Evolution and modelling of *Listeria spp.* growth in a κ-carrageenan matrix

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Introduction

In recent years, the evolution of microorganisms encapsulated in hydrogel supports has been a subject of major interest and consequently, different theoretical approaches supported by experimental results have been proposed. Traditionally, immobilized cell systems have been considered as a uniform distribution of biomass through the whole support, thus a cell development similar to that observed in free cultures has been supposed (Dervakos, 1991). However, notorious discrepancies between the behaviour of free and immobilized cells (Meldrum, 2003) suggest that the same kinetic is unsuitable to describe both conditions, since the diffusional limitations associated to the solid support cause concentration gradients of viable cells, substrates and products (Laca, 1998). In this sense, models including diffusion and reaction processes and describing the evolution of cells, substrates and products with the spatial variable as well as the temporal one, would be clearly desirable.

On the other hand, the immobilized cell systems are closer to what can be considered as a heterogeneous structure with two clearly differentiated phases (support and colonies) than to a homogeneous system, so more complex models, like pore diffusion or granule models, should be taken into account. Another phenomenon to be included in the boundary conditions of the model would be a possible release of cells out the support or cell escape due to the support damage, since the colonies pressing against the support cover, the external agitation or even the own composition of the culture medium could alter the stability of the support (Wijffels, 1995). In this sense, the support swelling and shrinking, caused by the superficial colonies and the lost of humidity, respectively, involve a modification in the assumed geometry of the system and strictly, this would give rise to the need for complex 3D models. In Figure 1, a schematic summary of possible aspects concerning immobilized cell systems, assuming a hypothetical sphericity of the support, are shown.

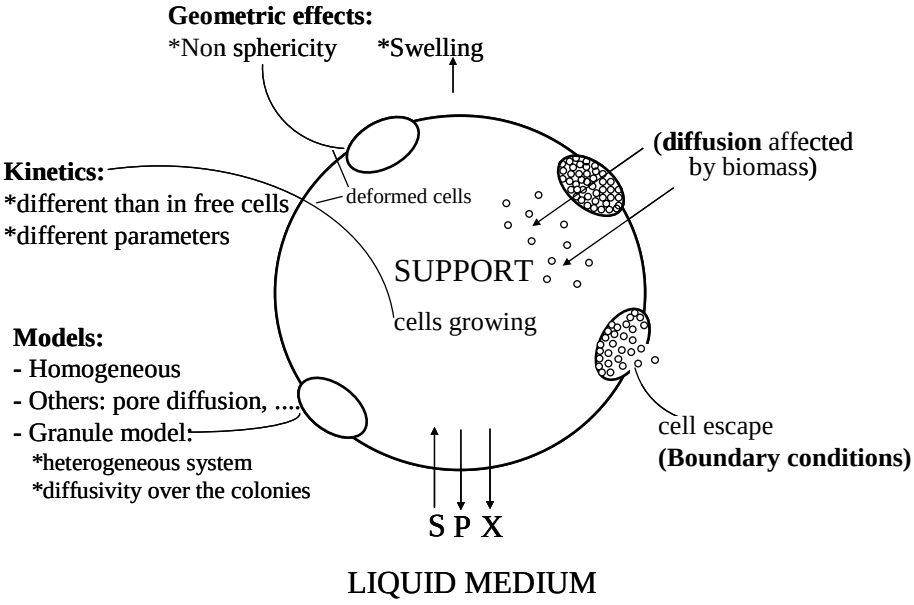

Figure 1. Schematic summary of all effects to be considered in immobilised cell systems

According to this figure, the large number of variables to be considered would imply mathematical expressions with an unacceptable level of complexity and a limited practical use. So, those parameters with a significant influence on the system behaviour should be previously selected, thus the model results as simple as possible and suitably describes the system. In this work, a model that combines diffusion and reaction terms was developed like a first approach to predict the evolution of *Listeria monocytogenes* within a homogeneous κ-carrageenan matrix with cylindrical geometry, assuming the oxygen as limiting substrate of cell growth. The equations, initial and boundary conditions are below indicated:

-Biomass balance	*Initial and boundary conditions*
$\frac{\partial C_X}{\partial t} = D_X \frac{\partial^2 C_X}{\partial z^2} + r_X$	$t = 0,\ C_s = C_{s\,sat};\ C_x = C_{xo}$
-Substrate balance	$z = L$ (surface), $C_s = C_{s\,sat}$
$\frac{\partial C_S}{\partial t} = D_S \frac{\partial^2 C_S}{\partial z^2} + r_S$	$z = 0$ (bottom), $\frac{\partial C_X}{\partial z} = 0,\ \frac{\partial C_S}{\partial z} = 0$

where, C_x and C_s are cell and oxygen concentrations respectively in the solid medium, t is the time, $C_{s\ sat}$ is the saturation oxygen concentration in the solid at 25ºC, D_x and D_s are cell and oxygen diffusion coefficients in the solid, r_x and r_s are cell growth and oxygen consumption rates and z is the position. A FORTRAN program was used to solve this differential equations system. *Listeria innocua* growth, as reference of *L. monocytogenes* behaviour, was monitored in BHI broth, in function of oxygen availability, and also in the κ-carrageenan matrix, contrasting these experimental results with the theoretical ones.

Materials and methods

Microorganisms culture in liquid medium

Depending on desired oxygen availability for cell growth, three types of assays were carried out in BHI (37 g l-1):

- Aerobic conditions: 1 l Erlenmeyer flasks containing 200 ml of medium (medium/air 1:5) were incubated in an orbital shaker (New Brunswick G25), at 250 rpm and 25 ºC.
- *Hypoxic conditions:* 1 l full closed bottles with screw tops were cultured at 25 ºC without shaking.
- *Anoxic conditions*: Initial dissolved oxygen was removed from the medium by injecting sterile nitrogen, and bottles were incubated under the same conditions that the hypoxic culture.

The inoculum was adjusted by dilution of a sample of the corresponding preinoculum in peptone salt water (NaCl, 8.5 g l^{-1}; peptone, 1 g l^{-1}) to give an initial concentration of about 10^5 CFU ml^{-1}. Cell growth was determined by measuring optical density at 600 nm and plating on BHI plates. These values were related to dry weight using the corresponding calibration curves. Dissolved oxygen concentration was also measured by employing a dissolved oxygen meter (model 58, YSI).

Microorganisms culture in the κ-carrageenan matrix

The solid medium was prepared by adding 0.8 % (w v^{-1}) κ-carrageenan to BHI broth. This medium homogeneously inoculated was solidified into sterile glass assay tubes (16 cm of length and 1.5 cm of diameter) closed with swaps and incubated at 25 ºC. Inoculum size was similar to that adjusted in the experiments in liquid medium. Experimental data of cell growth were obtained by dissolving sections taken from different longitudinal positions (surface, middle and bottom) of the solid cylinder in NaCl 0.9 % (w v^{-1}) at 40 ºC with gently shaking for about 15 min, and plating on BHI plates.

Results and Discussion

Cell growth in liquid medium

In spite of the different aeration levels, microbial growth took place in all the assayed cases. A higher availability of oxygen in the culture medium increased the cell concentration achieved at stationary phase, although specific growth rate and lag time values were almost not affected by oxygen presence/absence. However, significant differences were not found between maximum cell concentration under hypoxic and anoxic conditions, which shows that the initial presence of low oxygen concentrations in the medium almost do not change the cell development. Experimental data were fitted to Ricatti's equation indicated below and the values employed for the fitting are shown in Table 1:

$$r_x = \frac{dC_x}{dt} = K\,C_x\left(1-\tau\,C_x\right)$$

where K and τ ($1/C_{x\ \text{stationary state}}$) are kinetic parameters.

Table 1. Kinetic parameters from fitting of growth curves in BHI broth to Ricatti´s equation

Conditions	**Parameters**	
	K (s^{-1})	**τ** (ml mg^{-1})
Aerobic (7.6-8.0 ppm O_2)	1.57 10^{-4}	0.84
Hypoxic (0.2-2.6 ppm O_2)	1.52 10^{-4}	1.92
Anoxic (< 0.01 ppm O_2)	1.66 10^{-4}	2.28

Values of parameter τ, related to the maximum cell concentration achieved at stationary phase, decreased as oxygen concentration in the culture medium rose. As regards parameter K, it was similar under all assayed conditions, since, as mentioned before, the specific growth rate at the exponential phase was hardly affected by the oxygen availability in the medium. Both of these kinetic parameters will be considered as a starting point to model the microbial behaviour in solid medium.

Cell growth in solid medium

The evolution of the cell concentration profiles along the solid matrix is shown in Figure 3.

