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Immobilised Particles in Gels. Homogeneous porous media model

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Abstract

A model of diffusion assuming a homogeneous cell distribution in a gel was improved by considering the effect of tortuosity. By theoretical analysis and numerical modeling it was shown that the tortuosity of a gel with immobilised cells is the product of two factors: 1) tortuosity generated by the cells, T_c, and 2) tortuosity of the gel matrix, T_g, both variables being a function of cell volume fraction, ϕ_c. Total tortuosity is thus $T_\Sigma = T_c \cdot T_g$. Based on this approach, it was possible to analyse diffusivity data for gels with immobilised cells. It was shown that, in these systems, the diffusivity $\eta = D_e / D_0$ is a complex function of: 1) diffusivity in the gel, η_g and 2) diffusivity in immobilised cells, η_c. The developed model allowed for the description of the dependence of D_e / D_0 on ϕ_c. Comparison with numerous published experimental data showed a good fit of the developed model. Observed deviations might be explained by non-homogeneous cell distributions inside the gel matrix.

Introduction

One of the main problems in industrial technology is the use of operations where mass transfer is the limiting step. This is the case in immobilised cell systems and in the separation of macromolecules.

Considering D_e the effective diffusion coefficient in an immobilised cells system, D_g the effective diffusion coefficient in pure gel and ϕ_c the volume fraction of cells in the gel, several researchers investigated the diffusion phenomena in gels and in gels with immobilised cells and described the diffusion of solutes in the gel matrix by the relation $D_e / D_g \sim (1-\phi_c)^2$. This means that tortuosity is assumed to be proportional to $1/(1-\phi_c)$ (*1 – 4*). However, not all the data agree with this approach. Westrin and Axelsson (*1*), and Hannoun and Stephanopoulos (*5*) pointed out the possibility of modification of gel properties due to cells immobilisation. Assuming in this work that tortuosity of the gel matrix will be influenced by the presence of immobilised cells and accounting for the effect of cells in the overall tortuosity, we shall try to improve the available diffusion models for immobilised cells in gel systems.

Data, model analysis and discussion

To analyse diffusion in gels, two situations may be considered, according to the existence or not of immobilised cells in the gel.

Diffusion in pure gels. The available data for the effective diffusion coefficient, D_g, of sugars in calcium alginate gels show that the ratio $\eta_g = D_g / D_0$ (where D_0 is the diffusion coefficient in water) varies in the range 0.67 – 1.0, depending on the gel concentration and on the mode of preparation (*2, 5 – 7*).

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For diffusion of oxygen in gels (*8*), D_g values obtained were 81-86% of those measured in pure water. Increasing gel concentration led to a decrease in the effective diffusion coefficient, as previously reported (*5*). Gel pore size was estimated to be of the order of 150 Å. The observed decrease in the effective diffusion was tentatively related with the increased diffusion pathway.

The effective diffusion coefficient of bovine serum albumin (BSA) in alginate from *L. digitata* was measured to be 1.02×10^{-6}, 0.82×10^{-6}, and 0.54×10^{-6} cm^2/s for Na-alginate concentrations of 1.9, 2.5 and 4 %, respectively. At the same time, the dependence of D_e on the alginate source was shown (*9*). For glass beads (internal porosity $\varepsilon = 0.56$) filled with 1% Ca-alginate gel the glucose effective diffusion coefficient D_e was determined as $D_e = 2.2 \times 10^{-6}$ cm^2/s at 30°C and the overall tortuosity, T, was calculated as $T = 1.7$ (*6*).

The interpretation of experimental data for gel beads may be rather complicated since gradients of gel concentration may occur inside beads. This is often related with the method of gel preparation (*9*). The analysis of the three methods used for determining the effective diffusion coefficient D_e in different types of immobilization systems showed that the membrane and cylindrical techniques give more accurate results for D_e than the bead method (*10*). Thus, for further analysis, data determined with the membrane method will be used.

Data presented for mass transfer in gels by several authors (*3*, *5 – 7*, *11*, *12*) show that the increase in gel concentration is accompanied by a decrease in diffusivity $\eta_g = D_g / D_0 = \varepsilon_g / T_g$, where ε_g is the gel porosity or void fraction, and T_g is the gel pore network tortuosity. Moreover, the effect of porosity when $T_g = 1.0$ is assumed is not enough to explain the decrease in diffusivity.

