SHORT PROTOCOL No. 39

Measuring the Oxygen Transfer Rate (OTR) of Eppendorf Fermentation Bioreactors

Ying Yang, Marcus Deinstadt, and Ma Sha
Eppendorf, Inc., Enfield, CT, USA.
Contact: bioprocess-experts@eppendorf.com

Abstract

This short protocol describes the detailed procedure of how to measure the oxygen transfer rate (OTR) of the Eppendorf fermentation bioreactors. OTR is often listed as one of the bioreactor specifications and describes the rate of oxygen delivery from gas into liquid. It is important to measure OTR under industry standard conditions with 1 vessel volume per minute (VVM) of air sparging only with no oxygen enrichment. Higher VVM or oxygen sparging will significantly inflate OTR values. In this protocol, we used the industry standard sulfite depletion method for our measurement under air sparging and maximum agitation. As an example, we took a BioFlo® 320 3 L glass bioreactor to demonstrate the process. We calculated the average OTR to be 366.3 mmol O_2/(L x h). We also compared OTR measurements when using different types of dissolved oxygen (DO) sensors. This short protocol can serve as a reference for the bioprocess professionals who are interested in OTR measurement of the large collection of Eppendorf fermentation bioreactors.

Content

Fig. 1: BioFlo 320 bioreactor control system.

1. Introduction

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

The OTR of a bioreactor provided by most manufacturers represents the maximum oxygen transfer capability of this specific bioreactor under one VVM air sparging. There are many factors that affect the bioreactor's OTR including the physical properties of the gas and liquid, operating conditions, and geometric characteristics of the bioreactor. The mass transfer of oxygen is usually a limiting factor in aerobic fermentation bioprocesses. Under oxygen limitation, cell growth and product formation can be affected [2]. To assess their oxygen transfer capabilities, the Eppendorf applications team determined the OTR of many fermentation bioreactors.

Here in this short protocol, we describe in detail how we measure the OTR of an Eppendorf fermentation bioreactor [3]. We use the industry standard sulfite depletion method for our measurement [1]. Maximum agitation and 1 VVM air flow provide oxygen transfer to the system. With the addition of sodium sulfite (Na_2SO_3), oxygen then rapidly reacts as the oxidizing agent 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 to oxidize sulfite into sulfate, the reaction rate of sulfite oxidation represents the oxygen transfer rate of the bioreactor. OTR can be calculated by measuring the recovery of the dissolved oxygen (DO) in the bioreactor after sodium sulfite depletion. To demonstrate the process, we used a BioFlo 320 3 L glass bioreactor as an example to calculate its OTR. We also compared the performance of different DO sensors in the process. We compared an analog polarographic sensor from Mettler-Toledo®, a digital Mettler-Toledo ISM® polarographic sensor, and a digital Mettler-Toledo ISM optical sensor to evaluate if there was a difference in response rate for OTR measurements. For this experiment a BioFlo 320 5 L glass bioreactor was used.

2. Materials

2.1 Equipment

> An Eppendorf autoclavable glass bioreactor or a BioBLU® f Single-Use Bioreactor. In this study, we used a 3 L and a 5 L autoclavable glass bioreactor with stainless-steel dished bottom and direct drive for BioFlo 320.
> BioFlo 320 control station
> Mettler Toledo analog polarographic DO sensor (12 mm diameter with 220 mm insertion depth)
> Mettler Toledo analog polarographic DO sensor (12 mm diameter with 320 mm insertion depth)
> Mettler Toledo ISM polarographic DO sensor (12 mm diameter with 320 mm insertion depth)
> Mettler Toledo ISM optical DO sensor (12 mm diameter with 320 mm insertion depth)

2.2 Chemicals

> Sodium sulfite anhydrous (Na_2SO_3, Fisher Scientific®, S430-10)
> Copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) solution at 80 g/L concentration (J.T. Baker, 1843)

2.3 Other materials

Weigh dish, lab balance, stopwatch, funnel, transfer pipettes, Easypet® 3 electronic pipette controller.

3. Experimental procedures

3.1 Bioreactor assembly

Assemble the glass bioreactor as per the user manual or take a ready-to-use BioBLU f Single-Use Bioreactor. Fill the bioreactor with deionized (DI) water to its maximum working volume.

