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Review

Oxygen uptake rate in microbial processes: An overview

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ABSTRACT

In aerobic process oxygen must be continuously supplied in order to achieve acceptable productivities, Since the role of oxygen in microorganism growth and its metabolism is of vital importance, both the oxygen consumption by the cell and the oxygen transfer rate (OTR) into the system have to be understood.

The main function of a properly designed bioreactor is to provide a controlled environment and a concentration of nutrients (dissolved oxygen, mainly) sufficient to achieve optimal growth and/or optimal product formation in a particular bioprocess. Dissolved oxygen in the broths is the result of a balance of its consumption rate in the cells, and the rate of oxygen transfer from the gas to the liquid phase. Monitoring dissolved oxygen in the broth is mandatory because often oxygen becomes the factor governing the metabolic pathways in microbial cells.

In this work the oxygen uptake rate (OUR) in different fermentation broths is examined. Experimental techniques have been compiled from the literature and their applicability to microbial processes reviewed. The reciprocal influence of OUR and OTR is presented and an analysis of rate-limiting variables is carried out.

Mathematical models are a fundamental tool in bioprocess design, optimisation, scale-up, operation and control at large-scale fermentation. Kinetic models describing aerobic bioprocesses have to include an oxygen balance taking into account OTR and OUR. Many different specific rate expressions for cell growth, substrate consumption, product formation and oxygen uptake have been developed and incorporated in the models, and simulations of different bioprocess have been carried out. Some of them are presented here.

Contents

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Nomenclature

a	specific interfacial area (m^{-1})
C_j	concentration of compound j ($kg\,m^{-3}$ or $mol\,m^{-3}$)
C_X	cell concentration ($kg\,m^{-3}$)
Da	Damköhler number
D_i	oxygen diffusion coefficient in layer i ($m^2\,s^{-1}$)
DO	dissolved oxygen
d_b	bubble diameter (m)
E	biological enhancement factor
H	Henry constant ($mol\,m^{-3}\,atm^{-1}$)
Ha	Hatta number
J	flux density molar ($mol\,O_2\,m^{-2}\,s^{-1}$)
K	consistency index in a power-law model ($Pa\,s^n$)
k_L	mass transfer coefficient ($m\,s^{-1}$)
K_La	volumetric oxygen mass transfer coefficient in presence bio-transformation (s^{-1})
k_La	volumetric oxygen mass transfer coefficient in cell free medium (s^{-1})
m_{O_2}	dissolved oxygen consumption coefficient for maintenance ($mol\,O_2\,kg^{-1}\,X\,s^{-1}$)
N	stirrer speed (s^{-1} or rpm)
n	Flow index in a power-law model
OTR	oxygen transfer rate ($mol\,O_2\,m^{-3}\,s^{-1}$)
OUR	oxygen uptake rate ($mol\,O_2\,m^{-3}\,s^{-1}$)
Q	gas flow rate ($m^3\,s^{-1}$)
q_{O_2}	specific oxygen uptake rate ($mol\,O_2\,kg^{-1}\,s^{-1}$)
t	time (s or h)
t_e	exposure time (s)
V	volume of the liquid in the vessel (m^3)
V_S	superficial gas velocity ($m\,s^{-1}$)
X_{BDS}	percentage of desulphurization (% 2-hydroxybifenyl)
Y_G	macroscopic yield on the substrate ($kg\,X\,kg^{-1}\,S$)
Y_{ij}	macroscopic yield of compound i into compound j ($kgi\,kg^{-1}j$)
Y_{XO}	biomass yield on oxygen ($kg\,X\,mol^{-1}\,O_2$)
Y'_{XO}	overall biomass yield on oxygen ($kg\,X\,mol^{-1}\,O_2$)
z	film thickness or distance from the gas–liquid interface (m)

Greek letters

α	parameter for growth associated product formation
β	parameter for non-growth associated product formation
ε	energy dissipation rate ($W\,kg^{-1}$)
η	effectiveness factor
ϕ	gas hold-up
μ	specific culture growth rate (h^{-1}); viscosity (Pa s)
ν	generalised degree of reduction
ρ	density ($kg\,m^{-3}$)
σ	interfacial tension ($N\,m^{-1}$)

Subindexes

BDS	referred to biodesulphurization
d	relative to dynamic measurement
G	relative to gas phase
L	relative to liquid phase
m	relative to cell monolayer
max	relative to maximum value
O_2	relative to oxygen
p	relative to process measurement
S	relative to substrate
s	relative to surfactant
X	relative to biomass

Superindexes

c	relative to the intermediate
in	relative to inlet
out	relative to outlet
*	relative to equilibrium value in each phase
o	cell free medium

1. Introduction

In aerobic bioprocesses, oxygen is a key substrate employed for growth, maintenance and in other metabolic routes, including product synthesis. Due to its low solubility in broths, which are usually aqueous solutions, oxygen must be continuously provided by a gas phase, and thus the knowledge of oxygen transfer rate (OTR) is needed for bioreactor design and scale-up. The concentration of dissolved oxygen in the broth, a suspension of respiring microorganisms, depends on the OTR from the gas to the liquid phase, and on the rate of its consumption by the microorganism, the oxygen uptake rate (OUR).

Both oxygen mass transfer from the gas to the liquid phase and its consumption by microorganism has a decisive importance, since in many processes oxygen transfer is the controlling step for the microbial growth, and can affect the evolution of bioprocesses. This fact has been identified and reviewed early in the development of biochemical engineering [1–3] and work in this matter has been continued till these days [4–11].

Oxygen transfer rate is not a privative characteristic of bioreactors, but rather belongs to classical chemical engineering. For pneumatically agitated reactors one of the first correlations can be found in the book of Sherwood and Pigford [12] and those have been improved over the years to include more detailed influence of fluid properties and equipment geometry on k_La; the correlation by Akita and Yoshida [13] remains as a reference. For stirred tank reactors, the early work was directed towards expressions the ties between power input, liquid properties and geometric characteristics [14,15]. Extensive literature on the oxygen transfer rate in bioreactors is nowadays available and a considerable part of it has been published in the last years [6,8,16–26].

The OTR in a bioreactor depends on the liquid side mass transfer coefficient, k_L, the total specific surface area available for mass transfer, a, and the driving force in terms of concentrations. Since the two parameters, k_L and a, can not be measured easily individually, they are usually lumped together as one single parameter, called volumetric mass transfer coefficient, k_La. The available information on k_La in bioreactors is extensive. Many empirical correlations have been proposed for k_La estimation; and there are several reviews on this subject [17,21,27,28]. There are also models based on fundamental approaches, using concepts based on surface renewal models, where the parameters needed for those models are based on fluid-dynamic considerations in the bioreactor [28–31].

The oxygen uptake rate is one of the fundamental physiological characteristics of culture growth and has been used for optimising the fermentation process [32,33]. Usually the specific oxygen uptake rate, q_{O_2}, is calculated from OUR which is determined experimentally. OUR measurement has recently received the due attention in different bioprocess studies, such as in production of xanthan gum [34], toluene hydroxylation [35], bio-insecticides [36], xylitol production [37], benzaldehyde lyase production [7] and also in biodesulphurization processes [38–40]. The OUR monitoring is also important for assessment of the viability of the culture [41–43].

Pirt [44] found that the actual substrate consumption rate can be expressed as the sum of two terms: one representing the theoretical rate of substrate consumption for biomass synthesis and one showing the rate of substrate consumption for maintenance of the culture [45,46]. This implies the definition of two parameters: the theoretical yield, and the maintenance rate. Some of the works previously quoted model the OUR using these pioneer concepts [5,7,36,39,40,47–51], but only a few works present a model of the evolution of DO concentration along the bioprocess [38–40,50,52].

OUR is consecutive to OTR, and the slower step is that controlling the overall rate. However, the consumption of oxygen can affect OTR. In biological systems, where gas (oxygen) absorption is followed by a biochemical reaction, two steps can control the overall rate: the mass transfer of gas to liquid phase and the biochemical reaction in the cells. Sometimes, the transport of substrates into the microorganism occurs at a rate which is considerably faster than the rate of the biochemical reactions inside. However, if the biochemical reaction phenomenon is faster than the mass transfer, the concentration profile of the absorbed gas may be affected by the biochemical reaction, and the absorption rate into the pseudo-homogeneous liquid be enhanced because oxygen is consumed while it diffuses into the liquid. The extent of this enhancement can be derived from different theories for mass transfer; the film model is the most used because its simplicity [31,53–55].

This interaction of OTR and OUR should be included in the modelling of aerobic microbial processes, which implies a mathematical description based on physical–chemical principles. The model allows making predictions and gaining insights into the underlying phenomenon. The oxygen available in the broth is fundamental in the microbial process if the biocatalyst used is a strict

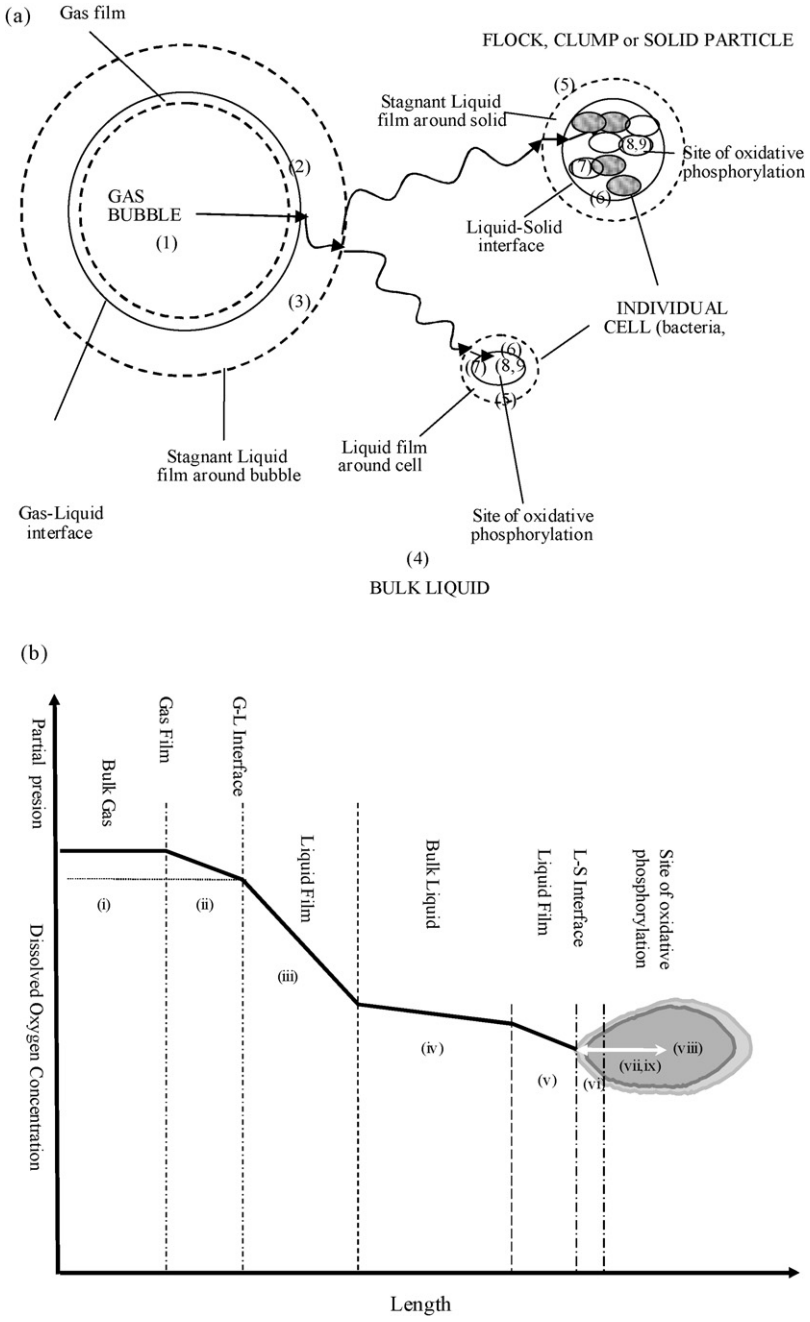


Fig. 1. (a) Steps of oxygen transfer from gas bubble to cell (adapted from Blanch and Clark [57]). (b) Oxygen concentration profile from gas bubble to cell.

aerobic microorganism. Therefore, the kinetic model that describes the growth of the microorganism, the nutrients consumption and the product formation has to consider the influence of the DO concentration on the bioprocess [5,39,40,56].

