Volumetric oxygen transfer coefficients ($k_L a$) in batch cultivations involving non-Newtonian broths

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Abstract

The oxygen transfer in non-Newtonian fermentation broths of Aspergillus awamori, during batch cultivations in conventional 10 l bioreactor has been investigated. Values of the volumetric oxygen transfer coefficient ($k_L a$), obtained at various impeller speeds, air flow rates and at distinct initial substrate concentrations were correlated with operational variables, geometric parameters of the system and physical properties of the broths utilising rigorous techniques in order to obtain a set of reliable and accurate data. An experimental device was constructed for on-line rheological measurements, and the apparent dynamic viscosity was determined from the broth rheograms. In order to measure power requirements, a torque meter was developed and non-Newtonian fluids with rheological characteristics similar to the Aspergillus fermented broths were utilised to obtain reference curves and correlations in the fermentor where the cultivations took place. Gas balancing method and a modified dynamic method were utilised simultaneously to determine $k_L a$ values. The rigorous methods thus developed allowed adequate evaluation of the oxygen transfer in the cultivations and also permitted good fits of four different traditional correlations for $k_L a$ to the experimental data. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Volumetric oxygen transfer coefficient; Power consumption; Broth rheology

1. Introduction

Fermentation broths containing mycelial cells frequently exhibit a pseudoplastic non-Newtonian rheological behaviour, which can be described by the power-law model. This behaviour exerts a profound effect on the bioreactor performance, affecting mixing pattern, power requirement, heat and mass transfer processes [1]. The increase in the broth apparent viscosity ($\mu_{ap}$) during aerobic fermentations can be partially compensated by increments in the operating conditions ($N$ and $Q$), in order to maintain adequate $k_L a$ values. Nevertheless, high impeller speeds ($N$) lead to the formation of high shear zones close to the impellers, with consequent physical damage to the cells and a reduction in the process productivity [2]. Due to the importance of the volumetric oxygen transfer coefficient ($k_L a$) in the performance and scale-up of conventional bioreactors, the literature describing various correlations for $k_L a$ in Newtonian fluids is rather extensive. However, particular care should be taken when applying these correlations to non-Newtonian systems containing electrolytes such as fermentation broths [3].

Two types of correlations have been proposed for the volumetric oxygen transfer coefficient ($k_L a$). The first does not make use of any dimensional criterion. In these correlations, $k_L a$ is related to the gassed power consumption per unit volume of broth ($P_g/V$) and the superficial gas velocity ($v_s$), as originally proposed by Cooper et al. [4]:

$$k_L a \alpha \left( \frac{P_g}{V} \right)^{a_1} \left( v_s \right)^{b_1}$$

(1)

