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CELLULAR AUTOMATA AS AN EFFECTIVE TOOL FOR MODELLING OF BIOFILM MORPHOLOGY

Mathematical model of the biofilm growth and morphology dynamics has been presented based on the cellular automata theory. All processes occurring in a biofilm have been modelled in a discrete manner. The two-dimensional distributions of microorganisms density and concentrations of substrates were obtained from the simulation. One-dimensional distributions of microorganisms density and biofilm porosity dependent on the growth time have been determined. It was shown that the biofilm morphology varies significantly over the process time. This phenomenon can be used for determining the age of a growing biofilm.

1. INTRODUCTION

Issues of protection of surface water in Poland and worldwide belong to the strategic research programs. Microbiological methods to reduce water pollution are ones of the recognized and effective tools used for this purpose. There are two groups of technical and procedural issues related to the microbiological water treatment. The former group incorporates biodegradation technologies of wastewater treatment: municipal and industrial. The latter group is related to the utilization and multiple use of water coming from aquacultures or treatment of drinking water. Each of these issues requires a different approach to both analysis and process design, as well as to the selection of the specific equipment, in particular, the construction and size of microbiological reactors. This selection is primarily based on: the type of the process, i.e., aerobic or anaerobic, the type of microorganisms, i.e. autotrophs or heterotrophs or their co-presence, the concentrations of substrates being subjects to microbial transformations and their volumetric flow rate.

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According to opinions of Loosdrecht et al. [1] and Eberl et al. [2], in aqueous environments typical of biodegradation processes, microorganisms always form a biofilm. This phenomenon is unavoidable. Hence, in a quantitative description of the microbial reactors, the presence of biofilms is to be taken into account and their influence on overall process rate occurring in the apparatus is to be evaluated. The presence of the biofilm is particularly important for operation of biofilters and fluidized-bed bioreactors, because in these apparatuses the share of biomass immobilized on a solid substratum is considerable.

Mathematical models of biofilms can be divided into two groups:

- continuous models which use the idea of continuum and differential calculus for modelling of biofilms,
- discrete models which treat time and space in a quantified manner.

Below, the theory of cellular automata has been discussed and used. This method belongs to the latter mentioned group. It has been applied for the modelling of biofilm morphology, in particular to determination of density and porosity distributions in the biofilm, because both quantities have a principal influence on the transport of substrates and rate of the processes occurring in the biofilm. No matter which method is used, these quantities are necessary for modelling of the process in the biofilm.

The new element of this work is the use of the cellular automata for modelling of microbial process following double-substrate kinetics with substrate inhibition. The microbiological degradation of phenol has been chosen as an example. Following premises were crucial in this choice:

- phenol is a strongly toxic compound, dangerous for aqueous organisms,
- this compound inhibits the growth of microorganisms,
- kinetics of this process is well described.

2. CELLULAR AUTOMATA AS A TOOL FOR MODELLING DYNAMICAL SYSTEMS

It is considered that Hungarian mathematician, Janos von Neumann, is the author of cellular automata (abbreviated CA). Polish mathematician, Stanisław Ulam, had also an important role for creation of this method. Thanks to Ulam, von Neumann has introduced discrete time and space into the model of self-reproducing machine [3].

Cellular automaton is a mathematic concept consisting of following elements [4]:

- grid of cells $\{i\}$ of D-dimensional space,
- set $\{s_i\}$ of single cell's states,
- transition function F, i.e., a set of rules determining the cell's state at moment $t + 1$ depending on the state of that cell and cells surrounding it at moment t:

$$s_i(t+1) = F\left[\left(s_j(t)\right)\right], \qquad j \in \Omega(i) \tag{1}$$

where $\Omega(i)$ is a neighborhood of i-th cell. On the 2-D rectangular grid, von Neumann or Moore neighborhood (Fig. 1) is most often used.

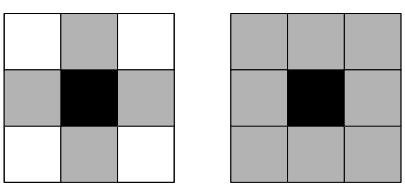

Fig. 1. Von Neumann neighborhood (left) and Moore neighborhood (right), grey color denotes cells which are neighbors of the central cell

Game of life created by John Conway is one of the most known cellular automata. This cellular automaton has following elements [5]:

- two-dimensional rectangular grid,
- two-element set of states: 0 and 1,
- Moore neighborhood,
- the following rules: if three living cells exist in cell's neighborhood (without taking it into account) at moment t, then at moment $t + 1$ this cell is alive. If at moment t the cell is alive and two living cells exist in its neighborhood, then it stays alive at moment t +1. In other cases, the cell is dead at moment $t + 1$.

