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Full Length Article

Study on the Tortuosity of the Extracellular Mass Transfer Passageway in Biofilms

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Abstract

Two-dimensional micro-structure model of biofilms was reconstructed based on the Monte-Carlo method to simulate the mass transport in biofilm. This kind of biofilm consisted of two phases, including microbial phase (M phase) and intercellular phase (IS phase), and was established according to the average porosity of biofilm and the agglomerate coefficient of microbial clusters. The effects of average porosity and the agglomerate coefficient of microbial clusters on mass transport resistance in biofilms were studied by connecting the average porosity and the agglomerate coefficient of microbial clusters with the tortuosity of the extracellular mass transfer passageway in biofilms. The results showed that with the increase of average porosity, the tortuosity of the extracellular mass transfer passageway in biofilms decreased, and then mass transport resistance in the extracellular mass transfer passageway of biofilms decreased with the decrease of its tortuosity, that is mass diffusion coefficient increased with the increase of average porosity. In addition, with increase of the agglomerate coefficient of microbial clusters in biofilms, the tortuosity of the extracellular mass transfer passageway in biofilms also decreased, and then mass transport resistance in the extracellular mass transfer passageway of biofilms also decreased, that is mass diffusion coefficient increased with the increase of the agglomerate coefficient of microbial clusters in biofilms.

Keywords: Biofilm; Reconstruction model; Agglomerate coefficient of microbial clusters; The average porosity of biofilm; Tortuosity of the extracellular mass transfer passageway

Introduction

Biofilms are porous and complicated microecological structures consisting of microbial cells and the extracellular space. The biofilms play a significant role in many fields, such as, water and waste water treatment, biotechnology, environmental science and so on. They are the critical component of various biofilm reactors. The structures of biofilms will determine the performance and costs of bioreactors, because substrates transport and take part in biotransformation reaction in biofilms. The transportation of mass in bioreactor is the precondition of biotransformation reactions. The mass transfer of the substrates in bioreactor is usually including the following processes: first, the substrates are transferred from the flow bulk to the outer boundary layer of the biofilm; and then these enter into the interface between the liquid phase and the biofilm; finally, mass transfer occurs inside the biofilm. Among the above three mass transport process, mass transport in biofilms is the slowest mass transfer process, and it will limit the biotransformation reactions that will take place in biofilms. Therefore, it is relatively significant to gain more knowledge about the mass transfer in the biofilms, which is usually the main factor of determining the reaction speed. Studying the mass transfer in biofilms has an important effect on enhancing mass transport and the performance of the biofilm reactor.

Studying the mass transfer in biofilms by means of experiment is not only high cost and long period, but also difficult to distinguish the effects of various influencing factors. On the contrast, computer simulation can just make up for these weaknesses. At present, there are some studies on the simulation of mass transfer in biofilms based on the macro-models (Beyenala and Lewandowskia, 2005; Fang and Govind, 2008; Wang *et al.*, 2011). The macro-models, which don't consider the detailed porous structure in biofilms and then simulate mass transfer in biofilms only by using an empirical formula or some of the mean empirical parameters, are not able to be accurate to stimulate the mass transfer in the biofilms. However, lots of researches have shown that the reaction and mass transfer rate in the porous medium have a great relationship with porous medium microstructure (Kim and Pitsch, 2009; Lange *et al.*, 2010; Zhang *et al.*, 2017). Hence, more researchers pay attention to the microstructure of the biofilms. Yun *et al.* (2006) studied the effects of various growth environments on the

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microstructure inside the biofilms by Confocal Laser Scanning Microscope (CLSM) diagram, especially the effect on biofilms porosity. Yang *et al.* (2000) reconstructed the two-dimensional microstructure of the biofilms by electron microscopy, and studied the diffusion distance for a cluster (the distance from the cells in the cluster to interstitial space). Garny *et al.* (2010) investigated the effects of the growth time on porosity in biofilms. Lewandowski (2000) reconstructed the three-dimensional microstructure of the biofilms and proposed the calculation method of the biofilm porosity by electron microscopy at different depths in biofilms. In this condition, if the correlations between porosity and mass transfer resistance were established, the simulation of the mass transfer and biotransformation reaction in the biofilms under the given culture conditions and growth time would be more accurate, and then bioreactor design would be more accurate.

In order to establish a more accurate relationship between porosity and mass transfer resistance, the three-dimensional microstructure lattice models of the biofilms were reconstructed by Monte Carlo method according to the average porosity of the biofilms. And the tortuosity of extracellular mass transfer passageway in biofilms, which was a most frequently-used parameter for characterizing the mass transfer resistance, was calculated by Non-sorbing tracer diffusion theory (Latour *et al.*, 1993) at different average porosity. In addition, considering the agglomeration of microbial in biofilms, a series of three-dimensional microstructure lattice models of agglomerated biofilm were established to study the effects of microbial agglomeration on the tortuosity of extracellular mass transfer passageway in biofilms.