The aim of this work is to examine the oxygen uptake rate (OUR) in several systems taking into account different experimental techniques and to model those systems involving oxygen consumption for maintenance, cell growth and product formation, presenting an adequate description of the actual oxygen concentration profiles. The information collected in the present work will be useful for a better understanding of the importance of DO concentration in aerobic microbial processes and of the influence of biochemical reactions on oxygen transfer phenomena (and *vice versa*), as well as for finding better operation conditions for oxygen transfer and for scale-up in bioreactors.

2. Phenomenology of aerobic bioprocesses

Bioreactors are heterogeneous, gas–liquid–solid systems and oxygen is one of the most important substrates in aerobic bioprocesses. It is essential to study the influence of the gas–liquid transport in bioreactors, and this is true not only in aerobic systems, where oxygen transport is obviously focussed, but also in the case of anaerobic systems, where usually the transport of other gases such as methane or carbon dioxide takes place. Transfer of oxygen from a gas bubble to a cell can be represented by the following steps (as schematised in Fig. 1a and b) [57]:

(i) transfer from the interior of the bubble to the gas–liquid interface;
(ii) movement across the gas–liquid interface;
(iii) diffusion through the relatively stagnant liquid film surrounding the bubble;
(iv) transport through the bulk liquid;
(v) diffusion through the relatively stagnant liquid film surrounding the cells;
(vi) movement across the liquid–cell interface; if the cells are in a flock, clump or solid particle, diffusion through the solid to the individual cell;
(vii) transport through the cytoplasm till the site where the reactions take place;
(viii) biochemical reactions involving oxygen consumption and production of CO_2 or other gases;
(ix) transfer of the produced gases in the reverse direction.

Steps (i)–(vii) and (ix) correspond to physical phenomena, in principle better known and easier to describe than the biochemical phenomena.

Vigorous agitation is required to insure homogeneous distribution of the nutrients. In shake flasks scale, oxygen transport by aeration and agitation are accomplished by the rotary or reciprocating action of the shaker apparatus. In laboratory and pilot scale, oxygen is generally supplied as compressed air and distributed by a gas distributor, and mechanical devices are used to improve mixing of the broth, for better distribution of gas bubbles and to rupture large bubbles into small ones obtaining a larger interfacial area. The oxygen is transferred from a suspended gas bubble into a liquid phase, where it is taken up by the microorganism and finally transported to the site of reaction inside the cell. The power input, which generates the mixing, is however restricted by physical, biological and mechanical constrains, which become more severe as the scale increases. The cells may be damaged by hydrodynamic stress when agitation and/or aeration are too vigorous. Typical aerobic large-scale cultures, 10,000 L or greater, are oxygen limited. In this case gas–liquid interfacial area and bubble residence time are the limiting steps in the process of oxygen delivery to the microor-

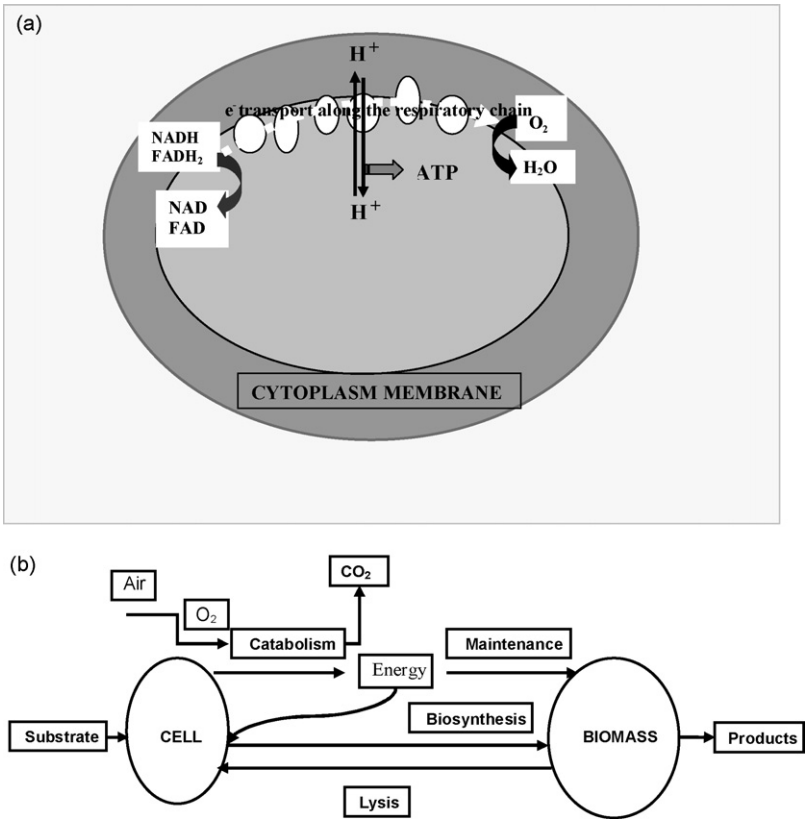
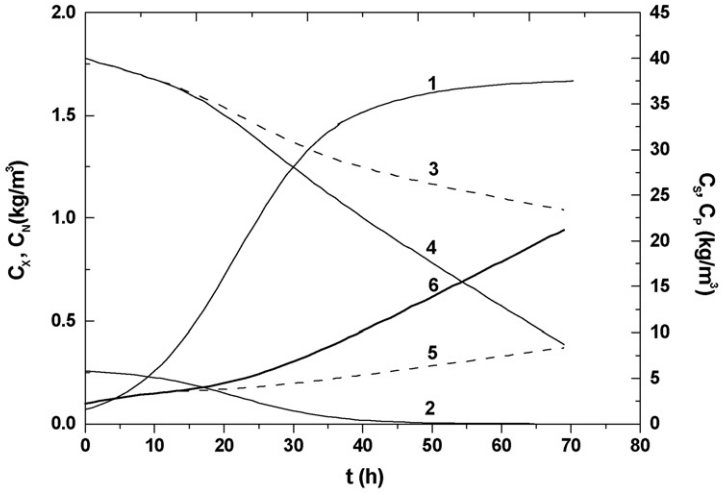


Fig. 2. (a) Pathway of chain respiratory aerobic bacteria. (b) A schematic of the biological reactions expected to occur in an aerobic culture.



Concentration	Biomass (C_X)	Nitrogen (C_N)	Sucrose (C_S)		Xanthan gum (C_P)	
DO (% sat)	Any	Any	10	20	10	20
Key	1	2	3	4	5	6

Fig. 3. Simulation of microbial growth curve for *Xanthomonas campestris* culture in batch operation for two constant values of dissolved oxygen concentration (10 and 20% saturation) (adapted from Garcia-Ochoa et al. [5]).

ganisms. The classic example of large-scale aerobic bioprocess is the production of penicillin by a specific mould, where commercial vessel sizes from 40 to 200 m^3 are used. The operation is semibatch, that is, the nutrient, lactose or glucose, is fed at controlled rates to an initial batch of liquid nutrients and cell mass. Reaction time is 5–6 days. Since oxygen is essential for growth, DO concentration must be kept at a high level for the organism to survive, and this is accomplished by continuous aeration of culture. Aeration also serves to agitate the mixture and to sweep out the CO_2 and any noxious volatile by-products that are formed. Air supply is in the range of 0.5–1.5 vvm. Mechanical agitation is needed to break up the gas bubbles but must avoid rupturing the cells. Power input by agitation and air sparging is 1–4 W L^{-1}. An increase of the power input could substantially improve the OTR, but would be detrimental for the process as a whole because the higher fluid-dynamic forces generated could damage the microorganisms. These effects on filamentous microorganisms have been indicated early on [58].

In aerobic processes the energy is generated by substrate oxidation. A simple scheme is presented in Fig. 2a; the energy is used for the generation of ATP, which is employed in production of new biomass, product formation and in other functions that are not directly related to growth, commonly referred to as maintenance activities. This includes re-synthesis of damaged cellular material, provision of the necessary concentration gradients across the cell membrane, cell motility and other non-growth related processes (Fig. 2b).

Simulations of typical batch microbial culture carried out with a metabolic kinetic model [5] and varying DO concentrations are shown in Fig. 3. Biomass growth, substrate (carbon and nitrogen sources) consumption and product (xanthan) formation patterns are shown. The growth patterns show a slow initial phase (lag phase) followed by a fast exponential phase (log phase), and then a stationary phase where no growth occurs due to nutrient limitations. A shortened lag phase is desirable for more economical large-scale cultures.

A kinetic model that describes the growth of the microorganism has to consider the influence of the dissolved oxygen in the broth in the set of differential equations representing the kinetic model of the process. This requires knowledge of the oxygen transport rate and of the oxygen consumption rate by the microorganisms, expressed as the volumetric mass transfer coefficient (k_La) and the specific oxygen consumption (q_{O_2}).

3. Oxygen uptake rate

Given the low solubility of oxygen in aqueous solutions, DO in the broth can be the limiting nutrient. Pinches and Pallent [47] described the fall of DO concentration in broths due to the high demand during fast growth, until the microorganism reaches the stationary phase and the DO concentration increases, because demand becomes smaller. This evolution fits the behaviour of most microorganisms cultures available in literature, such as *Xanthomonas campestris* [34], *Escherichia coli* [59], *Rhodococcus erythropolis* IGTS8 [60].

However, in some cultures, oxygen demand is so high that the DO concentration decreases until it approaches zero, and does not increase even when the culture reaches the stationary phase, in spite of improvement in oxygen transfer rate (by increasing gas flow rate, stirrer speed or the oxygen concentration in gas phase). *Pseudomonas putida* for example has a very high OUR and the DO concentration in the broth drops to 5% of the oxygen saturation value before 2 h of growth [39]. *X. campestris* has a slower OUR and reaches a 5% of oxygen saturation only after 10 h of growth [5]. In the first case, on *P. putida* growth, DO concentration is practically equal to zero till the end of the production of biodesulphurization enzymes [39]. In the second case, *X. campestris* cultures for the production of xanthan gum, it is necessary to increase oxygen transfer rate, and to achieve this stirrer speed and/or gas flow are increased during the bioprocess [5].

The specific oxygen uptake rate (q_{O_2}) is characteristic for each microorganism and is usually considered constant during the microbial growth, although experimental results are not in agreement with this assumption [5]. A typical evolution of both OUR and q_{O_2} as well as a typical oxygen concentration profile during fermentation is shown in Fig. 4. OUR increases in the exponential growth phase, because in this step a high substrate consumption rate takes place; after that OUR decreases because of the decreasing in metabolic activity of cells. On the other hand, a decrease of

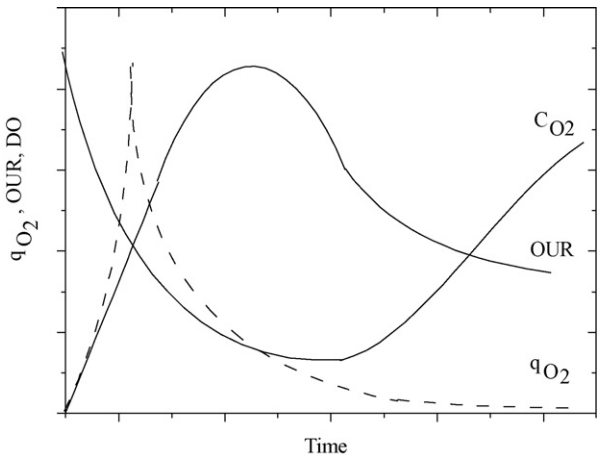


Fig. 4. Typical evolution of oxygen uptake rate (OUR), specific oxygen uptake rate (q_{O_2}) and DO concentration (C_{O_2}) in time course of fermentation.

the DO concentration is often observed in the first moments of the bioprocess due to the high specific oxygen demand by the cells at the beginning, in the lag phase [38–40]; after reaching a minimum, oxygen concentration profile gradually increases until the end of the growth and/or the production process.

OUR can be easily measured, giving important information about the metabolic activity of the cells in the culture. It has been employed for on-line estimation of viable cell concentration and growth phase of insect and mammalian cell cultures [32,61], for characterization of the organic composition in waste treatments [41,43] or its activity [62–64]; and it can be used as an indicator to adjust the amino acids feeding in animal cell culture [65]. Recently OUR has been proposed as a factor indicating the possible hydrodynamic stress, cell damage and cell death [66,67].