To illustrate this point, an example of the diffusion in an alginate membrane taken from the experiment (*5*) will be considered. According to these authors, the diffusivity of glucose and ethanol in 2% alginate membrane was $\eta_g = 0.88$ and 0.91, respectively, whereas, in a 4% gel, $\eta_g = 0.71$, for glucose and $\eta_g = 0.7$ for ethanol. Based on the author's estimations for gel porosity, ε_g of 0.98 and 0.96 for 2% and 4% gels, respectively, the tortuosity can be calculated by the expression $T_g = \varepsilon_g / \eta_g$. Hence, we obtain, for a 2% alginate gel $T_g = 1.08 - 1.11$ and for 4% gel $T_g = 1.352 - 1.37$. Therefore, the increase in tortuosity with gel concentration is much higher than the decrease in porosity.

The differences found in this example may be due to the fact that, with a growing polymer concentration in the gel, a more tortuous pathway for the diffusing species may be expected. Or, in other words, the structural changes induced in the gel matrix by the addition of alginate may give rise to a significant increase in tortuosity.

Diffusion in gels with immobilised cells. For diffusion in gels with immobilised cells, a large amount of data available in bibliography can be used for data analysis (*2*, *4*, *10*, *13*). Gels with immobilised inactivated and activated cells may be analysed in different ways (*13*). A gel with immobilised inactivated cells can be considered as a system with homogeneous cell distribution. When cell growth occurs in the matrix, cells may concentrate near the gel matrix surface and/or form micro colonies. This system should be treated as a porous medium with non-homogeneous cell distribution or/and as bi-disperse materials. Micro colonies may be considered as microporous particles (*2*).

Depending on the type of microorganisms, the following situations can be expected: 1). Cells with small size distribution can be simulated as a monosized system (*2*). 2). Cells with a wide range of size distribution (mixture of large, moderate and small particles) may form dense porous media with a porosity smaller than 0.4 (See for example (*14*)).

Equations used to explain diffusion phenomena in gels with immobilised cells could be classified in two types: a). Equations that include the function D_e / D_0 (D_e is the effective diffusion coefficient for gel with immobilised cells). For a volume fraction of immobilised cells ϕ_c = 0, the boundary condition is $D_e / D_0 = D_e / D_g < 1$. b). Equations that include the function D_e / D_g. In this case the models present a large deviation for large cell volume fractions.

The tortuosity dependence on cell volume fraction as well as on polymer concentration is the main problem when modeling immobilised cells systems in a gel matrix. According to the diffusion theory, the dependence of D_e / D_g on tortuosity can be represented as follows:

$$D_e / D_g = \varepsilon_c / T(\varepsilon_c) \tag{1}$$

where $T(\varepsilon_c)$ is the tortuosity factor expressed as a function of porosity or cell volume fraction $\varepsilon_c = 1 - \phi_c$.

Attention must be paid to the fact that, for the region where ϕ_c = 0 – 0.4 (which is the most usual situation), we have crumbly porous media. Most biological cells can be mainly represented as spherical or with a spheroid shape. For these porous media, the dependence of T on ε_c expressed as $1/\varepsilon_c$ should not be expected (*15*).

Depending on porous media structure, the tortuosity may have other representations. Tortuosity was determined by (*16*) as $T = 1 - 0.5\ln(\varepsilon)$. For packed beds the ratio $\eta = D_e / D_0$ was determined from the analogy with electrical conductivity (*17*) as $\eta = \varepsilon / (\varepsilon + k(1-\varepsilon))$, where for spherical particles k = 1.5 (shape factor). In turn, using $D_e = \varepsilon \cdot D_0 / T$, (*18*) the tortuosity was calculated with the expression $T = 1.5 - \varepsilon / 2$ (*18*).

Proposed model to characterize transport properties in gels – tortuosity analysis

Diffusion in pure gels. As was mentioned above, gel properties are sensitive to polymer concentration. Therefore the effective diffusion coefficient in gel D_g should be estimated through the expression used in mass transfer processes:

$$D_g = D_0(1-\phi_p) / T_g = D_0 \varepsilon_g / T_g \tag{2}$$

where $\varepsilon_g \approx 1 - \phi_p$ is the gel void fraction (porosity), and T_g is the molecule path tortuosity in a gel matrix for a defined structure and a defined diffusing molecule. In addition, tortuosity can provide some information on gel structure. Based on microphotographs of different gel types with and without immobilised cells, we can simulate the gel matrix as a cellular structure with permeable holed walls (*9*, *12*, *19 - 24*). Examples of two-dimensional graphs are shown on Figure 1.