where the values of the constants $a_1$ and $b_1$ may vary considerably, depending on the system geometry, the range of variables covered and the experimental methodology used. Although initially developed for fluids very distinct from fermentation broths, this type of correlation has been widely used in fermentation systems [3,5–7]. In a more recent work, Montes et al. [8] determined values of $k_L a$ in yeast broths (Triatoma variabilis) over wide ranges of both impeller speeds and superficial gas velocities in three different mechanically-stirred, sparger-aerated and baffled bioreactors (2, 5 and 15 l) in order to consider the effect
<table>
<thead>
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<th>Nomenclature</th>
<th>Description</th>
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<tbody>
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<td>constants of Eqs. (1), (2) and (8) (( \ast ))</td>
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<tr>
<td>( c_2, c_3 )</td>
<td>constants of Eqs. (2) and (8) (( \ast ))</td>
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<tr>
<td>( C )</td>
<td>dissolved oxygen concentration in the broth (mmol O(_2) l(^{-1}))</td>
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<td>( C^* )</td>
<td>dissolved oxygen saturation concentration in the broth (mmol O(_2) l(^{-1}))</td>
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<td>( d_1 )</td>
<td>constant of Eq. (8) (( \ast ))</td>
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<tr>
<td>( D )</td>
<td>impeller diameter (m)</td>
</tr>
<tr>
<td>( D_t )</td>
<td>tank diameter (m)</td>
</tr>
<tr>
<td>( Fr )</td>
<td>((=D_t^3n^2b)) Froude number (( \ast ))</td>
</tr>
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<td>( GLA )</td>
<td>glucoamylase activity (U l(^{-1}))</td>
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<td>( k )</td>
<td>constant of Eq. (10) (( \ast ))</td>
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<td>( k_e )</td>
<td>electrode sensitivity (h(^{-1}) or s(^{-1}))</td>
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<td>( k_{O_a} )</td>
<td>volumetric oxygen transfer coefficient (h(^{-1}) or s(^{-1}))</td>
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<tr>
<td>( K )</td>
<td>consistency index (Pa s(^n))</td>
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<tr>
<td>( n )</td>
<td>flow behaviour index (( \ast ))</td>
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<tr>
<td>( n_{in} )</td>
<td>inlet dry molar gas flow rate (mol O(_2) h(^{-1}))</td>
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<td>( n_{out} )</td>
<td>outlet dry molar gas flow rate (mol O(_2) h(^{-1}))</td>
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<tr>
<td>( N )</td>
<td>impeller speed (rpm)</td>
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<tr>
<td>( N_p )</td>
<td>power number (( \ast ))</td>
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<tr>
<td>( P_0 )</td>
<td>ungassed power consumption (W)</td>
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<td>( P_g )</td>
<td>gassed power consumption (W)</td>
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<td>( P_{g0} )</td>
<td>gassed power consumption per impeller (W)</td>
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<td>((P_g/Q)^*)</td>
<td>dimensionless group of Eq. (8) (( \ast ))</td>
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<td>( Q )</td>
<td>air flow rate (m(^3) s(^{-1}))</td>
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<tr>
<td>( Q_{O_2} )</td>
<td>specific oxygen uptake rate (mmol O(_2) g(^{-1}) h(^{-1}))</td>
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<td>( Q_{O_2X} )</td>
<td>maximum specific oxygen uptake rate (mmol O(_2) g(^{-1}) h(^{-1}))</td>
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<td>global oxygen uptake rate (mmol O(_2) g(^{-1}) h(^{-1}))</td>
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<td>( R^2 )</td>
<td>correlation coefficient (( \ast ))</td>
</tr>
<tr>
<td>( Re_{imp} )</td>
<td>modified Reynolds number (( \ast ))</td>
</tr>
<tr>
<td>( S )</td>
<td>substrate concentration (g l(^{-1}))</td>
</tr>
<tr>
<td>( Sc )</td>
<td>(\ast) Schmidt number (g(^{-1}))</td>
</tr>
<tr>
<td>( Sh^a )</td>
<td>( k_{O_a}D_e / D_t ), modified Sherwood number (( \ast ))</td>
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<td>( Sh^a_\ast )</td>
<td>dimensionless group in Eq. (7) (( \ast ))</td>
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<tr>
<td>( v_s )</td>
<td>(=4Q/(\pi D_t^2)) superficial gas velocity (m s(^{-1}))</td>
</tr>
<tr>
<td>( V )</td>
<td>broth volume (l or m(^3))</td>
</tr>
<tr>
<td>( V_i )</td>
<td>broth volume per impeller (m(^3))</td>
</tr>
<tr>
<td>( X )</td>
<td>cell concentration (g l(^{-1}))</td>
</tr>
<tr>
<td>( Y_{CO_2} )</td>
<td>carbon dioxide molar fraction in outlet gas (( \ast ))</td>
</tr>
<tr>
<td>( Y_{CO_2}^o )</td>
<td>carbon dioxide molar fraction in inlet gas (( \ast ))</td>
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Greek letters

\( \alpha \) constant of proportionality
\( \Phi_{air} \) specific air flow rate (vvm: air volume per broth volume per minute) (min\(^{-1}\))
\( \gamma_{av} \) average shear rate (s\(^{-1}\))
\( \mu_p \) apparent dynamic viscosity (Pa s)
\( \mu_g \) air viscosity (Pa s)
\( \mu_w \) water viscosity (Pa s)
\( \nu_{air} \) apparent kinematic viscosity (m\(^2\) s\(^{-1}\))
\( \rho \) density (kg m\(^{-3}\))
\( \sigma \) surface tension of filtrate (N m\(^{-1}\))
\( \sigma^* \) \(=\sigma/(\rho \nu_{air} D_t^2)\) (Eq. (8) (\( \ast \))
\( \tau \) average shear stress (Pa)