3.1. Experimental determination of OUR values

The transport of oxygen and its consumption have usually not been described together, and have been often measured by different methods in the past. Nowadays it is usual to obtain both experimental values using the same technique, i.e. simultaneous determination of both OUR and OTR (or K_La) in the same experiment.

A number of methods have been developed to calculate OUR in cultures. The main experimental techniques employed to measure oxygen uptake rate are:

(i) Mass balance by inlet and outlet gas phase oxygen concentration measurement.
(ii) The dynamic technique.
(iii) Techniques based on the yield coefficient method.
(iv) From the oxygen concentration profile data, knowing OTR.

The mass balance for the dissolved oxygen in the assumed well-mixed liquid phase can be written as

$$\frac{dC}{dt} = K_La \cdot (C^* - C) - q_{O_2} \cdot C_X \quad (1)$$

where dC/dt is the accumulation of oxygen in the liquid phase, the first term on the right hand side is the oxygen transfer rate (OTR) and the second term is the oxygen uptake rate (OUR). This last term can be expresed by the product $q_{O_2} \cdot C_X$.

3.1.1. Gas balancing method

This method uses a gaseous oxygen analyzer to measure the oxygen concentration of the gas streams entering and leaving the bioreactor. This technique is the most reliable and accurate, but also requires very accurate instruments. The OTR can be determined from gas oxygen mass balance on the bioreactor as follows:

$$OTR = \frac{Q}{V} \cdot \left(C_{O_2}^{in} - C_{O_2}^{out}\right) \quad (2)$$

where Q is the oxygen gas flow, V the volume of bioreactor, and C^{in} and C^{out} the oxygen concentration measured at bioreactor inlet and outlet, and well-mixing of the gas phase has been assumed. The OUR is then calculated from:

$$\text{OUR} = \text{OTR} - \text{Accumulation} \quad (3)$$

$$\text{OUR} = \frac{Q}{V} \cdot (C_{O_2}^{in} - C_{O_2}^{out}) - \frac{\Delta C_L}{\Delta t} \quad (4)$$

where it is assumed that OUR, and therefore the metabolic state of the culture, remains constant along the Δt considered. A sample is withdrawn from the vessel to determine biomass concentration (this is common to all methods). Therefore, this method requires a gaseous oxygen analyzer to measure the oxygen content of the inlet and outlet gas streams and a probe for measuring the DO concentration in the liquid. This method may not be the best choice in case of small bioreactors, where the difference between and C^{in} and C^{out} may be very small because of the short contact time.

3.1.2. Dynamic method

The dynamic method is based on the respiratory activity of organisms which are actively growing inside the bioreactor: the airflow inlet to the fermentation broth is interrupted for a few minutes (few, in order to avoid influences on process evolution), and a decrease of DO concentration is observed, which can be recorded by an oxygen probe. Afterwards, air is reintroduced under the same previous operational conditions, thus ensuring the same oxygen transfer rate [68]. The OUR is determined from the depletion in the DO concentration after stopping the air flow, and the procedure can be repeated several times during the production process. Under these conditions, the Eq. (1) can be simplified to:

$$\left(\frac{dC}{dt}\right)_d = -q_{O_2} \cdot C_X = \text{OUR}_d \quad (5)$$

obtaining OUR_d from the slope of the plot of DO concentration versus time after stopping air flow. Biomass concentration must be known in this point in order to relate the OUR_d calculated to biomass concentration. The underlying assumption is that no interchange of oxygen between gas and liquid occurs during the test period, and that the change in fluid dynamics associated to stopping the gas circulation does not affect the OUR. When the aeration is turned on again, the DO concentration increase until it reaches a steady-state concentration. In this conditions both the oxygen transfer and oxygen uptake rate terms apply. The slope of the response curve at a given point is measured to get dC/dt, and Eq. (1) can be solved for K_La because all the other values are known [5]. For the application of this method the response time of the oxygen electrode must be considerably lower than the characteristic time for the mass transfer process. If the response time of the electrode is not negligible, it can be taken into account in the modelling and in the elaboration of the experimental data. A profile of dissolved oxygen concentration from Eq. (1) during a cycle of turning aeration off and on is shown in Fig. 5.

A rapid review of the recent literature shows that, the dynamic method is still the most commonly used in the determination of OUR, due to its simplicity and reproducibility [5,7,9,10,36,43,65]. However, this method can be difficult to apply to certain situations in which OUR can not be determined correctly when the gas supply is turned off, because when the DO concentration is very low, the changes are difficult to evaluate. In this case a modified dynamic method can be used [39,69]. The modification consists in using a

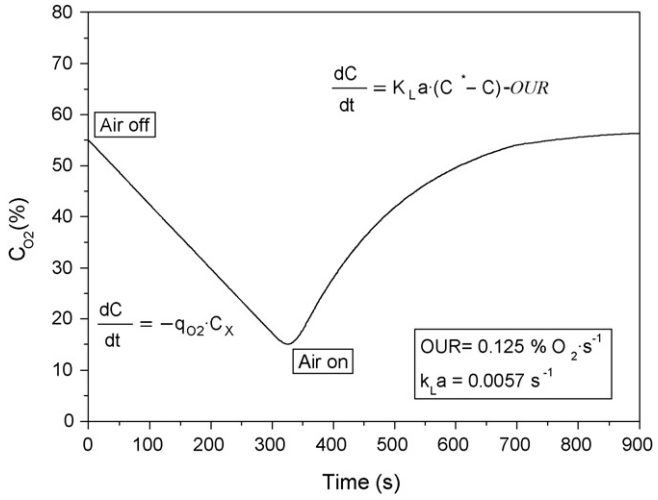


Fig. 5. Simulation of response of DO for dynamic measurement of OUR and $K_L a$ during fermentation.

step change of air to pure oxygen in the inlet gas stream or *vice versa*. The evolution of DO concentration in the transition between two pseudo-steady states allows the OUR and $K_L a$ calculations.

The integration of Eq. (1) in oxygen absorption process with the boundary conditions: $t = 0 \therefore C^* = C_0^*$ and $C = C_{L_0}$; $t = t_1 \therefore C = C_L$, yields the following equation:

$$C_L = \left(C_0^* - \frac{OUR}{K_L a}\right) - \left(C_0^* - C_{L_0} - \frac{OUR}{K_L a}\right) \cdot e^{-K_L a \cdot t} \quad (6)$$

while, if the desorption mode is applied, the integration of Eq. (1), now with the boundary conditions: $t = t_1 \therefore C^* = C_1^*$ and $C = C_{L_1}$; $t = t_2 \therefore C = C_L$, results in:

$$C_L = \left(C_1^* - \frac{OUR}{K_L a}\right) + \left(C_{L_1} - C_1^* + \frac{OUR}{K_L a}\right) \cdot e^{-K_L a \cdot t} \quad (7)$$

Eqs. (6) and (7) describe the evolution in time of the DO concentration in the liquid phase from a starting concentration, C_{L_0} or C_{L_1}, after changing from air to pure oxygen or from oxygen pure to air. According to the above procedure, OUR and $K_L a$ values can be determined during the bioprocess, at several cellular ages, by fitting of the above equations to the experimental data using non-linear regression techniques.

Several modifications of the dynamic method have been proposed, some of which stand out because their ingenuity. Thus, Linek et al. [70] proposed a dynamic pressure method for $k_L a$ measurement, where the step in oxygen concentration is replaced by a step in the total pressure. Mignone [71] proposed to make rather an agitation-step for $k_L a$ measurement. In both cases the oxygen concentration is the variable followed and analysed, in a manner similar to that given above.

3.1.3. Yield coefficient method

This technique was found to be a reliable and quite satisfactory method for estimating the oxygen uptake rate by microorganisms during bioprocesses. It is based on the oxygen uptake rate of the organism rather than the rate of depletion of oxygen in the gas or liquid phase. By using a stoichiometric balance of oxygen in the biomass together with the kinetic model for the growth rate, the following relationship during growth can be obtained:

$$OUR = \frac{\mu \cdot C_X}{Y'_{XO}} \quad (8)$$

where μ is the specific growth rate of the organism, and Y'_{XO} presents the overall yield of cell on oxygen.

This method for oxygen uptake rate determination is very simple; however, Eq. (8) is based on the assumption that substrate is completely converted into carbon dioxide, water and biomass, and this can be in doubt in many cases.

Initially the overall yield was introduced as a constant. However, cultivations of microorganism under growth rates much lower than μ_{max} showed that it was dependent on the specific growth rate. This is explained in terms of the endogenous respiration, or maintenance, concept [72,73]. In this concept it is assumed that maintenance of cellular function requires the availability of a flow of Gibbs energy for restoring leaky gradients, preservation of the optimal intracellular pH, the replacement of denatured proteins, ATP consumption in futile cycles, cellular mobility, etc.

The relationship between Y'_{XO} and μ can be established as

$$\frac{1}{Y'_{XO}} = \frac{1}{\mu} m_{O_2} + \frac{1}{Y_{XO}} \quad (9)$$

where m_{O_2} is the oxygen consumption coefficient for maintenance and Y_{XO} the yield of oxygen consumed for cell growth. If both, m_{O_2} and Y_{XO}, are practically constant, a plot of $1/Y'_{XO}$ versus $1/\mu$ is linear; from the slope, m_{O_2} and from the intercept, Y_{XO} could be determined. Note that for an assumed constant value of m_{O_2}, Eq. (9) predicts that any variation in μ will affect observed values of the overall growth yield.

On the other hand, Y'_{XO} can be estimated from experimental data of growth and volumetric oxygen mass transfer. Assuming that Y'_{XO} is constant for a short time interval between time t_1 to t_2, by integrating and rearranging Eq. (1) and inserting in Eq. (8), the following relationship is obtained [49]:

$$Y'_{XO} = \frac{\mu \left(C_{X_2} - C_{X_1}\right)}{\int_{t_1}^{t_2} K_L a (C^* - C)\, dt + (C_1 - C_2)} \quad (10)$$

If the specific growth rate, μ, and the volumetric mass transfer coefficient, $K_L a$, are known, Eq. (10) can be numerically solved for each time interval, calculating Y'_{XO} values.

Heijnen and Roels [74] showed that a macroscopic analysis based on elemental and enthalpy balances successfully describes the relationship between biomass yield on oxygen, according to:

$$\frac{1}{Y_{XO}} = \frac{\nu_S}{\nu_S^c} \cdot \frac{1}{Y_{XO}^c} + \frac{\nu_S - \nu_S^c}{4} \cdot \frac{\nu_X}{\nu_S^c} \therefore m_{O_2} = \frac{\nu_S}{\nu_S^c} \cdot m_{O_2}^c \quad \nu_S > \nu_S^c \quad (11)$$

$$\frac{1}{Y_{XO}} = \frac{1}{Y_{XO}^c} \therefore m_{O_2} = m_{O_2}^c \quad \nu_S \le \nu_S^c \quad (12)$$

where ν_S and ν_X are generalised degrees of reduction of substrate and of biomass, respectively, ν_S^c is the degree of reduction of an intermediate which is coupled to ATP generation, and Y_{XO}^c and $m_{O_2}^c$ are the yield of biomass and maintenance on oxygen which are used in oxidations reactions coupled to energy generation.

The model agrees with data available in literature and the most probable parameters values ν_S^c and Y_{XO}^c are 4.0 and 2, respectively. The yield coefficients of biomass on oxygen (Y_{XO}) (and its corresponding storage products, Y_{PO}), which is a stoichiometric constant, is not temperature dependent, assuming that the molecular composition does not change with temperature. This is in contrast with the maintenance coefficient; Heijnen and Roels [74] estimated a value for the activation energy of 9000 kcal mol^{-1} from the available data on maintenance requirements of a great variety of microorganisms.

3.1.4. OUR determination from oxygen concentration profile data knowing OTR

When oxygen transfer rate is known, either from an empirical equation or using a predictive model and the oxygen concentration

profile in the liquid phase measured during the course of fermentation, oxygen uptake rate during the process can be obtained from Eq. (1), as

$$\mathrm{OUR_p} = K_L a \cdot (C^*_{O_2} - C_{O_2}) - \left(\frac{dC_{O_2}}{dt}\right)_p \quad (13)$$

Therefore, experimental $\mathrm{OUR_p}$ values can be calculated from OTR (determined from volumetric mass transfer coefficient values and the oxygen concentration profile) and the values of the derivative of oxygen concentration versus time curve [40,43,75].

