Mass transfer properties of glucose and O_2 in *Saccharomyces cerevisiae* flocs

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Abstract

The use of flocculent microorganisms in fermentations is a very promising application. However, it has some drawbacks of which the most important ones are related to mass transfer limitations within flocs. Their mechanical fragility and their shape present extra difficulties when evaluating parameters such as effective diffusivity (D_e) and external mass transfer coefficient (K_c). This work focuses on the determination of D_e and K_c for glucose and oxygen transport in aggregates of *Saccharomyces cerevisiae*. A modified diffusion cell was used in order to avoid the destruction of the flocs and an image analysis technique was applied for floc size determination. D_e and K_c were calculated using two methods: an analytical one, based on the solutions of Fick's law of diffusion and a numerical one, based on general mass balances of a component in flocs and the bulk phase. Diffusion coefficients were found to be 1.10×10^{-10} m^2 s^{-1} for glucose and between 0.049×10^{-10} m^2 s^{-1} and 0.21×10^{-10} m^2 s^{-1} for oxygen. These values are around 17% and 0.2 to 1% of the respective diffusivities in pure water. They were not affected by the initial solute's concentration.   © 1998 Elsevier Science S.A. All rights reserved.

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1. Introduction

Biotechnological processes have progressed vigorously over the last decades and one of the main goals of bioprocess engineering is to increase their productivity. Such objective may be achieved using high cell density systems [1–5]. Among these, the use of flocculent microorganisms cultivated in bioreactors where their sedimentation characteristics can be exploited is a very promising application, having low associated costs and design simplicity as the main advantages [6,7].

Nevertheless, one of the major problems limiting both the application of modern control techniques to the above-mentioned bioprocesses and the overall performance of bioreactors is the lack of data on mass transfer properties of the various substrates and products in living cell aggregates. Despite the relative abundance of references dealing with other high cell density systems (of which cell immobilisation in alginate beads is the most used) [8–12], very little work has been done in this area with flocs [7,13,14] and existing data on diffusivities of glucose and oxygen do not usually refer to the case of cell aggregates [15]. A very simple yet

valid reason for this situation is their very fragile nature: flocs are very difficult to handle as they are easily destroyed. Further, this problem becomes more acute with the increase in size, namely when dealing with diameters of 2–3 mm. Also their geometry seldom is, as usually assumed, that of a perfectly defined sphere; instead, flat cylinders and ellipsoids are more common shapes.

Diffusion is probably the most important mechanism of solute transport through cell aggregates and it is generally described using a single parameter, the effective diffusivity (D_e). D_e relates the gradient of the characteristic concentration ($c(a,t)$) to the average diffusive solute flux (J_D) across the volume of the object in study, which is expressed by Fick's law:

$$J_D = -D_e \cdot \nabla c(a,t) \tag{1}$$

There are two distinct approaches for the calculation of effective diffusion coefficients which are widely employed: in the first one, the effective diffusivity in the aggregates can be determined analysing the data by means of a reaction–diffusion model; in the second, the assessment is made using Eq. (1) in the absence of reaction. The techniques used in both approaches have merit but also drawbacks [12,15] and, therefore, it is necessary to choose very carefully the suitable

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experimental procedure according to the features of the investigated systems.

In general, the diffusion coefficient of a component in a solvent is considered to be a function of some parameters such as temperature [16], pressure (in gaseous systems) and medium composition [17]. Further, characteristics such as floc dimensions and shape are of extreme importance to the assessment of D_e. Particularly, if the aggregates are assumed to be spherical or cylindrical, the determination of their diameter is crucial for the final result [18] and the best procedure to do this in a non-destructive way is by image analysis. Such technique applied to yeast flocs has been developed by Vicente et al. [19] and has been used in the present work. Also, the structure of the aggregates plays an important role: diffusivity determinations in alginate beads with immobilised cells cannot be used as a substitute for those in the aggregates. The values obtained are quite different in both cases, as shown by Ananta et al. [13], who used plant cell aggregates and found values for O_2 diffusivity as low as 2% of the value in pure water. Further, it is important to mention that while the beads' porosity values reported are usually around 0.80 to 0.95 [17,20–22], depending on the cell fraction in the gel, the corresponding value, e.g., for yeast flocs is of 0.50 [14] and for a biofilm may vary from 0.72–0.75 in the top layers to 0.35–0.44 in the bottom layers [23]; this property strongly influences D_e.

