Chemical Engineering Science 152 (2016) 586–590

Contents lists available at ScienceDirect

Chemical Engineering Science

journal homepage: www.elsevier.com/locate/ces

Application of a new model based on oxygen balance to determine the oxygen uptake rate in mammalian cell chemostat cultures

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HIGHLIGHTS

- Use of a model based on total O_2 balance to estimate the OUR in mammalian cells.
- The estimation of the OUR is more accurate when the proposed model is used.
- OUR and q_{O2} values differ depending on the model used.
- In chemostat at 33 °C, O_2 transfer through the headspace must be considered.

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form
3 June 2016
Accepted 21 June 2016
Available online 23 June 2016

Keywords:
Mammalian cells
Mathematical model for OUR determination
Dynamic method
k_La

ABSTRACT

The application of dynamic methods for determining the oxygen uptake rate (OUR) in continuous mammalian cell cultures frequently ignores contributions to the oxygen balance such as the oxygen content in the culture medium inlet and outlet and the oxygen transfer between the culture and the headspace through the culture surface. We develop a mathematical model that allows OUR determination in mammalian cell chemostat cultures through a dynamic method, incorporating these neglected variables, as well as the application and validation of this model at two culture temperatures, 37 °C and 33 °C.

The proposed model was compared with a model that only includes the OUR term (typical model). The standard error of the proposed model was less than that of the typical model, making the proposed model more accurate. The results showed that at 37 °C, the results significantly differed depending on which model was used. At 33 and 37 °C, the specific oxygen uptake rate (q_{O2}) values obtained with the proposed model were within the range usually reported for mammalian cells. It was also shown that the OUR value was underestimated if the oxygen transfer through the headspace-medium interface was not considered under mild hypothermia conditions. The model could be applied for developing strategies based on OUR monitoring and controlling for recombinant protein production under conditions of mild hypothermia.

1. Introduction

In aerobic cultures, oxygen concentration is a key variable whose level depends on the solubility in the medium, oxygen transfer rate (OTR) and oxygen uptake rate (OUR). The OUR is a good indicator of cellular metabolism, allowing the quantification of the number of viable cells (Garcia-Ochoa et al., 2015; Hao et al., 2009) and it is essential to estimate the oxygen requirements of a culture as well as to scaling-up (Garcia-Ochoa et al., 2010). Different experimental procedures have been applied to measure the OUR in mammalian cell cultures (Garcia-Ochoa et al., 2010). One of the most used and least expensive procedures to measure the OUR

Abbreviations: (*s*) and (*b*) subscripts, Denote the medium-headspace air and medium-bubbles interfaces, respectively; C_L, Dissolved oxygen concentration in the culture medium; C_{L0}, Dissolved oxygen concentration in the feed; F, Volumetric flow of culture medium in the feed; D, Dilution rate; C^*, Equilibrium concentration of oxygen in the culture medium; k_La, Volumetric oxygen transfer coefficient in the liquid phase; q_{O2}, Specific oxygen uptake rate; T, Dead time of oxygen probe; $E(t)$, Signal sent by the oxygen electrode at time "t"; $y_{O2}b$, O_2 Molar composition in the gas fed through the diffuser; $y_{O2}S$, O_2 Molar composition in the gas fed through the headspace; DOT, (dissolved oxygen tension)

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http://dx.doi.org/10.1016/j.ces.2016.06.051
0009-2509/

in mammalian cell cultures has been the dynamic method of Humphrey, which is based on the mass balance of oxygen in the liquid phase (Fontova et al., 2009; Casas López et al., 2006). Considering that mammalian cells have low OUR values compared to bacterial cell cultures (Berrios et al., 2011), it is necessary to evaluate the oxygen mass balance more accurately than has commonly been done in order to reach an accurate estimation of the OUR in these cultures (Fontova et al., 2009; Casas López et al., 2006). Taking into account that the dynamic method has been widely applied in batch cultures of mammalian cells (Kirk and Szita, 2013; Lin et al., 2002; Singh, 1996) but has been scarcely used in continuous cultures (Lin et al., 2002; Ducommun et al., 2002; Heidemann et al., 1998), the aim of this work was the development and validation of a mathematical model to calculate the OUR in CHO cell chemostat cultures. This mathematical model had the particular characteristics of considering the oxygen contributions of the culture medium feed and outlet and the oxygen transfer between the headspace and the culture medium surface to the OUR estimation.

2. Materials and methods

2.1. Cell line and culture medium

A Chinese hamster ovary cell line TF 70R kindly provided by Torsten Björlig Pharmacia & Upjohn S.A., Sweden, was cultivated using HyClone serum-free culture medium supplemented with 20 mmol/L glucose; 5.5 mmol/L glutamate, 25 mmol/L HEPES (Sigma-Aldrich, USA), and 1.25 g/L of "Pluronic L-92".