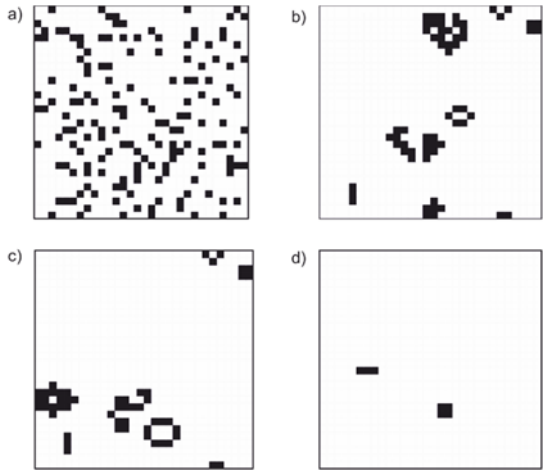

Fig. 2. Evolution of Game of life, white cells are dead, black are alive: a) $t = 0$, b) $t = 50$, c) $t = 100$, d) $t = 200$

The evolution of Game of life for randomly chosen cells' states at initial moment is shown in Fig. 2. This simple cellular automaton has directed attention of the physicists on the possibility of using the cellular automata for simulations of physical systems.

The cellular automata have been popularized mostly by Stephen Wolfram, the author of Mathematica software. He is also the author of the oldest and most popular classification of cellular automata. According to this classification, cellular automata are divided into four classes, numbered with increasing complexity, and every class differs from the others in certain features. CA Game of life belongs to fourth class.

Cellular automata have successfully been used for modelling of complex dynamical systems. The biofilm, i.e., multicomponent biological structure containing microorganisms attached to solid surface or porous material or to each other belong to such systems. Biofilm is a complex object, both regarding its composition, morphology and regarding processes occurring inside.

3. EMPIRICAL FACTS CONCERNING MORPHOLOGY OF BIOFILMS

One of the pioneering papers concerning biofilms morphology has been published by Trulear and Characklis [6]. The authors claimed that increase in substrate load causes the increase in biofilm density, however further investigations did not confirm this statement. In another paper, concerning heterogeneity of biofilms Tang and Fan [7] have shown that the mean thickness of a biofilm and the average diffusion coefficients of substrates depend on the total thickness of a biofilm. Whereas Kwok [8], discussing properties of the three-phase airlift bioreactor, proved that upon increasing small-grain carrier's content, the biofilm density increases and that upon decreasing the substrate load, the biofilm thickness decreases. It is commonly known that the biofilm structure, i.e., its thickness, density and morphology, is strongly influenced by three elements, i.e., the concentration of the substrates, hydrodynamic conditions in the apparatus, and microbial species [9].

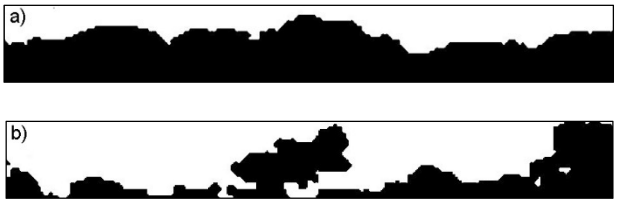

Fig. 3. Structures of 7-day old biofilms:
a) *Pseudomonas putida*, b) *Pseudomonas aeruginosa*,
based on microscopy images presented by Heydorn et al. [9]

It arises from the experimental examinations that biofilms may form various structures, from flat to strongly irregular. Figure 3 presents two biofilm structures, formed by different microbial species, i.e. *Pseudomonas aeruginosa* (Fig. 3a) and *Pseudomonas putida* (Fig. 3b). These figures have been drawn based on microscopic images presented by Heydorn et al. [9].

Lewandowski [10] pointed out a significant role played by the internal biofilm porosity to the mathematical modelling of the biofilm. It is related with the fact, that the values of the effective diffusion coefficients in the biofilm are even several times lower than in water [11]. Hence, the biofilm porosity influences the rate of substrate transport. Figure 4 presents one-dimensional distribution of the biofilm porosity, obtained by means of a confocal microscope [10].

