Three-dimensional multi-species mathematical model of the aerobic granulation process based on cellular automata theory

Guoqiang Zheng, Kuizu Su, Shuai Zhang, Yulan Wang and Weihong Wang

ABSTRACT

Aerobic granular sludge is a kind of microbial polymer formed by self-immobilization under aerobic conditions. It has been widely studied because of its promising application in wastewater treatment. However, the granulation process of aerobic sludge is still a key factor affecting its practical application. In this paper, a three-dimensional (3D) multi-species mathematical model of aerobic granular sludge was constructed using the cellular automata (CA) theory. The growth process of aerobic granular sludge and its spatial distribution of microorganisms were studied under different conditions. The simulation results show that the aerobic granules were smaller under high shear stress and that the autotrophic bacterial content of the granular sludge interior was higher. However, the higher the dissolved oxygen concentration, the larger the size of granular sludge and the higher the content of autotrophic bacteria in the interior of the granular sludge. In addition, inhibition of toxic substances made the aerobic granule size increase more slowly, and the spatial distribution of the autotrophic bacteria and the toxic-substance-degrading bacteria were mainly located in the outer layer, with the heterotrophic bacteria mainly existing in the interior of the granular sludge.

Key words | aerobic granulation, cellular automata, inhibition, mathematical model

INTRODUCTION

Aerobic granular sludge is a kind of biological polymer with a regular shape and dense structure formed by self-immobilization under aerobic conditions. Aerobic granular sludge technology overcame the defects in the structure of floc sludge and anaerobic granular sludge, and it has the advantages of good sedimentation, stronger impact loading capacity and a small footprint (Adav et al. 2008). The proposed aerobic granulation mechanism comprises four steps, which are cell-to-cell contact, micro-aggregate formation, excess production of extracellular polymeric substances (EPS), and hydrodynamic compacting of the granule matrix (Annadurai et al. 2002; Liu & Tay 2002). Recent literature has confirmed this mechanism (Liu & Tay 2015). Dissolved oxygen and hydraulic shear stress are two important factors affecting aerobic granulation (Rittman 1982; Tay et al. 2001b; Liu & Tay 2004).

Mathematical simulation, as an evaluation tool, is of great significance in the aerobic granulation process. Based on the individual-based model and a simultaneous storage and growth model, Xavier et al. (2007) and Ni et al. (2010) established two different models, respectively, to describe the granulation process. Su et al. (2015) developed a one-dimensional mathematic model to simulate the granulation process considering all the key steps. In addition, Kagawa et al. (2015) established a model to quantitatively describe the granulation process, by coupling a reactor-scale model and a granule-scale model. However, these models are too complex to put into practice. Therefore, a mathematic model with a simple structure and few parameters is highly desired.

Cellular automata (CA) theory is a dynamic system model that is discrete in time, space and state. The cells are scattered in a regular grid in finite discrete states and updated in accordance with certain local rules. The composition of CA includes cells, cellular space, cellular neighbours, and cellular rules. It can be divided into one-dimensional and multidimensional CA based on the dimension of cellular space.

CA plays an important role in the study of wastewater treatment. In 1998, Picioreanu et al. (1998) simulated the
biomembrane structure under different environmental conditions, such as surface shape, roughness, porosity, and so on, by means of a hybrid, discrete differential CA. After them, Laspidou & Rittmann (2004a, 2004b) proposed a unified multiple-component cellular automaton (UMCCA), established a two-dimensional biofilm model and studied the density and EPS of biomembranes. Hai et al. (2014) simulated the aerobic granular sludge wastewater treatment process based on CA in a sequencing batch reactor (SBR). It was found that CA could not only intuitively describe the adsorption and degradation process of the particles but also simulate the removal of organic compounds and the growth process of microorganisms. In addition, CA is also used in the growth mechanism of tumour cells (Alarcon et al. 2005), the epidemic propagation process (Holko et al. 2016), road traffic (Maerivoet & De Moor 2005), urban sprawl simulation (Ke et al. 2016) and other research fields.

Based on previous studies, the CA theory was applied to constructing a 3D multi-species mathematical model for the growth and distribution of the internal microbial population of a single aerobic particle in this study.

### NUMERICAL MODEL

**Biological reaction model of aerobic granular sludge**

This study uses the multi-species inhibition dynamics model based on ASM3 as the biological reaction module of the 3D multi-species mathematical model to simulate the biological processes of aerobic granular sludge. The aerobic granular sludge biological reaction model is mainly used to simulate the growth and decay rate of microorganisms and their intracellular storage in different sizes of particles in each layer (Zhang et al. 2017).