of the fermentor scale-up on \( k_{O_a} \). Experimental data were fitted using the correlation proposed by Cooper et al. [4] and the values for the parameters \( a_1, b_2 \) and proportionality constant were 0.35, 0.41 and 3.2 \times 10\(^{-3}\), respectively. Due to the fact that most of the yeast broths behave as slightly non-coalescent fluid, according to the authors, the correlation improved the prediction of \( k_{O_a} \) values with respect to other generic correlations usually developed for strong coalescent and non-coalescent fluids. Extended forms of this first type of relationship have been proposed in the literature, incorporating terms like impeller speed (\( N \)) and the fluid apparent viscosity (\( \mu_{ap} \)). For example, Ryu and Humphrey [9] observed the influence of the broth apparent viscosity (\( \mu_{ap} \)) on \( k_{O_a} \) in penicillin fermentation and proposed the following correlation:

\[
k_{O_a} = \left( \frac{k_{O_a}}{ \left( \frac{v}{V} \right)^{0.25} } \right)^{0.4} \left( v_s^{0.5} (N)^{0.5} \right)^{0.65} \mu_{ap}^{0.25} \mu_{g}^{0.65}
\]

(3)
Additionally, Shin et al. [11] verified that in high cell density cultures of fast-growing aerobes such as recombinant E. coli where the cell mass increases up to more than 70 g/l, the oxygen availability can be the rate-limiting factor for the fermentation process. In that way, the following correlation for \( k_{L,A} \) incorporating the effect of cell density \( (X) \) in oxygen transfer has been proposed:

\[
k_{L,A} = 0.0192 \left( \frac{P_g}{P} \right)^{0.55} (u_0)^{0.64} \times (1 + 2.12X + 0.20X^2)^{-0.25}
\]  

(4)

The second type of correlation for \( k_{L,A} \) is based on dimensional analysis. This approach presents certain advantages because the correlations obtained for a known system can be used to estimate \( k_{L,A} \) in other systems with different dimensions. Yagi and Yoshida [12] proposed the following correlation, valid for both Newtonian and non-Newtonian fluids, for the standard six-bladed turbine in standard configuration in a vessel of 0.25 m diameter:

\[
Sh^* = 0.060(Re_s)^{0.60} (Sc)^{0.56} (Pr)^{0.19}
\]  

(5)

This correlation fitted to the experimental data in a reasonable extent, with maximum deviations of 30 and 80%, between experimental and calculated \( Sh^* \) values, for Newtonian and non-Newtonian fluids, respectively. In order to determine the combined effects of the variables, including operational conditions, liquid and gas properties and geometry of the tank, García-Ochoa and Gómez [10] employed a similar dimensionless equation to fit the set of experimental data of \( k_{L,A} \) obtained in the same system under operational conditions above specified. Li et al. [13] proposed a relationship among \( k_{L,A} \), the impeller speed \( (N) \) and the apparent viscosity \( (\mu_p) \), for fermentation broths of \( \text{Aureobasidium pullulans} \) and \( \text{Xanthomonas campestris} \) in 1001 fermentor, which correlated very well the great variety of experimental data, as follows, respectively:

\[
Sh^* = 5.40 \times 10^4 (Pr)^{0.65} (\mu_p)^{-0.33}
\]  

(6)

\[
Sh^* = 3.32 \times 10^4 (Fy)^{0.23} (\mu_p)^{-0.58}
\]  

(7)

Zlokarnik [14] suggested, also through dimensional analysis, a general dependence of \( k_{L,A} \) on physico-chemical properties and operating conditions, as follows:

\[
k_{L,A} = \left( \frac{Q}{V_{r}} \right)^{-1} \left[ \frac{L}{\mu_p} \right]^{-0.33} (Sc)^{0.56} (P)^{-0.64} (Sh^*)^{0.64}
\]  

(8)

where the dimensionless variable \( Sh^* \), not exactly defined, is related to the coalescence behaviour of solutions. However, this correlation presents a feature not found in the others since the variable \( P_e \) represents the gassed power consumption per impeller, this equation can be applied to systems with different numbers of impellers and, therefore, with different geometries. This possibility was verified by Jurecic et al. [3], investigating submerged cultures of \( \text{Bacillus licheniforms} \) for bacitracin production in bioreactors of 1001 and 67.5 m³ with two and four turbine impellers, respectively.

