Two-Dimension Dynamic Simulations on Biofilm Forming and Developing*

LI Tian-cheng[1,2], *LI Xin-gang*[2], *ZHU Shen-lin*[1]
(1. The State Key Laboratory of Chemical Engineering //Department of Chemical Engineering, Tsinghua University, Beijing 100084, China;
2. School of Chemical Engineering, Tianjin University, Tianjin 300072, China)

Abstract: It played an important role for biofilm microstructure and morphology on wastewater biological treatments. Multi-species microorganisms were able to form varieties of biofilm on adaptive carriers, and the process of biofilm to form and develop on carriers was multi-dimensional and dynamic. In this article, substrate transfer, biofilm formation and development in a two-dimension space were dynamically simulated with a combined discrete-differential method. The micro structural and morphological characteristics, such as biofilm thickness, density and porosity, were obtained as model output by using this new-type model. Meanwhile, the effects of the growing time and initial inoculation number of microorganisms on biofilm microstructure and morphology were also discussed. Firstly, the thickness of biofilm was increasing along with the growing time of biomass extending. At the time of 30 h for biofilm formation and development, it was not completely covered for carrier by biomass, and the fluctuation of biofilm was very ambient; but at the time of 60 h, biomass had covered the carrier entirely, and the fluctuation of biofilm tended to gentleness. Secondly, as biomass growing time was 30 h, it was not covered completely for carrier by biomass with different initial inoculating number of 50, 150 and 250. And the covering degree of biomass was improving along with initial inoculating number increasing. Especially, as initial inoculating number was 250, it was nearly covered for carrier by biomass.

Key words: multi-species microbes; biofilm; Microstructure; morphology; dynamic simulation
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1 Introduction

As an effective technology of wastewater biological treatments, the applications of biofilm process become wider and wider along with the aggravation of water resource and the rise of appeal to pollution control. While the core for this process is biofilm, the microstructure and surface morphology of biofilm are directly related with the activity of multi-species microbes and the transfer of substrate in biofilm. In a word, it will have an obvious effect on water qualities in outlet.

A biofilm can be defined as a layered structure of microbial cells and cellular products, like extracellular polymers, attached to a solid surface. A biofilm can thus be seen as the accumulation product of naturally immobilized microorganisms at an interface.

The main parameters on biofilm include: density, pore distribution, porosity, specific surface area, adhesion strength, tortuosity factor, diffusion coefficients and fractal dimensions[1,2]. The structural and morphological characteristics of biofilm are very important for the overall performance of a biofilm reactor. And these factors strongly affect the biomass hold-up and biomass transfer in a biofilm reactor.

It has been observed as follows by using co-focal scanning microscope[3]: Heterogeneous biofilm is composed of microfloras embodied in extracellular polymers substrates (EPS), which are adhering to adaptive carriers, and there are varieties of voids, micro pores and channels. On one hand, extracellular polymer materials can avoid microbes to contact the potential poisons or antiseptic compounds; on the other hand, it is capable of limiting the free movements of microbes[4]. In general, the main factors to influence microbe microstructure and morphology include species of microorganism, transfer of substrate, flowing characters of fluid, and so on[5,6].

Biofilms can form lots of morphologies, such as compact or thickening layer-type, patching or villous mushroom-type. However, it is common for fluctuating and continuous layer biofilm[7]. See Fig. 1 and Fig. 2.

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作者简介: 李天成 (1970 年生), 男, 博士, 助理研究员; 通讯联系人: 朱慎林, E-mail: zhusl@mail.tsinghua.edu.cn