Materials and Methods

Reconstruction of the Biofilm Model

Typical biofilms composed of microorganisms and intercellular spaces are shown in Fig. 1A. Therefore, Monte Carlo method (Wei *et al.*, 2006) was used to reconstruct the two-phase model of biofilms including microbial phase (M phase) and intercellular phase (IS phase). The nutrients required for the survival of microorganisms, the reactants involved in the biotransformation reaction, and the cell secretions were all in the interstitial phase. A geometrical array including 200 × 200 × 200 elements across the biofilm thickness was used to represent a representative part of the biofilm in this model. In the array, each element represents a cube region in the biofilm, the size of which is similar to the size of a single microorganism in the biofilm, and each element consists of a single phase (M phase or IS phase). The biofilm microstructure model was reconstructed beginning with the reconstitution of the microbial clusters. We assumed that the microorganism in the biofilm existed in the form of spherical clusters, and the number of microorganisms in each cluster was equal. The number of microorganisms contained in the microbial clusters was defined as the agglomeration coefficient of the clusters in the biofilm φ. The amount of microbial clusters could be calculated according to the volume fraction of a given average porosity ε of biofilm and the agglomeration coefficient φ of the microbial clusters. The detailed reconstruction method of microbial spherical clusters was the same as that of Pt/C spherical clusters in Ref. Zhang (Zhang *et al.*, 2017).

Next, the above microbial clusters were randomly put into the simulation area. If the region, which would be placed in the microbial cluster, was partially or wholly occupied by former placed microbial clusters, the sites needed to be reselected to place microbial clusters. If part of the microbial cluster fell outside the simulated area, the microbial lattices outside the simulated area should be discarded. And the problem that the volume fraction of microorganism in the model was smaller than the target value will be solved by appropriately reducing the given porosity when generating the microbial clusters. Repeat the above processes until all the microbial clusters are placed in the simulation area. At this point, the lattices unoccupied by the microbial clusters represented the IS phase lattices. If the relative error of the average porosity in the biofilm to given value was less than 1%, the model was done. And a typical randomly reconstructed model is presented in Fig. 1B. Otherwise, the model should be regenerated.

Calculations of the Tortuosity

In this paper, we just consider the tortuosity of the mass transfer in the intercellular space. Therefore, the tortuosity of the IS phase was calculated by solving the diffusion equation. According to the Kozeny-Carman model (Latour *et al.*, 1993), the relationship between the tortuosity of porous biofilm (τ) and the effective diffusion coefficient was expressed as follows:

$$\tau = \frac{D_{bulk}}{D_{\text{eff}}} \tag{1}$$

Where, D_{eff} was the effective diffusion coefficient of the tracer in the IS phase and D_{bulk} was the effective diffusion coefficient of tracer in IS phase when its volume fraction was 1. D_{eff} and D_{bulk} were calculated by the non-sorbing tracer diffusion theory (Latour *et al.*, 1993). The detailed calculation method and calculator program was provided by Cao (2007).

The simulation area we constructed was the cube region with a length of a. Therefore, the variation of tracer concentration c with diffusion time t can be described as follows:

$$\frac{\partial c}{\partial t} = D_{\text{eff}}\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2}\right) \text{ for } 0 \le x, y, z \le a \tag{2}$$

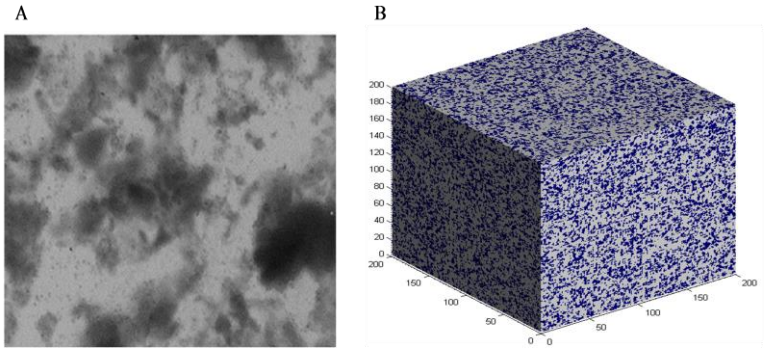

Fig. 1A: A typical microscope image of biofilm (Yang *et al.*, 2001) (Dark and white areas correspond to microbial and interstitial space, respectively; **B**) A typical randomly reconstructed biofilm model (gray: biomass; blue: interstitial space)