3.2 DO sensor calibration

Install a DO sensor in the bioreactor. Calibrate the sensor as described in Short Protocol 40 [4].

3.3 OTR measurement

We kept the temperature at 30 °C and sparged gas at 1 VVM with 100 % air. The experimental conditions are summarized in Table 1.

At the beginning of the experiment, DO is stable at 100 %. Open a port on the bioreactor head plate and transfer 80 g/L copper sulfate stock solution as the catalyst at a volumetric ratio of 2 mL per 1 L of working volume into the bioreactor. The copper is the reaction catalyst and when

Table 1: Process parameters applied in OTR measurement

Parameter	Configuration
Vessel	Autoclavable glass bioreactor with stainless-steel dished bottom or BioBLU f Single-Use Bioreactor
Drive	Direct drive (glass bioreactor); magnetic drive (BioBLU f Single-Use Bioreactor)
Working volume	Maximum working volume of the bioreactor
Agitation	Maximum agitation of the bioreactor
Gassing	100 % air at 1 VVM
Temperature	30 °C
Impeller	Rushton impellers
Sparger	Ringsparger for the glass vessel or macrosparger for the BioBLU f Single-Use Bioreactors

added sufficiently, it gives the solution a light blue color. It is important to investigate and if necessary, order fresh chemicals if this color is missing. Weigh Na_2SO_3 powder and add it all at once to the bioreactor through a funnel as quickly as possible to reach a final Na_2SO_3 concentration of 11 g/L. Leave the ports used to add chemicals open during the experiment to allow maximum exhaust gas outflow. Some preliminary studies in our lab showed that there is an 8 % decrease in OTR when measured with all ports closed after chemical addition vs. ports left open. Track time from the time point when DO reaches 50 % on the downward trend to the time point when DO bounces back to 50 % on the way up. The completion of 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, hence, automatic DO control is not used. Read the present values of DO manually on the control interface of BioFlo 320 and track time using a stopwatch or read them through the trend graph auto-collected by the unit. Carry out the experiment twice to provide an average OTR of the bioreactor. The detailed bioreactor setup is illustrated in Figure 3.

The oxygen transfer rate can be calculated using the following equation (W_{Na2SO3} 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):

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

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

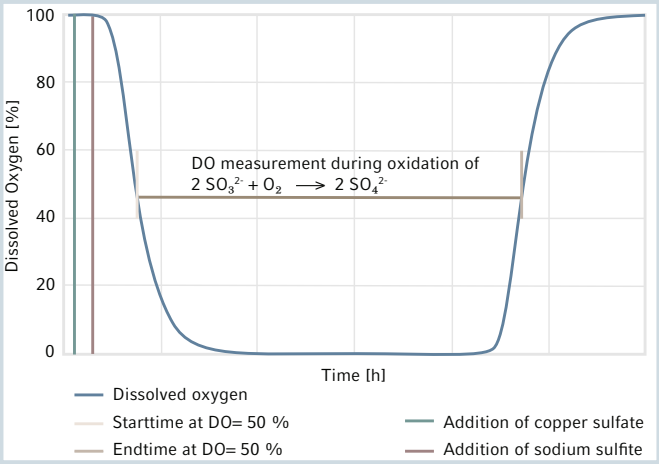

Fig 2. Time tracking according to the DO change during the sulfite oxidation reaction.

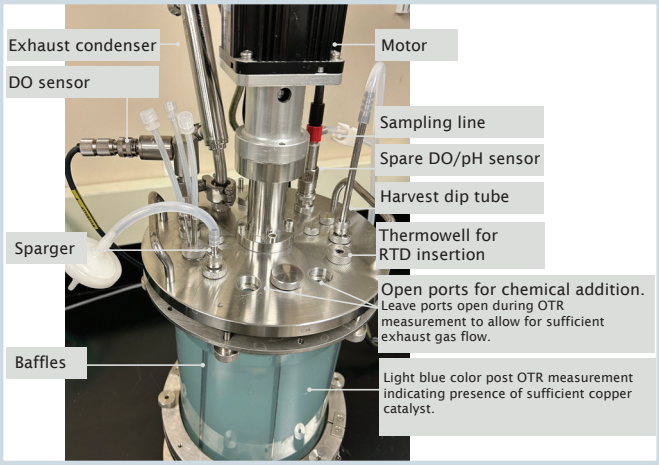

Fig 3. Standard bioreactor setup and components for OTR testing.