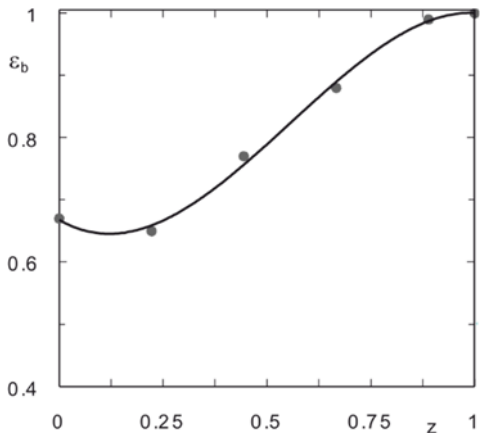

Fig. 4. Distribution of the biofilm porosity obtained using confocal microscopy (based on [10])

4. MATHEMATICAL MODEL OF THE BIOFILM BASED ON THE THEORY OF CELLULAR AUTOMATA

Two different approaches to the quantitative description of diffusion and reaction processes are used in biofilm models based on cellular automata. Differential diffusion--reaction equations are solved when the former of these approaches is employed. Papers by Picioreanu et al. [12] and Xavier et al. [13] can be given as examples. In the latter approach, the diffusion process is simulated using an algorithm of random walks (e.g., papers by Pizarro et al. [14, 15], and Chang et al. [16]). In the model presented in this work, the latter of the mentioned approaches has been used.

In this work, which concerns double-substrate processes, two-dimensional overlaying grids with square cells have been used. Similar approach has been used in work concerning single-substrate kinetics, by Pizarro et al. [14]. The substrate's grids contain information about the concentrations of oxygen and carbonaceous substrate, while grid for the biomass contains information about the presence and biological state of microorganisms. In a previous study [17], the algorithm was compared with the shooting method, for a different number of grids for each of the substrates. Criterion for the accuracy of the calculation was the value of the effectiveness factor of the biofilm. The calculations in this work were carried out using the same number of grids for each of the substrates, equal $J = 2$.

Neighborhood proposed by von Neumann (Fig. 1) has been applied in the presented model. Periodic conditions were used on the borders of the grid. At the fixed distance from the front of the biofilm at each iteration, the state of the cells was determined equal to c_i^c ($i = A, T$). This is to simulate the boundary layer and the liquid phase of constant concentrations of oxygen and carbonaceous substrate. The thicknesses of boundary layers were determined from the mass transfer coefficients of the reagents, i.e., oxygen and phenol from the liquid to the biofilm.

The state of cells for the substrate can be a value in the range $[0,\ c_i^c]$, ($i = A, T$). For the biomass, the values are μ from the set $\mu \in [-\rho_b^{\max}, \rho_b^{\max}]$. A negative value indicates that the microorganisms are dead, and a positive one that the microorganisms are active. Zero represents the absence of biomass. The states of cells were marked with shades of gray color (Fig. 5).

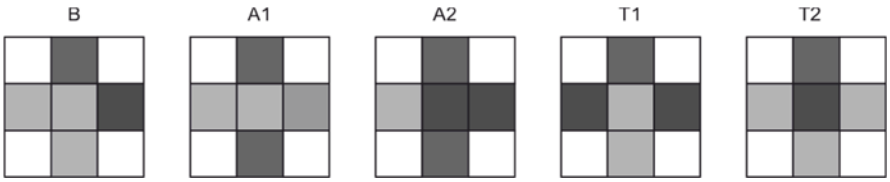

Fig. 5. Graphical representation of grids and cells' states, the darker color, the larger concentration of a substrate or biofilm density, white color denotes cells which are not neighbors of the central cell, B – grid representing the biomass, A1, A2 – grids representing the carbonaceous substrate, T1, T2 – grids representing oxygen

Since the reaction and diffusion processes take place at a much higher rate than the growth of the biofilm, it is assumed that the distributions of reagent concentrations reach a pseudosteady state [14]. This fact results in a separation of the two time steps in the algorithm of biofilm dynamics, significantly differing in values of them. The time step Δt, of the order of miliseconds, relates to the reaction and diffusion processes [14], while Δt_g time step, of the order of hours, refers to the processes of growth, decay and detachment of the biofilm [17, 18]. Such an approach was used in many studies on biofilm modeling based on the theory of cellular automata, also in the latest works [19].