Although several correlations have been developed for different systems, most of them are not specific to fermentation broths or, when developed with such a purpose, they do not determine all the variables throughout the cultivation. Surface tension of the liquid \( (\sigma) \), for instance, is considered to be constant throughout the whole cultivation and, therefore, it is not considered in the correlations. Furthermore, in the development of \( k_{L,A} \) correlations in cultivations involving filamentous microorganisms, particular care should be taken when measuring some variables, like the rheological properties of the broth and the \( k_{L,A} \) itself, when traditional equipment and methodologies are utilised. In recent works, Badino Jr. et al. [15], as well as Svhila et al. [16], verified that the use of conventional bench rheometers, such as the concentric cylinder, to determine rheological properties of viscous mycelial suspensions, is often unsatisfactory. The main problems are caused by pellet’s size, generally of the same order of magnitude as the annulus, as well as the tendency of the suspension to become heterogeneous due to settling and particle interaction. Thus, they propose the use of rotational rheometer provided with a helical impeller to obtain reliable and accurate rheological data for filamentous fermentation broths. In addition, Olsvik and Kristiansen [17] concluded that on-line continuous measurement of broth rheology enables the uniform treatment of the samples and statistically more “correct” measurements.

The aim of this work is to investigate the influence of operating conditions on the rheological properties of the fermented broth and on the oxygen transfer during batch cultivations of \( \text{A. awamori} \) in a bench scale fermentor. Methodologies for on-line rheological measurements and for determination of both power consumption and \( k_{L,A} \) have been developed in order to obtain a set of reliable and accurate data. Four classical correlations mentioned previously (Eqs. (1), (2), (5) and (8)) for the volumetric oxygen transfer coefficient \( (k_{L,A}) \), including the operating conditions, geometrical parameters of the system and physical properties of the broth (apparent viscosity and also surface tension of the filtrated broth), were fitted to the experimental values.

2. Materials and methods

2.1. Microorganism and culture media

A spore suspension \( (2 \times 10^7) \) spores per millilitre of \( \text{A. awamori} \) NRRL 3112, stored in cryotubes (glycerol 20% v/v) at \(-20^\circ C\), was used throughout this work.
Growth medium was composed of (in g/l): total reducing sugars (TRS) from cassava flour and assayed as glucose, 20.0; yeast extract, 0.2; MgSO$_4$·7H$_2$O, 1.0; (NH$_4$)$_2$SO$_4$, 10.0; Na$_2$HPO$_4$·12H$_2$O, 7.56; KH$_2$PO$_4$, 7.0; pH 5.0. Two fermentation media with different compositions were utilised. The first had the same composition as the growth medium and the second had initial TRS concentration ($S_0$) of 40.0 g/l, with addition of silicone antifoam (0.08 ml/l) to both.

2.2. Fermentation equipment and cultivation procedure

Nine batch cultivations were conducted at 35°C and pH 5.0 in a 10 l Microferm MF-14 fermentor (New Brunswick Sci. Co. Inc., USA) provided with four baffles in order to favour turbulence and to prevent vortex formation. For each fermentation, spores were initially germinated in five 1 l Erlenmeyer flasks, each holding 200 ml of growth medium. Flasks were inoculated with 1 ml of the spore suspension from the cryotubes and inoculated in a rotary shaker (New Brunswick Sci.) for 24 h at 35°C and 300 rpm. The contents of the flasks were transferred to the fermentor with 9 l of fermentation medium, performing a total of 10 l of fermentation broth. The cultivations were carried out at different operational conditions. The ranges of impeller speed ($N$, 300 < $N < 700$ rpm) and specific air flow rate ($\phi_{air}$, 0.2 < $\phi_{air}$ < 1.0 vvm) are frequently utilised during filamentous fungal cultivations in bench-scale bioreactors, in order to provide adequate mixing and oxygen supply to the cells. The labels E20-7-3, E20-5-6, E20-7-9, E40-5-2, E40-5-6, E40-5-10, E40-7-2, E40-7-6 and E40-7-10 were used to designate the experimental conditions: $S_0 = 20$ g/l, $N = 700$ rpm and $\phi_{air} = 0.3$ vvm, respectively. Samples were withdrawn each 3 h for analytical determinations.