The objective of this work is to determine the effective diffusivities of two important substrates (glucose and O_2) in aggregates of a highly flocculent strain of *Saccharomyces cerevisiae* (NRRL Y265) employed in fermentation processes. The values have been assessed measuring the exhaustion of those substrates in medium with inactivated suspended flocs. Both of the herein mentioned mathematical procedures for data analysis were applied in order to confirm the calculated values as well as the suitability of the experimental design: the analytical procedure ignores external mass transfer while the numerical procedure incorporates external mass transfer. The results of both procedures are compared, allowing for an assessment of the external mass transfer importance in transport phenomena associated with flocs. We are not aware of other such comparisons in the recent literature. Further, and in order to confirm the validity of the method used, the effective diffusivities of glucose and O_2 in calcium alginate beads with and without cells were also measured, being the values obtained compared with those published in the literature.

2. Materials and methods

2.1. Yeast flocs

The flocs used in this work were obtained from a highly flocculent strain of *S. cerevisiae* (NRRL Y265), grown continuously in an airlift bioreactor at 30°C and pH 4. The composition of the medium was as follows (g l^{-1}): KH$_2$PO$_4$, 5.0;

(NH$_4$)$_2$SO$_4$, 2.0; MgSO$_4 \cdot$7H$_2$0, 0.4; yeast extract, 1.0. Glucose was used as the carbon source, its concentration being adjusted according to specific needs. Details on the reactor and the conditions of operation have been described elsewhere [6,19,24].

2.2. Experimental procedure

After sampling, the aggregates were washed thoroughly with tap water and exposed overnight to an aqueous solution of ethyl acetate (1:13 v/v) in order to obtain non-viable, deactivated cells. The effectiveness of the deactivating procedure has been confirmed by adding fresh medium to the flocs and monitoring the metabolic activity of the culture: there has been no measurable ethanol production, gas evolution or cell growth during a period of at least 3 h. It should be stated that other inactivators such as sodium azide have been tried but the cells were still fermenting glucose after the treatment, despite the fact that about 99% of the cells appeared blue when stained with methylene blue. The stirring conditions were adjusted as during the experiments in order to maintain floc size and morphological characteristics. Finally the flocs were washed again with tap water to eliminate ethyl acetate.

In order to determine the influence of glucose adsorption on cell surface, blank experiments were made using inactivated cells of a non-flocculating yeast strain. These experiments confirmed that the glucose concentration in solution remained constant and equal to the initial value, as well as permitted a further confirmation of the effectiveness of the cell inactivation procedure in preventing glucose uptake.

The measurements of glucose diffusion coefficients were performed in 250 ml stirred glass Erlenmeyer flasks, placed in a laboratory incubator with orbital agitation at 150 rpm. All the floc suspensions were stabilised at a temperature of 30°C. At time zero, a defined volume of a solution of glucose with a known concentration was added to the suspension (final concentration of 10, 20 and 130 g l^{-1}, corresponding to experiments 1 to 3) which contained already all the other components of the medium and the inactivated flocs. The chronometer was then started. Samples of 100 μl were withdrawn at 60 s intervals during 30 min.

Oxygen diffusion coefficients were determined using the method and equipment described by [25], which allows to work with such aggregates with minimal mechanical stresses, avoiding their destruction. The device consisted mainly of an isolated measuring chamber with a polarographic dissolved oxygen probe and a thermostated reservoir. Both vessels were kept hermetically closed during the course of the experiments. The reservoir was filled with medium, closed, and pure N$_2$ was bubbled in the liquid in order to displace the dissolved oxygen. Meanwhile, a suspension of flocs was placed in the measuring chamber and the liquid phase was drained, under N$_2$ atmosphere. The flocs were deoxygenated by transferring oxygen free medium from the reservoir to the measuring chamber, until a low ($\leq 2\%$), stable value of dissolved oxy-

gen was obtained. The liquid remaining in the reservoir was then saturated with air (replacing the oxygen free medium in the measuring chamber) and the data acquisition was started. Experiments were performed (experiments 4 to 6) at different agitation rates (175, 200 and 300 rpm), giving rise to different floc sizes. The signal from the oxygen probe was acquired every 2 s via a Labtech (USA) data acquisition card supported with a corresponding Labtech Notebook Build Time (USA) software installed in a personal computer.

Also, determinations of glucose and oxygen diffusivities were made with calcium alginate beads both without and with (20% w/v) cells of *S. cerevisiae*, inactivated by the same procedure used with yeast flocs, in order to confirm the suitability of the system used in this work by comparison with published data. Details on the preparation of the beads are given elsewhere [24].

2.3. Analytical methods

Glucose concentrations of the samples were determined by HPLC equipped with a 830-RI (Jasco, Japan) refraction index detector and a 880-PU pump (Jasco). Commercial standards were used for the calibration of the column (Polyspher® CH CA, Merck, Germany) at 85°C with ultra pure water as eluent (0.5 ml min^{-1}).