In order to have an estimation of the tortuosity variation for different situations, let us then consider a simplified beehive compartment model representing three gel structures with slight alterations (see Figure 1). Structure a) will then be a stretched gel structure, structure b) is a relaxed gel matrix, whereas structure c) will represent a simplified compressed matrix.

Using this simple geometrical approach it is now easy to calculate the maximal geometrical pathway tortuosity by the ratio L / L_0. Considering a hexagonal geometry, $L_0 = a\sqrt{3}/2$ and $L_0 = ka\sqrt{3}/2$ for configurations (a) and (c). The value for k will be greater than the unity for all the stretched structures and lesser than 1 for the compressed structures.

Therefore, the tortuosity may be calculated as

$$L / L_0 = \sqrt{L_0^2 + (3a/2)^2} / L_0 = \sqrt{1 + 3/k^2} \tag{3}$$

The resulting boundary (maximal) tortuosity for different k values may now be calculated: (a) – 1.32 (k = 2), (b) – 2.0 (k = 1), and (c) – 2.78 (k = 0.667). This means that

the gel tortuosity may vary by more than two-fold, simply by stretching or compressing the gel compartments, which might explain in part the results reported by (*5*).

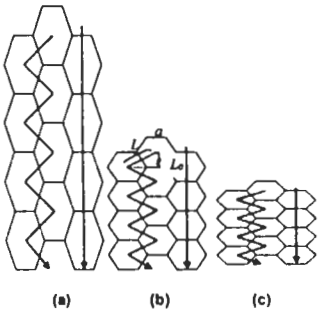

Fig. 1.

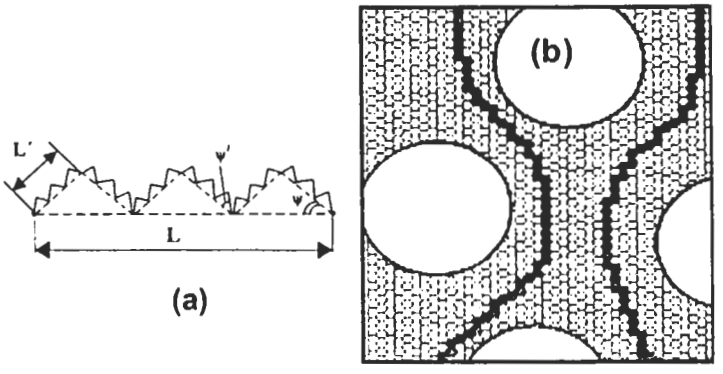

Fig. 2.

Figure 1. Simplified geometrical gel structure interpretation. (a) Low polymer concentration; (b) Moderate polymer concentration; (c) High polymer concentration. Straight arrows correspond to the minimal diffusion path length in the gel matrix. Folded arrows - maximal diffusion path length in the gel matrix.

Figure 2. Schematical representations of a gel structure. (a) Simplified pore channel. (b) Gel matrix with immobilised cells: dotted line is the tortuosity created by cells in the gel matrix and gray units chain is the total path in the gel matrix with cells.

Diffusion in gels with immobilised cells. In this case, tortuosity values can be estimated by adding the tortuosity related with cells embedded in the matrix space to the tortuosity increase of gel matrix described above.

Let us now consider a triangular zigzag configuration as a simplified description of a gel matrix filled with cells (Figure 2a). A molecule entering the gel free system from the left, in the presence of cells only, will move throughout a path with a tortuosity T_c (large dotted triangles), which will be defined as **large-scale tortuosity**. Large-scale tortuosity is $T_c = L_{cc} / L = 1/\cos(\psi)$ where L_{cc} is the molecule path length due to the presence of cells, Figure 2a, corresponding to the broken dotted line with fragments of L' length. The entire gel matrix thickness is represented by L. The molecule pathway in the pure gel matrix is represented by the solid broken line formed by the small triangles. This pathway has a tortuosity T_g that we will define as **small-scale tortuosity**. In the latter case, the tortuosity is $T_g = L_\Sigma / L_{cc} = 1/\cos(\psi')$, where L_Σ is the overall molecule path length in gel with immobilised cells. The total molecule path length in the channel is shown on Figure 2a as a broken solid line. So, the total tortuosity will be

$$T_\Sigma = L_\Sigma / L = \frac{L_\Sigma}{L_{cc}} \cdot \frac{L_{cc}}{L} = T_c \cdot T_g \qquad (4)$$

In the particular case of Figure 2a, the overall tortuosity is $T_\Sigma = \{1/\cos(\psi')\} \cdot \{1/\cos(\psi)\}$. When assuming $\psi' = \psi$, then $T_\Sigma = T^2 = 1/\cos^2(\psi)$.

