twice to provide a mean OTR of the vessel.

4. Results

The oxygen transfer rate can be calculated using the following equation ($W_{Na_2SO_3}$ is the weight of Na_2SO_3 powder used in the experiment, 126 g/mol is the molecular weight of Na_2SO_3, and t is the elapsed time in hours tracked for DO recovery):

$$\text{Amount of oxygen captured by the reaction} = n_{O_2}\,[\text{mmol}] = \frac{W_{Na_2SO_3}\,[\text{g}]}{126\left[\frac{\text{g}}{\text{mol}}\right]} \times \frac{1000\left[\frac{\text{mmol}}{\text{mol}}\right]}{2}$$

$$\text{OTR}\left[\frac{\text{mmol}}{\text{L} \times \text{h}}\right] = \frac{n_{O_2}\,[\text{mmol}]}{V_{\text{operation}}[\text{L}] \times t\,[\text{h}]}$$

4.1 An example of OTR measurement using the 3 L autoclavable glass vessel

We first assembled the 3 L BioFlo 320 fermentation vessel as per the user manual and filled the vessel to its maximum working volume of 3.75 L DI water in this case. Then we installed the polarized analog DO sensor onto the vessel head plate and calibrated the sensor. We set the temperature at 30 °C and agitation at 1200 rpm which is the maximum agitation rate of the vessel recommended by the user manual, and sparged gas composed of 100 % nitrogen into the vessel at 3.75 SLPM (1 VVM). When DO reading stabilized after 10-30 minutes, zero (0 %) was set. Then we changed the gas composition to sparge 100 % air into the vessel at 3.75 SLPM, and waited for another 10-30 minutes for the DO reading to stabilize to set the span (100 %). This completed the DO sensor calibration.

We kept the temperature at 30 °C and gas sparging at 3.75 SLPM with 100 % air for the OTR measurement. At the beginning of the experiment, DO stayed at 100 %. We opened a Pg 13.5 port on the vessel head plate and transferred 7.5 mL of the 50 g/L cupric sulfate stock solution into the vessel as the catalyst. We then weighed 41.25 g Na_2SO_3 dry powder and added them all to the vessel through a funnel as quickly as possible to reach a final Na_2SO_3 concentration of 11 g/L. Time was tracked by a stopwatch from the time point when DO reached 50 % on the downward trend to the time point when DO recovered to 50 % on the way up, as shown in Figure 2. We read the present values of DO manually on the control interface of BioFlo 320 or through the trend graph auto-collected by the unit, both at a data logging rate of every 5 seconds. The experiment was carried out twice to provide a mean OTR of this 3 L glass vessel.

There was only modest variation in the values in the two runs. The elapsed time t for DO recovery was 7m 7s and 7m 11s, corresponding to 0.1186 and 0.1197 h, respectively. The weight of Na_2SO_3 powder was 41.25 g. Based on this, using the equations shown above, we calculated the mean OTR to be 366.4 mmol O_2/(Lxh) for the 3 L autoclavable BioFlo 320 glass vessel.

Discussion

The sulfite depletion method used in this study is a reliable and relatively easy way for OTR determination under non-sterile conditions, although the process can be tedious and for large vessels, a great amount of chemicals is required.

There can be some human error in time tracking using the stopwatch but it was still the preferred method, because the controller's data logging was set to be every 5 seconds and the rate of oxygen depletion and oxygen recovery in this experiment can be relatively fast. However, the error from the timing technique can be minimized with practice and the elapsed time can be double checked by studying the trend graph collected by the BioFlo 320 control unit. We find both

timing techniques work equally well.

In summary, this short protocol describes the method for measuring and calculating OTR of a fermentation vessel under industry standard OTR measurement conditions using the sulfite depletion method. A fermentation vessel with a high OTR can effectively support robust microbial growth and desired product yield. For example, Eppendorf demonstrated very high *E. coli* fermentation biomass, approximately 215 ODs in a 1 L glass vessel [4] and 240 ODs in a BioBLU 3f single use vessel [5.] Therefore, this short protocol can serve as a methodological reference for those who are interested in OTR measurement of Eppendorf's large collection of fermentation vessels.

Literature

[1] Garcia-Ochoa F, Gomez E. Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. Biotechnology Advances (27), 153–176. 2009
[2] Hall SM. Rules of Thumb for Chemical Engineers (Sixth Edition). 2018
[3] Kelleher B, Nori C, Voll K, Siddiquee K, and Sha M. Evaluation of the BioFlo® 320 process capabilities. **Eppendorf Poster**. 2014
[4] Li B, Siddiquee K, and Sha M. The Eppendorf BioFlo® 320 Bioprocess Control Station: An Advanced System for High Density *Escherichia coli* Fermentation. **Eppendorf Application Note No. 340.** 2015.
[5] Yang Y and Sha M. A Beginner's Guide to Bioprocess Modes – Batch, Fed-batch, and Continuous Fermentation. **Eppendorf Application Note 408**. 2019

Ordering information

Description	Order no.
BioFlo® 320, all configured units include the same base control station	
Base control station	1379963011
Vessel Bundle, for BioFlo® 320, stainless-steel dished bottom, direct drive, 3 L	M1379-0301
DO sensor, Mettler Toledo® InPro 6830, angled T-82 connector, L 220 mm	P0720-6282
Eppendorf® Easypet® 3, single-channel, incl. mains/power supply device, wall mounting device, shelf stand, 2 membrane filters 0.45 µm, 0.1 – 100 mL	4430000018

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