The method proposed by Vicente et al. [19] was used to determine the floc size. In short, this method allows the determination of the average floc projected area by adjusting a Gauss curve to the floc size distribution data obtained by a computer aided image analysis technique; assuming that the projected area of a floc has a circular shape (which is in good agreement with the actual floc projected shape) the flocs' mean diameter is readily obtained.

Floc volume was assessed as the difference between the total volume of a floc suspension containing all the flocs used in a given experimental run and the volume of the liquid phase only. This 'bulk' method of volume determination was used due to the impossibility of assessing the total number of flocs present in each experiment.

Floc thickness was measured by means of a micrometer, taking the average of 20 measurements for every sample.

In order to avoid unnecessary stresses, the determinations of floc size, volume and thickness were performed only at the end of the experiments.

2.4. Data treatment

2.4.1. Analytical approach

A simple, reliable and very frequently discussed approach for data treatment is based on the analytical solutions for Fick's diffusion equation (Eq. (1)) [12,26,27]. The form of these solutions depends, among other factors, on the geometry of the object under study, on the data available and on the boundary conditions imposed.

In the present work, the chosen initial and boundary conditions were:

$$t=0 \quad \forall a \quad c(a,t)=c_0 \text{ and } C(t)=C_0 \tag{2}$$

$$t>0 \quad a=R,L \quad c(a,t)=c(t) \tag{3}$$

$$t>0 \quad a=0 \quad \frac{\partial c(a,t)}{\partial a}=0 \tag{4}$$

where condition (3) implies perfect mixing of the liquid phase around the particle, i.e., no external mass transfer resistance. For this set of conditions, and depending on the geometry ($f= 1$, 2 or 3 for a plane sheet, an infinite cylinder or a sphere, respectively), the analytical solutions for Eq. (1) are of the form [27]:

$$\frac{C(t)}{C_\infty}=1+\sum_{n=1}^{\infty}\frac{2 \cdot f \cdot (1+a)}{f^2+f^2 \cdot a+\alpha^2 \cdot q_n^2}\exp\left(-\frac{D_e \cdot q_n^2 \cdot t}{a^2}\right) \tag{5}$$

In the case of objects having a cylindrical geometry but in which the condition $R \ll L$ is not valid, there is the possibility to use the theorem of multiplied solutions [28]. The theorem states that the solutions of Eq. (5) for $f= 1$ and 2 (for a plane sheet and an infinite cylinder, respectively), already dimensionless, should be multiplied, representing the intersection of those two geometries (Fig. 1).

For large diffusion times the terms of Eq. (5) corresponding to $n \geq 2$ may be neglected [27]. D_e value can be obtained applying a suitable optimisation procedure. In the case of dissolved oxygen, oscillating data were previously smoothed using a Fast Fourier Transformation (FFT) technique.

A more general analytical solution including external mass transfer resistance is also available [27]. Nevertheless, convergence problems arose during the simultaneous assessment of D_e and K_c values rendering impossible their evaluation as a suitable fitting procedure could not be found.

2.4.2. Numerical approach

Disadvantages of the analytical approach can be avoided applying numerical methods for the calculation of the diffusion coefficient [9,29,30] or by the moment analysis of the
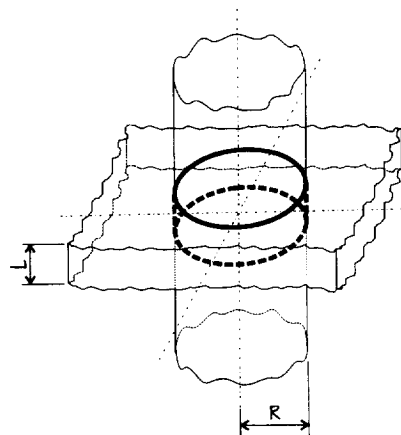

Fig. 1. Illustration of the theorem of multiplied solutions applied to the case of a cylinder in which the condition $R \ll L$ is not valid. The intersection of an infinite cylinder with a plane sheet gives the approximate shape of the cylindrical yeast flocs. This intersection is mathematically expressed as the product of the solutions of Eq. (5) for a plane sheet and an infinite cylinder.

experimental concentration time response for a pulse-type input in a batch system using Laplace transformation of Eqs. (6)–(10) [31].