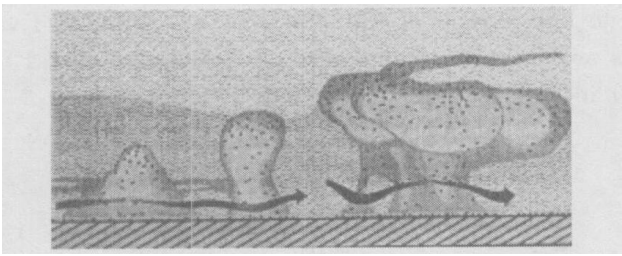

Fig 1　Mushroom or fingertip biofilms

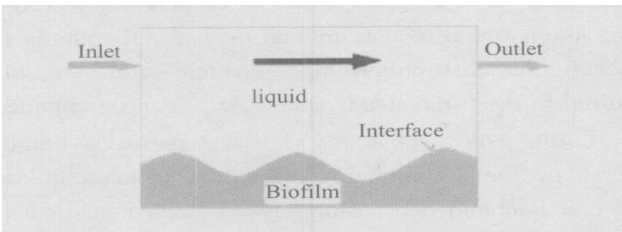

Fig 2　Continuous layer biofilms

In recent years, there are many literatures on biofilm simulating abroad. Initially, some modeling on biofilm regarded biofilm as mono-species homogeneous steady-state and localized in one-dimension substrate transferring and biochemical reaction[8,9]. Later, there are dynamic models developed, and they are capable of describing multi-species hierarchical biofilm and multi-substrates transfer[10,11]. These one-dimension models can reflect the interactions for microorganisms in biofilm, but can not display the morphological characters, such as biofilm thickness, density and surface shape. And the morphological characters are the inputs, not the outputs of model.

Nowadays, along with the improvements of mathematics theory and calculating ability, it is possible to carry out multi-dimension dynamic simulation for biofilm microstructure and morphology, combined with behaviors of fluid hydrodynamics, transfer of substrates and metabolism of microbes. Moreover, it can quantitatively describe the morphological characteristics of biofilm and the dynamic characters of microbes growing, propagating and sloughing[12]. In this article, two-dimension dynamic simulations for multi-species aerobic biofilm formation and development have proceeded on the basis of predecessors, and the main influencing factors on formation and development of biofilm are discussed in detail.

2　Mathematics modeling

Here, the discrete-differential combined method is adopted to dynamically simulate the forming and developing process of biofilm adhered to plane plate. Compared with traditional models on biofilm, the difference for this new-type model is that the micro-structural and morphological characters including biofilm porosity, thickness and density are outputs of model.

The physical space is represented by a rectangular uniform grid. Square elements are used as tiles to fill the 2-D space. In the 2-D cartesian grid of $N_X \times N_Y$, coordinates of square elements are given by a vector $(X, Y) \in (0 \cdots N_X - 1, 0 \cdots N_Y - 1)$. The substrate mass balance can be solved with reasonable accuracy on a 2 to 4 time coarser grid, of $N_{XS} \times N_{YS}$ elements.

The assumptions for this model are:

(1) As microbial cells are inoculated, they adhere to plane plate and grew. Under excellent aerobic conditions, varieties of biofilm are formed.

(2) Microstructures are determined by the net rates of biomass growth and slough dynamically[13].

(3) The states for biofilm forming and developing are reflected by the non-dimensional concentration of substrate (C) and biomass density (B).

(4) Variable (P) is used to stand for the occupying states of constituents (Biomass or liquid). For certain of grid element, if occupied by biomass, the existing state of P will be 1; otherwise, if occupied by liquid phase, including the voids in biofilm and elements in liquid phase, the existing state of P will be 0.

(5) In 2-D space, there is a linear infinite reservoir of substrate paralleling to carriers with a distance of Y_b, and its non-dimensional concentration is 1. Moreover, this substrate reservoir always keeps a certain distance with the maximum altitude of biofilm (Y_{fmax}) so as to guarantee of a certain extra mass transfer resistance.

Figure 3 (a, b) shows the 2-D space used to carry out biofilm simulation. And its size was 2 mm × 0.4 mm.

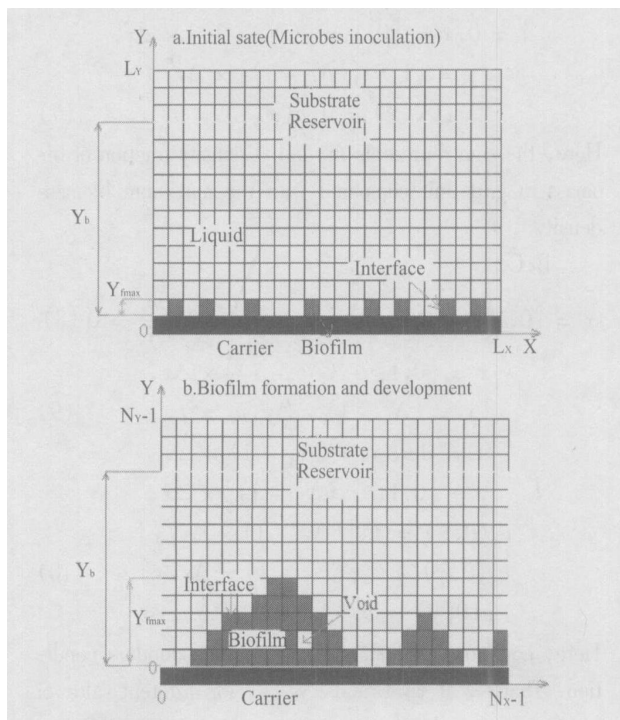

Fig 3　Simulation of biofilm formation and development

The 2-D space shown in figure 3 is in discretion with square grid unit. And the layer at the bottom represents carrier, on which inoculated biomass are growing and propagating to form biofilm. Regard solved oxygen as substrate, its reaction-diffusion mass balance is

$$\frac{\partial c_S}{\partial t} = D_S\left(\frac{\partial^2 c_S}{\partial x^2} + \frac{\partial^2 c_S}{\partial y^2}\right) - r_s(c_s, c_x) \quad (1)$$

