Characterization of Multiporous Structure and Oxygen Transfer Inside Aerobic Granules with the Percolation Model

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The characteristics of aerobic granules for wastewater treatment are greatly related to their complex internal structure. However, due to the limitation of characterizing methods, information about the granule internal morphology and structure is very sparse, and mechanism of mass transfer process is yet unclear. In this work, the internal structure of aerobic granules was explored using nitrogen adsorption method and confocal laser scanning microscopy technique. It was found that aerobic granules had multiporous structure with cross-linked gel matrix structure. With a consideration of the hydrodynamic regime and the porous structure of granules, a two-dimensional percolation model was established to describe the mass transfer in granules. With the approaches, interesting and useful results regarding the pore distribution and mass transfer in aerobic granules have been obtained. The results demonstrate that the intragranule convection could enhance mass transfer, hence ensure an efficient and stable operation of aerobic-granule-based reactors. Such approaches might also be applicable to characterizing the multiporous structure and mass transfer of other microbial aggregates for wastewater treatment.

Introduction

Aerobic granular sludge has attracted increasing interest due to its many advantages over the conventional microbial flocs

(1–7). They have dense and compact structure, which is found to influence the mass transfer inside granules. As the mass transfer efficiency of aerobic granules can significantly affect treatment performance as well as system stability, an understanding of the granule internal structure and its influence on mass transfer is essential. It has been reported that such a mass transfer process is related to the porosity and permeability of granules (4, 5). Nevertheless, none of these studies have offered a deep and comprehensive insight into the complex internal structure of aerobic granules. As a result, the correlation between the mass transfer and the granular structure is unclear yet.

Confocal laser scanning microscopy (CLSM) analysis can serve as an important basis for understanding and exploring microbial aggregates like aerobic granules (8–10). CLSM imaging is able to quantitatively describe the microbial aggregate morphology (9). In addition, the imaging data could be used in mathematical models to improve model predictions on mass transfer and substrate conversion in microbial aggregates (10). Xavier et al. (11) carried out a model evaluation on biofilm through comparing the model-predicted structure with that observed from two experimental data sets using the CLSM imaging. The use of CLSM technique with different staining protocols can provide useful information to identify the distribution of microbial extracellular polymeric substances (EPS) and cells in aerobic granules (2, 6).

The presence of meso-pores (<100 nm) in aerobic granules can also influence their mass transfer inside, which cannot be distinguished by the CLSM observation alone. The nitrogen adsorption method is well-known for extracting geometric, connectivity, and spatial distribution of pore size and the structure of porous materials (12, 13). With the nitrogen adsorption methods and the CLSM technique, we can achieve new insights into the internal structure of aerobic granules at different scales. These experimental results can provide useful information about the mass transfer in aerobic granules.

The hydrodynamic regime, aggregate geometrical characteristics, substrate loading rate and diffusivity have effects on the mass transport rate inside the microbial aggregates (14). In the past few years, a mathematical model on microbial aggregate formation using individual based modeling has been developed to simulate the microbial aggregate structure with a framework for multispecies (14–16). Additionally, several mathematical models about aerobic granules have been established based on the one-dimensional diffusion models (1, 17). However, the effects of granule porous structure and outflow dynamic conditions on mass transport and conversion rates have not been taken into consideration in these models. Percolation theory is a powerful tool for theoretical analysis of many natural systems, e.g., flow phenomena in porous media, conductor–insulator composites, propagation of star formation in galaxies (18–20), mechanical properties of random elastic networks (21), and electrical properties of random resistor networks (22). Since in the percolation model the behavior of permeability near the percolation threshold and various macroscopic parameters of porous materials are taken into account together, it is appropriate for analyzing the transport phenomena inside porous microbial aggregates like aerobic granules.

The main objective of this work was to establish a new methodology to elucidate the mass transfer phenomenon in aerobic granules with a consideration of the hydrodynamic regime and the porous structure of granules. Since aerobic granules have a very complex inner structure and the

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information regarding the pore distribution and mass transfer in aerobic granules is sparse, a new approach was developed to sort out the problems above in this work. The CLSM technique was used to visualize the internal structure of aerobic granules, and the nitrogen adsorption method was employed to explore the pore structure at a mesoscale where beyond the reach of the CLSM method. On the basis of the granule structure investigation, a two-dimensional percolation model was established to simulate the mass transfer process inside granules. The influence of the outflow hydrodynamic condition on the oxygen transfer in granules was evaluated, and the validity of the model for describing the oxygen transfer was confirmed using the experimental data.