The particles were layered in the model assuming that the concentration of each component is homogeneous in each layer. With the increase in the aerobic granule size, the number of layers increases. Five kinds of biomass, namely, heterotrophic bacteria, autotrophic bacteria, intracellular storage of heterotrophic bacteria, degrading bacteria and intracellular storage of degrading bacteria, were considered in the model.

The Monod model described the growth of microorganisms in a single substrate. In the presence of toxic substances, microbial growth will be inhibited, so the modified Monod model was used. As a type of toxic refractory substance, thiamethoxam (TMX) has a noncompetitive inhibition effect on the growth of microorganisms in granular sludge, and the kinetic expression is described as follows:

\[
    r_S = \frac{\mu_{\text{max}} S_S}{(K_S + S_S)(1 + (S_T/K_T))}
\]

(1)

where \( \mu_{\text{max}} \) is the maximum specific growth rate; \( K_I \) is the inhibition coefficient; \( S_S \) is the readily biodegradable substrate concentration; \( S_T \) is the TMX concentration; and \( K_S \) is the half-saturation constant.

In addition, the TMX-degrading microorganisms in the reactor that utilize TMX as a single substrate for growth will engender substrate inhibition, and the kinetic expression is:

\[
    r_T = \frac{\mu_T S_T}{K_T + S_T + (S_S^2/K_T)}
\]

(2)

where \( \mu_T \) is the maximum specific growth rate of the TMX-degrading microorganisms; and \( K_T \) is the half-saturation constant of the TMX-degrading microorganisms.

The TMX inhibitory constant was \( K_I = 90 \), and the effective diffusion coefficient of TMX was \( D_{e,\text{TMX}}^{\text{MX}} = 2.0 \times 10^{-10} \) (Zhang et al. 2017).

**Three-dimensional model of aerobic granular sludge**

**Basic hypothesis**

To simplify the model calculation, the hypothesis is as follows:

1. Aerobic granular sludge is made of many small elemental particles, each of which represents the microbial flora.
2. The size of elemental particles is uniform and does not change with time and space.
3. The aerobic granule size is increased by the number of various particles.

**Cell and cellular space**

Based on the above assumptions, the 3D space region is divided into a uniform cube grid. The size of each cube is \( d \), and a single cube is considered to be a cell element. The space set of all the cubes is defined as the cellular space (Figure S1(A)). In the 3D Cartesian coordinate system \( N \times M \times L \), the centre of the cube coordinate \( (x, y, z) \in \{-(N-1)/2 \ldots (N-1)/2, -(M-1)/2 \ldots (M-1)/2, -(L-1)/2 \ldots (L-1)/2\} \). If the space mesh is fine, it will increase the running time of the model. Considering the actual situation, the \( d \) of the model is...
0.075 mm, and N, M, L are all 3 mm. As shown in Figure S1(B), six adjacent cellular locations, including above and below, left and right, front and rear, are selected as cellular neighbours. (Figure S1 is available with the online version of this paper.)

**Cellular state**

Each cell has a finite discrete state, and the state of the cell is described by 3D matrix C. As shown in Table 1, C_{x,y,z} represents the state of the cell in the 3D coordinates (x, y, z) with a total of six states. The toxic substance study was carried out using the TMX example. There is only one particle at most in each cell. The dynamic growth and distribution of the internal microorganisms of the aerobic granular sludge are realized by the evolution of the different states of the cell.

**Growth rule**

The growth and decay of the biomass per layer of microorganisms and their intracellular storage are calculated by the aerobic granular sludge biological reaction model. Assuming that the particles represent a fixed amount of biomass, the growth or decay of the microorganisms and their intracellular storage in each layer is converted to an increase or decrease in the number of various particles in the layer. Compared with the growth process, the decay process of the microorganisms is relatively simple, consisting of randomly selecting particles that need to be removed from the position, so that the cell is in a blank state. The particles are randomly selected in the layer to grow or decay according to the rules.

The growth rules are as follows:

2. The particle was divided into two identical particles.
3. The first particle was placed in its original position, and the other particle searched for a new position in the ortho position.
4. If there was only one vacancy in the ortho position, a vacancy was selected for the new particle.
5. If there were multiple vacancies in the ortho position, a vacancy was selected randomly to place the new particle.
6. If there was no vacancy in the ortho position, a particle in the ortho position was placed at random.
7. The particles that are placed followed (4)–(6) steps to find the position.