4. Results

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

We first assembled the 3 L BioFlo 320 fermentation bioreactor as per the user manual and filled the bioreactor with DI water to its maximum working volume which was 3.75 L in this case. Then we installed the polarized analog DO sensor in the bioreactor head plate and calibrated the sensor as follows. We set the temperature at 30 °C and agitation at 1200 rpm which is the maximum agitation rate of the bioreactor recommended by the user manual,

and sparged gas composed of 100 % nitrogen into the bioreactor 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 bioreactor at 3.75 SLPM, waited for another 10–30 minutes for the DO reading to stabilize to set the span (100 %). The DO sensor calibration was then completed.

We kept the temperature at 30 °C, agitation at 1200 rpm, and gas sparging at 3.75 SLPM with 100 % air for the OTR measurement. At the beginning of the experiment, DO was stable at 100 %. We opened a Pg 13.5 port on the bioreactor head plate and transferred 7.5 mL of the 80 g/L copper sulfate stock solution into the bioreactor as the catalyst. We then weighed 41.25 g Na_2SO_3 powder and added it to the bioreactor through a funnel as quickly as possible to reach a final Na_2SO_3 concentration of 11 g/L. The ports were left open after chemical addition. Time was tracked by a stopwatch from the time point when DO reached 50 % on the downward trend to the time point when DO bounced back 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 bioreactor.

In this duplicated experiment, the elapsed time t we tracked for DO recovery was 427 s and 431 s, corresponding to 0.1186 h 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 366.3 mmol O_2/(L x h) for the 3 L autoclavable BioFlo 320 glass bioreactor (Table 2).

Table 2: BioFlo 320 3 L glass bioreactor OTR measurements

Experiment #	Time (s)	OTR (mmol O_2/L×h)	Average
Experiment #1	427	368.01	366.31
Experiment #2	431	364.60	

4.2 DO sensor type comparison for OTR measurements

For these experiments, we evaluated different types of DO sensors to compare their performances in OTR measurement. We first assembled a BioFlo 320 5 L fermentation bioreactor as per the user manual and filled it with DI water to its maximum working volume, which is 5.625 L. The sensors were polarized and calibrated together using the process stated previously in Short Protocol 40 [4].

Two experiments were designed for such comparisons. We first compared the analog DO sensor with the digital ISM sensor. We installed the two sensors in the same bioreactor for the experiment, removing the pH sensor to allow for the extra DO sensor in the process. This experiment was repeated three times so an average OTR can be calculated for each sensor. We then ran a second experiment comparing an analog DO sensor with the optical DO sensor. All experimental conditions were set the same as described below.

The bioreactor temperature was set to 30 °C and gas sparging was set to 5.625 SLPM corresponding to 1 VVM with 100 % air for the OTR measurement. Agitation was set at the maximum of 1200 rpm. Pg 13.5 ports were opened on the headplate for chemical addition. Then 11.25 mL of the 80 g/L copper sulfate solution were added to the bioreactor, followed by the addition of 61.875 g Na_2SO_3. The ports were left open after chemical addition. Time was tracked by a stopwatch from the time point when DO reached 50 % on the downward trend to the time point when DO bounced back to 50 % on the way up as shown in Figure 2. The calculated OTRs of the 5 L glass bioreactor using these different DO sensors are shown in the Tables 3 and 4. The averages and standard deviations of these OTR measurements are further illustrated in Figure 4.

Table 3: BioFlo 320 5 L glass bioreactor OTR measurements using an analog and ISM DO sensor, respectively.

Sensor type	Time (s)	OTR (mmol O_2/L×h)	Average
Analog DO sensor run 1	408	385.15	379.02
Analog DO sensor run 2	415	378.66	
Analog DO sensor run 3	421	373.26	
ISM DO sensor run 1	407	386.10	378.74
ISM DO sensor run 2	416	377.75	
ISM DO sensor run 3	422	372.38	

Table 4: BioFlo 320 5 L glass bioreactor OTR measurements using an analog and optical DO sensor, respectively.