Materials and Methods

Aerobic Granules. Aerobic granules were cultivated in a laboratory-scale sequencing batch reactor (SBR) using the diluted effluent from a laboratory-scale anaerobic acidogenic reactor, which was rich in volatile fatty acids such as acetate, propionate and butyrate. The influent chemical oxygen demand (COD) to the SBR was kept at approximately 1200 mg L⁻¹. The medium added to the SBR was composed of (in mg L⁻¹): NH₄Cl, 1000; KH₂PO₄, 140; CaCl₂, 52; MgCl₂, 31; and FeSO₄·7H₂O, 10. The influent pH was adjusted to 7.0 ± 0.1. The reactor was operated sequentially as 5 min of influent filling, 349 min of aeration, 1 min of settling, and 5 min effluent withdrawal. The reactor superficial upflow velocity was kept at 1.1 cm s⁻¹. With a shift from small and loose sludge flocs to large and dense granules, a stable and clear outer surface was gradually formed after operation of 3 months. Matured aerobic granules with a mean size of 3.2 mm and sludge volume index of 50.8 mL g⁻¹ were cultivated in this SBR. The specific gravity and settling velocity were 1.010 ± 0.001 g cm⁻³ and 20.1 ± 2.1 m h⁻¹, respectively.

Pore Structure Analysis. Nitrogen adsorption measurements were performed using a volumetric adsorption analyzer (ASAP 2020M, Micromeritics Co., USA) at a temperature of 77.4 K and relative pressures of 10⁻⁷ to 1 atm. Granules were prepared following the pretreatment method for scanning electron microscopy analysis as described by Weber et al. (3). In order to avoid the influence of vacuum and higher temperature conditions, granules were first stabilized using 2.5% glutaraldehyde and 75 mM lysine in 0.1 M cacodylate buffer for 10 min to minimize the structural damages resulting from the analytical procedure (3). Samples were degassed at 393 K for 10 h before analysis. In order to exclude the influence of the moisture inside the granule pore channels, a degassing temperature of 433 K was adopted for comparison. Adsorption isotherms were used to analyze the surface areas and pore structure. The specific surface area was calculated using the Brunauer–Emmett–Teller (BET) equation (23). The total surface area and total pore volume were calculated from the single point adsorption at relative pressures of 0.30 and 0.995 atm, respectively (24). Pore size distribution was obtained using the density functional theory (DFT) method.

CLSM Imaging. Aerobic granules for CLSM analysis were first fixed with 4% paraformaldehyde in phosphate-buffered saline to minimize the structural damages in the subsequent slice up process. Then, the granules were stained using different fluorescences dyes of SYTO 63, calcofluor white and FITC, respectively. The staining details could be found in our previous paper (2). The stained granules were embedded for cryosectioning and frozen at -20 °C; after that, 20-μm sections were cut using a cryomicrotome and mounted onto the microscopic slides for observation (2). CLSM (TCS SP2, Leica GmbH, Germany) was used to visualize the internal structure of granules. In the acquisition of CLSM images, appropriate vision fields were selected in order to collect the

structural information for entire granule. More than 20 images were sampled for each slide. Granules were imaged with a 10× objective and analyzed using Leica confocal software.

Model Development. Two-Dimensional Percolation Model. The model for describing the pore connectivity structure of aerobic granules is based on two-dimensional site percolation. In the percolation structure, the fluid flows through the sample-spanning cluster connecting the two opposite edges of the network. The modeling zone occupies $L \times L$ square lattice with the probability of p , and each lattice has a size of $\delta l \times \delta l$. The computational domain is set as a rectangle instead of the round contour of granules. In the percolation model, pores in structure are open (or connected) with probability of p ($0 \leq p \leq 1$) and closed (or isolated) with probability of $(1-p)$. In our simulation, the p value determines the porosity of granules and p_c is the percolation threshold (for two dimension point percolation network model, $p_c = 0.5927$ (25)). At $p = p_c$, the fluid pathway exhibits a fractal structure, where there are obstacles for a granule with all sizes (up to the size of the system) (25). A contiguous component of open pores is called as an open cluster. The substrates will reach the granule if there is an open cluster joining its center with the periphery. Below the threshold a phase of only small, noninterconnected clusters exists. The change in the connectivity may lead to a significant change in macroscopic property.

