IMPLEMENTATION OF A COMBINED DIFFERENTIAL–DISCRETE CELLULAR AUTOMATA MODEL FOR CELL GROWTH IN GEL BEADS

ZORAN TODOSIJEVIĆ[1]
BOJANA OBRADOVIĆ[1]
VIKTOR NEDOVIĆ[2]
BRANKO BUGARSKI[1]

[1]Department of Chemical Engineering, Faculty of Technology and Metallurgy, Belgrade, Serbia and Montenegro

[2]Faculty of Agriculture, Zemun, Beograd, Serbia and Montenegro

Immobilized cell systems are traditionally described by one–dimensional mathematical models that take into account diffusion and conversion of substrates as well as cell proliferation in immobilization matrices and biofilms. All properties (substrate concentration, biomass density, porosity etc.) are assumed to vary only in the direction from the bulk liquid to the carrier interior. However, development of cell clusters inside the support matrices and formation of channels and filaments in biofilms result in significant spatial variability of all significant parameters, thus requiring multidimensional modeling (Picioreanu et al., 1999). Cellular structures have been modeled by cellular automata rules. For bacterial colonies, diffusion limited aggregation (DLA) model used by Fujikawa (1994) and different random walk models by Schindler and Rataj (1992) predicted complex growth patterns similar to those experimentally observed, but no explicit conversion of nutrients has been included. Combined differential–discrete models were successfully developed for modeling diffusion–reaction–microbial growth in gel beads and biofilms (Picioreanu et al., 1998, 1999) Basis of these models is combination of differential–discrete models with hard cellular automata rules. Thus, the substrates are represented in continuous field and determined by numerical methods from the reaction–diffusion mass balances, whereas discrete mapping is used for cell arrangement. Cell growth in spherical gel beads was determined as substrate limited and distribution and spreading of cells were modeled by a discrete cellular automation algorithm. The differential–discrete–cellular–automata (DDCA) model was successfully applied for prediction of oxygen concentration profiles and cell distributions in gel beads (Picioreanu et al., 1998). In the present work we have modified the proposed DDCA model by addition of a rule for cell spreading and applied it to the same experimental data reported by Picioreanu et al. (1998).

Author address: Z. Todosijević, Department of Chemical Engineering, Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, Serbia & Montenegro

Paper presented as a poster.

MODEL DEFINITION

Rectangular uniform grid is used to represent the physical space. Cubic volume elements are used to fill 3D volume. In N x M x L 3D Cartesian grid, coordinates of volume elements are given by vector (x, y, z) ∈ (0...N–1,0...M–1,0...L–1).

Two basic variables are chosen to represent the state of the system: the soluble limiting substrate concentration (S) and the biomass density (C) (both in dimensionless forms, varying in the range 0 to 1). Third variable (c) is used for storing information about the occupation state of space elements with cells and takes two values: 0 for unoccupied space (gel matrix) and 1 for occupied space (biomass placement).The substrate concentration in grid elements depends on the balance between transport mechanisms and consumption rate by immobilized cells. In the present model only diffusive transport of the substrate within the gel matrix is considered. Consequently, the mass balance for substrate over time in the general 3D system is:

$$\frac{\partial c_S}{\partial t} = D_S \left(\frac{\partial^2 c_S}{\partial x^2} + \frac{\partial^2 c_3}{\partial y^2} + \frac{\partial^2 c_S}{\partial z^2} \right) - r_S (c_S, c_X) \qquad (1)$$

where c_S and c_X are the substrate and biomass concentrations within the gel beads, respectively, D_S is the substrate diffusion coefficient and r_S is the substrate conversion rate usually defined as a Monod–like saturation function:

$$r_S (c_S, c_X) = \left(\frac{\mu_m}{Y_{XS}} + m_S \right) \cdot c_X \frac{c_S}{K_S + c_S} \qquad (2)$$

where μ_m is the maximum specific growth rate of cells, Y_{XS} is the cell growth yield from substrate, m_S is the maintenance coefficient and K_S is the Monod saturation constant. The net rate of biomass formation, r_X, can be then expressed as:

$$r_X (c_S, c_X) = Y_{XS} (r_S (c_S, c_X) - m_S c_X) \qquad (3)$$

Considering the biomass accumulation to be the net result of biomass growth and biomass decay, the biomass balance will be:

$$\frac{\partial c_X}{\partial t} = r_X (c_X, c_S) \qquad (4)$$

The equations (1), (2), (3) and (4) define the differential part of the model that is the substrate transport and biomass accumulation.