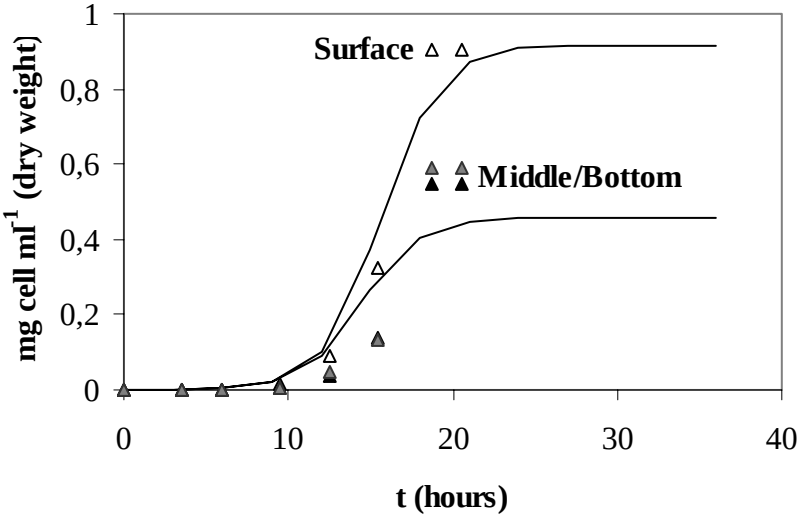

Figure 3. *L. innocua* growth in the κ-carrageenan matrix. Experimental (symbols) and model results (solid lines).

Microbial growth evolved throughout the solid, resulting biomass levels on the surface almost two-fold higher than those inside the solid. These concave profiles of cell concentration are probably due to different availability of oxygen in the surface and inside the food, resulting the diffusion of the oxygen through the solid a key parameter in the development of the bacterium.Theoretical results obtained from the simulation are also shown in Figure 3. As kinetic equation for cell growth (r_x), Ricatti's equation was employed and the kinetic parameters, K and τ were deduced from experimental data obtained in BHI broth under several aeration conditions (see Table 1). The oxygen uptake rate (r_s) was expressed as $r_s = -YC_sC_x$. Diffusivity values were taken from the literature (Wijffels et al., 1995). It is necessary to point out the uncertainty involved with some of the parameters employed for the fitting, for instance, the saturation concentration of oxygen in κ-carrageenan (7.9 ppm) that is not an exact value since it has been estimated from the respective value in water and the gel porosity, and it is probable that the real value is lower or even that the oxygen saturation concentration is not maintained on the surface during the culture. A small reduction of the oxygen saturation value in the food from 7.9 to 6.4 ppm gives a theoretical maximum cell concentration on the surface in agreement with experimental data. Values employed in the simulation are indicated in Table 1.

Table 1. Parameters and initial conditions considered in the modelling

Parameters	**Initial conditions**
$Dx = 3.0\ 10^{-14}\ m^2\ s^{-1}$	$Cxo = 1.210^{-4}\ mg\ ml^{-1}$
$Ds = 1.5\ 10^{-9}\ m^2\ s^{-1}$	$Cs\ sat = 6.4\ 10^{-3}\ mg\ ml^{-1}$
$K = 1.58\ 10^{-4}\ s^{-1}$	
$\tau = -177.1\ ml^2\ mg^{-2}\ x\ Cs + 2.2\ ml\ mg^{-1}$	
L = 6 cm	
$Y = 0.014\ ml\ mg^{-1}\ s^{-1}$	

This model including empirically based approaches describes quite properly the real behaviour in immobilized cell systems. In this sense, when modelling it is necessary to take into account that the goodness of the fit does not always involve the existence of a suitable modelling, since different and not necessarily correct assumptions for the same phenomenon could adequately describe the results. Therefore, more in-depth knowledge of the processes taking place throughout the system would provide the basis for a really proper modelling.

Conclusions

κ-carrageenan is an interesting matrix for the growth of different target microorganisms. Diffusional control of oxygen through the solid medium causes a reduction in the cell growth until distances of 6 mm. From this point with oxygen depleted, the cell concentration profiles homogenize because of the anaerobic growth. Both types of growth correspond with predictable values from experiments in BHI broth in absence and presence of oxygen. The model including diffusion and reaction processes provides a quite proper simulation of growth processes in solid supports.

References

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