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Modeling the Geometric Regularity in Proteus Mirabilis Colonies

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Modeling the Geometric Regularity in Proteus Mirabilis Colonies

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Bin Zhang

B.S. Nanjing University, 2014

Advisor: Minsu Kim

An abstract of

A thesis submitted to the Faculty of the

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2016

Abstract

Modeling the Geometric Regularity in Proteus Mirabilis Colonies

By

Bin Zhang

The colonies of *Proteus Mirabilis* exhibit a geometric regularity. There are three phases involved in the colony expansion, namely the lag phase, the swarming phase and the consolidation phase resulting in periodicity properties both in space and time domain. As the repetition of swarming and consolidation phases goes on, the pattern of the colony is concentric rings with higher and lower cell density alternately in space. The measurement of the repetition time of swarming and consolidation is periodic. We investigate this spatiotemporal regularity using a one-dimensional reaction-diffusion model. We analyze the influences of the thresholds in two categories, nutrient and cell density. The thresholds are added to the reaction-diffusion model as Heaviside functions. We found that the thresholds in these two categories together can provide the period of *P. mirabilis* colony expansion in the simulation. However, they are not sufficient to maintain an unchanged period in time as observed in the experiments.

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Chapter 1

Introduction

1.1 Proteus Mirabilis

Proteus mirabilis is a rod-shaped, gram-negative bacillus, which belongs to the Enterobacteriaceae family. This kind of bacteria is widely distributed in soil and water in nature and prefers moist habitats. *P. Mirabilis* is a significant pathogen in human P. species infections, especially in urinary tract infections [1]. First, *proteus mirabilis* colonized in bladder, and this bacterium can hydrolyze urea to ammonia (NH_3), thus forming an alkaline environment in bladder. Then, the increased alkalinity results in cystitis in bladder. Finally, it could cause urinary tract infection and stones in kidney.

The colonization of *P. mirabilis* is the first step in human urinary tract infection. Thus, the study of the mechanism of colonization is essential to both basic microbiology understanding and human health for preventing infection. In the first stage of the urinary tract infection, the *P. mirabilis* could swarm by differentiating from rod-shaped bacteria of a few micrometers to elongated multinucleate swarming cells which express thousands of flagella of characteristic length of several tens micrometers.

1.2 Regularity of the Growth in Proteus Mirabilis

Proteus mirabilis, which is a kind of rod-shaped bacterium of roughly 2 micrometers, form growth colonies that have fascinated microbiologists for over a century [3]. On solid media, their swarming and consolidation pattern exhibits striking geometry regularity [4]. These patterns are characterized by circular symmetry and concentric zones or terraces [5]. These terraces of several centimeters in space and a few hours' scale in time indicate periodic event in colony growth [5]. A typical colony of *P. Mirabilis* is shown in Figure 1-1 [4]. A video of the colony development can be found online at the following link from the James Shapiro's Lab at University of Chicago. (https://www.youtube.com/watch?v=K69Yn8tvGh4&feature=youtu.be)

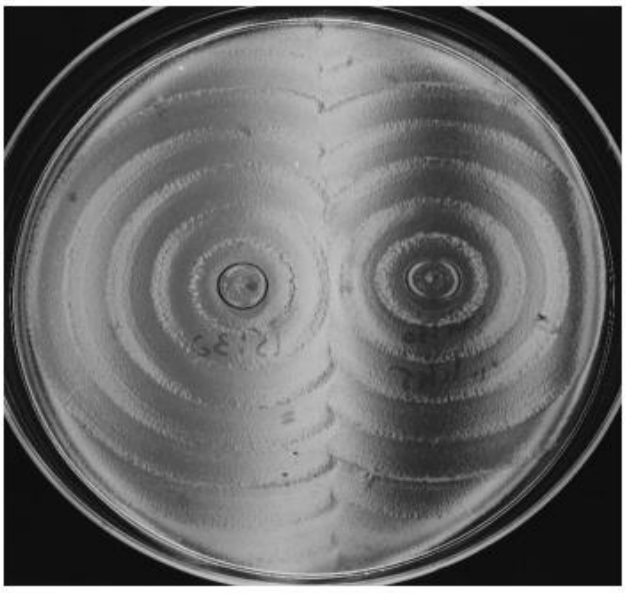

Figure 1-1 The geometry Regularity of the P. Mirabilis Colony (Two terrace P. mirabilis colonies displaying spatially periodic structures. These PRM1 colonies were inoculated at 1-h

interval and then incubated for a future 42h at 32 Celsius-degree on standard medium containing 2% agar. Note that the colonies displayed the same periodicity but remained out of phase with each other throughout development.) [4]

1.3 Phases in the Growth of the Proteus Mirabilis

The growth process is cyclic repetition of the alternating phases: the lag phase, the swarming and the consolidation phases [3]. The swarming process is active migration [6], which is a dynamic process involving movement over the solid substrate by multicellular rafts of specially differentiated swarmer cells [7]. The swarmer cells are elongated and hyper flagellated but have the same DNA/length ratio as the short oligo flagellated swimmer cells characterized as liquid population [4,8]. The consolidation phase is defined as the growth without movement of the colony perimeter [4,9]. The periods of the lag phase depend on the inoculation density of the cells [4]. These phases are shown in Figure 1-2.

According to the experimental observation by Shapiro's group, the different controlling parameters in the experiment will result in the duration of different phases. The inoculation density has a significant influence in the lag phase [4,10], the temperature and the medium enrichment has something to do with the regularity of the terrace formed in the growth process [4,11,12]. When the nutrient (glucose) is depleted, *P. mirabilis* change their behaviors and prepare for the starvation [4].