The rules governing the proposed cellular automaton are as follows.

RULE 1. DIFFUSION OF THE SUBSTRATES

The diffusion process was simulated using the algorithm of random walks in a modified version. The probabilities of movement of the mass quantum of oxygen in the liquid phase in all four directions on the grid are the same and are P_{dTw}, and the sum of these probabilities is equal to one. Mentioned modification is the assumption that the probability of movement of the mass quantum of oxygen is dependent on the presence of biomass in the starting and the target cell. It was calculated as follows

$$P_{dT} = P_{dTw}\left(\frac{n_w}{2} + \frac{D_{eT}}{D_{Tw}}\frac{n_b}{2}\right) = 0.25\left(\frac{n_w}{2} + \frac{D_{eT}}{D_{Tw}}\frac{n_b}{2}\right) \tag{2}$$

The above equation is useful for both the water and the biofilm, where n_b is the number characterizing the system of two cells: the starting and the target one. It specifies the sum of the cells in this doublet, which represent the biomass. In turn, n_w is the number related to the presence of water in the same doublet of cells. Both n_b and n_w can take values from the set {0, 1, 2}.

Similarly as was done above for the oxygen transfer process, the relationship defining the probability P_{dA} of the displacement of the mass quantum of the carbonaceous substrate is

$$P_{dA} = P_{dAw}\left(\frac{n_w}{2} + \frac{D_{eA}}{D_{Aw}}\frac{n_b}{2}\right) = 0.25\,\frac{D_{Aw}}{D_{Tw}}\left(\frac{n_w}{2} + \frac{D_{eA}}{D_{Aw}}\frac{n_b}{2}\right) \tag{3}$$

It was assumed that the ratio of the probability of the mass quantum displacement of the carbonaceous substrate in water to the probability of the mass quantum displacement of oxygen in water is equal to the ratio of diffusion coefficients in water, $P_{dAw}/P_{dTw} = D_{Aw}/D_{Tw}$.

RULE 2. UTILIZATION OF THE SUBSTRATES IN THE BIOFILM

The computations were made on the example of the aerobic biodegradation of phenol following double-substrate kinetics of Seker et al. [20]. The rates of utilization of the carbonaceous substrate r_A and oxygen r_T in the biofilm are described by

$$r_A^b\left(c_A^b, c_T^b\right) = \frac{1}{w_{BA}} f_1\left(c_A^b\right) f_2\left(c_T^b\right) \rho_a \tag{4a}$$

$$r_T^b\left(c_A^b, c_T^b\right) = \frac{1}{w_{BT}} f_1\left(c_A^b\right) f_2\left(c_T^b\right) \rho_a \tag{4b}$$

The functions f_1 and f_2 take the form of the equations

$$f_1(c_A) = \frac{kc_A}{K_A + c_A + \dfrac{c_A^2}{K_{in}}} \tag{5a}$$

$$f_2(c_T) = \frac{c_T}{K_T + c_T} \tag{5b}$$

The value of the substrate concentration in the cell indexed (k, l) is determined as the arithmetic mean of concentrations in the cells (k, l) in each grid for this substrate, according to

$$c_A^b\left(k,l\right)=\frac{1}{J}\sum_{j=1}^{J}c_A^{b,j}\left(k,l\right) \tag{6a}$$

$$c_T^b\left(k,l\right)=\frac{1}{J}\sum_{j=1}^{J}c_T^{b,j}\left(k,l\right) \tag{6b}$$

where J denotes the number of grids for each substrate.

Using the equation defining the reaction rate related to the particular substrate A, we have

$$r_A^b=-\frac{dc_A^b}{dt}\approx-\frac{\Delta c_A^b}{\Delta t} \tag{7}$$

Based on the above relationship, the expressions determining the increases in the concentrations of substrates during the time step Δt can be obtained. These expressions are as follows

$$\Delta c_A^b\left(k,l,t\right)=-\Delta t\times r_A^b\left[c_A^b\left(k,l,t\right),\ c_T^b\left(k,l,t\right),\ \rho_a\left(k,l,t\right)\right] \tag{8a}$$

$$\Delta c_T^b\left(k,l,t\right)=-\Delta t\times r_T^b\left[c_A^b\left(k,l,t\right),\ c_T^b\left(k,l,t\right),\ \rho_a\left(k,l,t\right)\right] \tag{8b}$$