3.1.5. Comparison of OUR values determined by the dynamic method and from the oxygen profile data

Oxygen uptake rate is commonly measured by the dynamic method; however, in some cases the use of a non-aerated period may interfere on microbial growth and lead to underestimation of the OUR value. When OUR is measured by the 'dynamic method' and modelled as above indicated, some differences in results have been observed, because Y_{OX} values determined by the classical dynamic method are different from those obtained by fitting a metabolic kinetic model to experimental values of oxygen concentration versus time. Santos et al. [40] have demonstrated in a biodesulphurization microbiological system, that when the dynamic technique is employed to measure OUR the oxygen consumption decreases, which can explain the differences observed. Fig. 6 compares experimental OUR values obtained from the oxygen concentration profile data and those obtained using the dynamic technique for two bioprocess, *Xanthomonas campestris* and *Rhodococcus erythropolis* IGTS8 cultures. As it can be seen, experimental OUR values obtained from the DO concentration profile are higher than those obtained by the dynamic method. This discrepancy could be explained using the "cellular economy principle": during the time that oxygen is not transferred, microorganism cells consume oxygen at a lower rate [76,77].

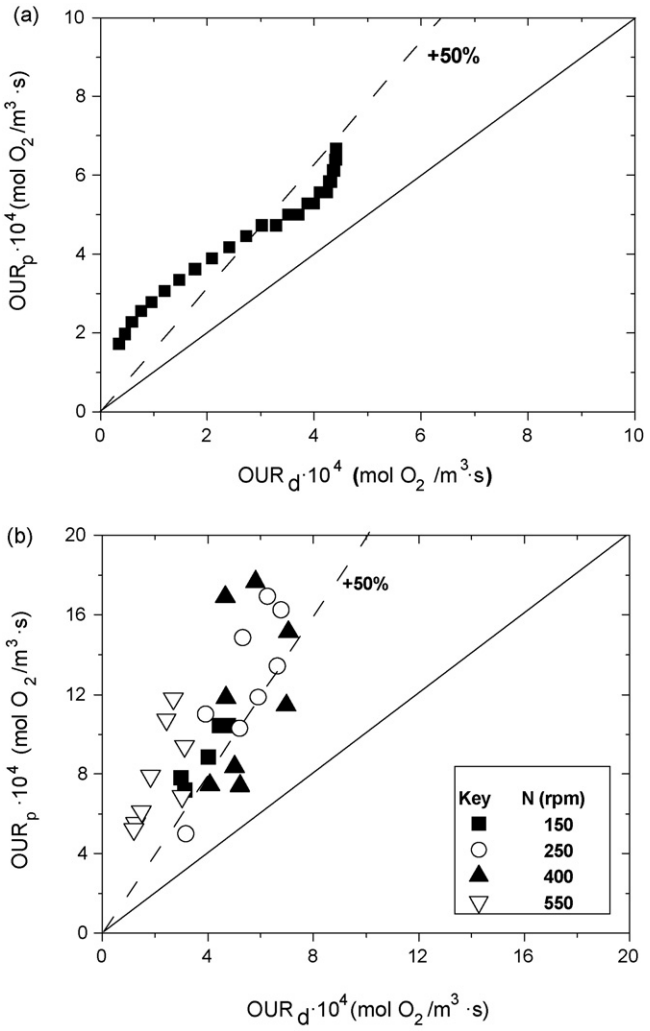


Fig. 6. Differences between oxygen uptake rate experimental values obtained from dynamic technique ($\mathrm{OUR_d}$) and from oxygen profile data ($\mathrm{OUR_p}$) for different stirrer speed (a) in *Xanthomonas campestris* culture (b) in *Rhodococcus erythropolis* IGTS8 culture (adapted from Santos et al. [40]).

4. Kinetic modelling of our in bioprocesses

The concept of a maintenance energy coefficient for substrate consumption was first proposed by Pirt [72] in the study of the culture of *Aerobacter cloacae* grown in chemostat. Pirt model [44] is considered valid for maintenance description; it applies to almost any substrate involved in cellular energy metabolism and is supported by experimental data and energetic considerations.

According to that, OUR has been usually related both to biomass concentration in the broth – oxygen necessary for biomass maintenance – and to biomass production rate – oxygen necessary for growth – [5,7,36,39,40,47–51], as follows:

$$\mathrm{OUR} = q_{O_2} \cdot C_X = m_{O_2} \cdot C_X + \frac{1}{Y_{XO}} \cdot \frac{dC_X}{dt} \quad (14)$$

where C_X is the biomass concentration, m_{O_2} is the oxygen consumption coefficient for maintenance and Y_{XO} the yield of oxygen consumed for cell growth.

Assuming that the growth rate of microbial cells can be modelled according to a logistic equation:

$$\frac{dC_X}{dt} = \mu \cdot C_X \cdot \left(1 - \frac{C_X}{C_{X_{max}}}\right) \quad (15)$$

in an aerobic process where oxygen is only consumed for cell maintenance and cell growth, the above equations lead to:

$$\mathrm{OUR} = \left[m_{O_2} + Y_{OX} \cdot \mu \cdot \left(1 - \frac{C_X}{C_{X_{max}}}\right)\right] \cdot C_X \quad (16)$$

being $Y_{OX} = 1/Y_{XO}$; and therefore:

$$q_{O_2} = \left(m_{O_2} + Y_{OX} \cdot \mu\right) - \frac{\mu \cdot Y_{OX}}{C_{X_{max}}} \cdot C_X \quad (17)$$

From the slope and the intercept of the plot of q_{O_2} versus C_X both oxygen consumption for maintenance and yield of oxygen consumed for cell growth can be obtained. Both relations have been used for determination of the characteristic parameters of consumption [38–40].

Experiments show that OUR values present an increase during the lag stage and especially during the exponential growth stage; afterwards the values of OUR decrease slowly till reaching a practically constant value during the stationary stage. On the other hand, the specific oxygen uptake, q_{O_2}, increases dramatically in the lag and first exponential stages of growth, until a maximum value is reached. At the beginning of the process q_{O_2} increases mainly due to the increasing of biomass production and substrate consumption rates. At longer fermentation times, when the substrate consumption and biomass production rates decrease q_{O_2} decreases as well.

It has been shown that the maximum OUR value in *Xanthomonas campestris* culture was obtained in the middle of the exponential phase with values ranging from 1.15 to 1.84×10^{-3} mol O_2 m^{-3} s^{-1}, depending on the biomass concentration during the experi-

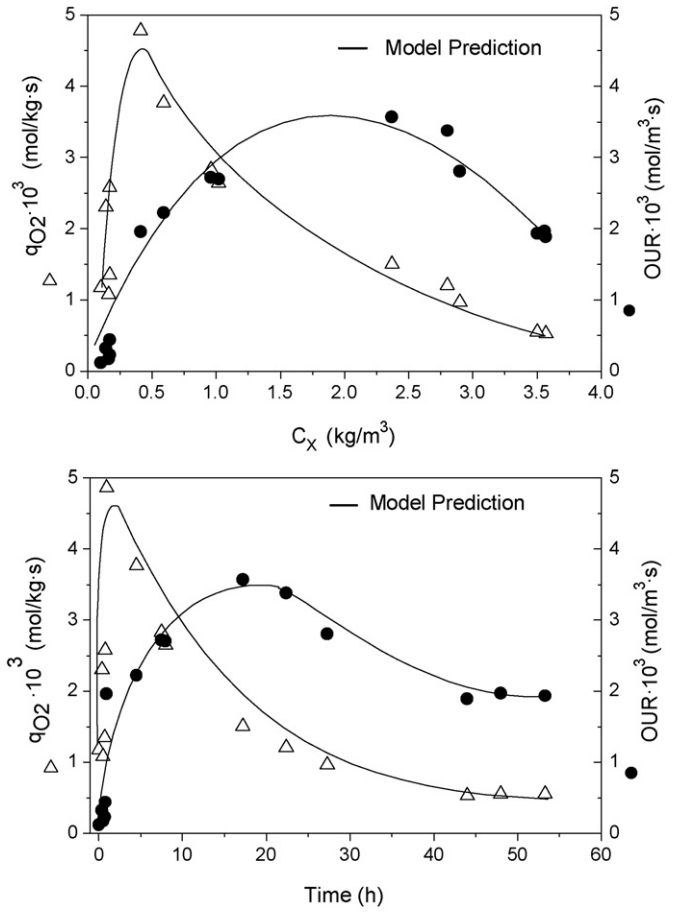


Fig. 7. Variation of experimental values (symbols) and prediction model (lines), of q_{O_2} (Δ) and OUR (●), using Eqs. (16) and (17), with biomass concentration and the time course of fermentation of *Pseudomonas putida* (a genetically modified bacteria able to carry out the DBT biodesulphurization process) (N = 200 rpm; $V_S = 2.5 \times 10^{-3}$ m s^{-1}) (adapted from Gomez et al. [39]).

ment; the maximum value of specific OUR, $q_{O_2\,max}$, was around 4.2×10^{-3} mol O_2 kg^{-1} s^{-1}, which would be taken as the end of the lag phase [5]. In the case of *Rhodococcus erythropolis* cultures, OUR seems to depend on culture growth phase: firstly increasing during lag stage and especially during the first exponential growth stage, taking a maximum value (from 5 to 7×10^{-4} mol O_2 m^{-3} s^{-1}) for a biomass concentration of 1 kg m^{-3}; afterwards the values of OUR decrease slowly at the stationary stage [40]. In *Pseudomonas putida* culture [39] the maximum OUR value was also obtained in the middle of the exponential phase, with values ranging from 3.0 to 3.5×10^{-3} mol O_2 m^{-3} s^{-1}, depending on the biomass concentration; the maximum value of the specific OUR, $q_{O_2\,max}$, was around 5×10^{-3} mol O_2 kg^{-1} s^{-1}, and was observed at the end of the lag stage.

Fig. 7 show the evolution of OUR and q_{O_2} with the biomass concentration and time course of *P. putida* fermentation, for a stirrer speed of 200 rpm and superficial gas velocity of 2.5×10^{-3} m s^{-1} (gas flow of 2 L min^{-1}) [39]. In this figure values predicted by Eqs. (16) and (17) are also presented. It can be seen that the equations describe satisfactorily experimental data of OUR and q_{O_2}.

There are not many experimental values of these oxygen consumption parameters in the literature; some values for yeasts and bacteria are shown in Table 1. The parameter m_{O_2} presents a constant value independently of the conditions of oxygen transport that prevail in the broth, whereas parameter Y_{XO} can be influenced by oxygen transport. For this reason different values can be obtained for this last parameter if the transport conditions are altered, as it occurs in the case of using the dynamic method [5,40]. In this table results obtained by macroscopic model proposed by Heijnen and Roels [74] for m_{O_2} and Y_{OX} estimation are also shown. Although fluctuations are considerable in both parameters, it is clear that in general available data in literature agree well with predicted values.

The modelling of OUR using several metabolic functions has been considered in different ways depending on the characteristic of bioprocess. Koutinas et al. [52] considered the OUR for growth, maintenance and glucoamylase production (Y_{OG}) by fungal cells in submerged cultures of *Aspergillus awamori*. Giordano et al. [42] assumed that in aerobic biodegradation of contaminated sediments OUR was the sum of two terms, related, respectively, to exogenous and endogenous respiring, modelling the OUR profile according to this assumption. Çalik et al. [59] studying the effects of pH on benzaldehyde lyase production by *Escherichia coli* and Kocabas et al. [9] for the L-tryptophan production by thermoacidophilic *Bacillus acidocaldarius*, established that oxygen is consumed for three purposes: cell growth, by-product formation and maintenance.

Indeed, in cases of product synthesis a new term must be introduced in Eq. (14), yielding the following expression:

$$\mathrm{OUR} = m_{O_2} \cdot C_X + \frac{1}{Y_{XO}} \frac{dC_X}{dt} + \frac{1}{Y_{PO}} \frac{dC_P}{dt} \tag{18}$$

The last term corresponding to oxygen consumption for product formation can be expressed according to the following equation [78]:

$$\frac{dC_P}{dt} = \alpha \frac{dC_X}{dt} + \beta \cdot C_X \tag{19}$$

where α is the term for growth associated product formation and β is the term for non-growth associated product formation. This model was derived on the basis of an analysis of lactic acid fermentation, but it is in principle valid only for metabolic products that are formed as a direct consequence of the growth process, as shown by Yamani and Shiotani [79]. However, the model may in some cases be applied to other products. Heijnen et al. [80] described a formal approach to modelling of secondary metabolite formation in the penicillin fermentation, which is not growth related.