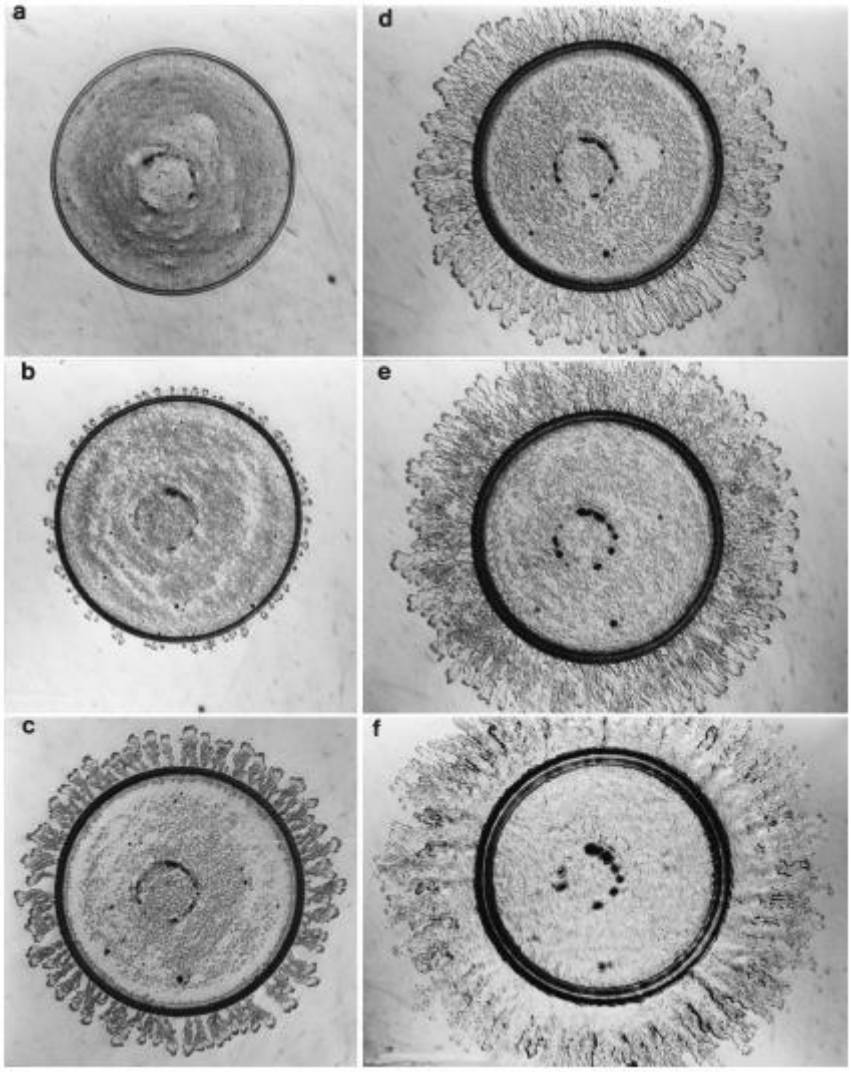

Figure 1-2 Phases in the Growth of the P. Mirabilis Colony (First swarming phase of an older PRM1 inoculum. (a) Before emergence of first rafts (photographed 4.75h post inoculation); (b) initial emergence of rafts at the start of the first swarming phase (photographed 7.75h post inoculation); (c and d) expansion of the swarming population (photographed 9.25h and 10.5h post inoculation respectively); (e) initiation of cellular multiplication from the edge of the inoculation zone as swimmer cell spreading slows down (photographed 11.3h post inoculation); (f) continued expansion of cellular multiplication as edge movement cases and the first consolidation phase begins (photographed 12.5h post inoculation). The inoculation spot measured 5mm in diameter.) [4]

1.4 Controllable Parameters in the Experiment

There are several factors important for their colony expansion [4]. The temperature of the *P. mirabilis* colonies growth environment, the nutrient level, the concentration of the agar, the inoculation status and so on. This can be divided into several categories, and the study of the growth of the colony by Shapiro's group revealed that the temperature and medium enrichment determine the regularity of the *P. mirabilis* colonies [4]. Meanwhile, the inoculation density serves as an initial condition of the growth process, and this determines lag phase of the whole process [4]. Kim's experiments on lag time dependence on nutrient level suggested that there's a threshold cell density that drives switch to swarming.

The nutrient is essential for cell growth. The colonies can change their behaviors according to different levels of the nutrients: when the nutrient is sufficient, the colonies have the ability to consume the nutrient and increase their population; when the nutrient is not sufficient, the population increase in *P. mirabilis* colony is not preferred, the colonies tend to consume less nutrient and reduce their metabolism rate for surviving.

Another important factor in the colony development is the agar concentration in the dish. The mean swarming mean speed <V> exhibits a strong dependence on agar concentration [13]. A theoretical study by Harry L. Swinney's group

indicated that an increase in agar concentration results in less water being extracted from the gel and forming a thinner lubricating layer. Meanwhile, the bacteria in that thin layer could produce about the same amount of the extracellular materials. Hence this increase in agar concentration results in a higher viscosity, which decrease the speed [13]. When the mobility of the cell changes, the swarming speed of the cells changes, thus it has some influences of the swarming phase.

On the solid medium, the cell density is another essential parameter in the colony growth. Based on the experimental observation [4], the pattern of the colony is a series of alternative rings with higher and lower cell density, which is an indication that the cell density plays an important role and tunable parameter in the development of the colonies.

Quorum sensing (QS) is employed and ubiquitous by many species of bacteria. QS is a system responding to population density in bacterium colony. Many species of bacteria use QS to coordinate gene expressing according to the density of their local population [14]. These QS molecules have their own dynamic. Thus quorum sensing serves as a crucial factor for tuning the development of the bacteria colonies.

1.5 Questions and Hypothesis

According to the experiment observation by Shapiro group, the periodicity in

time does not change when the concentration of agar changes [4]. The mechanism underlying this phenomenon is still elusive. We explore the reason the existence of the constant period is a question to be explored.

The hypothesis of this unchanged periodicity in time mentioned above is that when the agar concentration decreases which determines the mobility of *P. mirabilis*, the mobility will be slower thus the swarming phase will be shortened. The period in time is determined by the warming and time for consolidation $\mathrm{T} = T_S + T_C$. Because of the compensation of the consolidation time, the total time will stay unchanged.

To test the hypothesis, we develop a mathematical model considering nutrient and cell density factors. The goal of this simulation is trying to understand this special phenomenon, i.e., the unchanged period in time when the agar concentration changes. Typically, the thresholds could result in the fluctuation between two values or even periodic phenomenon. Especially, in this thesis, the influences of thresholds in nutrient level and cell density these two categories in reaction diffusion model are explored.

Table-1 Parameters in the P. Mirabilis Colony Growth Experiment

Controllable Parameters	Influences	Modeling
Temperature	Regularity	NA
Glucose(Nutrient) Level	Biomass Production Standard (Growth)	Y
Agar Concentration	Cell mobility	Y
Quorum Sensing	Cell Communication	NA
Inoculation Density	Duration of Lag Phase	NA
…	…	NA

Chapter 2

Methods, Models and Discussion

2.1 General Diffusion and Growth Model

The growth colony pattern in homogeneous lab medium is mostly angular symmetric [4], so the dimension can be reduced. Thus one-dimensional description is sufficient. So we can start the model, growth dynamic, using the one-dimensional model in Cartesian Coordinate system, considering only the radical expansion of the colony. Bacterial swarming motility has been commonly modeled as a random diffusion process [16].