When, the diffusion process starts i.e., t = 0, a large number of tracer particles uniformly distributed in the porous media, and at this moment, the initial concentration of the tracer in the medium was C_0. Then the initial condition can be expressed as the following equation:

$$c(x,y,z,t) = C_0 \text{ at } t = 0 \quad (3)$$

Assuming the tracer particles disappear once moving out of the cube boundary, then the boundary condition of the equation can be written as:

$$c(x,y,z,t) = 0 \text{ at x, y, or } z=0 \text{ or } a \quad (4)$$

When the tracer particles all remove from the simulated region i.e., $t \to \infty$, its concentration in the simulated region is 0, then

$$c(x,y,z,t) = 0 \; t \to \infty \quad (5)$$

Solving Eq. (2) by method of variables separation, and then we can get the relationship between the tracer's number which leave out the biofilm $M(t)$ and time t. If time is long enough, all tracer particles will leave out the biofilm, thus

$$\text{When } t \to \infty, M(\infty) = c_0 a^3 \quad (6)$$

Where, $c_0 a^3$ is the initial number of the tracers; Thus, the relationship between $M(t)$ and t can be written as:

$$M(t) = c_0 a^3 - \int_0^a \int_0^a \int_0^a c(x,y,z,t)dxdydz$$

$$= M(\infty) - \frac{512M(\infty)}{\pi^6}\left[\sum_{n_1=1,3,5,7...}^{\infty} \frac{\exp\left(-\frac{D_{eff}\pi^2 n_1^2 t}{a^2}\right)}{n_1^2}\right]^3 \quad (7)$$

Where, $\int_0^a \int_0^a \int_0^a c(x,y,z,t)dxdydz$ is the concentration of tracers, which keep in the biofilm at the time t. With the increasing t, $M(t)$ goes to convergence rapidly, so we just keep the first five items in Eq. (7). And then, t and Deff were changed into dimensionless parameter. Thus, Eq. (7) was written as:

$$M(\theta) = \frac{512M(\theta_{\max})}{\pi^6}\left[\frac{\pi^6}{512} - \exp(-K_{eff}\theta) - \frac{1}{3}\exp\left(-\frac{11K_{eff}\theta}{3}\right) - \frac{2212}{18225}\exp(-9K_{eff}\theta) - \frac{1}{27}\exp\left(-\frac{19K_{eff}\theta}{3}\right)\right] \quad (8)$$

θ is dimensionless time, which is given by

$$\theta = \frac{t}{t'} \quad (9)$$

K_{eff}, is dimensionless effective diffusion coefficient of the tracer in the IS phase, which is given by

$$K_{eff} = \frac{3\pi^2 D_{eff}}{a^2} t' \quad (10)$$

t' is unit time, we use the method of random walk to simulate non-sorbing tracer diffusion process, and we can get a series of correspondence between $M(\theta)$ and θ. And then according to Eq. (8), fit these data, we can get the value of K_{eff}. Using the same method, we can get the value of K_{bulk}, which can be written as:

$$K_{bulk} = \frac{3\pi^2 D_{bulk}}{a^2} t' \quad (11)$$

Thus, we can calculate tortuosity by

$$\tau = \frac{D_{bulk}}{D_{\text{eff}}} = \frac{K_{\text{bulk}}}{K_{\text{eff}}} \quad (12).$$

Results

As shown in Fig. 2, with increasing the average porosity in the biofilm ε, the tortuosity of extracellular mass transfer passageway τ gradually decreased. Correspondingly, the effective diffusion coefficients of the transfer substances (i.e., carbon source, nitrogen source, oxygen) in the extracellular mass transfer passageways were increased (The effective diffusion coefficient of substances under different porosity might be calculated by Eq. 1 and the intrinsic diffusion coefficients of the substances.). But, However, the slope of the ε~τ curve decreased gradually with increasing average porosity in the biofilm ε. When average porosity in the biofilm was larger than 0.7, the influence of porosity of biofilm on the extracellular mass transfer passageways can be ignored. As shown in Fig. 3, with the average porosity of the biofilm increasing, the effective diffusion coefficients of oxygen, sodium nitrate, sodium chloride in biofilms gradually increased. In addition, the effects of microbial agglomeration on the tortuosity of extracellular mass transfer passageway τ were studied and we found that the tortuosity of extracellular mass transfer passageway τ decreased with the increase of the agglomeration coefficient of microbial clusters φ when average porosity ε was constant, as shown in Fig. 2. Also, with the increasing of the biofilm average porosity ε, the reduction of the tortuosity of extracellular mass transfer pathway in biofilms caused by the greater agglomeration coefficient of the microbial clusters was gradually decreased.