If a layer needs to grow a particle of A in one iteration, its growth process is shown in Figure S2 (available online).

**Aerobic granular sludge shear model**

Due to the relative velocity between the gas or liquid phase and the sludge particles, as well as the collision of particles, shear stress in the SBR reactor will be produced. Hydraulic shear stress plays an important role in the formation of aerobic granular sludge. Aerobic granular sludge will desorb under shear stress. The following formula is used to calculate the shear detachment rate of granular sludge in this chapter (Rittman 1982).

\[ R_S = -0.0421 \rho_s d_p r^{-0.58} \]  

(3)

\( R_S \) is the shear detachment rate (mg cm\(^{-2}\) d\(^{-1}\)), and \( d_p \) is the diameter of the granular sludge (cm) in the formula. \( \rho_s \) refers to the density of the 1.034 g cm\(^{-3}\) measured particles. The shear stress (\( r \)) is taken as 0.01 N m\(^{-2}\) to simplify the calculation without considering the change in shear stress. The detachment biomass was calculated by the surface area of the granular sludge, and the number of shear particles could be calculated by the detachment biomass divided by the biomass. The formula is as follows:

\[ N_S = \frac{R_S \pi d_p^2}{m} \]  

(4)

\( N_S \) is the number of shear particles, and \( m \) (estimated at 2.6 \times 10^{-6} \text{ mg} \) is the biomass of the individual particles. According to the number of shear particles, particle removal from the outermost layer of the granular sludge is used to simulate the influence of the shear force.

**Model solving**

The 3D multi-species mathematical model of aerobic granular sludge is composed of an aerobic granular sludge
biological reaction model, a 3D model of aerobic granular sludge and an aerobic granular sludge shear model. The 3D model is based on CA, combining the biological reaction model to calculate the microbial growth and decay rate, which simulates the 3D dynamic growth process of the aerobic granular sludge. Then, the shear model is added to calculate the shear detachment biomass and simulate the influence of shear stress on the aerobic granular sludge. The 3D multi-species mathematical model is realized by MATLAB (ver. 2009a, MathWorks, Natick, MA) software programming.

RESULTS AND DISCUSSION

Simulation of aerobic granulation process

The formation process of the aerobic granular sludge is very complex, and the granulation process is affected by many factors. In this section, taking three kinds of biomass of heterotrophic bacteria, autotrophic bacteria and intracellular storage of heterotrophic bacteria into consideration, the growth process and biomass of the aerobic granular sludge were simulated using the established mathematical model.

Figure 1 shows the simulation results of the aerobic granular sludge growth within 42 days. The 3D map of the simulation of the entire granular sludge is on the left, and the cross section of granular sludge in the Z = 0 plane is on the right. In the early stages, the growth of the granular sludge was slower. The aerobic granule size was smaller, and the shape was regular at 10 days. At 20 days, the granular sludge began to take shape, and the aerobic granule size was 1.1 mm. Heterotrophic bacteria and autotrophic bacteria rapidly grew, and the distribution of the microorganisms in the particles increased. However, at 30 days, the aerobic granule size was further increased, reaching 1.7 mm. The interior autotrophic bacteria began to grow in the outer layer, and the autotrophic bacteria in the centre of the granules decreased gradually. At 42 days, the aerobic granule size reached 3 mm. It can clearly be seen that autotrophic bacteria are mainly located in the outer layer of the aerobic granular sludge. However, heterotrophic bacteria and the intracellular storage of the heterotrophic bacteria were distributed in the inner layer and outer layer of the granular sludge, and the intracellular storage was distributed evenly in the space position of the heterotrophic bacteria. As a result, the larger aerobic granule size limited the diffusion of dissolved oxygen in the particles, resulting in a lack of dissolved oxygen in the interior particles. Low concentrations of dissolved oxygen in the centre of a particle are not conducive to the growth of autotrophic bacteria with a higher dissolved oxygen saturation coefficient, but heterotrophic bacteria can also grow without dissolved oxygen. The distribution of the internal microorganisms in aerobic granular sludge was basically consistent with that of Ni et al. (2008).