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} \text{.....(1)}$$

$$\frac{\partial N}{\partial t} = D_N \frac{\partial^2 N}{\partial x^2} \text{.....(2)}$$

Where $\mathrm{C}(x,t)$ is the cell density and $\mathrm{N}(x,t)$ is the nutrients (glucose) and D_C and D_N are the diffusion coefficient of cell and nutrient respectively. Initially, the nutrient is homogenously distributed $\mathrm{N}(x,0) = N_0$. The swarming of the bacteria occurs at a much slower timescale than the diffusion of the nutrient. Thus the nutrient level can be considered as a steady state compared to the swarming speed of the *P. mirabilis*.

The equations (1,2) describe the cell swarming process and cell could grow simultaneously, hence a growth term should be added to the general diffusion equations. The bacterial growth as a function of nutrient and cell number can be described as $\mathrm{G}(C,N)$.

1. Linear

$$\mathrm{G}(C,N) = g_0 CN$$

2. Monod

$$\mathrm{G}(C,N) = g_0 \frac{N}{K+N} C$$

3. Fisher

$$\mathrm{G}(C,N) = g_0 NC(1-C)$$

Considering the cell swarming and growth, the growth term can be added to the right hand side of the diffusion equations.

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} + G(C,N) \text{.....(3)}$$

$$\frac{\partial N}{\partial t} = D_N \frac{\partial^2 N}{\partial x^2} - pG(C,N) \text{.....(4)}$$

Where g_0 is a constant and K is a parameter in Monod form, and the parameter p is the nutrient consumption rate.

2.2 Forward Time and Centered Space Method

The Forward Time Centered Space (FTCS) method is a common technique in numerical solution for PDEs [20,21].

$$\frac{\partial u}{\partial t} = \frac{u_{n,j+1} - u_{n,j}}{\triangle t} + O(\triangle t) \text{.....(3)}$$

$$\frac{\partial u}{\partial x} = \frac{u_{n+1,j} - u_{n-1,j}}{2\triangle x} + O(\triangle x^2) \text{.....(4)}$$

Based on the equations above, the arbitrary order partial derivatives could be explained in the form of the finite difference. Taking the diffusion equation for example, the second order part in space could be expressed as equation (5).

$$\frac{\partial^2 u}{\partial x^2} = \frac{u_{n+1,j} - 2u_{n-1,j} + u_{n-1,j}}{\triangle x^2} + O(\triangle x) \text{.....(5)}$$

Combine the factors mentioned above, the diffusion equation (6) could be expressed as the finite difference form.

$$\frac{\partial u}{\partial t} = D\frac{\partial^2 u}{\partial x^2} \text{.....(6)}$$

$$\frac{u_{n,j+1} - u_{n,j}}{\triangle t} = D\frac{u_{n+1,j} - 2u_{n,j} + u_{n-1,j}}{\triangle x^2} \text{.....(7)}$$

$$u_{n,j} = -\beta u_{n-1,j+1} + (1+2\beta)u_{n,j+1} - \beta u_{n+1,j+1} \text{.....(8)}$$

$$n = 1, 2, \ldots, N-1 \qquad \beta = \frac{D\triangle t}{\triangle x^2}$$

This method can be applied to solve this one dimensional diffusion reaction equations and get a stable numerical solution when $\beta \leq \frac{1}{2}$.

2.3 Influences of the Thresholds

In this thesis, the influence of the thresholds in nutrient and cell density is to be explored. The general rules of the different situations with different thresholds are shown in Table-2.

Table-2 General Rules of Thresholds in Explored Scenarios

Scenario	Threshold Number	General Rule
1	1 Threshold in Nutrient 0 Threshold in Cell Density	Nutrient Depletion: Cells Stop Growth
2	1 Threshold in Nutrient 2 Thresholds in Cell Density	Nutrient Depletion & C>C2: Cells Stop Growth C1: Switch On Cells Diffusion
3	2 Threshold in Nutrient 2 Thresholds in Cell Density	Nutrient Depletion & C>C2: Cells Stop Growth C1 & N<N1: Switch On Cells Diffusion
4	1 Threshold in Nutrient 2 Thresholds in Cell Density (2 thresholds for diffusion control)	Nutrient Depletion: Cells Stop Growth C1: Switch On Cells Diffusion C2: Switch Off Cells Diffusion

2.4 Diffusion Reaction Model with One Threshold in Nutrient Cannot Generate Periodic Patterns

The parameters g and p stand for the growth rate and nutrient consumed rate in this process.

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} + gCN \text{.....(9)}$$

$$\frac{\partial N}{\partial t} = D_N \frac{\partial^2 N}{\partial x^2} - gpCN \text{.....(10)}$$

The nutrient and the cell density are coupled through these diffusion-reaction equations [8]. In this model, there is an inherent threshold in nutrient, i.e., when the nutrient is depleted locally, the cell can no longer increase their population any more. The simulation results were given by the Mogilner group [16]. As shown in Fig 2-1 [16], both the cell density (normalized) and nutrient concentration have a traveling wave solution. The cell density and nutrient concentration also anti-correlated: when the concentration of the nutrient is depleted locally, the cell density will reach to the maximum value 1. The pattern formed by this model determined by these equations (3,4) is an expanding solid round disk (in 2-dimensional scenario), the radius is associated with the time $\mathrm{R} = v_p \mathrm{t}$.