The model is described in terms of the percolation structure and the transfer of solute nutrients. The isolated or dead-end pore space in the granule was set to be solid. The first computational step is to solve the hydrodynamics. This initially necessitates solution of the equations of continuity (eq 1) and the Navier–Stokes momentum balance (eq 2) for an incompressible Newtonian liquid flowing through the pore space:

$$\nabla \cdot u = 0 \quad (1)$$

$$\frac{\partial u}{\partial t} + u \cdot \nabla u = -\frac{1}{\rho} \nabla P + \nu \cdot \nabla^2 u \quad (2)$$

where u is the local velocity vector, P is the pressure, and ν is the kinematic viscosity of the liquid. The SIMPLE method is used to solve these equations (26).

A matrix *sor1* is used to distinguish the computational domain and the solid:

$$\text{sor1} = \begin{cases} 0 & \text{solid} \\ 1 & \text{pore space} \end{cases}$$

At the solid–liquid interfaces, no-slip wall conditions are assumed.

The resulting velocity field of liquid flowing between the solid components of the porous medium is then used to calculate the mass transport of different nutrient species consumed by different microorganisms based on a microbial concentration field. The fluid is assumed to be a constant and homeothermic Newton fluid. Mass transport of nutrient solutes includes advective transport coupled with Fickian diffusion and a reaction source term:

$$\frac{\partial C}{\partial t} = -u \cdot \nabla C + D \nabla^2 C + r_s = 0 \quad (3)$$

where C is the local nutrient species concentration within the granules, D is the diffusivity. The consumption of a single substrate (e.g., oxygen) by microorganisms is described by a Monod eq 3:

$$r_s = -q_{\max} X \frac{C}{K_s + C} \quad (4)$$

FIGURE 1. Nitrogen adsorption isotherms for aerobic granules.

where $q_{\max}$ is the maximum specific uptake rate, X is the biomass concentration, and K_s is the half-saturation constant.

$$\text{The boundary condition is: } C = C_0 \quad (5)$$

One-Dimensional Diffusion Model. The diffusion model was developed with a consideration of classical issues concerning oxygen diffusion into a sphere with radius of R . The transport equation has the general form:

$$\nabla(D\nabla C) = g(C) \quad (6)$$

where $g(C)$ is proportional to the reaction rate, and in this study it is considered to be a Michaelis–Menten model reaction rate. In dimensionless variables the diffusion kinetic equation could be written as follows:

$$\varepsilon \left(\frac{d^2 C}{dr^2} + \frac{2}{r} \frac{dC}{dr} \right) = \frac{C}{C + k_s} \quad (7)$$

where

$$\varepsilon = \left(\frac{D_{\text{eff}} C_0}{X q_{\max} R^2} \right)$$

Simulation Parameters. The dissolved oxygen (DO) inside an aerobic granule is often a limiting factor for its growth and stability (1, 17). In this work, the percolation mass transfer model was developed, with the oxygen transfer inside a granule as an example. A series of simulations were carried out to analyze the effect of the granule structure and the outflow conditions in the oxygen transfer process. The diffusion coefficient of oxygen D_{eff} was measured to be $1.58 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ following Chiu et al (6). From a nonlinear regression for the oxygen consumption rate ($d\text{DO}/dt$) with time, $q_{\max}$ and K_s were calculated to be $5.79 \times 10^{-6} \text{ s}^{-1}$ and 1.91 g m^{-3} , respectively. The biomass concentration X of granules was measured as 4350 g m^{-3} .

Results and Discussion

Multiporous Structure of Aerobic Granules. The nitrogen adsorption isotherms of aerobic granules are shown in Figure 1. According to the IUPAC classification, these isotherms belonged to Type II curve, which was typical for macroporous and nonporous adsorbents (27). Point B in Figure 1 indicates the completion of the monolayer formation, and the adsorption at point B was equal to the monolayer capacity. The isotherms showed a sharp increase in nitrogen adsorption at higher relative pressures, suggesting the presence of mesoporous structure and occurrence of mesopore ad-

FIGURE 2. Pore size distribution of the aerobic granules calculated by the DFT method from the nitrogen adsorption isotherms.

