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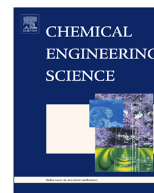

Specific oxygen uptake rate as indicator of cell response of *Rhodococcus erythropolis* cultures to shear effects

Felix Garcia-Ochoa*, Sebastian Escobar, Emilio Gomez

Department of Chemical Engineering. Faculty of Chemistry. Universidad Complutense de Madrid, 28040 Madrid, Spain

HIGHLIGHTS

- Hydrodynamic stress on *R. erythropolis* cultures in STBR is analysed.
- OTR and OUR and possible shear stress due to the fluid dynamic are jointly studied.
- A simple and fast method to determine favourable level of shear stress is proposed.
- This method is based on the specific oxygen uptake rate measurement.

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ABSTRACT

Specific oxygen uptake rate, q_{O_2}, on *Rhodococcus erythropolis* IGTS8 cultures has been measured under different fluid dynamic conditions in a STBR, changing stirrer speed, between 100 and 700 rpm, and air flow rate, between 1 and 10 L min^{-1}. Experimental results indicate that q_{O_2} values for *R. erythropolis* cultures can be influenced by the fluid dynamics inside the bioreactor. Under non oxygen-limited conditions, the oxygen consumption rate by the cells does not depend on the oxygen transfer rate; hence, the specific oxygen uptake rate is constant. However, when the stirrer speed is increased over a limit value, around 450 rpm in the culture studied, a change is clearly detected and, as higher stirrer speeds are used, specific oxygen uptake rate suffers a significant decrease. Nevertheless, when the hydrodynamics are varied changing the air flow rate, between 1 and 10 L min^{-1}, this variable does not affect the oxygen uptake rate. According to these results, specific oxygen uptake rate measurement provides a good indication of whole stress generated by the hydrodynamic conditions into the bioreactor. A simple method to determine changes into the cell response due to the fluid dynamic environment in the bioreactor is herein proposed. According to this method, shear effects on the culture can be detected by the change on specific oxygen uptake rate measured at laboratory scale, employing the usual gas in-gas out dynamic method. This assay combines oxygen transfer, oxygen uptake rate and possible cell damage in a one simple method that can considerably simplify and accelerate fundamental research on the development and scale-up of bioprocesses.

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1. Introduction

In aerobic bioprocesses, oxygen is a key substrate employed for cell growth, maintenance and other metabolic routes, including product synthesis. The dissolved oxygen (DO) concentration in the broth (a suspension of respiring cells) depends on the oxygen transfer rate (OTR) from the gas to the liquid phase and the oxygen uptake rate (OUR) by the cell culture. Both OTR and OUR have a decisive importance for bioreactor design, operation and scale-up, since in many processes oxygen transfer is the controlling step for cell growth and can affect the evolution with time of the bioprocess (Garcia-Ochoa and Gomez, 2009; Garcia-Ochoa et al., 2010; Ruffieux et al., 1998; Zou et al., 2009). OUR is one of the fundamental physiological characteristics of microorganism cultures, whose value changes during the course of the bioprocess due to the change in cell population.

On the other hand, OUR can also provide a considerable amount of information about the response of cells to the fluid dynamics into the bioreactor. Thus, OUR has been used for cell activity estimation, viable cell concentration and growth of cell cultures (Ganesh et al., 2006; Hao et al., 2009; Kamen et al., 1996; Palomares et al., 2004; Palomares and Ramirez, 1996; Schäfer et al., 2004).

* Corresponding author.
E-mail address: fgochoa@quim.ucm.es (F. Garcia-Ochoa).

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Therefore, in order to provide optimal environmental conditions for a successful bioprocess operation, a balance of several effects (mixing, mass transfer, substrate concentration and shear effects) needs to be achieved in order to obtain the maximal production and productivity of the desired products. When some variables of operation (such as stirrer speed or gas flow rate) are intensified with the aim of increasing the mixing and mass transfer, the hydrodynamic forces generated can affect the culture performance, especially in the case of plant or animal cells cultures, due to their relatively large size and the lack of a protective cell wall (Chisti, 2000, 2010; Nienow, 2006; Papoutsakis, 1991; Pohorecki et al., 2001). Bacteria, yeast and fungal mycelia are generally more robust and, therefore, not easily damaged under typical processing conditions. Nevertheless, there are classical references describing bacteria and yeast–fungi alterations by shear stress (Bronnenmeier and Markl, 1982; Merchuk, 1991), and even the most robust cells are damaged when subjected to intense shear fields and enough exposure time (Chisti and Moo-Young, 1986).

Shear effects can have many manifestations and different parameters have been employed as indicators of shear response of cells such as modification of morphology (Garcia-Ochoa et al., 2013; Peña et al., 2000), alteration of the metabolism and the productivity, which can affect the rates of growth, production and consumption of nutrients (Kao et al., 2007; Kunas and Papoutsakis, 1990; Meijer et al., 1994), the change of rheology for non-Newtonian broths (Rosa et al., 2005; Campesi et al., 2009) and loss of viability, necrosis and cell death (Al-Rubeai and Singht, 1998; Garcia-Ochoa et al., 2013; Hao et al., 2009; Sahoo et al., 2006; Wang et al., 1994). Although the previously cited stress indicators can be used to have an idea about the operating conditions that may damage or produce changes into the cell culture, it would be interesting to determine a critical value of some parameter or magnitude (stirrer speed, shear rate, shear stress, cumulative energy dissipated, etc.) causing a specified level of damage over a given exposure time or under certain operating conditions.