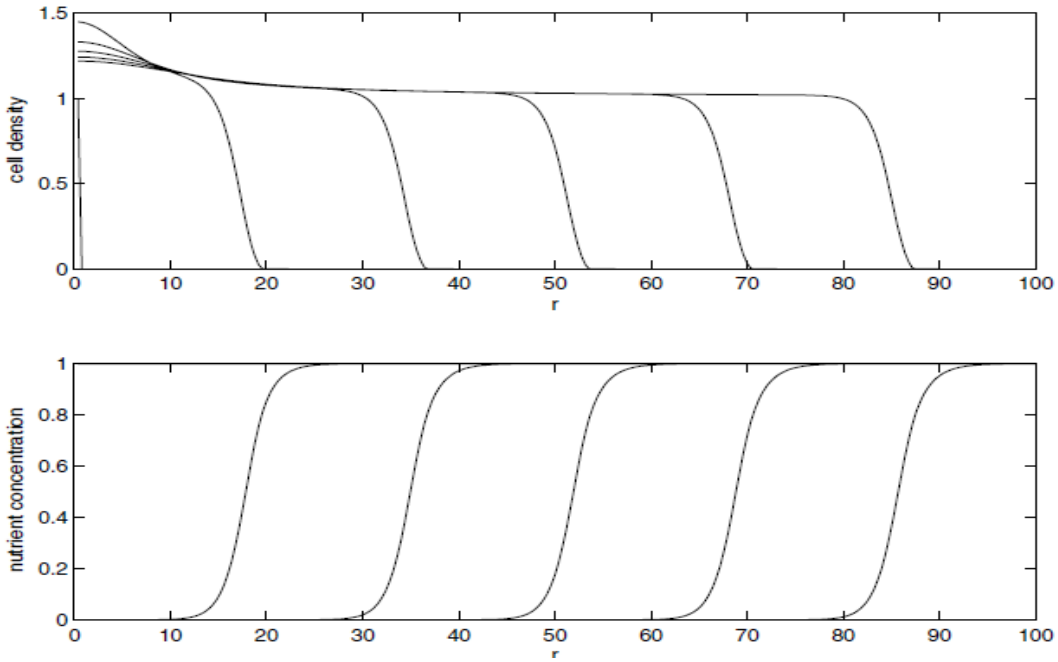

Fig 2-1 Spatially Distributed Cell and Nutrient Density versus time. (Results of numerical simulations of the reaction-diffusion equations (3,4) in the polar coordinate system describing cell and nutrient dynamics. Both densities and distance are in non-dimensional units. The densities are plotted at equation time intervals (at time $t = .02,30,60,90,120,150$ *units)) [16]*

This simulation revealed that with one threshold in nutrient to limit the growth, the periodic behaviors both in time and space of the repetition of swarming and consolidation as seen in *P. mirabilis* colony cannot be explained [4].

2.5 Diffusion Reaction Model with One Threshold in Nutrient and Two Thresholds on Cell Density Can Generate Periodic Pattern with Varying Period

The results above are the propagating wave frontiers of the growing colonies of *P. mirabilis* when only one threshold in nutrient is applied to the PDEs (9,10). When the thresholds in cell density are added to the equation, the equations become a little bit complicated:

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} H\left(C - C_1\right) + gCNH\left(C_2 - C\right) \text{.....(11)}$$

$$\frac{\partial N}{\partial t} = D_N \frac{\partial^2 N}{\partial x^2} - gpCN \text{.....(12)}$$

$\mathrm{H}(x)$ is the Heaviside step function in equation (11):

$$H\left(x\right) = \begin{cases} 1(x \geq 0) \\ 0(x < 0) \end{cases} \text{.....(13)}$$

C_1 and C_2 (C1>C2) are two thresholds in the cell density. When the cell density is below C1, cells keep growing. When the cell density reaches C1, the cells switch from growing to swarming, which we model as a diffusion process.

For analytical solutions, equation (11), Fourier transform can be used to infer the properties of this PDE. The Fourier transform for step function is given by equation (14) [17].

$$F\left[H\left(x\right)\right]=\int_{\infty}H\left(x\right)\cdot e^{-2\pi kix}dx=\frac{1}{2}\left[\delta\left(k\right)-\frac{i}{\pi k}\right]\text{.....(14)}$$

Where, $\delta\left(k\right)=\frac{dH\left(x\right)}{dx}$ is the delta function. As i appears in the equation, it indicates that the solutions have periodic property. In Figure 2-2 and 2-3, the simulation shows that the thresholds result in the period in time with the cell number.

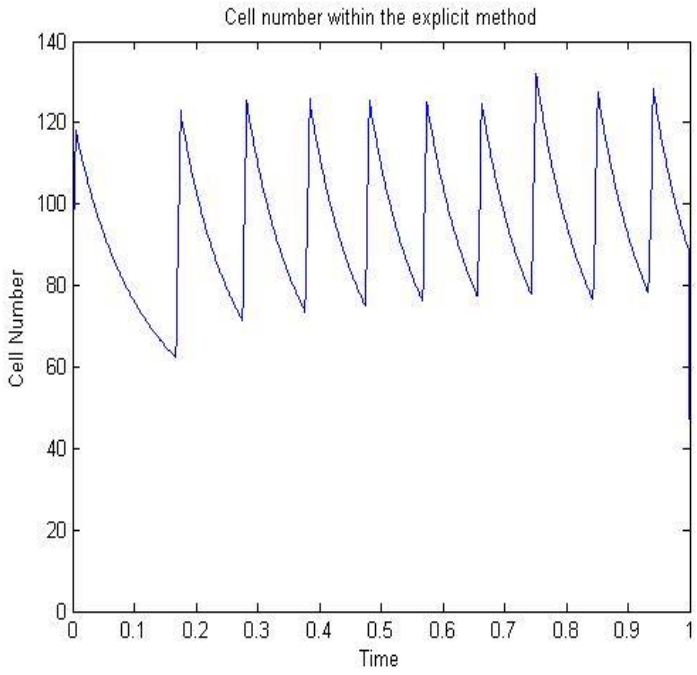

Figure 2-2 The cell number versus time at a certain point (x=1). In the simulation, the parameters in equation (11) are: $D_C = 0.000050,\ C_1 = 100$ *and* $C_2 = 50.$

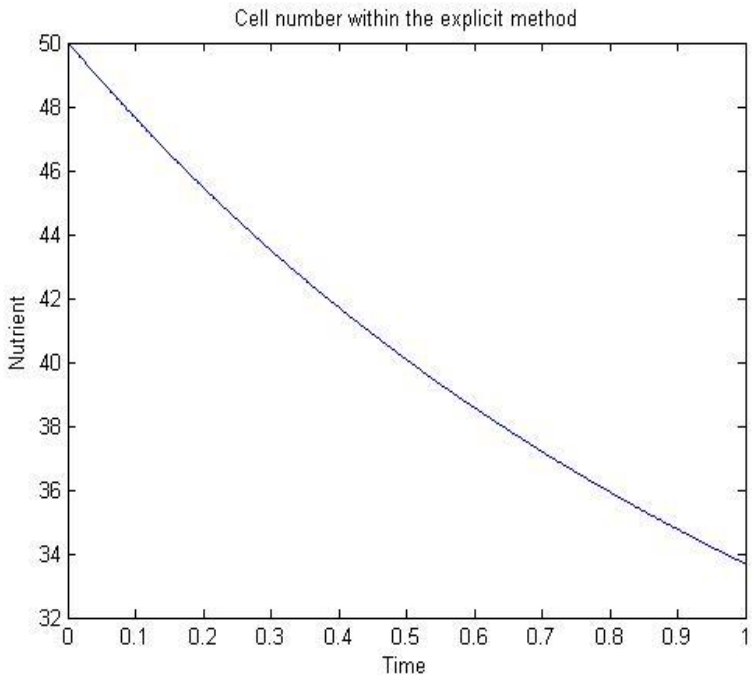

Figure 2-3 The nutrient level versus time at a certain point (x=1). In the simulation, the parameters in equation (12) are: $D_C = 0.000050,\ C_1 = 100$ *and* $C_2 = 50$