The general mass balance of a component for non-steady state diffusion across the differential shell of the particle, ∂a, is as follows:

$$\varepsilon_p \frac{\partial c(a,t)}{\partial t} = D_e \cdot \left(\frac{\partial^2 c(a,t)}{\partial a^2} + \frac{(f-1)}{a} \frac{\partial c(a,t)}{\partial a} \right) \qquad (6)$$

with initial and boundary conditions:

$$t = 0 \qquad \forall a \qquad c(a,t) = c_0 \text{ and } C(t) = C_0 \qquad (7)$$

$$t > 0 \qquad a = R,L \qquad K_c \cdot (C(t) - c(a,t)) = D_e \frac{\partial c(a,t)}{\partial a} \qquad (8)$$

$$t > 0 \qquad a = 0 \qquad \frac{\partial c(a,t)}{\partial a} = 0 \qquad (9)$$

The mass balance of the liquid phase involving external mass transfer resistance for a stirred batch reactor can be written in the following form:

$$\varepsilon_r \frac{dC(t)}{dt} = -K_c \cdot A \cdot (C(t) - c(a,t)) \qquad a = R,L \qquad (10)$$

Finally, Eqs. (6)–(10) were re-arranged and normalised using dimensionless parameters. The orthogonal collocation method according to Villadsen and Michelsen [32] with at least five internal collocation points was applied together with a fitting procedure with an optimisation routine especially developed for the determination of D_e and K_c in the present work.

3. Results

3.1. Floc size determination

For the assessment of the representative floc diameter by image analysis, more that 150 particles were measured for a single determination. In Fig. 2 a representative histogram of the floc area distribution is presented. Care was taken to ensure that the flocs were sufficiently separated when grabbing images, otherwise the area of a single detected object would be a multiple of the real floc projected area, biasing the results. Also shown is a Gauss curve fitted to the data, allowing for the determination of the mean floc projected area (μ). The floc size was quite uniform around the mean value
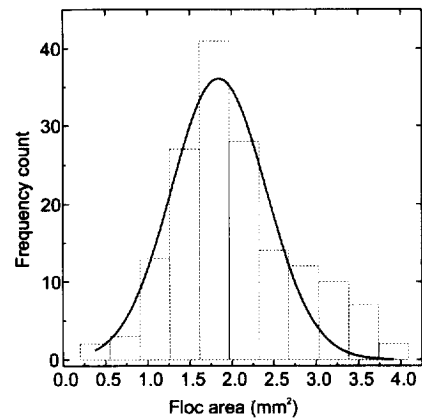

Fig. 2. Histogram of the floc projected area distribution calculated by image analysis, with the corresponding Gauss distribution curve.

as shown by the narrow normal distribution curve. Smaller flocs had an almost spherical shape; larger ones had a flat, circular disk shape. In both cases, however, the projected area was circular which permitted an easy calculation of the floc radius, R, using Eq. (11):

$$R = \sqrt{\frac{\mu}{\pi}} \qquad (11)$$

Table 1 summarises the main characteristics of the flocs used in the present work. These data were very important when choosing the proper factor f in Eqs. (5) and (6). Floc thickness was determined only in samples composed of aggregates with observable cylindrical shape.

3.2. Effective diffusion coefficient for glucose

Experiments 1 to 3 were performed in order to determine the effective diffusion coefficient for glucose in yeast flocs and the possible influence of the initial sugar concentration, varied from 10 to 130 g l^{-1}. The corresponding experimental points and the curves using the values calculated by the analytical and numerical approaches previously described are plotted in Fig. 3. Due to the similarity of the flocs used in these experimental runs, both in size and geometry, this figure represents the curves resulting from the data treated as if they were part of one single experiment. This reflects the absence of influence of glucose concentration on D_e.

In Table 2 the results of D_e and K_c estimations are summarised, including the 95% confidence interval (C.I.) values. The minimisation of the residual sum of squares (RSS) was

Table 1
Summary of the geometrical and shape characteristics of the flocs used in the experiments

Experiment	1	2	3	4	5	6
Floc shape	spherical	spherical	spherical	spherical	cylindrical	cylindrical
Floc radius $R \times 10^3$ [m]	0.44	0.46	0.48	0.49	0.78	1.21
Floc thickness $L \times 10^3$ [m]	–	–	–	–	0.7	1.1
Stirring speed [rpm]	300	300	300	300	200	175

Table 2
Calculated results of the non-linear fit of experimental data from experiments 1 to 3 using approaches I (analytical) and II (numerical)

Experiment	Approach	$D_e \times 10^{10}$ [$m^2 s^{-1}$]	95% C.I.	$K_c \times 10^8$ [$m s^{-1}$]	95% C.I.	RSS $\times 10^4$	$100 \times D_e/D_{w,gluc}$ [%]
1	I	0.47	±0.24	–	–	9.5	6.9
	II	1.08	±0.43	4.75	±0.36	10.6	15.9
2	I	0.51	±0.24	–	–	11.3	7.5
	II	0.98	±0.41	4.96	±0.37	54.6	14.4
3	I	0.54	±0.23	–	–	88.7	7.9
	II	1.22	±0.37	4.89	±0.36	22.0	17.9
Generalised	I	0.52	±0.26	–	–	109.5	7.6
	II	1.10	±0.47	4.87	±0.36	77.2	16.2