As indicated above, Santos et al. [40] observed differences between OUR values determined by the classic dynamic technique and from oxygen concentration profile. The fitting of the experimental values obtained from the first technique using Equation (14) provides the values of the oxygen consumption parameters related to maintenance (m_{O_2}) and growth (Y_{OX}). However, when the experimental the oxygen concentration profile obtained during batch growth is employed to determine OUR ('process method'), higher oxygen consumption rates were observed and consequently the authors described OUR using three parameters (Y_{OX}, m_{O_2} and Y_{OBDS}), according to:

$$\mathrm{OUR_p} = m_{O_2} \cdot C_X + Y_{OX} \frac{dC_X}{dt} + Y_{OBDS} \frac{dX_{BDS}}{dt} \tag{20}$$

where Y_{OBDS} is the yield of oxygen to biodesulphurization capability development and X_{BDS} desulphurization capability of whole cell that can be expressed by a simplified Luedeking–Piret equation:

$$\frac{dX_{BDS}}{dt} = \alpha \cdot \frac{dC_X}{dt} \tag{21}$$

Considering that the growth rate of the microbial culture can be modelled according to a logistic equation and that only the first term involving growth due to synthesis of desulphurization enzymes must be considered to influence oxygen uptake rate, Eq. (20) can be rearranged into:

$$\mathrm{OUR_p} = m_{O_2} \cdot C_X + (Y_{OX} + \alpha \cdot Y_{OBDS}) \cdot \mu \cdot C_X \cdot \left(1 - \frac{C_X}{C_{X_m}}\right) \tag{22}$$

Table 1
Parameter consumption values for some microorganism.

Microorganism	q_{O_2} (mol O_2 kgX^{-1} h^{-1})	m_{O_2} (mol O_2 kgX^{-1} h^{-1})	Y_{OX} (mol O_2 kgX^{-1})	Substrate (carbon source)	Reference
Xanthomomas campestris NRRL 1775	2–15	1.0	0.6	Sucrose	Garcia-Ochoa et al. [5]
Escherichia coli K 12[a]	0.9–23	2.4–6.4	12.5–520[b]	Glucose	Çalik et al. [7]
Bacillus acidocaldarius NRRC-207 F	3.1–31.2	2.2–16.6	0.3–43.8	Fructose	Kocabaş et al. [9]
Phaffia rhodozyma ENMS 1100	1.9	–	–	Glucose	Liu et al. [10]
Bacillus thuringiensis subspecies kurstaki HD-1	2–15.5	0.9	17.2	Glucose	Rowe et al. [36]
Rhodococcus erythropolis IGTS8[a]	0.2–4.3	0.8	16.4–20	Glucose, glutamic acid and citrate	Gomez et al. [38]
Pseudomonas putida CECT 5279	2–18	1.9	52.6	Glucose, glutamic acid and citrate	Gomez et al. [39]
Trigonopsis variabilis CBS 4095	2–3	0.03	13–16	Glucose	Montes et al. [49]
Escherichia coli K 12[c]	0.9–17.2	0.4–7.2	0.01–13.6*	Glucose	Çalik et al. [59]
Candida bombicola NRRL Y-17069	0.3–1	0.01	4.4	Glucose	Casas [104]
Hansenula anómala CBS6759	0.8	–	–	Glucose	Djelal et al. [105]
Biomass molecular formula $CH_{1.79}O_{0.5}N_{0.20}$	–	1.0	20.3	Glucose	Heijnen and Roels [74]. Estimated from Eqs. (11) and (12)

[a] Changing oxygen transport conditions.
[b] Overall biomass yield on oxygen (mol O_2 kgX^{-1}).
[c] Changing pH of the fermentation medium.

This equation has been employed to obtain the parameters of oxygen consumption in this bioprocess, and it was found that the values m_{O_2} and Y_{OX} were similar to those obtained by the dynamic method [40].

In summary, a complete description of a bioprocess includes the dynamic behavior of the oxygen, and therefore both OTR and OUR. OTR can nowadays be predicted, as shown in recent works [21]. On the other hand, OUR values can also be affected by OTR, or fluid-dynamic conditions: the following section is devoted to this possible influence.

5. Influence of OTR on OUR

Independently the mode of the aerobic process (batch, semi-batch, or continuous), oxygen must be continuously supplied if acceptable productivities are the objective. Sufficient aeration and agitation of the culture are important in promoting effective mass transfer to the liquid medium and good mixing in the bioreactor which are required for achieving optimal growth and/or product formation in the particular bioprocess focused.

The study of oxygen mass transfer characteristics, traditionally, has been separated into the parameters related to transport (studying the volumetric mass transfer coefficient, k_La, mainly) and the oxygen consumption by microorganism (determining OUR). However, adequate description of bioprocess must be made taking into account the relationship between both of them.

5.1. Oxygen transfer characteristics on microbial processes

Taking into account that the maximum value of oxygen concentration is limited due to the low solubility, most efforts has been focussed on the volumetric mass transfer coefficient. The influences of hydrodynamic parameters (physical properties of gas and liquid, operation conditions, geometric parameters of the bioreactor) on K_La have been widely investigated (see for references Garcia-Ochoa and Gomez [21]). There are many empirical equations to determine k_La, and recently great efforts have been made toward developing mathematical models able to predict this parameter in bubble columns of different types [28,29,81–83], and in stirred tank bioreactors [28,30,31,84]. These methods aim at predicting transport coefficient for bioreactors of different sizes and under different operation conditions.

Recently, prediction techniques based on theoretical models of gas–liquid contact and on turbulence description, have been proposed. For the prediction of k_L, theoretical models based on Higbie's penetration theory, which is widely accepted for description of gas–liquid transfer, were applied by several authors for Newtonian and non-Newtonian fluids [29,30,82,85]. According to this model, the mass transfer coefficient can be calculated as

$$k_L = 2 \cdot \sqrt{\frac{D_L}{\pi \cdot t_e}} \tag{23}$$

The evaluation of contact time, t_e, can be done according to Kolmogoroff's theory of isotropic turbulence, from two characteristic parameters of eddies, namely the eddy length and the fluctuation velocity. Both parameters depend on the rate of energy dissipation per mass unit, ε, and on the cinematic viscosity. The exposure time is usually calculated as the time spent by the bubble to travel a length equal to its diameter, and it is estimated using the ratio between the eddy length and the fluctuation velocity of Kolmogoroff.

If the rheological model of Ostwald–de Waele is adopted for description of non-Newtonian flow behaviour of fluids, the following equation for t_e and k_L are obtained [31,81]:

$$t_e = \left(\frac{K}{\varepsilon \cdot \rho}\right)^{1/(1+n)} \tag{24}$$

$$k_L = \frac{2}{\sqrt{\pi}} \cdot \sqrt{D_L} \left(\frac{\varepsilon \cdot \rho}{k}\right)^{1/2\cdot(1+n)} \tag{25}$$

For Newtonian media ($n = 1$; $k = \mu_L$), Eq. (25) is reduced to:

$$k_L = \frac{2}{\sqrt{\pi}} \cdot \sqrt{D_L} \left(\frac{\varepsilon \cdot \rho}{\mu_L}\right)^{1/4} \tag{26}$$

The specific interfacial area, a, is a function of the hydrodynamics and of the vessel geometry. Assuming spherical bubbles the specific interfacial area can be calculated from the average bubble size, d_b, and the gas hold-up, ϕ, by the following equation:

$$a = \frac{6\phi}{d_b} \tag{27}$$

Both hydrodynamic parameters, d_b and ϕ, can be estimated for stirred tank bioreactors using the followings equations [30]:

$$\frac{\phi}{1-\phi} = 0.819 \cdot \frac{V_s^{2/3} N^{2/5} T^{4/15}}{g^{1/3}} \left(\frac{\rho_L}{\sigma}\right)^{1/5} \cdot \left(\frac{\rho_L}{\rho_L - \rho_G}\right) \cdot \left(\frac{\rho_L}{\rho_G}\right)^{-1/15} \tag{28}$$

$$d_b = 0.7 \cdot \frac{\sigma^{0.6}}{\left(P/V\right)^{0.4} \cdot \rho_L^{0.2}} \cdot \left(\frac{\mu_L}{\mu_G}\right)^{0.1} \tag{29}$$

Oxygen absorption into a culture broth can be considered as the absorption of a gas into a liquid where it reacts, the suspended microorganisms being the consumer of the oxygen, and therefore an enhancement of oxygen mass transfer rate can take place. Indeed, Tsao [86] founded that the absorption rate of oxygen in a surface-aerated stirred vessel was higher than expected from physical absorption. The accumulation of microorganism near the gas–liquid interface was proposed by Tsao [86] as explanation of such as phenomenon. On the other hand, Yagi and Yoshida [87] working in a sparged stirred fermenter reported experimental evidence that the activity of microorganism does not affect the oxygen transfer rate. Both of these observations were reconciled by a mathematical model proposed by Merchuk [53] taking into account the influence of the different hydrodynamics conditions in the bioreactor operation. More recently, Çalik et al. [88] studying the oxygen transport effects in growth of *P. dacunhae* for L-alanine production found an enhancement on transport due to presence of microorganism. Similar results have been reported by Çalik et al. [7,59], in an investigation of the effects of oxygen transport on the synthesis of the enzyme benzaldehyde lyase in recombinant *E. coli*, and by Kocabas et al. [9] in culture of the thermoacidophilic bacteria *B. acidocaldarius* in L-tryptophan. On the other hand, other effects, such as blocking effects of cells on gas–liquid interface have been reported by Galaction et al. [17] for bacteria, yeasts and fungi broths.

To take into account the possibility of mass transfer rate enhancement in a biological system, a biological enhancement factor, E, is defined as the ratio of the absorption flux of oxygen due to the oxygen consumption by the cells to the absorption flux in its absence under the same hydrodynamic conditions and driving force, according to:

$$E = \frac{J}{J^0} \tag{30}$$

Some works in the literature have discussed the biological enhancement factor for oxygen absorption into fermentation broths, and several models with different cell concentration distribution have been proposed [53,54,86]. Garcia-Ochoa and Gomez [31] have proposed a model for estimation of the potential biological enhancement factor in bioreactors taking into account the substances usually added to the broths, such as surfactants, electrolytes, sugars, etc. Because oxygen has a very low solubility in water, it can often be assumed that no mass transfer limitation is observed within the gas phase and only the liquid phase mass transport resistance needs be considered. This model considers three layers in series. Therefore, three mass transfer resistances are considered to describe the oxygen transport: (i) the resistance at an interfacial surfactant film, (ii) the resistance of layers of microorganism adsorbed at the interface, next to the surfactant monolayer film and (iii) the liquid film resistance.

The different layer resistances are taken into account by the diffusion coefficient (D_i) in each layer and its thickness (z_i). If a linear relationship for the cell concentration in stagnant liquid layer is assumed [53] as a first approximation, the following equation is obtained for the biomass concentration profile (for details see Garcia-Ochoa and Gomez [31]):

$$C_X(z) = C_{X_L} - \frac{C_{X_m} - C_{X_L}}{z_L} \cdot (z - z_T) \therefore z_s + z_m \le z \le z_s + z_m + z_L \tag{31}$$

where C_{X_L} is the biomass concentration in the bulk, C_{X_m} is the biomass concentration adsorbed in the monolayer, z is the distance from the gas–liquid interface and z_T is the total thickness film.