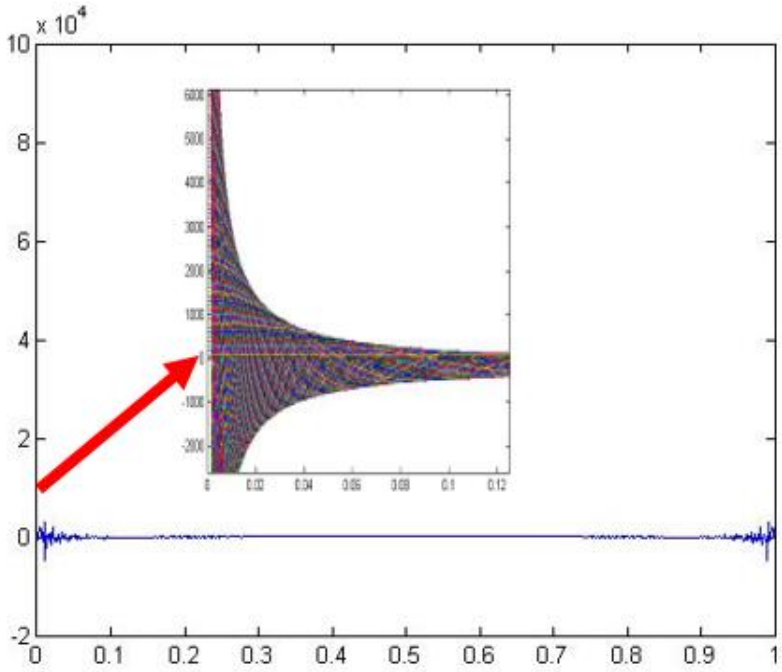

Figure 2-4 Fast Fourier Transform of the cell number versus time at a certain point (x=1).

In the simulation, the parameters in equation (12), $D_C = 0.000050$, $C_1 = 100$ *and* $C_2 = 50$

Figure 2-2 shows the cell number versus time at a certain point, which seems to be periodic. Figure 2-3 shows the nutrient level versus time at the same point. To testify the periodicity of this situation, a Fast Fourier Transform (FFT ignoring the imaginary part) is conducted on the cell number versus time and a delta-like shape appears in Figure 2-4, which is an evidence of periodicity. From the plot, we can define a parameter normalized frequency $\tilde{f}$ and normalized period $\tilde{T}$. Here, n is the number of the peaks in the graph.

$$\begin{cases} \tilde{f} = \mathrm{n} \\ \tilde{T} = \dfrac{1}{n} \end{cases} \quad \text{.....(15)}$$

However, when we change the diffusion coefficient in equation (5), which measures the mobility of the cell swarming: the larger the coefficient, the faster the *P. mirabilis* motility.

According to the experiment, the period remains the same when we change the diffusion coefficient [4]. However, the period of the *P. mirabilis* also changes as Figure 2-5 shows.

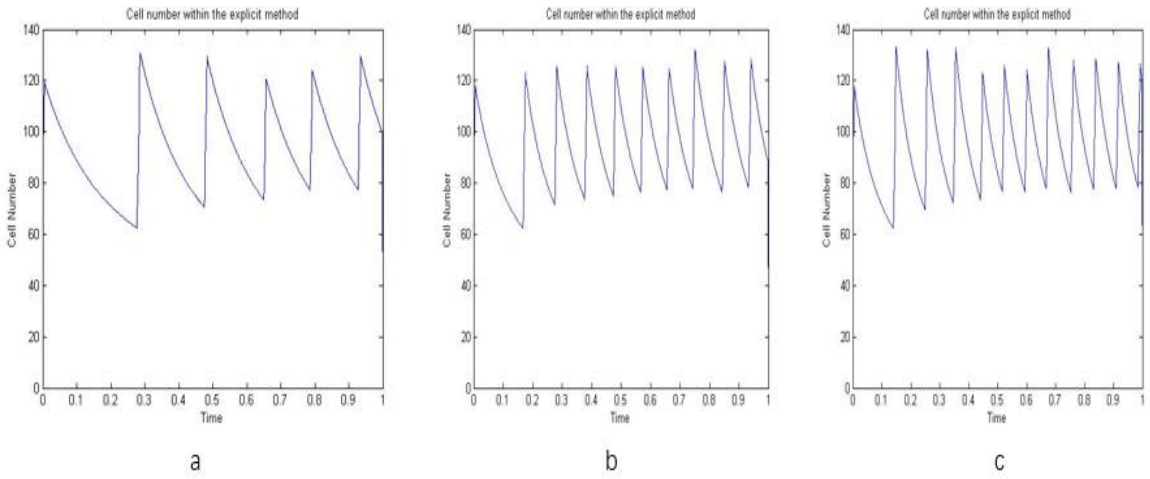

Figure 2-5 Relationship between period and diffusion coefficient. Change of the period when the diffusion coefficient of the cells changes. (a) $D_C = 0.000040$*; (b)* $D_C = 0.000050$*; (c)* $D_C = 0.000060$.

From Figure 2-5, as the mobility of the *P. Mirabilis* increases (D_C increases), which means the swarming speed will increase, the swarming time will be reduced thus result in the decrease of the period. When the parameter D_C changes, associated with changing the concentration of the agar which will change the mobility of the *P. mirabilis*. The time period T changes correspondingly. However, according to the experimental observation that the period in time T stays unchanged when the mobility of the bacteria changes, which indicates the thresholds in only one category is not sufficient to maintain the time period. Therefore, another category of thresholds in nutrient can be added to the modified diffusion equation (5).

2.6 Diffusion Reaction Model with Two Thresholds in Nutrient and Two

Thresholds on Cell Density Can Generate Periodic Pattern with Varying Period

For complicity in math, two thresholds in nutrients and two in cell density should be discussed.

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} H\left(C - C_1\right) H\left(N_1 - N\right) + gCNH\left(C_2 - C\right) \text{.....(16)}$$

$$\frac{\partial N}{\partial t} = N_0 - \int_0^t \alpha C\left(x,t\right) dt - p\alpha C\left(x,t\right) \text{.....(17)}$$

$$\frac{D \triangle t}{\triangle x^2} \leq \frac{1}{2} \text{.....(18)}$$

Also considering extremely short establishment time of the steady state in nutrient, the equation of nutrient can be written as equation (10), which depends on the local cell density which results in inhomogeneous nutrient consumption. From the stability of the numerical solution point of view in mathematics, the magnitude of diffusion coefficient is crucial. When it is too large, the Von Neumann stability criterion [18,19,20] cannot be satisfied, thus the solution is unstable. Under this condition and the actual physical situation in the experiment [10], the diffusion coefficient for cell density which is related with the slow cell migration speed can satisfy the criterion while the nutrient diffusion may not.