The results of the fits to the data altogether are also presented. $D_{w,gluc}$ (30°C) = 6.80×10^{-10} $m^2 s^{-1}$ [12].
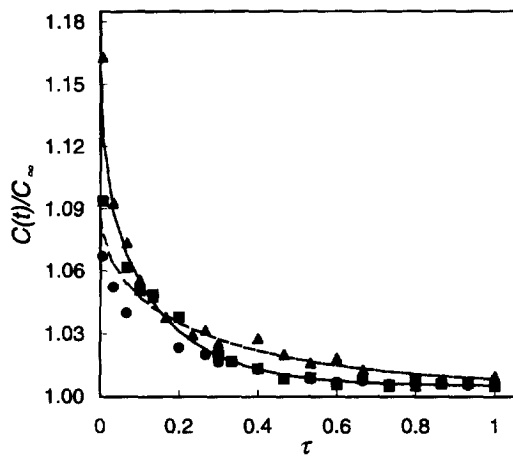

Fig. 3. Dimensionless concentration ($C(t)/C_\infty$) versus dimensionless time (τ) for experiments 1 (■), 2 (●) and 3 (▲) with initial glucose concentrations of 10, 20 and 130 g l^{-1}, respectively. Curves calculated from the results of the analytical (I) (- - -) and numerical (II) (———) approaches.

used as criterion for the non-linear fit of the experimental data. The experiments row marked 'Generalised' corresponds to the curves plotted in Fig. 3. A comparison of the values obtained for D_e is made with those quoted in the literature for the diffusivity of glucose in pure water ($D_{w,gluc}$).

3.3. Effective diffusion coefficient for oxygen

Experiments 4 to 6 were performed in order to determine the effective diffusion coefficient of oxygen in yeast aggre-
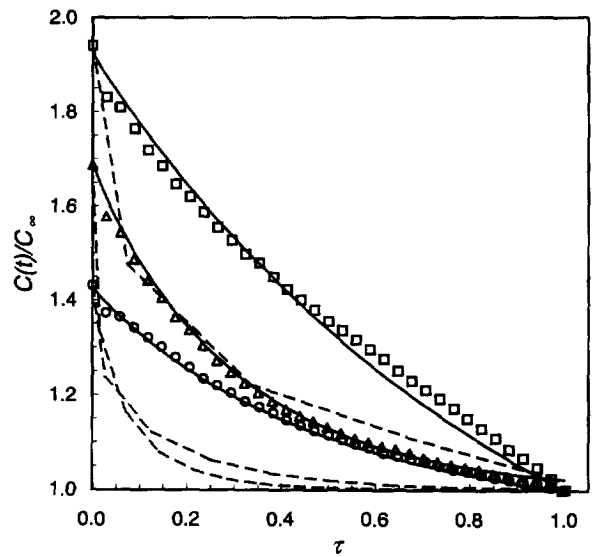

Fig. 4. Dimensionless concentration ($C(t)/C_\infty$) versus dimensionless time (τ) for experiments 4 (○), 5 (△) and 6 (□) with stirring speed of 175, 200 and 300 rpm, respectively. Curves calculated from the results of the analytical (I) (- - -) and numerical (II) (———) approaches.

gates as well as the influence of floc size at different mixing conditions.

Fig. 4 presents data from experiments 4 to 6; the curves have been calculated making use of the results given by the analytical and numerical approaches.

Table 3 summarises the results of D_e and K_c estimations by both of the approaches discussed above, including the

Table 3
Calculated results of the non-linear fit of experimental data from experiments 4 to 6 using approaches I (analytical) and II (numerical)

Experiment	Approach	$D_e \times 10^{11}$ [$m^2 s^{-1}$]	95% C.I.	$K_c \times 10^8$ [$m s^{-1}$]	95% C.I.	RSS $\times 10^4$	$100 \times D_e/D_{w,O_2}$ [%]
4	I	0.49	–	–	–	*	0.2
	II	0.65	±0.33	1.46	±1.21	3.81	0.3
5	I	2.10	–	–	–	*	0.8
	II	2.93	±1.05	1.03	±1.11	7.23	1.1
6	I	1.60	–	–	–	*	0.6
	II	2.49	±1.04	0.75	±0.65	54.1	1.0

D_{w,O_2} (30°C) = 2.56×10^{-9} $m^2 s^{-1}$ [9].

respective 95% confidence intervals. The RSS was, here too, used as the minimisation criterion for the non-linear fit of the experimental data. Very high values of RSS correspond, in Fig. 4, to the curves obtained through the analytical approach, and were replaced in Table 3 by asterisks (*). The calculated oxygen diffusivity in yeast flocs was compared to that in pure water (D_{w,O_2}).