According to this approach, the oxygen transfer flux can be evaluated at the boundary between the surfactant film and the cell monolayer, considering the uptake rate as zero order respect to the oxygen concentration [54,55], obtaining:

$$J_{O_2} = -D_m \left.\frac{dC}{dz}\right|_{z=z_s} = \left[\frac{D_m q_{O_2} C x_m z_m z_L}{D_L} + \frac{z_l^2 q_{O_2}\left(2C_{X_m} + C_{X_L}\right)}{6} + \frac{z_m^2 q_{O_2} C_{X_m} + 2(C^* - C_L)}{2}\right] \left[\frac{1}{\sum_i z_i/D_i}\right] \tag{32}$$

The absorption flux in absence of biochemical reaction ($q_{O_2} = 0$) at the same hydrodynamic conditions and driving force for mass transfer is then given by

$$J_{O_2}^0 = -D_m \cdot \left.\frac{dC}{dz}\right|_{z=z_s} = \frac{(C^* - C_L)}{z_L/D_L} \tag{33}$$

By combining Eqs. (30), (32) and (33), the following expression is obtained:

$$E = \left[1 + \frac{q_{O_2} C x_m z_m^2}{2D_m (C^* - C_L)} \cdot \left(1 + 2 \cdot \frac{z_l D_m}{z_m D_L} + \frac{2z_L^2}{3z_m^2}\right) + \frac{1}{3} \cdot \frac{q_{O_2} C_{X_L} z_L^2}{2D_L (C^* - C_L)}\right] \cdot \left[\frac{z_L/D_L}{\sum_i z_i/D_i}\right] \tag{34}$$

The first bracket in Eq. (34) is always ≥1 and can be written as a function of the *Hatta* number, which takes into account the biological enhancement factor due to the oxygen uptake by the microorganisms in the different layers, according to:

$$f(Ha) = \left[Ha_m \cdot \left(1 + 2\frac{z_L \cdot D_m}{z_m \cdot D_l} + \frac{2z_L^2}{3z_m^2}\right) + \frac{1}{3} Ha_L\right] \tag{35}$$

where Ha_m and Ha_L represent the dimensionless *Hatta* number in the cell monolayer and in the liquid layer, respectively, being defined as

$$Ha_i = \frac{q_{O_2} C_{X,i} z_i^2}{2D_i \cdot (C^* - C_L)} \quad (i = L, m) \tag{36}$$

The second bracket of Eq. (34) is always ≤1, representing the resistance to diffusion exerted by each of the three films defined previously. Since the resistances are in series:

$$g\left(\frac{z}{D}\right) = \left[\frac{z_L/D_L}{z_s/D_s + z_m/D_m + z_L/D_L}\right] \tag{37}$$

Depending on the relative values of both terms in Eq. (34) the enhancement factor E, can take values below, equal or above unity. This fact has been previously described in the literature; the final value of E depends on the presence of surfactants, the kind of microorganisms and the biomass concentration [17,54,87].

The oxygen mass transfer rate can be expressed as

$$\mathrm{OTR} = a \cdot J = a \cdot E \cdot J^0 = k_G a \cdot (p_G - p_i) = E \cdot k_L a (C_i - C_L) \quad (38)$$

where J is the absorption flux of oxygen in presence of microorganism, a is the interfacial area, k_G and k_L are mass transfer coefficient, p_G is the oxygen partial pressure in the gas phase, and C_L the oxygen dissolved concentration in the liquid phase; index i refers to the magnitude values at the gas–liquid interface.

Considering Henry's law, an overall volumetric mass transfer coefficient can be used, being it related to the individual phases coefficients according to:

$$\mathrm{OTR} = K_G a \cdot (p_G - p^*) = K_L a \cdot (C^* - C_L) \quad (39)$$

being

$$\frac{1}{K_L a} = \frac{1}{H \cdot k_G a} + \frac{1}{E \cdot k_L a} \quad (40)$$

where H is the Henry constant.

It can be observed that the overall volumetric mass transfer coefficient in the presence of a biochemical reaction, $K_L a$, is a lumped parameter comprising the resistances to mass transport of oxygen due to several films around the gas–liquid interface, and to the oxygen consumption, whose effect can be expressed by a biological enhancement factor, E. Taking into account that the gas phase resistance can usually be neglected, the overall resistance to transport can be written as

$$K_L a = E \cdot k_L a \quad (41)$$

Thus, the biological enhancement factor is the ratio between the volumetric mass transfer coefficient in the presence of biochemical reactions (consumption) and that under inert conditions, being frequently assumed to be equal to 1. When biochemical reactions do not take place, $E = 1$ and the overall mass transfer will be denoted $k_L a$ and the flux J^o.

5.2. Influence of oxygen transport conditions on OUR and q_{O_2}

In bioprocesses, OTR as well as the OUR values change not only with the presence of biomass but also with oxygen transport conditions (determined by hydrodynamics conditions in the bioreactors). Thus, OUR also can depend on oxygen mass transfer [7,9,38].

Typical time profiles of specific oxygen uptake rate and the enhancement factor predicted values obtained in different cultures are shown in Fig. 8. For E calculation, the liquid film thickness, z_l, can be calculated from the contact time – Eq. (24) – considering the influence of viscosity on the mass transfer coefficient through the rheological parameters k and n The film thickness of adsorbed cell monolayer, z_m, has been considered to be the average size of the microorganism used (bacteria or yeast); and for the film thickness of surfactant, a value of 1×10^{-7} m has been assumed; oxygen diffusion coefficient in bulk liquid, D_L, has been estimated from Wilke–Change correlation [89], while in the layer of microorganism adsorbed is calculated as $D_m = 0.3 D_L$ [90], and in the surfactant monolayer, D_s is assumed to be 4.2×10^{-9} m^2 s^{-1} [31].

Fig. 8a shows experimental values of specific oxygen uptake rate, q_{O_2}, and the variation of the biological enhancement factor, E, during the biomass evolution in time for a typical growth of the bacteria *Xanthomonas campestris* in the production of xanthan gum. Specific oxygen uptake rate seems to be influenced by the microorganism growth phase: there is an increase during the lag phase and the beginning of the exponential phase, and afterwards q_{O_2} decreases during the stationary growth phase. The enhancement factor, E, increases because of the increased OUR during the first stage of growth, as expected. The increase of E after this stage, in spite of the lower biochemical demand as shown by the descent of

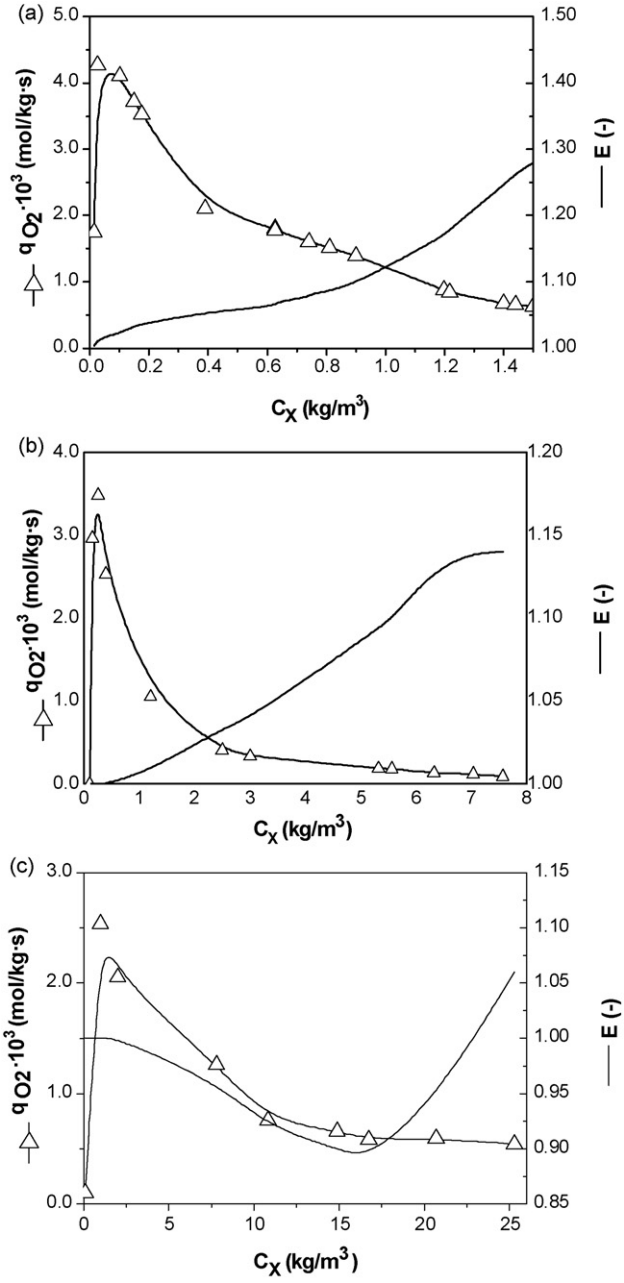


Fig. 8. Typical time course profiles of specific oxygen uptake rate (Δ) and enhancement factor predicted (–) by Eq. (34) in different cultures. (a) Production of xanthan gum by *Xanthomonas campestris*. (b) Growth culture of *Candida bombicola*. (c) Production of surfactant by *Candida bombicola* (adapted from Garcia-Ochoa and Gomez [31]).

q_{O_2}, is due to the influence of the viscosity of broth, which dramatically increasing and produces an increase of the resistance to mass transport (a decrease on the volumetric mass transfer coefficient). Since E is the ratio of the actual OTR to the hypothetical OTR that would be observed in a system of the same physical characteristics, but without biochemical activity, E continues to increase in spite of the decrease in q_{O_2}. This decrease is partially compensated in practice by increasing of the agitation speed from 250 to 500 rpm.

Fig. 8b shows experimental values of the specific oxygen uptake rate, q_{O_2}, and the variation of the biological enhancement factor, E, with biomass evolution in the course of a typical fermentation of the yeast *Candida bombicola*, in a medium where only growth is relevant. It can be observed that the specific OUR, q_{O_2}, in the lag phase and first exponential phase, achieves values of

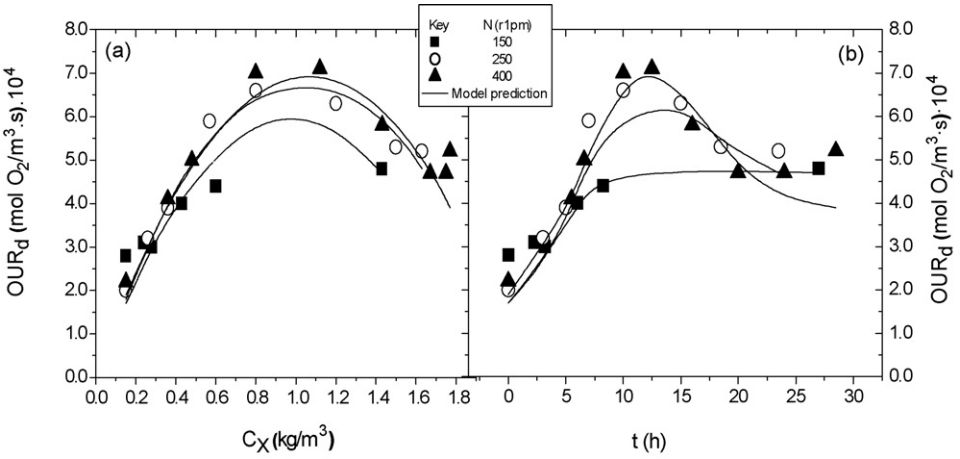


Fig. 9. Variation of oxygen uptake rate (experimental values as symbols, and predictions by Eq. (16) as lines) with biomass concentration (a) and growth time (b) for different stirrer speed on *Rhodococcus erythropolis* cultures (a bacteria able to carry out the DBT biodesulphurization process) (adapted from Santos et al. [40]).

3.5×10^{-3} mol kg^{-1} s^{-1} and then decreases with time. The E value however increases continuously due to the increasing of biomass concentration and OUR, reaching values of 1.14. During the production of sophorolipids by the same yeast shown in Fig. 8c, q_{O_2} values show the same tendency, but at one order of magnitude above. The biological enhancement factor E predicted by the model first decreases due to the accumulation of the sophorolipid produced, which is a new resistance to mass transfer (a new layer around the interface); it is considered that the surfactant layer increases from 0 to 10^{-7} m, but the increase of biomass concentration produces an increase in the OUR, and the enhancement factor fall into the range of higher than unity.

In Fig. 9 variations of OUR on *Rhodococcus erythropolis* cultures, under different transport conditions (changing stirrer speed of 150, 250 and 400 rpm) are shown. In all runs, OUR seems to be influenced by the stirrer speed employed and the microorganism growth phase (increasing during lag stage and especially during the first exponential growth stage, then taking a maximum value for a biomass concentration of 1 kg m^{-3}. After that, the values of OUR decrease slowly at the stationary stage because of decrease in metabolic activity of the cells. In general, the lowest OUR values are observed under oxygen transport limitations; however, higher oxygen transport conditions can produce a decrease in OUR due to growth inhibition by oxygen [7].

In Fig. 10 the relationship between specific oxygen uptake rate and biomass concentration under different transport conditions is shown. Experimental results of q_{O_2}, according to Eq. (17), show a linear relationship with C_X and increase when stirrer speed increases. Specific OUR has a minimum value that corresponds to maintenance; this value will be reached when C_X has a maximum value (that is, in stationary phase).