In this equation, $r_s(c_s, c_x)$ represents the consumption rate of substrate, and its kinetics equation is[14]

$$r_s(c_s, c_x) = \left(\frac{\mu_m}{Y_{XS}} + m_S\right) c_X \frac{c_S}{K_S + c_S} \quad (2)$$

And the growing rate of biomass is

$$r_s(c_s, c_x) Y_{XS}(r_s(c_s, c_x) - m_s C_X) \quad (3)$$

Previously, microbes are inoculated on the carrier randomly ($Y=0$, $Y_{fmax}=0$), and the initial number of inoculation is n_0. If there are only molecular diffusion and reactive transformation to take place, the corresponding initial and boundary conditions are

I. C.

$$t = 0,\ x = [0, 1, \cdots, (N_X - 1)] \times \Delta x,\ y = [0, 1, \cdots, (N_b - 1)] \times \Delta y,\ c_s(x, y) = c_{s0} \quad (4)$$

If there are n_0 integral numbers at random with equal probability as

$$a_1, a_2, \cdots, a_{n_0} \in [0, 1, \cdots, (N_X - 1)],$$

thus

$$t = 0,\ x \neq (a_1, a_2, \cdots, a_{n_0}) \times \Delta x,\ y = 0,\ c_X(x, y) = 0,\ p(x, y) = 0 \quad (5)$$

$$t = 0,\ x \neq (a_1, a_2, \cdots, a_{n_0}) \times \Delta x,\ y = 0,\ c_X(x, y) = \alpha \cdot c_{Xm},\ p(x, y) = 1 \quad (6)$$

$$t = 0,\ x = [0, 1, \cdots, (N_X - 1)] \times \Delta x,\ y = [1, \cdots, (N_b - 1)] \times \Delta y,\ c_X(x, y) = 0,\ p(x, y) = 0 \quad (7)$$

Here, the sign represents the initial density fraction of biomass in grid unit compared with the maximum biomass density.

B. C.

$$x = [0, 1, \cdots, (N_X - 1)] \times \Delta x,\ y = 0,\ \frac{\partial c_s}{\partial y} = 0 \quad (8)$$

$$x = [0, 1, \cdots, (N_X - 1)] \times \Delta x,\ y = (N_b - 1) \times \Delta y,\ c_s = c_{s0} \quad (9)$$

$$x = 0 \text{ 或 } x = (N_X - 1) \times \Delta x,\ y = [0, 1, \cdots, (N_X - 1)] \times \Delta x,\ c_X(0, y) = C_S[(N_X - 1) \times \Delta x, y],\ c_X(0, y) = c_X[(N_X - 1) \times \Delta x, y],\ p(0, y) = p[(N_X - 1) \times \Delta x, y] \quad (10)$$

Here, equation (10) stands for periodic boundary condition. That is, at $x=0$ and $x=L_X$, for different value of y, there are same concentrations of substrate, biomass and the value of occupied state so as to expand the simulating area to left or right at will. Meanwhile, normalized variables are introduced into above equations from (1) to (10) in order to save the amount of calculations.

$$X = \frac{x}{\Delta x},\ Y = \frac{t}{\Delta y},\ C = \frac{c_S}{c_{S0}},\ B = \frac{c_X}{c_{Xm}},\ R_s = \frac{r_S}{c_{S0}},\ R_X = \frac{r_X}{c_{Xm}} \quad (11)$$

As equation (1) of reaction-diffusion mass balance is normalized and in discretion, it can be numerically solved by using alternating direction implicit method. Therefore, the concentration distributions of substrate and the time required to reach the steady state (Δt_m) can be obtained.

Furthermore, equation (3) is also normalized and in discretion, the accumulating amount of biomass in each grid unit is obtained by using forward Euler numerical integration method.

$$B^{t+\Delta t_g}(X, Y) = B^t(X, Y) + R_X(C^{t+\Delta t_g}, B^t)\,\Delta t_g \quad (12)$$

Here, Δt_g stands for the interval of biomass growth time. In general, there is $\Delta t_m \ll \Delta t_g$.