Dissolved oxygen concentration was measured by a sterilisable galvanic electrode (Mettler-Toledo InPro6000 Series), bearing a 0.0254 mm thick FEP (fluorinated ethylene propylene) membrane. Readings of the oxygen ($Y_{O_2}$) and carbon dioxide ($Y_{CO_2}$) molar fractions in the outlet gas were furnished by gas analysers produced by Beckman Industrial (model 755) and Fuji Electric (model IR-730), respectively. The experimental apparatus is shown in Fig. 1.

2.3. Analytical determinations and surface tension measurement

Cell concentration ($X$) was evaluated as dry matter obtained after vacuum filtration and drying at 85°C for 6 h. Substrate concentration ($S$) was determined as equivalent glucose utilising Glucose Autoanalyser II (Bran-Luebbe), after the polysaccharide in the sample had previously been converted to glucose through an enzymatic hydrolysis [18]. Liquid surface tension of the filtrated broth ($\sigma$) was measured with a Fisher Scientific Co. ring tensiometer.

2.4. Rheological properties

Continuous on-line rheological measurements were recorded every 2 h of cultivation, utilising the experimental device constructed and calibrated by Badino Jr. et al. [15] based upon the original reports of Kemblowski and Kristiansen [19]. In order to determine flow curves of fermentation broths, a rotational helical ribbon impeller was connected to Brookfield rheometers, models LV-DVIII and RV-DVIII (Brookfield Eng. Lab. Inc., USA), which measured the impeller torque ($T$) at different rotational impeller speeds ($N$). The average shear rate, $\gamma_{av}$, and the average shear stress, $\tau_{av}$, around the impeller were calculated from the calibration equations, $\gamma_{av} = 28.91 N$ and $\tau_{av} = 10530 T$.

Fig. 1. Experimental apparatus.
For these continuous on-line rheological measurements, the fermentation broth was pumped out of the bioreactor through silicone rubber tubing and into the impeller rheometer by a peristaltic pump. The outlet of the impeller shaft in the rheometer was provided with a rotating labyrinth seal. Aseptic conditions were ensured by introducing sterile air through the glass tubing next to the head of the vessel. The average residence time in the loop was fixed at 90 s, in order to allow good measurements, without causing particle settling or even disturbing the performance of the cultivation.

The power law model (\( \tau_{av} = K \gamma^{n} \)) was fitted to on-line experimental data and the rheological properties, consistency index (\( K \)) and flow behaviour index (\( n \)), were estimated.

2.5. Power consumption

Ungassed and gassed power consumptions were estimated under the previously mentioned different operating conditions (\( V \) and \( Q \)), every hour during cultivation, by using traditional reference curves and correlations to interpret data from a purpose-built torque meter based on flat strain gauges of 120\( \Omega \) resistance. The new device was connected to the stirrer shaft inside the fermentor, to avoid frictional losses in the mechanical seal. In order to obtain the reference curves and correlations, non-Newtonian fluids of similar rheological characteristics to the fermentation broths were used [20]. Ungassed power consumption (\( P_{g} \)) was obtained through a graph of power number (\( N_{p} \)) as a function of modified Reynolds number (\( Re_{m} \), as proposed by Metzner et al. [21]) for non-Newtonian pseudoplastic fluids.

\[
N_{p} = \frac{P_{g}}{\rho N^{3} \left( \frac{D}{2} \right)^{5}}
\]

\[
Re_{m} = \frac{\rho N^{2-n} D^{5}}{K(Re^{n-1})}
\]

where \( k = 11.4 \), under the conditions considered.