Regarding consumption parameters in general, the coefficient of oxygen consumption for maintenance, m_{O_2}, and the yield of oxygen, Y_{XO}, depend on the conditions of oxygen transport that prevail in the broth. They may decrease dramatically when the microorganism is grown at high stirrer speed, due to metabolic changes or cell damage produced by hydrodynamic conditions [66].

5.3. Rate-limiting step analysis

A method similar to that employed for inter- and intra-phase transfer rate limitations in heterogeneous catalytic systems has been applied to compare relative rates of mass transfer and biochemical reaction and to determine the rate-limiting step. This methodology has been applied to complex bioprocess involving immobilised enzymes or cells, microbial agglomerates and single cells. Two dimensionless parameters, the observed effectiveness factor and the modified *Damköhler* number, which contain measurable parameters only, have been used to determine whether the overall reaction was limited by oxygen diffusion or by the biochemical reaction [7,38,59,91,92].

The effectiveness factor is alternative way of looking into the interaction of diffusion and reaction. The effectiveness factor, η, is defined as a ratio of reaction rates of substrate with and without diffusion limitations, while in the case of the enhancement factor the reference adopted is the rate of absorption in absence of chemical reaction.

Thus, in aerobic microbial processes, effectiveness factor for oxygen uptake rate can be expressed by the ratio of the observed biochemical reaction rate and the biochemical reaction rate without mass transfer resistance, according to:

$$\eta = \frac{\mathrm{OUR}}{\mathrm{OUR}_{\max}} \tag{42}$$

This parameter indicates the relative utilisation of oxygen by microorganisms in a bioprocess. If η is close to 1, the respiratory activity is not limited by oxygen diffusion. If $\eta < 1$, it indicates that the diffusion is affecting, even controlling, the overall rate.

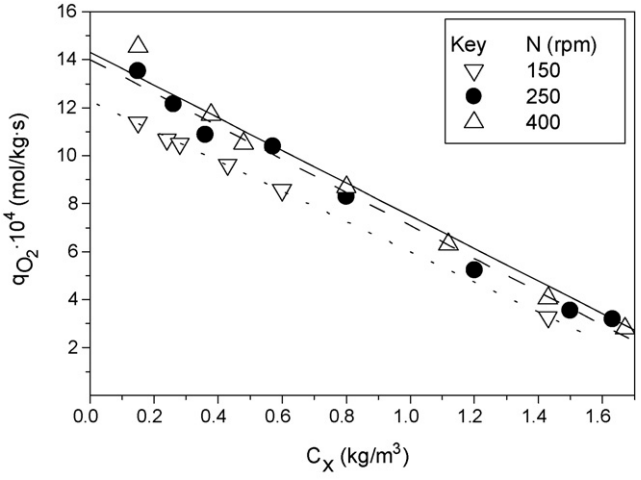


Fig. 10. Variation of specific oxygen uptake rate with biomass concentration for different stirrer speed on *Rhodococcus erythropolis* cultures.

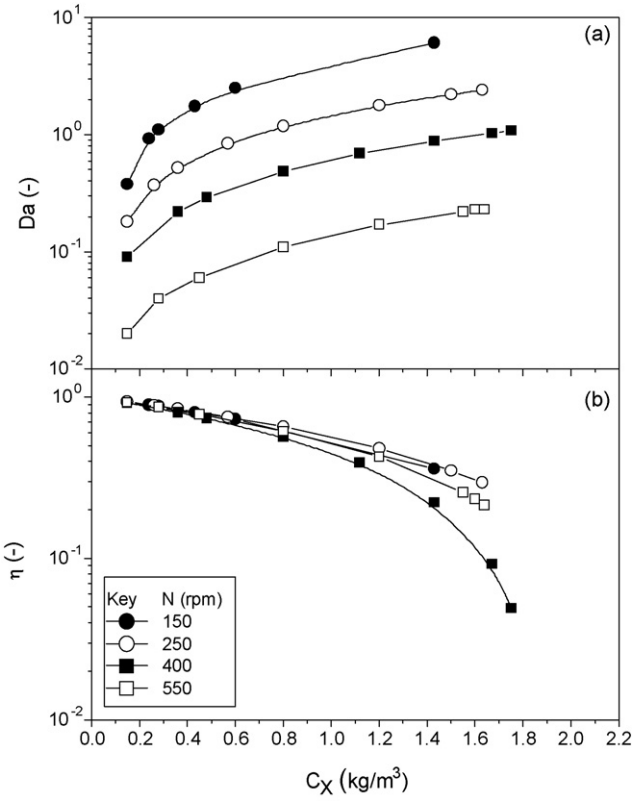


Fig. 11. Evolution of *Damköhler* number (a) and effectiveness factor (b) with biomass concentration for different mass transport conditions during *Rhodococcus erythropolis* growth (adapted from Gomez et al. [38]).

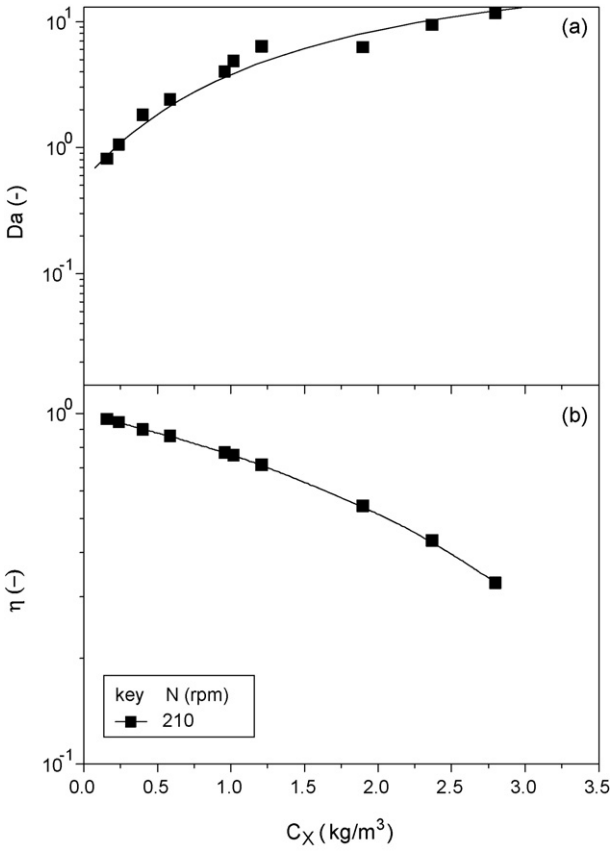


Fig. 12. Evolution of *Damköhler* number (a) and effectiveness factor (b) with biomass concentration for different mass transport conditions during *Pseudomonas Putida* CECT5279 growth.

In Eq. (16), the maximum oxygen uptake rate is given by

$$\mathrm{OUR_{max}} = \left(m_{O_2} + \frac{\mu_{max}}{Y_{XO}}\right) \cdot C_X \tag{43}$$

Considering the overall oxygen yield, Y'_{XO}:

$$\mathrm{OUR_{max}} = \frac{\mu_{max}}{Y'_{XO}} C_X \tag{44}$$

where de maximum demand of oxygen depends on the characteristic parameters of the microbial process and on biomass concentration.

During the growth phase the $\mathrm{OUR_{max}}$ is often greater than actual OUR because of the inability of the aeration system to provide sufficient oxygen transfer. However, in the stationary phase, OUR is based on the maintenance requirements and is therefore considerably less than maximum value.

Likewise, the maximum mass transfer rate from gas to liquid can be determined as

$$\mathrm{OTR_{max}} = E \cdot k_L a \cdot C^* \tag{45}$$

being C^* the saturation concentration in the liquid phase and E the enhancement factor due to biochemical reaction.

In order to compare the potential OUR and OTR maximum values and to find the rate-limiting step of the bioprocess, the modified *Damköhler* number can be used. The *Damköhler* number (Da) can be also expressed as [7,38,59,91]:

$$Da = \frac{\mathrm{OUR_{max}}}{\mathrm{OTR_{max}}} \tag{46}$$

This ratio indicates whether the bioprocess is transport-limited, biochemical reaction-limited, or in an intermediate operation regime. When $Da > 1$, the biochemical reaction rate is large compared to transport rate (mass transfer resistances become dominant); on the other hand, if $Da \leq 1$ the rate of mass transport is large compared to the biochemical reaction rate (biochemical reaction limitations are dominant).

In Figs. 11 and 12 the evolution of Da and η with biomass concentration, for different mass transport conditions during the growth of *Rhodococcus erythropolis* and *Pseudomonas putida* are shown. In Figs. 11a and 12a it can be seen that Da increases with the increase of biomass concentration. It can also be observed that Da decreases with increasing stirrer speed (Fig. 11a). In the runs performed at 150 and 250 rpm, when biomass concentration and oxygen uptake rate increase, mass transfer resistance is clearly the limiting step due to the low mass transfer rate, and Da takes values higher than 1 for biomass concentration of 0.2 kg m^{-3} (for growth time above 3 h). Under other operation conditions (with stirrer speed of 400 rpm), there is no transport limitation and Da takes values lower than 1. In Figs. 11b and 12b it can be observed that the effectiveness factor, η, estimated by the ratio between the observed biochemical reaction rate and the intrinsic reaction rate without mass transfer resistance, takes values close to 1 at the beginning of the bioprocess, indicating that the cells are consuming oxygen at such a high rate that the maximum possible oxygen utilisation values are approached. The decrease in η after that indicates that the bacteria are consuming oxygen at a rate that is below the maximum demand.

6. Scale-up and modelling of dissolved oxygen concentration

Aeration and agitation of culture are important in promoting effective mass transfer from gas phase to liquid medium and good

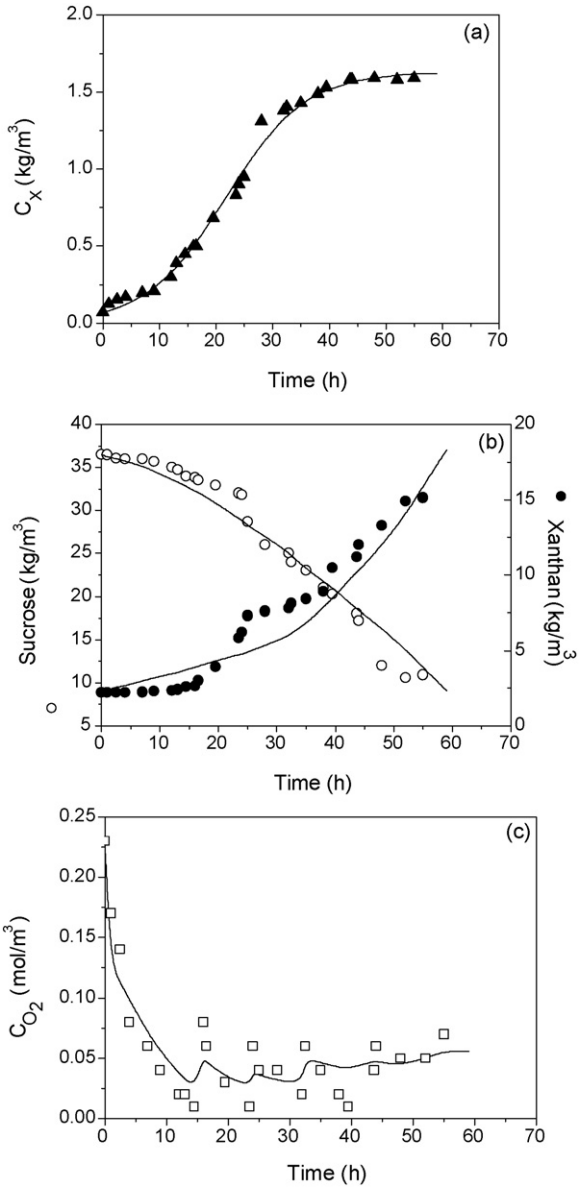


Fig. 13. Experimental values (points) and prediction by the model (lines) during time course bioprocess of the production of xanthan gum by *Xanthomonas campestris*: (a) biomass concentration; (b) sucrose and xanthan concentrations; (c) dissolved oxygen concentration (adapted from Garcia-Ochoa et al. [5]).

mixing into the bioreactor. Therefore the scale-up of a bioreactor must provide a controlled environment and an appropriated DO concentration to achieve the optimal microorganism growth and/or product formation in the particular bioprocess employed. The problem of bioreactor scale-up has been addressed many times in the literature The empirical approach integrated with the principle of similarity and dimensional analysis for scale-up puts forward the general principles of the equalities of specific power input (P/V), volumetric mass transfer coefficient (K_La); impeller tip speed of the agitator or shear rate of stress (ND), or mixing time (t_m). However, in general, it is impossible to scale-up a bioprocess keeping simultaneously all conditions (hydrodynamics or mass transfer) similar at different scales [93] and it is therefore necessary to choose a variable to be considered as the most important [21].