The new values of the cells' states in the grids for the substrates are

$$c_A^{b,j}\left(k,l,t+\Delta t\right)=c_A^{b,j}\left(k,l,t\right)+\Delta c_A^b\left(k,l,t\right)\frac{J}{n_A\left(k,l,t\right)},\ \left(j=1,\ 2,\ ...,\ J\right) \tag{9a}$$

$$c_T^{b,j}\left(k,l,t+\Delta t\right)=c_T^{b,j}\left(k,l,t\right)+\Delta c_T^b\left(k,l,t\right)\frac{J}{n_T\left(k,l,t\right)},\ \left(j=1,\ 2,\ ...,\ J\right) \tag{9b}$$

where n_A, n_T are the total number of cells whose state is determined by a number greater than zero in a given position in the grids of the substrate, while $c_A^{b,j}$ and $c_T^{b,j}$ are the mean concentrations of the substrates in the j-th substrate's grid.

RULE 3. GROWTH AND DECAY OF MICROORGANISMS
AND DETACHMENT OF THE BIOFILM

Increase in mass of active microorganisms in one time step Δt_g is determined by

$$\Delta\rho_a\left(k,l,t\right)=\Delta t_g r_B^b\left[c_A^b\left(k,l,t\right),\ c_T^b\left(k,l,t\right),\ \rho_a\left(k,l,t\right)\right] \tag{10}$$

New value of cell's state is then

$$\rho_a\left(k,l,t+\Delta t_g\right)=\rho_a\left(k,l,t\right)+\Delta\rho_a\left(k,l,t\right) \tag{11}$$

An algorithm of the biofilm growth was implemented, according to Picioreanu et al. [12]. The value of the probability of microorganisms decay was calculated from:

$$p_o=k_o\Delta t_g\ \frac{|\mu|}{\rho_b^{\max}} \tag{12}$$

To calculate the probability of the biofilm detachment, an equation was used, like that proposed by Chambless et al. [21].

$$p_{\text{det}}=K_{\text{det}}\Delta t_g\left[x(k,l)\right]^2 \tag{13}$$

5. RESULTS AND DISCUSSION

Figure 6 shows how the structure of the biofilm changes over time. The biomass density distributions are given for different values of the biofilm growth time *t*, i.e., 20 h, 50 h, 150 h, and 200 h. Different shades of green color represent the concentrations of biomass (living or dead microorganisms). Initially, in the last row of grid for biomass, i.e., in cells representing the biofilm in contact with the surface of the support, the value of one cell's state for every twenty was $\mu=\rho_b^{\max}$, and the value of remaining cells' state was $\mu=0$. The initial moment is not shown in Fig. 6. Initially the colonies of microorganisms grow without larger deformations (Fig. 6a). Next, microbial colonies join together forming a layer at the base of the biofilm with very low porosity, of the order $\varepsilon_b = 0.2$ (Fig. 6b, $t = 50$ h). After 150 h (Fig. 6c), the biofilm structure is very irregular. It arises from the Eq. (13) that the probability of the biofilm detachment is proportional to the square of the distance from the base of the biofilm. For this reason, the detachment does not deform the biofilm for its smaller thickness. Figures 6c and 6d point out that for a sufficiently long growth time, the thickness of the biofilm stabilizes.

Values of the model parameters, for which the computations were performed, are givrn in Table 1.