Moreover, the split of biomass in grid units are realized through cellular automation. When the dimensionless biomass concentration in a grid-cell reaches maximum value, it will divide in two equal parts. The first one will stay on the same place, whereas the other will be placed somewhere in an adjacent grid elements, "pushing" the neighbors according to discrete mechanism. The occupation matrix is then updated following a cellular automaton mechanism. The state of a site is marked as "occupied with biomass" when the corresponding biomass density is more than 0, as a result of the growth process.

In addition, the necessary parameters for biofilm simulation are listed in table 1[12].

Tab. 1 Parameters of dynamical simulation on biofilm formation and development

Parameter of model	Symbol	Value	Unit
Length of zones	L_X	2×10^{-3}	m
Width of zones	L_Y	0.5×10^{-3}	m
Normalized length	N_X	500	—
Normalized width	N_Y	100	—
Substrate concentration	C_{S0}	4×10^{-3}	$kg_S \cdot m^{-3}$
Initial inoculation number	n_0	50, 150, 250	—
Initial density fraction	α	0.5 或 0.7	—
Growing rate in ratio	μ_m	1.52×10^{-5}	s^{-1}
Biomass pruduction coefficient	Y_{XS}	0.045	$kg_X \cdot kg_S^{-1}$
Constant of Monod	K_S	3.5×10^{-4}	$kg_S \cdot m^{-3}$
Sustaining coefficient	m_S	3×10^{-5}	$kg_S \cdot kg_X^{-1} \cdot s^{-1}$
Maximum biomass concentration	c_{Xm}	70	$kg_X \cdot m^{-3}$
Diffusion Coefficient	D_S	1.6×10^{-9}	$m^2 \cdot s^{-1}$
Thickness of diffusing border	Y_b	4×10^{-5}	m

The overall simulation on biofilm formation and development is finished by using the program self-designed (Biofilm. for).

3 Results and discussion

3.1 Influence of biomass growing time on biofilm forming and developing

Figure 4 (a, b) describes the simulating result of biofilm microstructure and morphology for different biomass growing time (30 h or 60 h) as initial inoculating number is 250. It can be seen that the thickness of biofilm is increasing along with the growing time of biomass. At the time of 30 h for biofilm formation and development, it is not completely covered by biomass for carrier, and the fluctuation of biofilm is very ambient; but at the time of 60 h for biofilm formation and development, biomass has covered the carrier entirely, and the fluctuation of biofilm tends to gentleness. This indicates that the growing time of biomass has an obvious influence on biofilm microstructure and morphology. Before approaching to the maximum thickness for aerobic biofilm, biofilm thickness increases as the extending of growing time. Meanwhile, biofilm density, porosity and surface roughness are alternating along with the growing time of biomass.

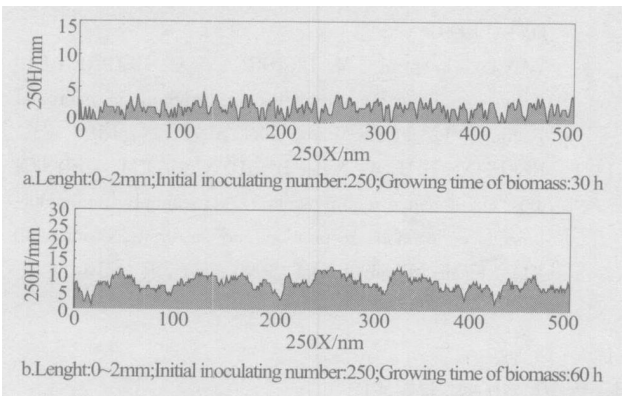

Fig 4　Biofilm formation and development in 2-D space

3.2 Influence of initial inoculating number on biofilm forming and developing

As biomass growing time is 30 h, Figure 5 (a, b, c) shows the dynamic simulating results of biofilm microstructure and morphology for different initial inoculating number of 50, 150 and 250, respectively. It can be seen that as biomass growing time is 30 h, it is not covered completely by biomass for carrier with different initial inoculating number of 50, 150 and 250. Moreover, the covering degree of biomass is improving along with initial inoculating number increasing. And as initial inoculating number is 250, it is nearly covered for carrier by biomass. Furthermore, compared with biomass growing time of 60 h, the fluctuation of biofilm formed on plane-plate carrier is rather severe within short time of biomass growth. In conclusion, initial inoculating number of microbes directly affects the microstructure and morphology of biofilm.