The *cellular automata* rules for cell growth are as follows:

If the biomass concentration in a defined space element increased to a maximum level (i.e. the threshold for cell division):

1. The biomass in the volume element is divided into two equal parts (representing two cells). The first stays at the same site, and the second cell must be placed in another space element.

2. Search for a free space elements in the neighborhood considering search depth.

3. If a free space element is not in the nearest neighborhood push randomly chosen nearest neighborhood element into the direction of the free space found and make one nearest neighborhood element free.

4. Place the second cell in the nearest free neighborhood space element.

These rules present a modification of the previously proposed DDCA model by Picioreanu et al. (1998). In that model, the free space element was randomly chosen regardless the search depth, while in the present model it is assumed that the cell will find the nearest free space so that the number of cells that have to be "pushed" will be the lowest. Cell movements towards free space elements by described cellular automata rules (1–4) are schematically presented in Figure 1. In addition, in the present model, the most simple biomass detachment approach is used. If the space, in which the newly formed biomass has to be placed, is located outside of the gel sphere then the microorganisms are simply removed. In the future, different detachment rules could be applied considering hydrodynamic environments around the cell carriers.

The boundary conditions are set for the case in which external mass transfer limitations could be neglected, that is the substrate concentration is maximal and constant outside of the bead: S = 1 = constant (at any t) for where r is a radial distance from the center of the spherical bead and Rbead is the bead radius.

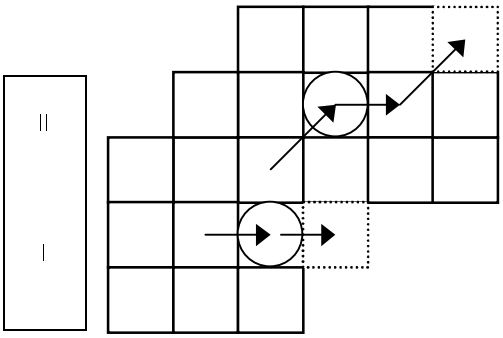

Figure 1. A simple chosen for cellular automata division rules for two cases: I) the nearest space element is free; II) the nearest free space is found such that the new cell has to "push" randomly chosen cells towards that space element.

At time zero, substrate is also at the maximal concentration and uniformly distributed in the space: for all (x, y, z) elements. In addition, an initial number (n_0) of virtual microbial cells are randomly distributed throughout the gel sphere.

The algorithm for determination of microbial cell growth and diffusion gradients of substrate is as follows:

Initialization. Specify initial substrate field and seed the bead volume with microorganisms, randomly distributed in the sphere. Initial count of microorganism cells is n_0.

*Step*1. Find the substrate distribution after a defined time interval. An iterative super–relaxation algorithm solves the balance equations (1) – (2).

*Step*2. Solve the biomass balance (3) – (4). The biomass growth rate is used in each grid element occupied by biomass to calculate the amount of biomass produced at a given time interval.

*Step*3. Check each element that contains biomass to determine if the threshold for cell division has been reached. If yes then redistribute the biomass according to the automation rules.

Then the algorithm goes back to the step 1 to find the substrate distribution for the new state of biomass matrix.

MODEL PARAMETERS

Parameters for microbial growth and diffusive substrate transport, applied in the previously proposed DDCA model (Picioreanu et al., 1998), were also used in this study as summarized in Table 1.

Table 1. Parameters used in simulations of substrate concentration profiles and growth of Nitrosomonas europaea immobilized in carrageenan gel beads (Picioreanu et al., 1998)

Parameter	Symbol	Value	Units
Total number of volume elements	N–M–L	10^6	(–)
Substrate concentration in bulk liquid	C_{S0}	$3.84\cdot10^{-3}$	(kg m^{-3})
Biomass maximum density in colonies	C_{Xm}	70	(kg m^{-3})
Initial number of "cells"	n_0	210	(–)
Bead diameter	d	$2.00\cdot10^{-3}$	(m)
Diffusion coefficient of substrate	D_S	$2.00\cdot10^{-9}$	(m^2 s^{-1})
Monod saturation constant for substrate	K_S	$3.50\cdot10^{-4}$	(kg m^{-3})
Maintenance coefficient	m_S	$3.00\cdot10^{-5}$	(kg$_S$ kg$_X^{-1}$ s^{-1})
Maximum specific growth rate	μ_m	$1.52\cdot10^{-5}$	(s^{-1})
Growth yield from substrate	Y_{XS}	0.045	(kg$_X$·kg$_S^{-1}$)