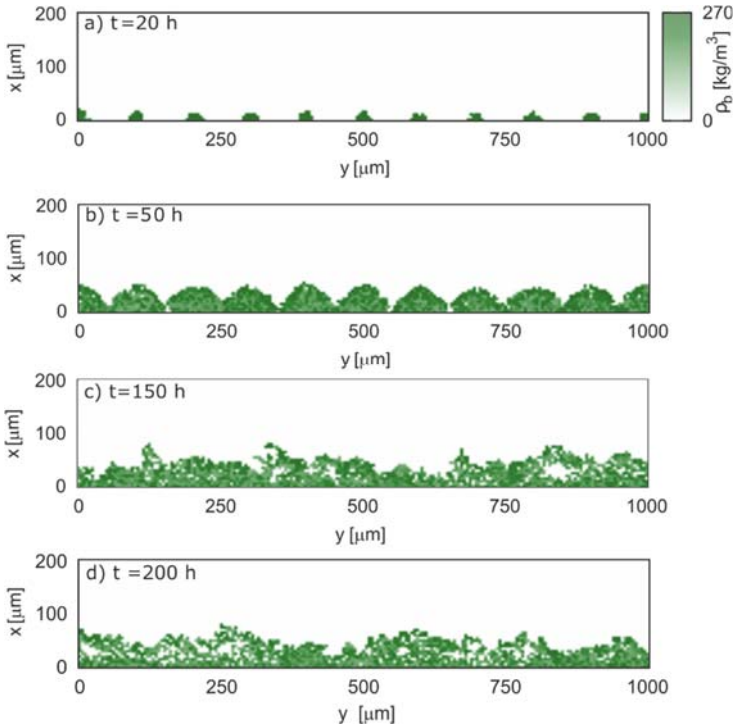

Fig. 6. Two-dimensional distribution of the biofilm density dependent on time: a) t = 20 h, b) t = 50 h, c) t = 150 h, d) t = 200 h

Table 1

Values of the parameters used in the simulation

Parameter	Value	Parameter	Value
c_A^c, kg·m^{-3}	$3\cdot10^{-2}$	K_{det}, h^{-1}·m^{-2}	$2\cdot10^{7}$
c_T^c, kg·m^{-3}	$8.29\cdot10^{-3}$	K_{in}, kg·m^{-3}	0.09937
D_{eA}, m^2·h^{-1}	$3.3\cdot10^{-6}$	K_T, kg·m^{-3}	$4.8\cdot10^{-5}$
D_{eT}, m^2·h^{-1}	$8.28\cdot10^{-6}$	w_{BA}, kg B·[kg A]$^{-}$	0.521
k, h^{-1}	0.569	w_{BT}, kg B·[kg T]$^{-1}$	0.338
k_o, h^{-1}	$2.5\cdot10^{-4}$	Δt, h	$7.55\cdot10^{-7}$
k_{sA}, M·h^{-1}	0.144	Δt_g, h	5
k_{sT}, m·h^{-1}	0.277	$\rho_b^{\max}$, kg·m^{-3}	270
K_A, kg·m^{-3}	0.01854		

Figure 7 shows one-dimensional distributions of biofilm porosity ε_b and biofilm density ρ_b for the process time $t = 20$ h (Fig. 7a, b), and for $t = 200$ h (Fig. 7c, d). The distributions of the density were obtained by averaging the biofilm density for each row in the grid of the cellular automaton. The biofilm porosity for each row in the grid was determined by dividing the number of cells of the state 0 to the number of all cells in the row.