4. Discussion

In this work, two different mathematical approaches were used to analyse experimental data: an analytical one, which is a solution of Fick's law of diffusion and neglects external mass transfer resistance, and a numerical one which, along with the internal diffusion, considers also an external resistance to mass transfer.

It is not difficult to find in the literature [27] an analytical solution that also considers external mass transfer resistance. The problems arise when, from experimental data, one attempts to calculate simultaneously D_e and K_c, once their values strongly influence each other, making virtually impossible the convergence of the solution by any iterative method. To our knowledge, no papers were found in the literature describing this approach to calculate both D_e and K_c.

If the hydrodynamic conditions around the objects are well characterised, it is possible to apply one of the widely known empirical correlations to calculate K_c. This possibility, however, demands also the shape of the object to be very well defined. In other words, correlations are available only for sphere, infinite cylinder and plane sheet geometry which, together with the need of very well defined hydrodynamic conditions, strongly limits the use of this method and rendered impossible its application during the course of this work. Therefore, a numerical approach had to be considered.

The presence of an external resistance to mass transfer is often negligible, if perfect mixing around the particles can be provided. Nevertheless, due to their very fragile structure, yeast flocs demand a very gentle agitation during the experiments to keep their integrity, which is hardly compatible with a perfect mixing assumption around the particles. Internal mass transfer resistance (by diffusion) and external mass transfer resistance (by convection) are the inverse values of D_e and K_c, respectively. Being so, the overall resistance (diffusion + convection) will be obviously greater than the value of the resistance by diffusion alone if the resistance by convection is considerable. On the contrary, if it is possible to neglect external resistance, then the overall resistance and the internal resistance alone will have approximately the same values. In other words, D_e value calculated ignoring the existence of external mass transfer resistance (approach I) will represent the overall resistance and will be lower than the value of D_e considering the existence of external mass transfer resistance (approach II). D_e values will be approximately the same in both cases if external resistance is negligible. Therefore, it was found that calculating the effective diffusivity

both ignoring the existence of external mass transfer resistance and including it, and comparing the two values obtained, it would be possible to conclude about the appropriateness of neglecting that resistance. Such a comparison has been made and a glance at Table 2 for glucose and Table 3 for oxygen allows the inference that there would be a considerable error, in the conditions of this work, to undervalue K_c. In fact the Biot number (Bi) was found to be around 0.20 for glucose and 0.21 for oxygen transfer; these values are well below the usually accepted limit of 50, indicating that external mass transfer has to be accounted for.

4.1. Effective diffusion coefficient for glucose

In experiments 1 to 3, corresponding to the determination of the effective diffusion coefficient for glucose, the stirring speed was kept constant to ensure an uniform size of the flocs. Being so, and making the reasonable assumption that the floc internal structure is similar for aggregates of approximately the same size, the change in the initial glucose bulk concentration is not expected to influence the results, as neither D_e nor K_c depend on it. Fig. 3 and Table 2 confirm these expectations: the normalised data points, although belonging to different experiments, behave as if they were part of the same run as D_e and K_c values show no significant difference between different experiments. This is the reason why, along with an individual treatment for each run, in a so-called 'Generalised' experiment, data points were also treated altogether (Table 2).

To experiments 1 to 3 corresponds the smaller size of flocs (Table 1), that is, the higher stirring speed. Therefore, a very significant difference between the curves calculated from the results of approaches I and II is not to be expected (Fig. 3). In fact, the RSS values are even lower for approach I in experiments 1 and 2, but are higher for experiments 3 and 'Generalised'. In this case, the K_c value, although not negligible, has a smaller influence on the overall behaviour of the system.

A comparison between D_e and $D_{w,gluc}$ shows values of around 7 to 17% of those in water, depending of whether or not external mass transfer resistance was neglected and are in accordance with the values obtained by De Backer et al. [33] for a high yeast cell concentration immobilised in a 4% Ca–alginate membrane.

4.2. Effective diffusion coefficient for oxygen

Having concluded that the initial solute concentration has no effect on D_e and K_c, then the effect of the floc size was studied. Changes of the size, itself, are not expected to influence the values of D_e, but changes in the floc internal structure certainly do, as they mean changes in tortuosity. In experiments 4 to 6, where the floc size was increased (Table 1) by means of a decreasing stirring speed, there is a noticeable change both in the values of D_e and K_c. There is no unambiguous dependence of D_e on floc size. On the other hand K_c

diminishes, meaning an increase in the external resistance (by convection) to mass transfer (Table 3).