Gassed power consumption (\( P_{g} \)) was estimated through a traditional correlation, proposed by Michel and Müller [22], obtained for the experimental system used in cultivations:

\[
P_{g} = 0.832 \left( \frac{P_{g} N P_{g}}{\rho n^{0.44}} \right)^{0.44}
\]

2.6. Oxygen transfer

Global oxygen uptake rate (\( Q_{O2} \), \( X \)) as well as the volumetric oxygen transfer coefficient (\( k_{L,a} \)) were obtained by both steady-state and dynamic methods. By the gas balancing method, \( Q_{O2} \times X \) was determined from an oxygen mass balance in the gas phase by the following expression:

\[
Q_{O2} \times X = \frac{\dot{m}_{a} \Delta C_{O2}^{*} - \dot{m}_{a} \gamma C_{O2}^{*}}{V}
\]

The inlet molar gas flow rate (\( \dot{m}_{a} \)) was measured utilising a mass flowmeter model 5811-N (Brooks Instrument Division), and the outlet molar gas flow rate (\( \dot{m}_{out} \)) was calculated from the nitrogen mass balance in the gas phase by the following equation:

\[
\dot{m}_{out} = \frac{0.79 \dot{m}_{a} \frac{1}{1 - \frac{\gamma}{C_{O2}^{*}}} - \frac{\gamma}{C_{O2}^{*}}}{1 - \frac{\gamma}{C_{O2}^{*}}}
\]

The volumetric oxygen transfer coefficient (\( k_{L,a} \)) could be calculated for the steady-state condition, from the mass balance for dissolved oxygen as follows:

\[
k_{L,a} = \frac{Q_{O2} \times X}{C^{*} - C}
\]

where \( C \) is the dissolved oxygen concentration in the broth at the steady-state given by the galvanic electrode and \( C^{*} \) is the dissolved oxygen saturation concentration in the broth estimated by the method proposed by Schumpe et al. [23].

The volumetric oxygen transfer coefficient (\( k_{L,a} \)) was also evaluated through an alternative method, based on the traditional dynamic method taking into consideration the electrode sensitivity (\( k_{e} \)) [24]. The electrode sensitivity (\( k_{e} \)) is defined as the inverse of the time taken for the electrode to reach 63.2% of its final value when exposed to a step change in oxygen concentration. After each cultivation the electrode sensitivity (\( k_{e} \)) was determined in triplicate by adding xanthan gum solution 0.4% w/v at 35°C to simulate the fermentation broth.

3. Results and discussion

3.1. Rheological behaviour of fermentation broths

With the average shear rate (\( \gamma_{av} \)) ranging from 0.96 to 72.27 s\(^{-1}\), all fermentation broths at different cultivation times, behaved as non-Newtonian pseudoplastic fluids. Good fits between the power law model and the experimental data were achieved, showing that on-line rheological measurement is a consistent and reliable technique for mycelial cultivations. Fig. 2 illustrates typical flow curves (rheograms) obtained at different times for the run E40-7-6. Significant changes in the rheological parameters, fluid consistency index (\( K \)) and flow behaviour index (\( n \)) as depicted the Fig. 3 for the run E40-7-6, were observed as the fermentations proceeded: \( K \) increased rapidly while \( n \) decreased to values between 0.3 and 0.5. Interestingly, an added feature of the on-line technique was its ability to accurately anticipate the instant at which the culture medium is depleted of carbon source, especially when measurements are taken frequently. As can be observed in Fig. 3, immediately after the carbon source is exhausted (∼27 h), the \( K \) value decreases suddenly, probably as a consequence of both hyphae fragmentation and lysis.
3.2. Characteristics of oxygen uptake and oxygen transfer

Typical time course profiles of specific oxygen uptake rate \( (Q_{O_2}) \) and of dissolved oxygen concentration \( (C) \) obtained during an experiment with oxygen limitation (E40-5-2) is shown in Fig. 4. Considering all runs, the maximum values of specific oxygen uptake rate \( (Q_{O_2}^{\text{max}}) \) ranged from 2.8 to 4.5 mmol \( O_2 \) g\(^{-1}\) h\(^{-1}\). Metwally et al. [25], as well as Shuler and Kargi [26], both cultivating \textit{Aspergillus niger} strains, utilising glucose as the main carbon source, found \( Q_{O_2}^{\text{max}} \) values in the range 3–4 mmol \( O_2 \) g\(^{-1}\) h\(^{-1}\), which agrees very well with the present results.