Sensor type	Time (s)	OTR (mmol O_2/L×h)	Average
Analog DO sensor run 1	415	378.66	376.94
Analog DO sensor run 2	410	383.28	
Analog DO sensor run 3	426	368.88	
Optical DO sensor run 1	425	369.75	368.91
Optical DO sensor run 2	422	372.38	
Optical DO sensor run 3	431	364.60	

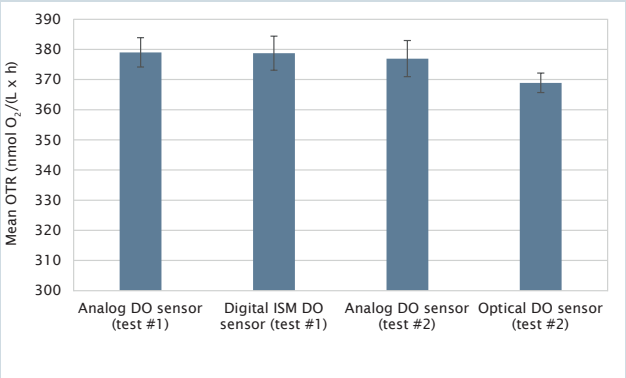

Fig. 4: The comparison of 5 L glass bioreactor OTR measurements among analog, digital ISM, and optical DO sensors (each column represents the mean of three independent measurements and the error bar represents the respective standard deviation). Test #1: Analog DO sensor and digital ISM DO sensor were compared. Test #2: Analolg DO sensor and optical DO sensor were compared.

We found that the analog and ISM sensors measured almost identically. For the BioFlo 320 5 L glass bioreactor, the average OTR calculated from the analog and ISM sensor measurements were 379.02 and 378.74 mmol O_2/(L x h), respectively. The OTR measurements based on optical DO sensor readings seemed to be slightly lower than the OTR measurements based on analog DO sensor readings, however the differences were not statistically significant.

5. 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 bioreactors, a great amount of chemicals is required.

There can be some human error in time tracking using a stopwatch because the data logging was set to be every five seconds and the rate of oxygen depletion and oxygen recovery in this experiment can be relatively fast. However, the error from 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.

When measuring OTR of a specific bioreactor using different types of DO sensors, it seemed that OTR based on optical DO sensor readings were slightly lower compared to OTR based on analog and digital ISM DO sensor readings. We would recommend following this comparison procedure against an analog sensor if using an alternative DO sensor in OTR measurements to ensure accuracy.

In summary, this short protocol describes the method for measuring and calculating OTR of a fermentation bioreactor under industry standard OTR measurement conditions using the sulfite depletion method. A fermentation bioreactor with a high OTR can effectively support robust microbial growth and desired product yield. Therefore, this short protocol can serve as a reference for the bioprocess professionals who are interested in OTR measurement of Eppendorf's large collection of fermentation bioreactors.

6. 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] Willard S. A Guide to Calibration on the BioFlo® 120 and BioFlo® 320: Dissolved Oxygen Sensors. Eppendorf Short Protocol No. 40. 2017.

Ordering information

Description	Order no.
BioFlo® 320, base control station	1379963011
Vessel Bundle, for BioFlo® 320, stainless-steel dished bottom, direct drive, 3 L	M1379-0301
Vessel Bundle, for BioFlo® 320, stainless-steel dished bottom, direct drive, 5 L	M1379-0302
DO sensor, Mettler Toledo® InPro 6830, angled T-82 connector, L 220 mm	P0720-6282
DO sensor, Mettler Toledo® InPro 6830, angled T-82 connector, L 320 mm	P0720-6283
DO sensor, Mettler Toledo® InPro 6850i, ISM®, L 320 mm	P0720-6654
DO sensor (Optical), Mettler Toledo® InPro 6860i, ISM®, L 320 mm	P0720-6661
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

Find out more about the BioFlo 320 bioreactor control station in our eshop at www.eppendorf.group/bioflo320

Your local distributor: www.eppendorf.com/contact
Eppendorf SE · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com/bioprocess