FIGURE 3. CLSM images of a stained aerobic granule: (A) β -polysaccharides (calcofluor white); (B) proteins (FITC); (C) total cells (SYTO 63); and (D) combined image of (A)–(C) (scale bar 200 μm).

ditionally capillary condensation. The DFT method was used to analyze the nitrogen adsorption isotherms of granules, and the pore size distribution is given in Figure 2.

In Figure 2, two peaks were observed in the meso-pore regime (2.3 nm) and the macro-pore regime (68.5 nm). The meso-pore fraction reached 66.4%, indicating that granules were highly mesoporous. On the contrary, the fraction of micropores in samples (<2 nm) accounted for only 0.4%.

A similar pore size distribution was obtained at 433 K. The peak in the macro-pore regime appeared at the same pore width as at 393 K, but the peak in the meso-pore regime showed a greater pore width than that at 393 K. A second predominant group was found at 3.2 nm. It implies that some of the pores in the meso-pore regime expanded at a higher degassing temperature, which might be attributed to the fact that the pores in the granules were gradually plugged by EPS (28). The macromolecules polymers inhabited the channels and isolated groupings of pores (7). After the degassing temperature was increased, the plug materials were removed and the channels became unblocked.

FIGURE 4. Oxygen concentration distribution inside aerobic granule at different Re values of 0, 3, 30, and 300: (A), (B), (C), and (D) for percolation probability p of 0.59; (E), (F), (G) and (H) for p of 0.80 (the unit for the color bar is mg L^{-1} , the coordinate axis represent the percolation square lattice, arrow is the flow direction).

CLSM Visualization of Granule Internal Structure. Figure 3 reveals the distribution of proteins (FITC), cells (SYTO 63), β -D-glucopyranose polysaccharides (calcofluor white) in granules, in addition to their combined images. The granules had intricate structure consisting of cell clusters, discrete aggregates of microbial cells in an EPS matrix, and many interstitial voids. Also, open channels were connected to the bulk liquid. EPS were filled in the intercellular space among the microcolonies present in granules (29). It is the most likely that EPS played an important role in maintaining the structural and functional integrity of aerobic granules.

Percolation Modeling of Mass Transfer Inside Granules. The pores with different sizes play distinct roles in mass transfer inside granules: the large pores are closely related to the advection, whereas the small ones are correlated with

the capillary function, detention, matrix suction, diffusion and chemical reactions (9). EPS can reversibly absorb and exude water or biological fluids (30). Their presence in granules can gently clog the pores, form isolated or dead-end pore spaces, and yield a significant reduction in the connection of intragranule channels. This implies that the mass transfer in granules could not be simply treated as a homogeneous pattern. Therefore, the widely used one-dimensional diffusion models are inadequate to describe the mass transfer inside aerobic granules.

The granules have a highly heterogeneous porous structure, and this property can be regarded as a percolation problem. A model based on the two-dimensional site percolation theory was developed to characterize the pore connectivity of aerobic granules. A comparison among several

FIGURE 5. Oxygen concentration distribution of the experimental results of Chiu et al. (6), one-dimensional diffusion models and two-dimensional percolation model of: (A) the phenol-fed granules; and (B) the acetate-fed granules.

different configurations through numerical simulation shows that the statistical properties of macroscopically homogeneous fluid are different from those in fractal porous media (31). In porous materials where all the void space contributes to the flow through it, the effective porosity is equal to the porosity. By contrast, for aerobic granules a large part of the total void space might be nonconducting. Thus, the effective porosity might be significantly smaller than the geometrical one.

The modeling zone occupies 50×50 square lattice (Figure 4) with the percolation probability of p . And two different percolation probability of $p = 0.59$ and $p = 0.80$ were selected to evaluate the effect of the structure difference on the mass transfer inside the granule with a diameter of 3 mm at Re values of 3, 30, and 300, respectively. The initial oxygen concentration was set as 6.0 mg L^{-1} . Additionally, the simulation results with the percolation model at p of 0.59 and 0.80 were compared in terms of the oxygen transfer process where only diffusion was taken into account (Figure 4, parts A and E). The DO concentration dropped to zero at nearly 500 μm beneath the granule surface (Figure 4A). However, as shown in Figure 4E, the DO concentration decreased gradually in the radial direction and the minimum value appeared at the granule center. This was because a bigger p value meant more porous structure in the percolation model.