With respect to \( k_L a \) determinations, the simultaneous use of the two measuring methods allowed reliable determinations of \( k_L a \) over a wide range (60.1–768 h\(^{-1}\)) showing that the gas balancing and the alternative dynamic method taking into account the electrode response time, should be used in a complementary manner: while the gas balancing method provides accurate measurements mainly after the cell concentration reaches higher values, the alternative dynamic method is particularly useful to evaluate \( k_L a \) at the beginning of the fermentation, when the differences in oxygen composition between inlet and outlet gas are quite small and the levels of dissolved oxygen concentration are very high, a situation at which the gas balancing and other methodologies are often inadequate. Nevertheless, dynamic methods for determinations of \( k_L a \) are useful only when the dissolved oxygen concentration is fairly above the critical value \( (C_{\text{crit}}) \); for lower values, the gas balancing method would be more appropriate [24].

It is known that besides the agitation \( (N) \) and aeration \( (Q) \) conditions, a variable that negatively affects \( k_L a \) is the broth apparent viscosity \( (\mu_{ap}) \), because it offers resistance to the oxygen transfer from the gaseous to the liquid phase. Values of apparent viscosity \( (\mu_{ap}) \) varied between 0.013 and 0.257 Pa s and the estimated maximum value of ungassed power consumption \( (P_0) \) was 187 W. The strong influence of the broth apparent viscosity \( (\mu_{ap}) \) on the volumetric oxygen transfer coefficient \( (k_L a) \) during experiment E40-5-10 is shown in Fig. 5. It can be observed that \( k_L a \) decreased from 381 to 124 h\(^{-1}\) when \( \mu_{ap} \) increased from 0.013 to 0.257 Pa s in the time interval from 0 to 37 h of cultivation. In addition, in Fig. 5 the time course of the relative surface tension of the filtrated broth \( (\sigma/\sigma_w) \) is depicted, where \( \sigma_w = 7.04 \times 10^{-2} \text{N m}^{-1} \) is the surface tension of water at 35°C [27]. The relative surface tension of the filtrated broth presented an ascendant behaviour, varying from 0.897 to 0.943 for the same time period considered before. Such variation is relatively modest and it is probably due to the consumption
of the dissolved nutrients from the original culture medium as the fermentation proceeds.

3.3. Correlations for $k_L a$

As mentioned before, four classical correlations have been utilised to correlate the volumetric oxygen transfer coefficient ($k_L a$) with the operation variables ($N$, $Q$, and $P_g$), geometric parameters of the system ($V$ and $D_i$) and physical properties of the broths ($\mu_{ap}$ and $\sigma$), as described by Cooper et al. [4], Ryu and Humphrey [9], Yagi and Yoshida [12] and Zlokarnik [14]. The correlations were fitted to the experimental values and the parameters were estimated through Marquardt’s procedure that utilises the least squares non-linear regression [28]. The criterion for the best fit and parameter optimisation was the sum of squares of residuals (SSR). No convergence problems were found in non-linear regression and all confidence intervals, computed for the 95% of probability, were below 25% of the estimated values. The fitted correlations, together with the respective correlation coefficients ($R^2$) and numbers of experimental values used in the regressions, are presented in Table 1. Figs. 6–9 illustrate the quality of the fits.

The simultaneous use of the two methods, gas balancing (GB) and dynamic method (DM), allowed a great number of $k_L a$ values to be obtained as previously discussed, with the obtention of similar values in the same operating conditions.

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Table 1

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<tr>
<th>Correlation</th>
<th>Reference</th>
<th>Regression coefficient ($R^2$)</th>
<th>Experimental values</th>
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<tbody>
<tr>
<td>$k_L a = 41.46 P_g / V^{0.44} V^{0.47}$</td>
<td>Cooper et al. [4]</td>
<td>0.90</td>
<td>286</td>
</tr>
<tr>
<td>$k_L a = 18.09 P_g / V^{0.53} V^{0.34}$</td>
<td>Ryu and Humphrey [9]</td>
<td>0.92</td>
<td>305</td>
</tr>
<tr>
<td>$Sh = 4.5 (R_e V^{0.46}) (k_L a V^{0.64}) (D_i^{0.64}) (Re)^{0.25}$</td>
<td>Yagi and Yoshida [12]</td>
<td>0.94</td>
<td>316</td>
</tr>
<tr>
<td>$k_L a = 1.45 \left[ (P_g / Q)^{0.54} \right] (k_L a V^{0.64}) (D_i^{0.64}) (Re)^{0.25}$</td>
<td>Zlokarnik [14]</td>
<td>0.96</td>
<td>318</td>
</tr>
</tbody>
</table>

Fig. 6. Comparison of the experimental data with those calculated using the correlation proposed by Cooper et al. [4] (DM: dynamic method, GB: gas balancing for $k_L a$ determination).