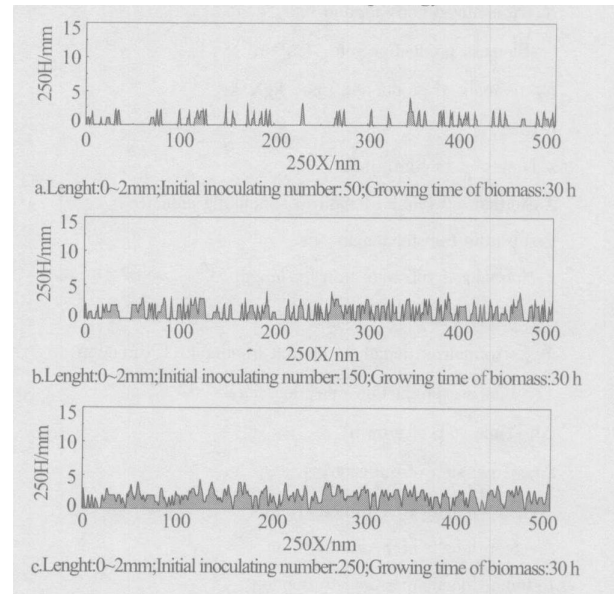

Fig 5　Biofilm formation and development in 2-D space

4 Conclusion

In summary, it is important for biomass growing time and initial inoculation number to biofilm formation and development, and this induces to emerging varieties of biofilm morphologies. Moreover, the micro structural and morphological characters are displayed by means of output variables of model, and this also provides a feasible way to theoretically predict biofilm morphologies.

Nomenclature

B-Density matrix of normalized biomass

c_S-Substrate concentration, $kg_S \cdot m^{-3}$

c_{S0}-Substrate concentration in reservoir, $kg_S \cdot m^{-3}$

c_X-Biomass concentration, $kg_X \cdot m^{-3}$

c_{Xm}-Maximum biomass concentration, $kg_X \cdot m^{-3}$

C-Concentration matrix of normalized substrate

D_S-Diffusion coefficients, $m^2 \cdot s^{-1}$

K_S-Monod saturated coefficients, $kg_S \cdot m^{-3}$

l_b-Thickness of diffusion boundary layer, m

L_X-Length of simulating space in X direction, m

L_Y-Length of simulating space in Y direction, m

m_S-Maintain coefficients, $kg_S \cdot kgX^{-1} \cdot s^{-1}$

n_0-Initial number of inoculation

N_b-Node number between biofilm frontier and substrate reservoir

N_X-Node number in discretion in X direction

N_Y-Node number in discretion in Y direction

p-Matrix of biomass occupied state

P-Normalized matrix of biomass occupied state

r_S-Consumption rate, $kg_S \cdot m^{-3} \cdot s^{-1}$

R_S-Normalized consumption rate, s^{-1}

r_X-Biomass production rate, $kg_X \cdot m^{-3} \cdot s^{-1}$

R_X-normalized production rate, $kgX \cdot kg_S^{-1} \cdot s^{-1}$

t-Time, s

x-Length of biofilm, m

X-Normalized biofilm length in simulating area

y-substrate transfer length, m

Y-Normalized substrate transfer length

Y_b-Normalized length of substrate reservoir in Y direction

Y_{fmax}-Normalized length of biofilm frontier in Y direction

Y_{XS}-Biomass production rate, $kg_S \cdot kg_X^{-1} \cdot s^{-1}$

Δt_g-Time step of growth, s

Δt_m-Time step of diffusion, s

Δx-Step length in X direction, m

Δy-Step length in Y direction, m

α-Initial inoculating density fraction

μ_m-Maximum specific growing rate, s^{-1}

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生物膜形成与发展二维动态模拟

李天成[1,2], 李鑫钢[2], 朱慎林[1]

(1. 清华大学化学工程系, 北京 100084;
2. 天津大学化工学院, 天津 300072)

摘 要: 生物膜微观结构与形态特征直接影响废水生物处理效果。混合微生物可在适宜载体表面形成各种各样的生物膜, 且其形成与发展是一个动态过程。采用差分—离散复合法动态模拟二维区域上的基质传递、生物膜形成与发展过程, 并探讨生物质生长时间与初始接种数等对生物膜结构与形态造成的影响。与传统生物膜模型不同之处在于其结构特性包括孔隙率、厚度和密度等都是模型输出量。

关键词: 混合微生物生物膜; 微观结构; 形态特征; 动态模拟

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