A model of a zigzag pore channel for a gel with immobilised cells is shown in Figure 2b. In Figure 2b, two hypothetical paths in the gel matrix with immobilised cells are shown. The left pathway has a tortuosity T_Σ = 1.23, when the tortuosity created by cells is only T_c = 1.11. For the right pathway, we have T_Σ = 1.203 and T_c = 1.07. The observed pathway when cells are present is also influenced by the gel matrix as was discussed above: $T_\Sigma = T_c \cdot T_g$. A good illustration of a gel matrix structure and its transformation due to polymer concentration or to the presence of immobilised cells presence is shown on microphotographs by (*20*). Gel matrix

structure changes are most significant in the case of cell growth inside a gel, since in this case there will be additional compression effects onto the gel matrix as soon as formation of new cells occurs.

Based on this approach, it is possible to analyze data for cells immobilised in a gel system, where tortuosity looks too large sometimes. Assuming that the most general case of tortuosity dependence of T on ε is $T \sim 1/\varepsilon^n$, where n is an empirical constant, usually close to 0.5, a quantitative analysis of the data will be done. By equation (4), $T_\Sigma = T_c \cdot T_g = 1/\varepsilon$, assuming that $T_c \sim 1/\sqrt{\varepsilon}$ and $T_g \sim 1/\sqrt{\varepsilon}$.

The overall diffusivity $\eta = D_e / D_0$ for this system is a complex function of partial diffusivities in gel $\eta_g = D_g / D_0$ and in immobilised cells structure $\eta_c = D_e / D_g$. Hence,

$$\eta = D_e / D_0 = \eta_c (D_g / D_0) = \eta_c \eta_g \tag{5}$$

The diffusivity in a pure gel (without immobilised cells) is represented by the equation

$$\eta_g = D_g / D_0 = \varepsilon_g / T_g \tag{6}$$

According to equation (5), for the total tortuosity of the gel matrix with cells

$$\eta_c = D_e / D_g = \frac{\varepsilon_c}{T_\Sigma} = \frac{\varepsilon_c}{T_c(\varepsilon_c) \cdot T_g(\varepsilon_c)} \tag{7}$$

where $T_g(\varepsilon_c)$ is the tortuosity of gel matrix filled with cells (small scale tortuosity) and $T_c(\varepsilon_c)$ is the tortuosity created in the matrix by the presence of cells (large scale tortuosity). Replacing equations (6) and (7) in (5), we obtain

$$\eta = \eta_g \eta_c = \frac{\varepsilon_g}{T_g} \cdot \frac{\varepsilon_c}{T_c(\varepsilon_c) \cdot T_g(\varepsilon_c)} \tag{8}$$

In the particular case where $T_g(\varepsilon_c) = T_c(\varepsilon_c) = T$, equation (7) becomes

$$\eta_c = \frac{\varepsilon_c}{T_c(\varepsilon_c) \cdot T_g(\varepsilon_c)} = \frac{\varepsilon_c}{T^2} \tag{9}$$

and, for $T = T_g = T_c = 1/\varepsilon^{0.5}$, we obtain

$$\eta_c = \frac{\varepsilon_c}{(1/\varepsilon_c^{0.5})^2} = \varepsilon_c^2 = (1-\phi_c)^2 \tag{10}$$

which is in good agreement with the existing models (*1 – 4*). For a pure gel, the boundary conditions of equation (7) are ε_c = 1.0, $T_g(\varepsilon_c)$ = 1.0 and $T_c(\varepsilon_c)$ = 1.0 and, as a consequence, $\eta = \eta_g = \varepsilon_g / T_g$.

Figure 3 represents the proposed model (equation 7) for the general case where the tortuosity created in the matrix by cells is $T_c(\varepsilon_c) = 1/\varepsilon_c^\beta = 1/(1-\phi_c)^\beta$ and the tortuosity of the gel matrix filled with cells is $T_g(\varepsilon) = 1/\varepsilon_c^\gamma = 1/(1-\phi_c)^\gamma$. Thus:

$$\eta = D_e / D_0 = \eta_g \cdot (1-\phi_c) \cdot (1-\phi_c)^\beta (1-\phi_c)^\gamma = \eta_g (1-\phi_c)^\alpha \tag{11}$$

$$\alpha = 1 + \beta + \gamma \tag{12}$$

where β and γ are values in the range 0 to 1.0.