The parameter N_1 is the nutrient threshold, below which the migration of the cell can happen. The parameter N_0 and α are the initial nutrient level in the dish and the nutrient consumption rate for each cell respectively.

Meanwhile, since $D_C \ll D_N$, the grid and time intervals can be tuned thus both of the diffusion coefficient can satisfy the Von Neumann stability criterion. So the

equation can be reduced to the combination of equation (12,15).

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} H\left(C - C_1\right) H\left(N_1 - N\right) + gCNH\left(C_2 - C\right) \text{.....(16)}$$

$$\frac{\partial N}{\partial t} = D_N \frac{\partial^2 N}{\partial x^2} - gpCN \text{.....(12)}$$

The results are plotted in Figure 2-6 with the same parameters used in Figure 2-2.

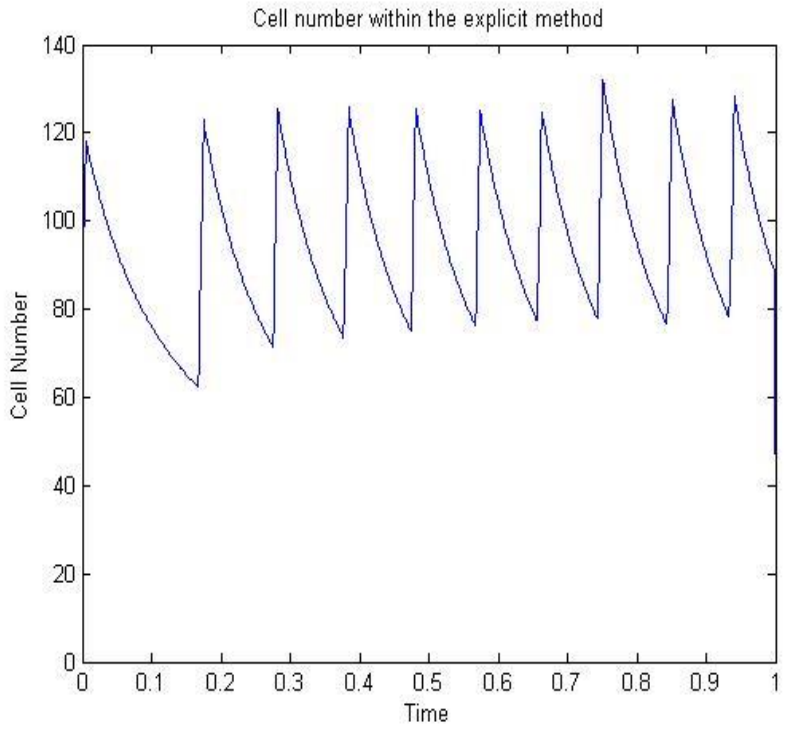

Figure 2-6 The cell number versus time at a certain point (x=1). In the simulation, the parameters in equation (5), $D_C = 0.000050$, $C_1 = 100$ *and* $C_2 = 50$.

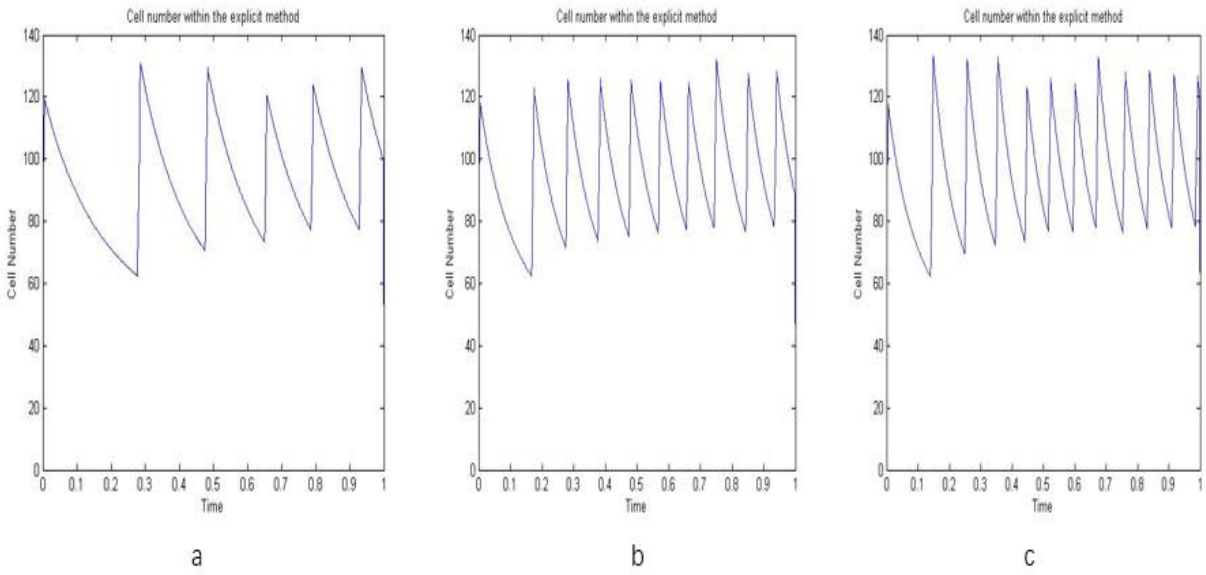

Figure 2-7 Relationship between period and diffusion coefficient. Change of the period when the diffusion coefficient of the cells changes. (a) $D_C = 0.000040$*; (b)* $D_C = 0.000050$*; (c)* $D_C = 0.000060$.

2.7 Diffusion Reaction Model with One Thresholds in Nutrient and Two Thresholds on Cell Density (for Diffusion Activation and Deactivation) Can Generate Periodic Pattern with Varying Period

In equation (16), the two Heaviside step functions which together serve as the activation of the diffusion, are in two different categories namely cell density and nutrient. If all the switches controlling the diffusion are in one categories, that is to say: if the high cell density (C1) serves as the activation switch for the cell diffusion and the lower one (C2) as the criterion deactivation.