Alternatively, when convection was taken into account, the minimum DO concentration was deviated from the model structure center in the upwind direction. The simulation results in Figure 4 clearly show that convection inside granules could effectively enhance the mass transfer. In addition, when Re was increased from 3 to 300, the mean DO concentration inside granules increased from 2.8 mg L^{-1} to 4.6 mg L^{-1} (Figure 5, parts B and D) and from 3.47 mg L^{-1} to 5.03 mg L^{-1} (Figure 5, parts F and H), respectively. The DO concentration inside the granules increased as the Re value increased, suggesting that the granule internal mass transfer was dependent on the external flow velocity to a certain degree. This is in accord with the results of mass fluxes in fungal biopellets reported by Hille et al. (10). They observed a significant influence of advection and turbulent diffusion in the outer zones of biopellets.

Microelectrode method has been employed to probe the diffusion process of granules in many studies (6, 17). Chiu et al. (6) estimated oxygen diffusivity by probing the DO level inside both a phenol-fed granule and an acetate-fed granule with velocity changes of the outflow stream. Their data were used to validate our percolation model through comparing it with the one-dimensional diffusion model (Figure 5, parts A and B). The parameters used for this simulation are listed in Table 1. For the granules tested, the phenol-fed granule had a diameter of 1.5 mm and the acetate-fed granule of 3.12 mm. Granules were set under the controlled Re conditions through changing the outflow stream velocity.

As shown in Figure 5, the DO concentration dropped quickly at the granule surface for both granules. The simulation results of the two-dimensional percolation models were more approximate to the experimental results than the one-dimensional diffusion model with the same parameter values in Table 1. For the percolation model simulation of the phenol-fed granules, the DO concentration decreased to almost zero at 125 μm beneath the granule surface (Figure 5A), which was close to the measured result. The same trends could be found for the acetate-fed granule (Figure 5B). The simulation results demonstrate that the two-dimensional percolation model was more appropriate to quantitatively characterize the mass transfer in aerobic granules, compared with the one-dimensional diffusion models.

Implication of This Work. The aerobic granules have complex internal structure and the mass transfer inside them is also a very complicated process, it is thus difficult to elucidate such a phenomena. In the present work, a novel two-dimensional percolation model is developed, and the simulation results suggest that mass transfer within aerobic granules is dependent strongly on their morphology as well as the actual fluid dynamic conditions outside them. All these show that the porous structure and the pore connectivity of granules should be taken into account to quantify the mass transfer process in granules. The percolation model has a potential to elucidate the nutrient and substrate supply mechanism in the internal granule regions. In addition, these results should be integrated into dynamic models with a consideration of microbial growth. The approach established in our work would provide an effective methodology to better explore the performance and mechanisms of aerobic-granule-based reactors.

TABLE 1. Simulation Parameters Used

granule	granule diameter d (mm)	Re	inlet oxygen concentration C_0 (kg/m ³)	D_{eff} (m ² /s)	reaction rate $X \cdot q_{\text{max}}$ (kg/m ³ ·s)	ϵ (diffusion model)
phenol-fed	1.5	4.44	3.06×10^{-3}	1.35×10^{-9}	1.07×10^{-4}	0.0686
acetate-fed	3.12	7.48	5.44×10^{-3}	5.95×10^{-10}	8.27×10^{-5}	0.0174

Appendix A

NOMENCLATURE

C	oxygen concentration (kg m^{-3})
C_0	oxygen concentration in the fluid (kg m^{-3})
d	aerobic granule diameter (m)
D	the diffusivity coefficient ($\text{m}^2 \text{s}^{-1}$)
D_{eff}	the effective diffusivity coefficient ($\text{m}^2 \text{s}^{-1}$)
K_s	the half-saturation constant (g m^{-3})
R	aerobic granule radius (m)
u	the fluid velocity (m s^{-1})
P	pressure (Pa)
p	percolation probability
q_{max}	the maximum oxygen uptake rate (s^{-1})
X	the biomass concentration (g m^{-3})

GREEK LETTERS

ε	parameter for the diffusion model
ρ	density of the fluid (kg m^{-3})
ν	kinematic viscosity ($\text{m}^2 \text{s}^{-1}$)

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