For tortuosity functions of the type $T_c(\varepsilon_c) = 1/\varepsilon^\beta$, β = 0.5 and $T_g(\varepsilon_c) = 1/\varepsilon^\gamma$, the gel matrix tortuosity in a gel system with immobilised cells can be estimated as summarized in Table 1. Proposed model functions from Figure 3 were used. The gel matrix tortuosity (in comparison with pure gel tortuosity), in the case of immobilised cells, increases due to the increase of polymer concentration in the gel, meaning that $\gamma = \gamma(\phi_p)$. In our case, $\gamma \sim 10\phi_p$.

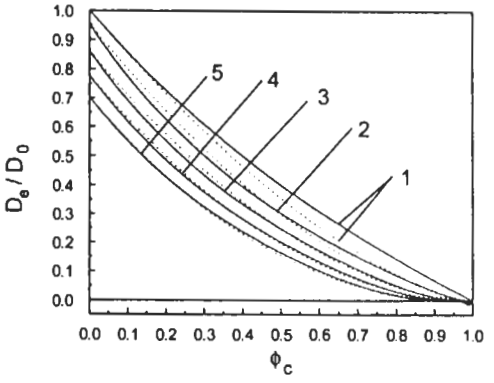

Fig. 3

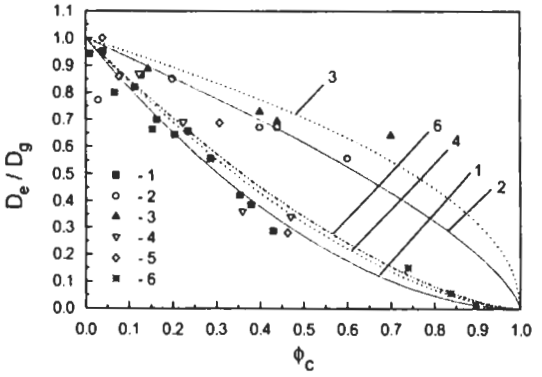

Fig. 4

Figure 3. Dependence of D_e / D_0 on ϕ_c for the model of Westrin and Axelsson (*1*) (solid lines) and for the model proposed in this work (dot lines). 1 – ϕ_p = 0.0, 2 – 0.01, 3 – 0.03, 4 – 0.05, and 5 – 0.07. Proposed model (equation 11): 1 – $D_e / D_0 = (1-\phi_c)^{1.32}$, 2 – $D_e / D_0 = 0.955(1-\phi_c)^{1.68}$, 3 – $D_e / D_0 = 0.86(1-\phi_c)^{1.8}$, 4 – $D_e / D_0 = 0.78(1-\phi_c)^2$, and 5 – $D_e / D_0 = 0.7(1-\phi_c)^{2.2}$.

Figure 4. Experimental data and the model plots of D_e / D_g vs. ϕ_c. 1 – 2% Ca-alginate gel, *Z. mobilis*, solute – galactose, (*2*). 2 – 2% collagen and 1% agar, EAT cells, solute – glucose, (*25*). 3 – Ca-alginate gel 20 kg/m^3, *B. amyloliquefaciens*, solute - oxygen (*8*). 4 – Lactose (*18*). 5 – 4% Ca-alginate gel, *S. cerevisiae*, solute - glucose (*13*). 6 – 2% Ca-alginate gel, baker's yeast, solute - acetophenone (*4*). Lines correspond to the model proposed in this work.

Table 1

Summarized data comparing the model of Westrin and Axelsson (*1*) and proposed model (equations 10 and 11) assuming $\beta = 0.5$

Gel concentration, ϕ_p	α	γ	η_g	T_g
0.00	1.5	0	1.0	1.0
0.01	1.68	0.18	0.955	1.037
0.03	1.8	0.3	0.86	1.128
0.05	2.0	0.5	0.78	1.218
0.07	2.2	0.7	0.7	1.329

We may see that, if the cells characteristics such as shape and size distribution - the function T_c - are known, then the model can be used to calculate the tortuosity trend in the gel with immobilised cells for the ratio D_e / D_0 as well as for D_e / D_g. The model parameters lie in the region corresponding to real measured values of gel tortuosity with different polymer concentrations, (*2*, *5* – *7*) for $T_g \sim 1.0 - 1.4$.

The model plots compared with experimental data are shown on Figures 4 – 6. The values of the power order in the model (Figure 4) are as follows: 1 - α = 1.9, 2 – 0.7, 3 – 0.5, 4 – 1.65 and 6 – 1.55. Full information about data shown in Figures 5 and 6 is available in (*1*).