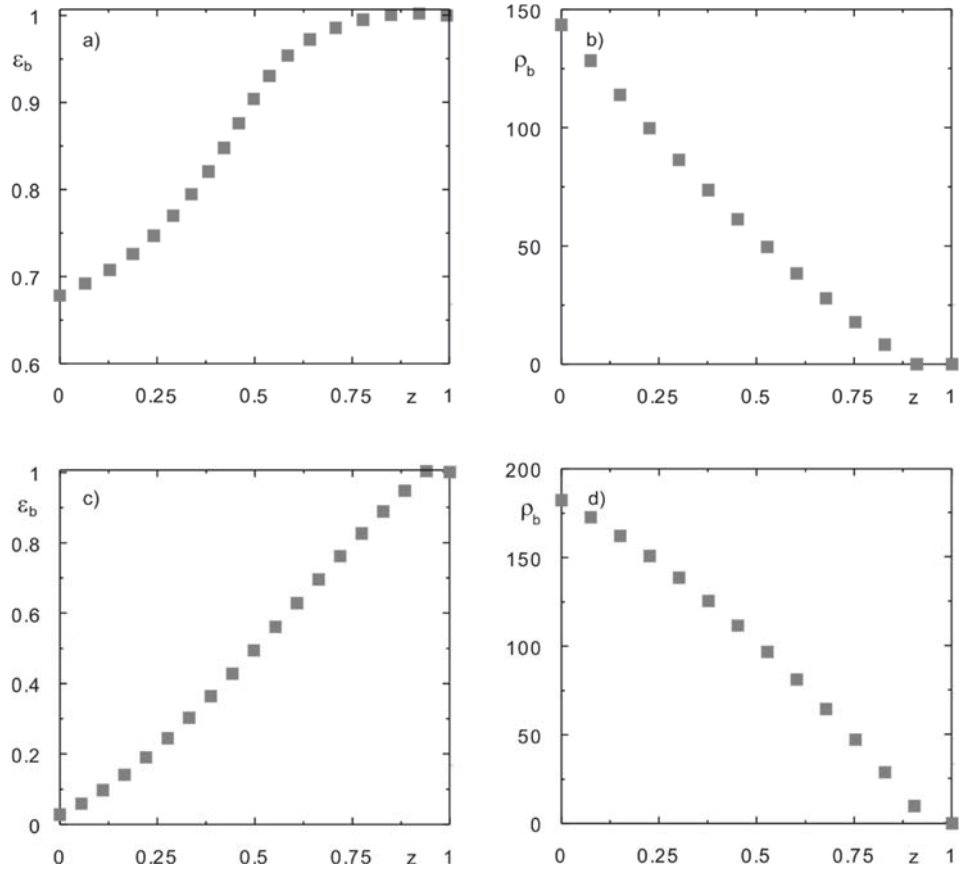

Fig. 7. One-dimensional distributions of biofilm porosity (left) and biofilm density (right) for two values of the growth time: a), b) $t = 20$ h, and c), d) $t = 200$ h

Figure 7 shows that as the biofilm growth time increases, a qualitative and quantitative changes occur in the distribution of biofilm density and porosity. This property can be used to determine the age of the biofilm. The qualitative and quantitative similarity between porosity distribution can be seen, in Fig. 4 and that shown in Fig. 7a. One can conclude that the porosity distribution shown in Fig. 4 was obtained for a "young"

biofilm. “Older” biofilms, as can be seen in Fig. 7c, are characterized by almost linear functions of $\varepsilon_b(z)$ and $\rho_b(z)$.

6. CONCLUSIONS

The paper presents a mathematical model of the biofilm growth based on the theory of cellular automata. This model was used to simulate the biofilm morphology and to determine the change in the morphology over time. The proposed model takes into account the diffusion and uptake of the substrates, the growth of microorganisms, their decay and detachment of the biofilm. The mathematical model can be used to simulate microbial processes following any kinetics, both single- and multi-substrate. One can also use it to determine the density distributions of the biofilm and the associated porosity of the biofilm, as well as concentrations of the reagents. On this basis, one can determine the rate of microbial processes inside the biofilm.

Changes in one-dimensional distributions, i.e. in relation to the depth of the biofilm, of its porosity and density were shown. The shape of these functions depends on the age of the biofilm. It is hypothesized that measuring of these distributions can be used to assess the age of the biofilm influencing, e.g., its resistance to biocides.

The computations were made on the example of the aerobic biodegradation of phenol. The literature suggests that the theory of cellular automata has not so far been applied to the modelling of microbial processes with inhibiting substrate. The mathematical model can be used to determine the effect of inhibition constant of the carbonaceous substrate on the biofilm morphology. So far, such research, both theoretical and experimental, has not been conducted.

SYMBOLS

c_A, c_T – concentration of the carbonaceous substrate and oxygen, respectively, kg·m^{-3}
D_e – effective diffusion coefficient in the biofilm, m^2·h^{-1}
J – number of grids for each substrate,
k – maximum specific growth rate, h^{-1}
k_s – liquid–biofilm mass transfer coefficient, [m/h]
K – saturation constant in kinetic equations, kg·m^{-3}
k_o – decay constant, h^{-1}
K_{det} – detachment probability constant , h^{-1}·m^{-2}
K_{in} – inhibition constant, kg·m^{-3}
w_{BA}, w_{BT} – yield coefficients, kg B·(kg i)$^{-1}$
P_d – probability of the mass quantum displacement
p_{det} – probability of the biofilm detachment
p_o – probability of microorganisms decay
r_A, r_B, r_T – consumption rate of the carbonaceous substrate, growth rate of bacteria and consumption
– rate of oxygen, respectively, kg·m^{-3}·h^{-1}
t – time, h

Δt – time step for reaction and diffusion processes, h
Δt_g – time step for processes of growth and decay of microorganisms, and biofilm detachment, h
x – space coordinate in the biofilm, μm
z – dimensionless coordinate of biofilm thickness
μ – state of a cell in the cellular automaton
ε_b – biofilm porosity
ρ – biofilm density, kg·m^{-3}

SUPERSCRIPTS

c – liquid phase
b – biofilm

SUBSCRIPTS

a – concerns active microbial cells
A – carbonaceous substrate
b – concerns biofilm
T – oxygen
w – concerns water

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