2.2. Cell cultures

Two chemostat cultures were performed in a Biostat A Plus bioreactor (Sartorius Stedim Biotech S.A., France), one each at 33 and 37 °C, maintaining a working volume of 500 mL and a pH level of 6.9 (HEPES buffer, Sigma-Aldrich) in both cases. The bioreactor was inoculated and operated in batch mode for 48 h, and then it was supplied with a sterile feeding of fresh culture medium using a peristaltic pump ($D=0.014\ h^{-1}$). A gas mixture of air and pure oxygen (41% oxygen in the gas) was sparged directly into the medium with a steady, total gas flow rate of 10 mL/min. The bioreactor was agitated at 100 rpm, and the DOT was measured using a polarographic oxygen probe (Oxyferm FDA 160, Hamilton Bonaduz AG, Switzerland). The DOT was controlled at 30% of the air saturation by using an on/off automatic control system over the gas sparging valve. Steady-state was reached when the number of viable cells and the glucose concentration remained constant (< 10% variation) after at least 3–4 residence times under the conditions of the experiment. Is very important to say that the use of 33 °C in this investigation was fundamented in the fact that in our group have evidences regarding that the use the mild hypothermia conditions have a positive effect in the recombinant protein production (Vergara et al., 2014; Berrios et al., 2009).

2.3. Analytical procedures

The number of viable cells was determined by counting in a hemocytometer employing the Trypan Blue exclusion method. Glucose concentration was determined using a biochemical analyser (YSI 2700, Yellow Spring Instruments, USA).

2.4. Experimental determination of the input parameters of the model

2.4.1. Determination of the volumetric oxygen transfer coefficient from the headspace to the liquid phase ($k_La_{(s)}$)

The $k_La_{(s)}$ was determined at 33 °C and 37 °C. The culture medium (500 mL) was sparged with nitrogen in the bioreactor until the DOT reached a value of zero. The nitrogen flow was stopped, and was continued with the agitated (100 rpm) without air sparging, leaving surface aeration as the only oxygen source for the liquid phase. Using an oxygen electrode (polarographic sensor), the DOT was recorded as a function of time. The response time of an electrode (τ) can be described as a first-order equation (Badino et al., 2000), as follows:

$$\frac{dE}{dt} = k(C_L - E) \tag{1}$$

where k ($=1/\boldsymbol{\tau}$) is the electrode's sensitivity. Eq. (1) relates the probe signal (E) to the real dissolved oxygen concentration (C_L). Using a modified version of Singh's method (Singh, 1996) and considering Eq. (1), the $k_La_{(s)}$ values were calculated from Eq. (2):

$$E(t) = E(\infty)\cdot\left(\frac{1}{\tau\cdot k_La_{(s)}-1}\cdot e^{-k_La_{(s)}\cdot t} - \frac{\tau\cdot k_La_{(s)}}{\tau\cdot k_La_{(s)}-1}\cdot e^{-\frac{t}{\tau}}+1\right) \tag{2}$$

Data were fitted to Eq. (2) using the *Levenberg–Marquardt* algorithm (Jorjani and Ozturk, 1999).

2.4.2. Determination of $C^*_{(s)}$ and $C^*_{(b)}$

$C^*_{(b)}$ and $C^*_{(s)}$ were determined by employing Henry's Law (Eq. (3)).

$$y_{O_2}\cdot P_T = H\cdot C^* \tag{3}$$

A total pressure (P_T) equal to 101.3 kPa, $y_{O2}{}^b$ equal to 0.21 mol/mol and $y_{O2}{}^S$ equal to 0.387 mol/mol were considered. It was used a Henry's constant value for water, which required applying a correction factor (Fc=0.9) suggested by Takagi and Ueda (1994), thus correlating the saturation concentration in the culture medium ($C^*_{(b)}$ or $C^*_{(s)}$) with that of water ($C^*_{(b)\ H2O}$ or $C^*_{(s)\ H2O}$), as described in Eqs. (4) and (5).

$$C^*_{(b)} = C^*_{(b)_{H2O}}\cdot 0.9\left[\frac{mg}{L}\right] \tag{4}$$

$$C^*_{(s)} = C^*_{(s)_{H2O}}\cdot 0.9\left[\frac{mg}{L}\right] \tag{5}$$

2.4.3. Determination of DOT profiles

During the steady-states, the sparging was stopped and then restored, preventing the DOT whenever decreased below 10%. DOT values were continuously registered and stored (Kyung et al., 1994). The DOT data saved during the time in which oxygen sparging was interrupted were considered for calculating the OUR using the mathematical model described in Section 2.5.

2.5. Development of a mathematical model for the determination of the OUR

A schematic representation of the oxygen balance in a chemostat culture is presented in Fig. 1.

Fig. 1 shows the elements of the oxygen balance for the development of the mathematical model. A mass balance for oxygen was determined in the liquid phase, obtaining the following equation for the variation of dissolved oxygen concentration (C_L) over time:

$$\frac{dC_L}{dt} = D\cdot C_{Lo} + k_La_{(b)}\cdot\left(C^*_{(b)} - C_L\right) + k_La_{(s)}\cdot\left(C^*_{(s)} - C_L\right) - D\cdot C_L - OUR \tag{6}$$