The model becomes equation (19)

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} H\left(C - C_1\right) H\left(N_1 - N\right) + gCNH\left(C_2 - C\right) \text{.....(16)}$$

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} \left(H\left(C - C_1\right) + H\left(C - C_2\right) H\left(-\frac{\partial C}{\partial t}\bigg|_{C_i} \right) \right) + gCN \text{.....(19)}$$

$$\frac{\partial N}{\partial t} = D_N \frac{\partial^2 N}{\partial x^2} - gpCN \text{.....(12)}$$

In equation (19), there are two step functions in cell density to control the diffusion. The first term $\mathrm{H}(C - C_1)$ shows when the cell density is larger than C1, the colony will diffuse; the second term $\mathrm{H}(C - C_2) H\left(-\frac{\partial C}{\partial t}_{Ci}\right)$ indicates that when the cell density is larger than C2 meanwhile it has a diffusion tendency (because of the diffusion, the cell density would have a negative slope as the time goes on) at a certain point C_i it will be in diffusion state. In other words, only when the cell density reaches C1, the cell will begin to diffuse, and when it diffused to the cell density of C2 (C2<C1), the diffuse stops, meanwhile, the growth of the cell will last until the nutrient is depleted. The results are shown in Figure 2-8.

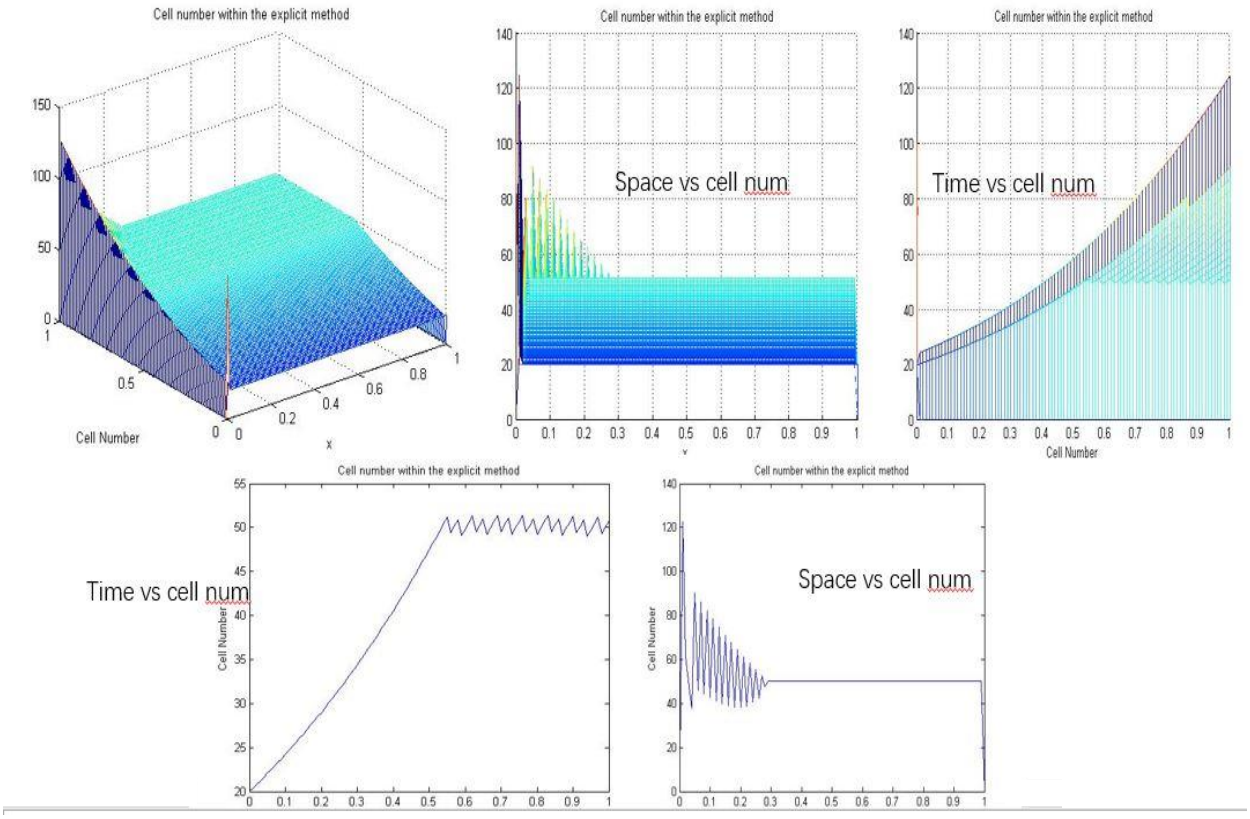

Figure 2-8 Spatiotemporal of the P. mirabilis colony modelling. (The graphs in the first row are cell density-time-space plot, cell density-space, cell density-time plot; The second row is the cell density-time (at a certain point point) and cell density-space (at a certain time) respectively)

It's a similar result, as Figure 2-6 when the nutrient is not depleted, that the periodicity can be established, however, as the cell diffusion coefficient changes, the periodicity will also change.

2.8 Analysis of the Time Period

From the Figure 2-2 and Figure 2-6, we can see the cell number sometimes

exceeds the maximum threshold which indicates the growth rate in this situation is two large, which is a drawback of this model that the growth rate is not well adjusted in the simulation.

Using the normalized period defined by equation (15) mentioned in chapter 2, a period versus diffusion coefficient curve are plotted in Figure 3-1.

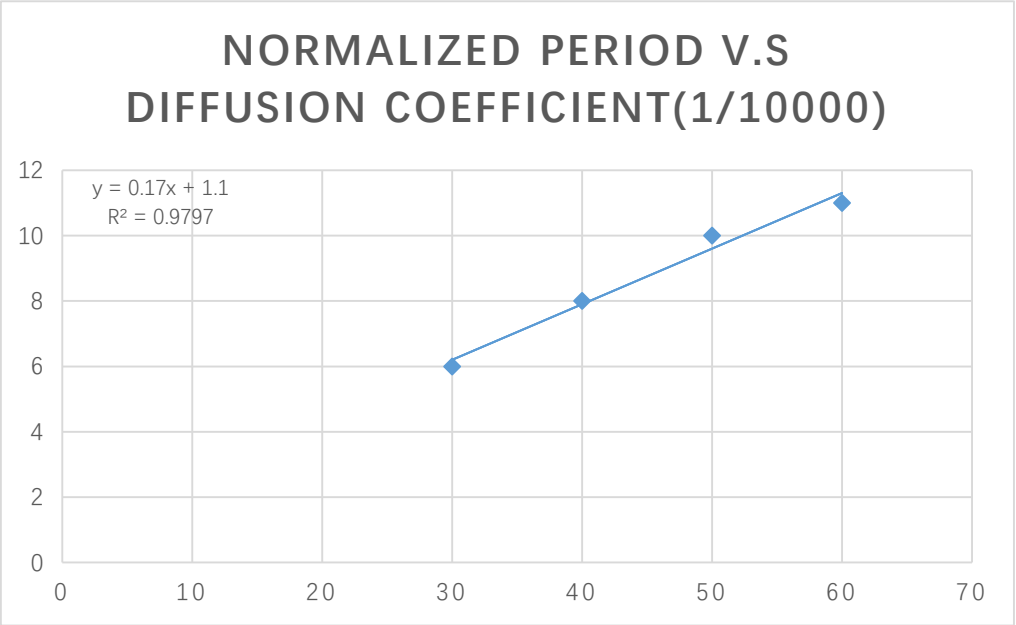

Figure 2-9 Normalized Period versus Diffusion Coefficient (The 3 and 4 thresholds situation share the same trend of the figure.)