The percentage of biomass each layer of the granule at 10 days, 20 days, 30 days and 42 days was determined using the simulation results. As shown in Figure 2, the biomass of the inner layer of granular sludge was mainly heterotrophic bacteria and their intracellular storage. With decreasing distance to the granular sludge surface, the percentage of autotrophic bacteria gradually increased. This was mainly due to the limiting effect of the granular sludge on the diffusion of dissolved oxygen, which led to the dissolved oxygen mainly being found in the outer layer of granular sludge. The inner autotrophic bacteria could not compete with the heterotrophic bacteria. The autotrophic bacteria could only compete with heterotrophic bacteria in the outer particle with a high concentration of dissolved oxygen and ammonia nitrogen.

Simulation of the effect of shear stress on aerobic granular sludge

Hydraulic shear force is an important factor in the formation of aerobic granular sludge. Referring to the related literature reports (Rittman 1982; Tay et al. 2001a; Ren et al. 2009), this section has selected the different shear stress levels of 0.001, 0.005 and 0.010 Nm\(^{-2}\) to study the effect of the hydraulic shear force on aerobic granular sludge using the model.

The results of the simulation of aerobic granular sludge growth at different shear stresses for 38 days are shown in Figure 3. To gain a better understanding of the distribution of microorganisms in the particles, a two-dimensional section of granular sludge in the Z = 0 plane is provided in the figure. As can be seen from the graph, the hydraulic shear force has a great influence on the aerobic granule size. With the increase in the shear force, the amount of detachment of the microbial biomass increased, and the aerobic granule size gradually decreased (Figure 4(a)). The effect of hydraulic shear stress on granulation is basically consistent with the simulation results of Chen et al. (2007).

Hydraulic shear stress also affects the distribution of microorganisms in the aerobic granular sludge to some extent (Figure 4(b)). With decreasing shearing force, autotrophic bacteria tended to grow outside of the granular sludge, and the percentage of the total biomass occupied...
Three-dimensional graph

Two-dimensional section (Z=0)

10 days

20 days

30 days

42 days

- Heterotrophic bacteria
- Autotrophic bacteria
- Intracellular storage of heterotrophic bacteria

Unit (mm)

Figure 1 | Model simulation results of the growth process of the aerobic granules.
by autotrophic bacteria decreased gradually. This may have been related to the diffusion limitation of dissolved oxygen. The larger shear forces made the aerobic granule size decrease, and the oxygen diffused into the whole particle. Sufficient dissolved oxygen is beneficial to the growth of autotrophic bacteria. When the shear force is
smaller, the larger aerobic granule size is not conducive to the diffusion of dissolved oxygen, and autotrophic bacteria can only grow in the outer layer of the granular sludge. In the model, the shear stress was considered for the detachment of the microbial biomass, but other effects, such as the production of EPS, were not considered.

**Simulation of the influence of dissolved oxygen on aerobic granular sludge**

Dissolved oxygen concentration is one of the key factors in the formation of aerobic granular sludge, and it also has a great influence on the distribution of microbial species in aerobic granular sludge. The penetration depth of the dissolved oxygen in aerobic granular sludge plays a key role in the conversion of the components and the removal efficiency of the nutrients. This section selects three different dissolved oxygen concentrations, 2, 4, and 6 mg L\(^{-1}\), to study the effect of dissolved oxygen concentration on aerobic granular sludge using the model.

Figure 5 is a two-dimensional cross-section of the granular sludge in the \(Z = 0\) plane at 42 days when the aerobic granular sludge was grown at a different dissolved oxygen concentrations. The effect of dissolved oxygen on the aerobic granule size and the spatial distribution of the microbial species in the particles are directly reflected in the figure. According to Figure 6, the aerobic granule size is relatively small at a low concentration of dissolved oxygen, due to the diffusion limitation of dissolved oxygen. The percentage of autotrophic biomass is relatively low, and only exists in the outer layer of granular sludge. When the concentration of dissolved oxygen is higher, the penetration depth of oxygen in the granular sludge is greater, and the microbial species can grow more uniformly.
sludge is deeper, the final aerobic granule size is larger and the proportion of autotrophic bacteria in the granular sludge increases.

In addition, it can be seen from Figure 6(a) that in the early stage of aerobic granular sludge growth, the aerobic granule size under different dissolved oxygen concentrations after the same growth time are not significantly different. However, with the increase in time, the difference between the aerobic granule size gradually expanded. The simulation results may be because dissolved oxygen at different concentrations can diffuse through the whole particle when the aerobic granule size is smaller. However, with increasing the aerobic granule size, the lower concentrations of dissolved oxygen cannot reach the inner layer of particles, which limits the growth of the particle. The effect of dissolved oxygen on granulation is basically consistent with the simulation results of Liu et al. (2009).