Despite the fact that hydrodynamic forces influence the behaviour of cells in suspension has been established long ago (Feugier et al., 2005; Garcia et al., 2005; Garcia et al., 2001; Han and Yuan, 2004; Hao et al., 2009; Kao et al., 2007; Legazpi et al., 2009; Merchuk, 1991; Yepez and Maugeri, 2005), there is a lack of proposals to describe this phenomenon together with the most appropriate measuring technique. However, it is an important challenge to integrate the physiological conditions of the cells and the hydrodynamics of the medium together for the development and scale-up of bioprocesses.

Rhodococcus erythropolis strain IGTS8 employed in biodesulfurization studies is a convenient experimental model to investigate the shear effects because the culture characteristics are well known (Etemadifar et al., 2008). In previous works (Olmo et al., 2005a; Olmo et al., 2005b), the growth rate and the biodesulfurization capacity of *R. erythropolis* IGTS8 for different media composition and under different operating conditions were studied, and a kinetic model was proposed. In a later work (Gomez et al., 2006) it was established that the growth rate and the percentage of biodesulfurization are affected by the OTR. A recent work (Garcia-Ochoa., 2013) has shown that an increment in the stirrer speed produces an increase in the growth rate and biodesulfurization capacity of the cells. However, when a critical value of stirrer speed is reached (400–450 rpm), cell damage caused by hydrodynamic stress in the turbulent bulk of the broth was observed together with a negative effect in the growth rate and the development of the biodesulfurization capacity. The mentioned varying operating conditions may change the physiological response of cells, such as the growth rate and the metabolism, and also the specific oxygen uptake rate. The study of the impact of an increase in power dissipation and oxygen transfer on physiological response and OUR is a hard task, and a lot of time and experimental work are necessary to assign the best conditions for *R. erythropolis* cultures.

The aim of this work is to propose a simple and fast method to determine a practical limit to check the effect on bioprocesses of the OTR, OUR and the possible cell damage generated using the measurement of the specific oxygen uptake rate measured by the gas in-gas out dynamic method in a broth. Bacterial cultures of *R. erythropolis* IGTS8 under different operating conditions, in a sparged and stirred tank bioreactor, have been used for this proposal. Thus, the identification of a level of shear admissible by the cells, based on the changes on the specific oxygen uptake rate values, can be achieved.

2. Theoretical background

Oxygen transport and oxygen uptake are two phenomena coupled in series. Therefore, the OTR value can affect the OUR values if OTR is the limiting step of the overall process rate. In sparged and stirred tank bioreactors (STBR) OTR depends mainly on stirrer speed and gas flow rate (together with other conditions such as broth properties, vessel geometry, etc.). The changes in hydrodynamic conditions (stirrer speed and gas flow rate) will provoke a change in OTR values, and perhaps in OUR values. Additionally, the hydrodynamic change can provoke some hydrodynamic stress or cell damage. The fluid dynamics complexity arises from the turbulence generated by both the stirrer speed and the gas flow. To understand both of these phenomena it is necessary to describe them separately and take into account that oxygen limitation (when OTR is the limiting step of the overall process rate) and hydrodynamic stress can have similar effects at a macroscopic level.

2.1. Oxygen transfer and uptake rates

Oxygen transfer rate depends on the mass transfer coefficient, k_L, the gas–liquid interfacial area, a, and the gradient between the concentration of the oxygen at the interface and that in the bulk liquid, according to

$$\mathrm{OTR} = k_L a \cdot (C^*_{O_2} - C_{O_2}) \tag{1}$$

being $C^*_{O_2}$ the equilibrium concentration in the liquid phase.

The maximum oxygen transfer rate from gas to liquid is achieved when the dissolved oxygen concentration in the bulk liquid, C_{O_2}, is zero, being obtained as follows:

$$\mathrm{OTR}_{\mathrm{max}} = k_L a \cdot C^*_{O_2} \tag{2}$$

OUR can be related both to biomass concentration in the broth (oxygen necessary for biomass maintenance) and biomass production rate (oxygen necessary for growth) (Garcia-Ochoa et al., 2000; Pinches and Pallent, 1986), as follows:

$$\mathrm{OUR} = q_{O_2} \cdot C_X = m_{O_2} \cdot C_X + Y_{\mathrm{OX}} \cdot \frac{dC_X}{dt} \tag{3}$$

where m_{O_2} is the oxygen consumption coefficient for maintenance and Y_{OX} is the yield of oxygen consumed for cell growth.

The growth kinetics can be described by a simple kinetic model, assuming a first order growth kinetics respect to the limiting substrate concentration nutrient and zero order respect to oxygen concentration, according to the following equation usually used (Luedeking and Piret, 1959; Weiss and Ollis, 1980;

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