In laboratory shake flasks, aeration and agitation are accomplished by the rotary or reciprocating action of the shaker. In pilot-scale and in production-scale, oxygen is generally supplied by compressed air, and mechanical devices are used to mix the broth and to facilitate the gas–liquid contact. OTR often can be most important in scale-up due to effect of DO concentration on cell growth and product formation. Furthermore, it can be the key to scale-up the bioreactor; when the criterion of constant OTR is used in scale-up, the underlying assumption is that OUR is equal to the OTR. Zou et al. [33] have used OUR as a scale-up parameter that was successfully applied in industrial bioreactors (132 m^3 and 372 m^3); the final erythromycin production was similar to that obtained at the pilot plant scale (0.050 m^3).

Changes in pressure with scale-up can influence the values of C^*, and consequently on C_L. Since for most aerobic bioprocesses the critical dissolved oxygen concentration is very low, C_L is assumed to be zero. This fact severely affects growth rate. Alternatively, although OUR (and K_La) can change dramatically over the course of the fermentation, an optimal value for C_L may be determined from laboratory studies and used for scale-up [94]. A value of C_L above 30–70% saturation usually assures adequate DO concentration in less mixed regions of a viscous mycelia broth; for a less viscous yeast fermentation broth, a value of C_L as low as 10–30% saturation can be adequate [95]. A similar result was observed during xanthan gum fermentation in stirred tank bioreactor, where a C_L over 20% saturation improves the biopolymer production [5].

The concentration of dissolved oxygen changes depending on the oxygen transfer rate from the air bubbles to liquid phase and on the oxygen uptake rate for growth, on maintenance, and also on the metabolic production by the cells. So, the study of the different variables affecting oxygen and its relation with growth and yield is of great importance. Çalik et al. [88] have shown that variation of specific growth rate values, μ, with DO concentration in the growth of *Pseudomonas dacunhae* for L-alanine production obeys a substrate inhibition model. Koutinas et al. [52] have shown that increasing of DO concentration (changing aeration rate and stirrer speed) causes an increasing in the yield of biomass on substrate and that the efficiency in glucose and oxygen consumption is hampered under oxygen limiting conditions; similar results have been obtained by Çalik et al. [7], determining that cell yield on substrate increased with increase in oxygen transfer rate. Also, Sampaio et al. [37] found that metabolism of yeast *Debaryomices hansenii* UFV-170 results to be practically inactive under strict oxygen limited conditions. Olmo et al. [60] have shown in aerobic bioprocess of biodesulphurization by *Rhodococcus erythropolis* that growth of the bacteria is strongly dependent on the availability of oxygen. Experimental results of Mantzouridou et al. [96] and Liu et al. [10] have shown in shake flask cultures of *Blakeslea trispora* and *Phaffina rhodozyma*, respectively, that growth and carotenoid production depend strongly on the oxygen supply.

For design and optimisation of bioprocesses, it is essential to have a mathematical model that represents the system kinetics, including the evolution and influence of DO concentration. Therefore, the kinetic models proposed to describe bioprocesses have to include as a key-compound in its set of differential equations the dissolved oxygen concentration, because very often this is the most important nutrient for success of the production process. Mathematical models incorporating oxygen uptake and dissolved oxygen concentration have been developed for different cultures [48,97–100] and different specific rate expressions for cell growth, substrate consumption, product formation and oxygen uptake have been utilised.

Monod type kinetic [101] has been frequently used for description of the specific culture growth rate when dissolved oxygen is

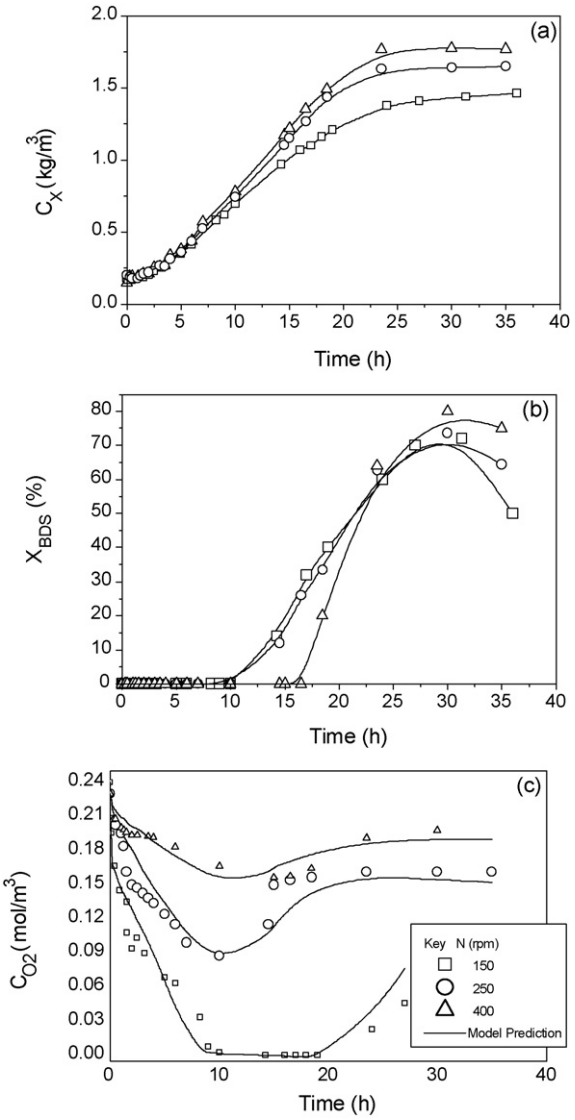


Fig. 14. Experimental values (points) and prediction by the model (lines) during time course bioprocess of the growth of *Rhodococcus erythropolis* under different mass transfer operational conditions: (a) biomass concentration; (b) desulphurization capability; (c) dissolved oxygen concentration (adapted from Gomez et al. [38]).

the limiting nutrient for growth:

$$\mu = \frac{\mu_{\max} \cdot C_{O_2}}{K_{O_2} + C_{O_2}} \quad (47)$$

and the same formalism has been used for specific oxygen uptake rate [49,97,102]:

$$q_{O_2} = \frac{q_{O_2\max} \cdot C_{O_2}}{K_{O_2} + C_{O_2}} \quad (48)$$

The above approach has been considered in the metabolic structured kinetic model proposed by Garcia-Ochoa et al. [99] for xanthan gum production by *X. camprestris*. This model takes into account five key compounds: biomass, carbon source, nitrogen source, xanthan gum and dissolved oxygen. The model is able to consider the influence of DO concentration on the bioprocess evolution and, therefore, the results obtained are more realistic and closer to experimental evidence. It should be noted that this model is also able to predict the influence of temperature on the evolution of all the described compounds. Experimental and predicted by model values for biomass concentration, consumption of sucrose, xanthan gun production and DO concentration during time are represented in Fig. 13. In a later work [5] this kinetic model was used for predicting the behaviour of the system in scale-up when a transport parameter (K_La, air flow and DO concentration) is changed. Results showed that increases of DO concentration produce fast carbon source consumption and favours the production of xanthan gum (see Fig. 3). It can be observed that, as found by other authors [103], the increase of DO concentration favours the production of biopolymer.

A kinetic model for DBT desulphurization has been proposed to describe the growth of *R. erythropolis* in batch operation and under different operation conditions [38,60]. Experimental and predicted values of biomass concentration, desulphurization capability, X_{BDS}, and DO concentration during the course of the bioprocess of biodesulphurization by *R. erythropolis* are shown in Fig. 14, for different OTR values, changing the stirrer speed. In Fig. 14a, it can be observed that the maximum cell concentration obtained increases when stirrer speed increases, that is when OTR increases. In Fig. 14b,

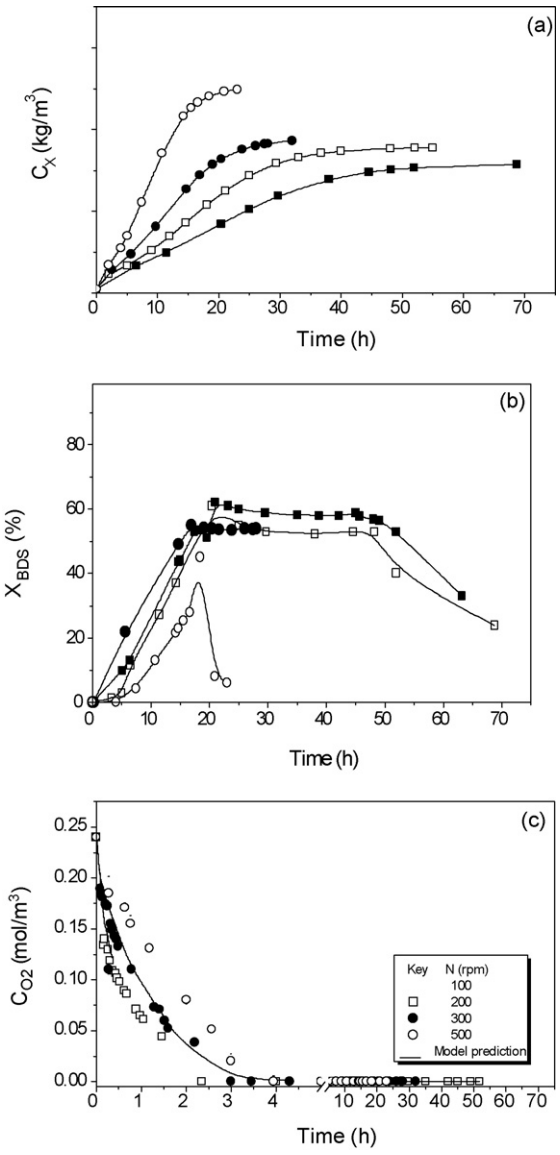


Fig. 15. Experimental values (points) and prediction by the model (lines) during time course bioprocess of the growth of *Pseudomonas putida* under different mass transfer operational conditions: (a) biomass concentration; (b) desulphurization capability; (c) dissolved oxygen concentration (adapted from Gomez et al. [39]).

desulphurization capability is represented in time for different transport conditions; it can be seen that the maximum percentage of desulphurization capability is similar for stirrer speeds between 250 and 400 rpm (approximately of 80% for 30 h of growth); after that, this capability decreases lightly. In the case at 150 rpm, this decrease is faster and the maximum is approximately 50% lower, most probably as consequence of the oxygen transport limitation during growth. Finally, experimental and predicted oxygen concentration values, under different operational conditions, are showed in Fig. 14c.

In a previous work [11], a kinetic model has been proposed to describe biomass growth rate of *P. putida* CECT5279 grown in several media compositions and under different operational conditions (gas air flow, temperature and pH). In cultures of *P. putida* CECT5279 (Fig. 15), DO concentration affects growth and BDS capability of the cells. While a high oxygen concentration increases the biomass growth rate (Fig. 13a), the desulphurization capability of the cultured cells decreases. Therefore, a macroscopic maximum of biocatalyst desulphurization capability is achieved at medium stirrer speeds, as 200–300 rpm (Fig. 15b). Experimental and predicted oxygen concentration values, under different operation conditions, are showed in Fig. 15c. As it can be seen, a reasonable agreement between the experimental data and the model-predicted values is found in all the cases cited above, for a wide range of operation conditions and in different bioprocesses.

7. Conclusions

The bioreactor is a key factor in biological process. A proper mechanical design of the bioreactor is aimed at maintaining the environmental and nutritional conditions (temperature, pressure, pH, nutrients, mixing, hydrodynamics stress, etc.) for optimal growth and/or a product formation. Among the various environmental and nutritional factors that influence in the bioprocess, oxygen is a critical parameter since it is the only nutrient that has to be provided continuously, even in batch cultures. In these conditions OUR (together OTR) is one of the key parameters involved on the design, operation and scale-up of bioreactors, since DO concentration is dependent on transport processes. For a satisfactory description of the aerobic microbial systems it is therefore necessary to know the dependence of the DO concentration on the many physical and biological parameters of the system, taking into account the intricate interconnections between them seen above.

Acknowledgements

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