**Simulation of the growth process of aerobic granular sludge of degrading toxic substances**

In this section, the growth of aerobic granular sludge was simulated by adding TMX-degrading bacteria and the intracellular storage of TMX-degrading bacteria. Figure 7 is a simulation result of the degrading process of TMX by the aerobic granular sludge at 83 days. The growth rate of the microorganisms in the granular sludge was inhibited after the addition of the toxic substance TMX. At 20 days, the aerobic granule size was only approximately 0.68 mm, and the particles were evenly distributed. With the rapid growth of the microorganisms, the aerobic granule size further increased. The simulation results show that the spatial position of the autotrophic bacteria, TMX-degrading bacteria and the intracellular storage of the TMX-degrading bacteria in the granular sludge gradually changes, which tends towards the outer layer of the granular sludge as shown in the granular sludge simulation Figure at 60 days. At 83 days, this phenomenon is very obvious. The inner layer of the granular sludge is mainly composed of heterotrophic bacteria and the intracellular storage of heterotrophic bacteria. The autotrophic bacteria, the TMX-degrading bacteria and the intracellular storage of the TMX-degrading bacteria are mainly located in the outer layer of the granular sludge. The effect of degrading toxic substances on granulation is basically consistent with the experimental results of Liu et al. (2003).

To further analyse the distribution of microorganisms in the granular sludge, the percentage of biomass in each layer of aerobic granular sludge is given in Figure 8(a). The growth of the TMX-degrading bacteria was treated with a single substrate, TMX. The special structure of the aerobic granular sludge limits the diffusion of TMX in the interior of the particles. The higher concentration of TMX in the outer layer of the granular sludge was beneficial to the growth of the TMX-degrading bacteria. However, it inhibited the reproduction of the heterotrophic bacteria and the autotrophic bacteria. Therefore, closer to the granular sludge particle surface, the percentage of autotrophic bacteria biomass, the TMX-degrading bacteria and the intracellular storage of TMX-degrading bacteria was higher, while the percentage of the total biomass containing heterotrophic bacteria and the intracellular storage of the heterotrophic bacteria was smaller. According to Figure 8(b), from the whole granular sludge, it can be seen that heterotrophic bacteria and TMX-degrading bacteria occupied the whole particle. Due to the restriction of oxygen diffusion and the inhibition of TMX, autotrophic bacteria
Figure 7 | Model simulation results of the growth process of aerobic granules degrading TMX.

- Blue: Heterotrophic bacteria
- Red: Autotrophic bacteria
- Green: Intracellular storage of heterotrophic bacteria
- Black: TMX degrading bacteria
- Pink: Intracellular storage of TMX-degrading bacteria
could not grow better in the inner or outer layers of the granular sludge.

CONCLUSIONS

In this paper, we describe three models: an aerobic granular sludge biological reaction model, an aerobic granular sludge shear model and a 3D aerobic granular sludge model. A 3D multi-species mathematical model of aerobic granular sludge was established. The three-dimensional spatial structure and evolution of the spatial distribution of microorganisms were simulated in a single aerobic granular sludge based on CA theory. The simulation results showed the following.

1. With the increase in the aerobic granule size, the autotrophic bacteria tended to grow in the outer layer of granular sludge.
2. The aerobic granule size was smaller under high shear force, but the content of autotrophic bacteria in the granular sludge interior was higher.
3. The high concentration of dissolved oxygen was not only beneficial to increasing the aerobic granule size but also to improving the autotrophic bacteria content of the granular sludge interior.
4. The results of the simulation of the effects of TMX on the granular sludge showed that it inhibited the growth of heterotrophic and autotrophic bacteria. After adding TMX, the average growth rate of the aerobic granule size dropped from 0.071 mm/d to 0.036 mm/d. With the increase in the aerobic granule size, the autotrophic bacteria, the TMX-degrading bacteria and the intracellular storage of the TMX-degrading bacteria tended to grow in the outer layer of the granular sludge. However, the inner layer of the granular sludge was dominated by heterotrophic bacteria and the intracellular storage of the heterotrophic bacteria.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 51378165 and No. 51668062).

REFERENCES


First received 9 January 2018; accepted in revised form 20 May 2018. Available online 12 June 2018