According to the Figure 2-8, the normalized period is linear associated with the diffusion coefficient of the *P. mirabilis*. The period of the growth process contains two parts, $\mathrm{T} = T_S + T_C$, the swarming period and consolidation period. From the Figure 2-2, 2-5, 2-6, 2-7 and 2-8, the change of the diffusion coefficient only makes a difference in altering the swarming period. Shapiro's group gave an

explanation that when the concentration of agar changed, the swarming period would change and meanwhile the consolidation period would also change accordingly. However, in the simulation above, the consolidation period hasn't changed obviously.

2.9 Modification of Growth Function and Rate

By analyzing the period above, the key factor is to the change the period in consolidation and this is determined by the growth rate g in equation which is linked with the nutrients.

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} H\left(C - C_1\right) H\left(N_1 - N\right) + gCNH\left(C_2 - C\right) \text{.....(16)}$$

$$\frac{\partial N}{\partial t} = D_N \frac{\partial^2 N}{\partial x^2} - gpCN \text{.....(12)}$$

There are several forms to modify the growth form in the equations (12,16) above. The Monod (equation (20)) [23] and Fisher's (equation (21)) [24] equation can be used.

$$g = g_0 \frac{N}{K + N} \text{.....(20)}$$

$$g = g_0 (1 - N) \text{.....(21)}$$

The numerical solution for the equation (16) and (12) by applying the growth

form (equation (20)). This kind of growth form is dependent on the nutrient level and it has a saturation when the nutrient N is approaching infinity. Meanwhile, the parameter K is a characteristic parameter of this form, which can be tuned in numerical solution.

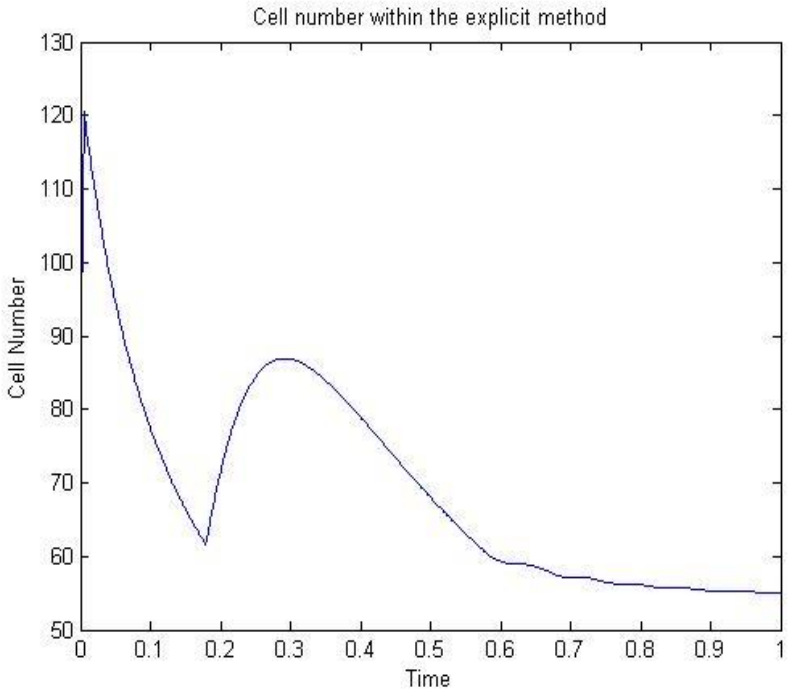

Figure 2-10 The Growth Rate Changes with Monod Form (equation (19))

By tuning the parameter K and g_0 in equation (20) and the proper thresholds in different categories, the period of both the swarming and consolidation can be change simultaneously, thus it may offer a way to simulate this phenomenon and achieve the unchanged period even when the concentration of the agar (the mobility) changes.

2.10 Conclusion

The behaviors *P. mirabilis* in lab medium exhibit striking geometry regularity [4]. The one dimensional simulation of this phenomenon is based on the modification reaction diffusion model. Adding thresholds to the equations, which can change the behaviors of the equation by switching to either diffusion or population increase, the periodic behaviors can be achieved. By simulation in one dimension, the only one threshold in nutrient could not provide the periodic numerical solution. Moreover, the thresholds in two different categories could provide the periodic regularity in this one dimensional simulation and the period in time domain is well defined and tested by FFT.

However, the two thresholds can only change the swarming period and meanwhile the consolidation period will almost remain the same. Thus, the total period $\mathrm{T} = T_S + T_C$ will not remain the same as we change the diffusion coefficient.

In conclusion, the thresholds can provide periodic behaviors but they are not sufficient to maintain a fixed period in time domain. Thus only by adding two categories thresholds to the diffusion reaction equations is not enough to solve this problem.

Chapter 3

Future Work

3.1 Quorum Sensing

Quorum sensing (QS) is employed by many species of bacteria [22]. In section 2, methods, models and discussion, the cells change their behaviors from consolidation to swarming by cell density. However, the cell density in the model is a global property of the colonies. However, quorum sensing is a system corresponding to the local population density. Thus it's a necessary and a promising method to separate cell density and quorum sensing molecule. The latter one has its own dynamics and its regulation is linked with the nutrient.

This property of the bacteria can be incorporated into this diffusion reaction model. Thus, the nutrient, cell density, quorum sensing, these factors are coupled by these reaction diffusion equations. By adding some thresholds in the QS, the influence of this property can be explored.

3.2 Modification of Growth Functions and Rates

In section 2.8, when the growth function changes, the consolidation time

T_C is significantly changed and based on the conclusion in section 2.9, only when the consolidation changes accordingly the total period will remain the same. There are several forms of the growth form and many parameters in the growth function. Thus further modification could be effective and essential for exploring this problem.

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