In this case, it was neither possible nor desirable to treat the experiments as a whole. Instead, the treatment was made to every single experiment and the curves calculated from the results of each of the mathematical approaches used. They show significant differences both between experiments and when a different approach (I or II) is applied to the same experiment (Fig. 4). The observed differences between experiments might be attributed to a distinct internal floc structure. As discussed before, size itself cannot influence D_e but this parameter is, in turn, strongly dependent on tortuosity, which is directly related to the structure that might change with floc size. Cell wall structure may behave as gels in gel exclusion chromatography [34], retaining smaller molecules (e.g., O_2) more than the bigger ones (e.g., glucose), thus making Stokes–Einstein relation not valid when comparing glucose and oxygen diffusivities in yeast cell flocs. All the above might account for the significantly lower values of oxygen diffusivity found in the present work when compared to those presented in the literature.

In fact, several correlations for prediction of diffusivity based on the cell volume fraction in gels containing immobilised cells are available [11,22,33]. Nevertheless, being the results of the present work not in agreement with these models, it must be stressed that the extrapolation of those correlations to cell aggregates which are only composed of cells and have no additional supporting matrix, is not likely to be valid. Interactions were found by Teixeira et al. [35] between a gel matrix and solute, indicating that interactions

between matrix and solutes may occur and have a decisive effect on mass transfer. In their work malic acid, although being a smaller molecule than glucose, had a lower diffusion coefficient than the one that could be obtained from the Stokes–Einstein relation. The values found by Teixeira et al. [35] for the ratio D_e/D_w are lower than those predicted by the above mentioned correlations. The same occurred with the work of Netrabukkana et al. [36], who determined the diffusion coefficients of glucose and glucitol in microporous and mesoporous catalysts, and also found values for that ratio far below those predicted by the Stokes–Einstein relation, demonstrating that it is not valid because of the small size of the pores where the solutes in question should penetrate. A similar analysis was applied by Dalvie et al. [37], while modelling a rotating continuous size exclusion chromatograph. They applied an equation for D_e/D_w which includes the Stokes–Einstein relation but also other parameters, giving much lower values for D_e. Being so, it was decided to apply the methodology used with the yeast flocs to well known systems: calcium alginate beads, without and with inactivated yeast cells (20% w/v) and compare the results. The values obtained are summarised in Table 4. They agree perfectly with both the Stokes–Einstein relation and with the data published for similar systems, confirming the appropriateness of the method. Therefore, it is possible to conclude that not only floc porosity, but essentially the cell wall components, are responsible for the obtained results. The explanation for these observations could only be advanced as a hypothesis.

The differences in the curves obtained when approaches I and II are applied to the same experiment can be interpreted

Table 4
Summary of the values of D_e for glucose and oxygen found in the literature

Support	Cell type	Temperature (°C)	Solute	$D_e \times 10^{10}$ [m² s⁻¹]	Refs.
Ca–alginate (4%)	yeast	30	glucose	1.0–3.2 [a]	[44]
Ca–alginate (3%)	yeast (32.5% w/v)	25	glucose	4.68	[31]
Ca–alginate (2%)	yeast (20% w/v)	22–26	glucose	6.1	[20]
Ca–alginate (3%)	–	37	glucose	6.21	[38]
Ca–alginate (2%)	–	30	glucose	6.22	[39]
Ca–alginate (2%)	–	30	glucose	6.79	[39]
Ca–alginate (2%)	–	20	glucose	6.8	This work
Ca–alginate (2%)	yeast (20% w/v)	20	glucose	5.5	This work
Ca–alginate (2 and 4%)	–	30	glucose	6.83	[12]
Aggregates	yeast	30	glucose	1.10	This work
Fermentation medium	yeast	22	oxygen	15.7–21.0 [b]	[40]
Ca–alginate (2.5%)	yeast	30	oxygen	20.8–22.2 [c]	[9]
Ca–alginate (3%)	–	37	oxygen	12.2	[38]
Ca–alginate (3%)	–	30	oxygen	3.66	[41]
Ca–alginate (2%)	–	20	oxygen	24	This work
Ca–alginate (2%)	yeast (20% w/v)	20	oxygen	19.1	This work
Polyurethane	–	30	oxygen	1.95	[41]
Mycelial pellet	fungi	–	oxygen	3.50	[42]
Aggregates	plant	25	oxygen	0.5–10 [d]	[13]
Aggregates	yeast	30	oxygen	0.049–0.21 [d]	This work