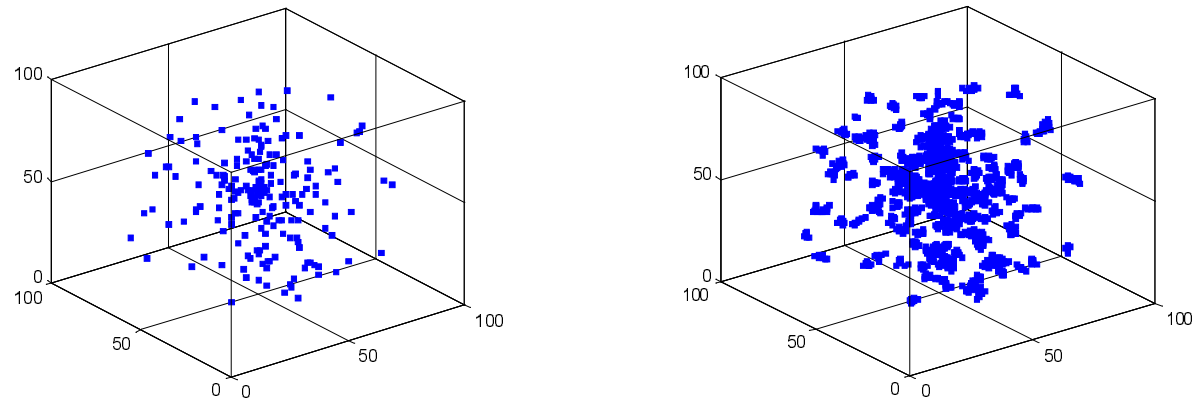

Figure 2. 3D occupation of space with microbial cells in a spherical gel bead (2 mm in diameter corresponding to 100 grid increments). a) Initial cell distribution (t = 0); b) Cell distribution after 15 days.

RESULTS AND DISCUSSION

Simulated growth of *Nitrosomonas europaea* immobilized in a spherical gel bead is presented in Figure 2. Development of microbial colonies of different sizes as functions of distance from the bead surface can be distinguished. The results of the simulation were compared with results obtained by the previously proposed DCCA model (Picioreanu et al., 1998). Predictions of the two models are overally similar with slightly different shapes of microbial colonies. The presented model is globally identical to the previous model but with altered cellular automata rules for cell division. The rules applied in this study take into account direction of the cell movement. In the present simulation it is assumed that the cell will take the nearest free space at the minimal number of already present cells to be "pushed". However, the applied rules also provide a possibility of governing the cellular division and movement in some particular direction e.g. towards the source of nutrients or other stimuli. In this way, the effects of a chosen cultivation parameter on cell growth could be modeled and estimated.

CONCLUSION

In this paper a automata–discrete–cellular–automata model was developed for prediction of substrate concentration profiles and growth of cells immobilized in gel beads. The model was based on a previously proposed DDCA model with modified cellular automata rules. The rules applied here provided modeling of cell movements towards nearest free sites. In addition, cell growth in the present model could be directed in some specific direction, which could be attractive for evaluation of the effects of growth factors or other biologically active molecules on growth kinetics and colony formation. Overally, the model predictions of cell distributions within the gel beads were in qualitative agreements with the results of the previously proposed model as well as with previously reported experimental data (Picioreanu et al., 1998).

NOMENCLATURE

c_S – Substrate concentration in volume elements (kg m^{-3})
c_X – Biomass concentration in volume elements (kg m^{-3})
r_S – Substrate consumption rate (kg m^{-3} s^{-1})
r_X – Biomass formation rate (kg m^{-3} s^{-1})
C – Dimensionless biomass density (–)
S – Dimensionless substrate concentration (–)
N, M, L – Sizes of matrices (–)

REFERENCES

[1] Fujikawa (1994) Diversity of the growth patterns of Bacillus suptilis colonies on agar plates. FEMS Microbiol. Ecol. 13. 159–168.

[2] Picioreanu et al. (1998) A new combined differential–discrete cellular automaton approach for biofilm modeling: Application for growth in cell beads. Biotechnol. Bioeng. 57 (6) 719–731.

[3] Picioreanu et al. (1999) Discrete–differential modelling of biofilm structure. Wat. Sci. Tech., 39. 115–122

[4] Schindler, J., Rataj, T. (1992) Fractal geometry and growth models of a Bacillus colony. Binary 4. 66–72.