It must be mentioned that the proposed model is based on the assumption of a homogeneous cell distribution within the gel matrix. Therefore some data, especially those related with cases where there is cellular growth, cannot be interpreted using this model in terms of average cell volume fraction. This problem will be addressed in a future work where $\alpha > 2.2$ and $\alpha < 1.8$. These ranges correspond to anisotropic non-homogeneous porous media and will need another approach.

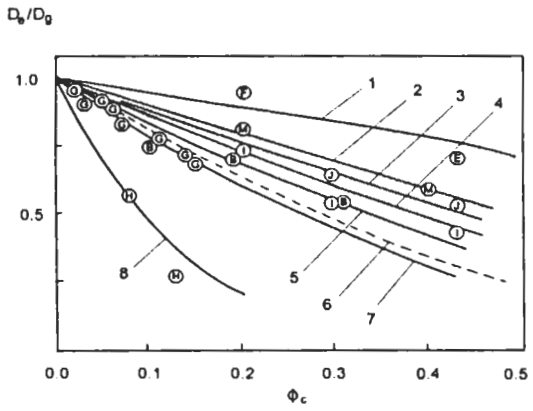

Fig. 5.

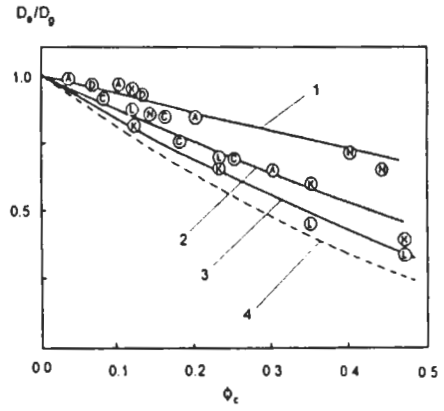

Fig. 6.

Figure 5. Comparison of experimental data from Figure 2 of (*1*) with the model proposed in this work. The values of α are: 1 – 0.45, 2 – 0.95, 3 – 1.1, 4 – 1.3, 5 – 1.6, 6 – 2.0 (by equation 9), 7 – 2.2, and 8 – 7.0. Data have symbols as in the original work: B – PMAAm gel + yeast, E – Ca-alginate gel + yeast for xylose, F – Ca-alginate gel + yeast for glucose, G – Ca-alginate gel + bacteria, H – Ca-alginate gel + plant cells, I – agar + mammalian cells for lactate, J – agar + mammalian cells for glucose, and M – *k*-carraginate + bacteria for oxygen.

Figure 6. Comparison of experimental data from Figure 3 of (*1*) with the proposed model. The value of α are in this case: 1 – 0.5, 2 – 1.15, 3 – 1.5, and 4 – 2.0 (by equation 9). Data have the same symbols as in the original work: A – PAA + bacteria for glucose, C – PAA + bacteria for ammonium fumarate, D – *k*-carrag. + bacteria for ammonium fumarate, K – Ca-alginate gel + yeast for lactose, L – Ca-alginate gel + yeast for ethanol, and N – Ca-alginate gel + bacteria for oxygen.

Conclusion

As compared with previous models, the proposed model shows a significant improvement in fitting real data, due to the introduction of a complex tortuosity value. Tortuosity is therefore a parameter necessary for modelling gel systems with immobilised cells. It was shown that tortuosity of a gel with immobilised cells is the result of two factors: 1) tortuosity generated by cells inclusion and 2) tortuosity of the gel matrix. Both variables are a function of cell volume fraction ϕ_c and can be described by an order function of ϕ_c.

The developed model gives the possibility of describing various types of dependence of D_e/D_0 on ϕ_c and also allows to control and optimise the diffusion inside a gel with immobilised cells. The most suitable way to reduce mass transfer resistance is to decrease the gel matrix tortuosity by controlling its structure. On the other hand, the model shows why, even with an ideal gel with tortuosity T_g = 1.0, the presence of cells in the gel matrix will give rise to a minimal tortuosity always above 1.0.

The model was compared with numerous published experimental data and a good approach was obtained. Nevertheless, not all experimental data can be explained based on a homogeneous model, when anomalously small or large values of D_e/D_0 vs. ϕ_c are measured. Although the present model considers the diffusivity in the gel, the diffusivity in the immobilised cells and the cell volume fraction, non-homogeneous cell distribution is probable in several cases. Factors such as cell mean size, cell distribution inside the gel and cell size distribution will be considered in a future work.

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