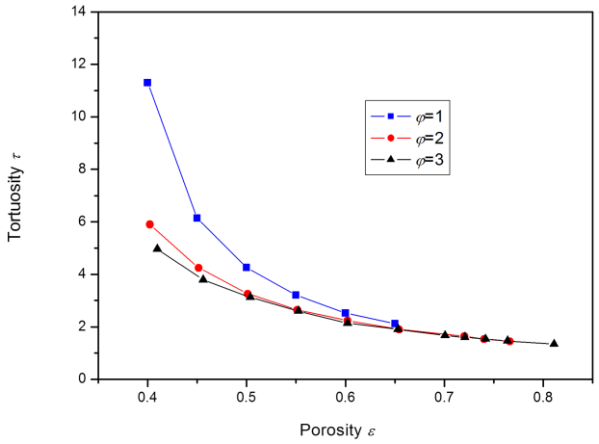

Fig. 2: Effect of porosity ε on tortuosity τ with different φ

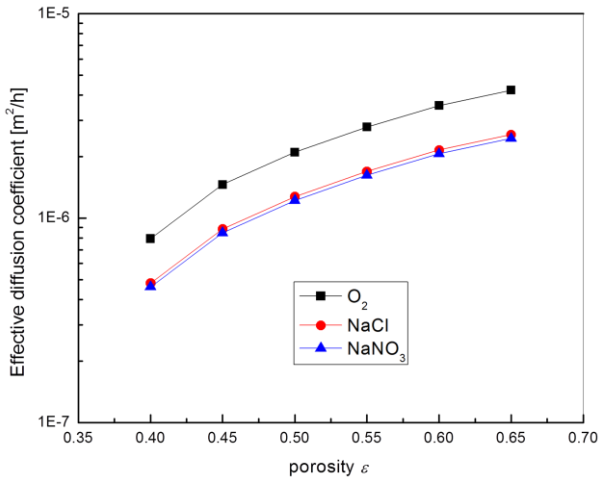

Fig. 3: Effect of porosity ε on effective diffusion coefficient of NaCl, O_2 and $NaNO_3$

Discussion

The tortuosity of extracellular mass transfer passageway τ gradually decreased with increasing the average porosity in the biofilm ε. This was because with the increasing of the average porosity in the biofilm ε, the extracellular mass transfer passageway connectivity would be better and mass transfer passageway would be widened, and then the extracellular mass transfer passageway tortuosity should be reduced. However, with the increase of average porosity in biofilm ε, the number of pore not in the transport way decreased. Therefore, with the increase of average porosity in biofilm ε, the impact of the average porosity ε on the tortuosity of extracellular mass transfer passageway τ weakened. As known, the porosity of the biofilm decreasing with the increase of grown time (Lewandowski, 2000), and then the tortuosity of extracellular mass transfer passageway τ increased with the increase of grown time. Moreover, it was known that the average porosity of biofilms were affected by the biofilm growth conditions (i.e., the concentration of nutrients, temperature) (Jackson *et al.*, 2001), microbial properties in biofilms (i.e., growth rate, anaerobic or aerobic) and other factors (Wäsche *et al.*, 2002). Thus we could calculate the effective diffusion coefficient in the porous medium by assaying the average porosity inside biofilms. And then, we can study the mass transfer rule in biofilms more convenient and more accurate.

The tortuosity of extracellular mass transfer passageway τ decreased with the increase of the agglomeration coefficient of microbial clusters φ when average porosity ε was constant. It was because the number of pore not in the transfer way caused by isolated by microbial decreased. With the increasing of the biofilm average porosity ε, the reduction of the tortuosity of extracellular mass transfer pathway in biofilms caused by the greater agglomeration coefficient of the microbial clusters was gradually decreased. It indicated the effect of microbial agglomeration on the tortuosity of extracellular mass transfer passageway τ, which was weakened with the increasing of porosity. In other words, with the increasing of porosity, the effect of microbial agglomeration on the mass transfer in biofilms was gradually decreased.

Conclusion

Two-phase lattice models (M phase and IS phase) were established and three-dimensional structure of biofilm was reconstructed according to the average porosity of biofilm and the agglomeration coefficient of microbial clusters. We connected the average porosity with the tortuosity of the extracellular mass transfer passageway in biofilms τ, also we studied the effects of the average porosity on the tortuosity of the extracellular mass transfer passageway in biofilms τ. The tortuosity of the extracellular mass transfer passageway in biofilms τ decreased when the average porosity was increased. However, the slope of the ε~τ curve decreased gradually with increasing average porosity, which indicated that the impact of the average porosity ε on the tortuosity of extracellular mass transfer passageway τ, was weakened with increasing average porosity ε. In addition, effects of microbial agglomeration on the tortuosity of the extracellular mass transfer passageway in biofilms indicated that at the same porosity, the tortuosity of the extracellular mass transfer passageway in biofilms decreased with increasing agglomeration coefficient of microbial clusters.

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