[a] Depending on the cell concentration in the membrane.
[b] Depending on the cell volume fraction in the medium.
[c] Depending on the cell density.
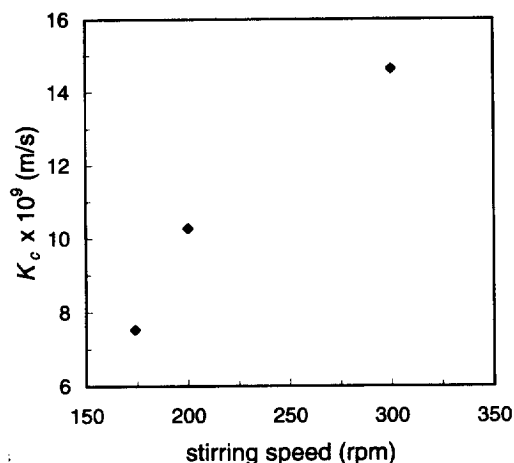[d] Depending on the size of the aggregate.

Fig. 5. Change in K_c values with the stirring speed for experiments 4 to 6. An increase in the stirring speed corresponds to a decrease in floc size.

as the error arising from neglecting external mass transfer resistance: when this is not considered (dotted lines) the disparity between the experimental points and curve is considerable (Fig. 4), giving rise to a very high RSS value (Table 3); when it is considered, curves (solid lines) and data points are in excellent agreement.

K_c values also change. In experiment 4, where stirring speed (and, consequently, floc size and shape) is the same as in experiments 1 to 3, K_c value is comparable to the ones obtained then. Nevertheless, when the stirring speed is set to a lower value (enabling the formation of bigger flocs with cylindrical shape), K_c decreases, as expected, as the external mass transfer resistance increases (Table 3).

Fig. 5 presents a plot of K_c versus the stirring speed, giving a very good idea of the magnitude of the changes induced by a different floc size/internal structure.

When comparing D_e with D_{w,O_2}, values of around 0.2 to 1% of those in water are obtained.

4.3. Comparison with other works

Finally a comment on the values of D_e found in the literature: as already mentioned, no references were found where such values for yeast flocs were dealt with. Nevertheless, a comparison with other immobilisation systems may be useful, and is presented in Table 4.

When comparing the results of this work with those of the literature it is possible to conclude that they are lower, in general, both for oxygen and glucose. This is not so surprising if the fact that the porosity of the yeast flocs (0.50) is much lower than that of other commonly used immobilisation systems (0.80–0.95) is taken into account, together with the differences in structure previously discussed. Further, the smallest value of D_e for oxygen in a plant aggregate [13] (the one that corresponds to a size of the plant aggregates similar to that of the biggest flocs used in the present work) is in quite good agreement with the value obtained for the yeast floc of about the same size, despite all the differences

that probably exist in the structure. Of all the systems quoted in Table 4, this is the one that is more closely related, in terms of floc structure and immobilisation technique [43], to the one studied in the present work.

5. Notation

a	radial (sphere, cylinder) or axial (plane sheet) coordinate in the floc, m
A	specific area, m^{-1}
Bi	Biot number ($K_c \cdot R/D_e$), dimensionless
$c(a,t)$	floc solute concentration in coordinate a and time t, g l^{-1}
$C(t)$	bulk solute concentration in time t, g l^{-1}
c_0	initial floc solute concentration, g l^{-1}
C_0	initial bulk solute concentration, g l^{-1}
C_∞	bulk equilibrium solute concentration, g l^{-1}
D_e	effective diffusion coefficient, $m^2 s^{-1}$
$D_{w,gluc}$	diffusion coefficient of glucose in pure water, $m^2 s^{-1}$
D_{w,O_2}	diffusion coefficient of oxygen in pure water, $m^2 s^{-1}$
f	geometry factor (1—plane sheet; 2—infinite cylinder; 3—sphere), dimensionless
J_D	diffusive flux averaged over local representative volume, $g\ m^{-2}\ s^{-1}$
K_c	convective mass transfer coefficient, $m\ s^{-1}$
L	floc thickness, m
q_n	non-zero positive roots of: $\tan q_n = -\alpha\ q_n$ for $f=1$
	$\alpha\ q_n\ J_0(q_n) + 2\ J_1(q_n) = 0$ for $f=2$
	$\tan q_n = 3\ q_n/(3+\alpha\ q_n^2)$ for $f=3$
	where $J_0(x)$ is Bessel function of the first kind of order zero
	$J_1(x)$ is Bessel function of the first kind of order one
R	floc radius, m
t	time, s
α	liquid/solid volume ratio, dimensionless
ε_p	floc porosity, dimensionless
ε_r	void fraction, dimensionless
μ	mean floc projected area, m^2
τ	dimensionless time ($t \cdot D_e/(\varepsilon_p \cdot R^2)$), dimensionless

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