Fig. 7. Comparison of the experimental data with those calculated using the correlation proposed by Ryu and Humphrey [9] (DM: dynamic method, GB: gas balancing for $k_L a$ determination).

Fig. 8. Comparison of the experimental data with those calculated using the correlation proposed by Yagi and Yoshida [12] (DM: dynamic method, GB: gas balancing for $k_L a$ determination).
consider the effects of the physical properties of the broth. Despite its simplicity, the correlation proposed by Ryu and Humphrey [9] gave also a good fit, practically as good as the more complex correlations proposed by Yagi and Yoshida [12] and by Zlokarnik [14] that need the knowledge of additional variables of the broth, such as apparent viscosity ($\mu_{ap}$) and surface tension ($\sigma$), for convenient application. However, if we intend to use a $k_v \alpha_a$ correlation in the design or scaling-up of conventional bioreactors, it is more convenient to use more complex correlations, such as those proposed by Yagi and Yoshida [12] and by Zlokarnik [14], which relate dimensionless variables.

In addition, it should be observed that the parameters estimated by the regressions are very close to the values found in the literature. The parameters of the correlation proposed by Cooper et al. [4], with $a_1 = 0.47$ and $b_1 = 0.39$, respectively, are in the ranges of values mentioned by Kawase and Mee-Young [29] for non-Newtonian systems, which are $0.37 < a_1 < 0.80$ and $0.20 < b_1 < 0.84$. The value obtained for the constant $c_2$ of the correlation proposed by Ryu and Humphrey [9] ($c_2 = -0.12$) is very different from that obtained in the original work ($c_2 = -0.86$). However, it should be pointed out that the broths studied had different physicochemical properties and the discrepancy may be due to the fact that different equations have been used to calculate the apparent viscosities ($\mu_{ap}$) of the fermentation media: while the power law model has been used in this work to describe the non-Newtonian behaviour of Aspergillus fermentation broths, Ryu and Humphrey [9] employed the Bingham plastic model. However, as expected, both of the values for the constant $c_2$ were coherently negative, indicating an inverse proportionality between $k_v \alpha_a$ and $\mu_{ap}$. The values obtained for the parameters from the correlation of Yagi and Yoshida [12] could not be compared with values found in the literature, because no published work has been found that applies this correlation to non-Newtonian fermentation broths. The parameters obtained from the correlation of Zlokarnik [14], presented values similar to those obtained by Jurecic et al. [3], employing non-Newtonian fermentation broths of B. licheniformis in pilot and industrial scales ($a_1 = 0.35$, $b_1 = -0.30$ and $c_1 = -0.50$). Also, it should be emphasised that although a lot of correlations for $k_v \alpha_a$ include dimensionless terms, in the literature investigated no works have been found that present measurements of parameters such as the surface tension of the filtrate ($\sigma$) during cultivation.

4. Conclusions

The main objective of this work was to investigate the influence of operating conditions on the rheological properties of the broth and on the oxygen transfer during batch cultivations of A. awamori in a bench scale fermentor. Four classical correlations found in the literature, which correlate the volumetric oxygen transfer coefficient ($k_v \alpha_a$) with the process operational variables ($V$, $Q$ and $P_a$) and the geometrical parameters of the system has been utilised. In particular, in some of these correlations, $k_v \alpha_a$ is also correlated with the physical properties of the broth (apparent viscosity and liquid surface tension), which are time dependent during the fermentation process.

Good fits were obtained for all the correlations tested, considering experimental data obtained with Aspergillus cultivation in quite distinct operational conditions, observing that the fit was even better for the more complex correlations, which take into account the physical properties of the broth. It is obvious, however, that the utilisation of such correlations means the necessity of more complex analytical determinations, which is not always possible.

Finally, it should be pointed out that the good fitted obtained are a consequence of the quality and accuracy of the generated experimental values, which are a result of carefully elaborated methodologies implemented in the cultivations.

References