# Simulation of Bacterial Self-Organization in Circular Container Along Contact Line as Detected by Bioluminescence Imaging

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***Abstract*—Simulation of quasi-one dimensional spatiotemporal pattern formation along the three phase contact line in the fluid cultures of lux-gene engineered *Escherichia coli* is investigated in this paper. The numerical simulation is based on a one-dimensional-in-space mathematical model of a bacterial self-organization as detected by quasi-one-dimensional bioluminescence imaging. The pattern formation in a luminous *E. coli* colony was mathematically modeled by the nonlinear reaction-diffusion-chemotaxis equations. The numerical simulation was carried out using the finite difference technique. Regular oscillations as well as chaotic fluctuations similar to experimental ones were computationally simulated. The effect of the signal-dependent as well as density-dependent chemotactic sensitivity on the pattern formation was investigated. The simulations showed that a constant chemotactic sensitivity can be applied for modeling the formation of the bioluminescence patterns in a colony of luminous *E. coli*.**

***Keywords*-chemotaxis; reaction-diffusion; pattern formation; whole-cell biosensor.**

## I. INTRODUCTION

Microorganisms respond to different chemicals found in their environment by migrating either toward or away from them. The directed movement of microorganisms in response to chemical gradients is called chemotaxis [1]. Chemotaxis plays crucial role in a wide range of biological phenomena, e.g. within the embryo, chemotaxis affects avian gastrulation and patterning of the nervous system [2]. Although chemotaxis has been observed in many bacterial species, *Escherichia coli* is one of the mostly studied examples. *E. coli* respond to the chemical stimulus by alternating the rotational direction of their flagella [1], [2].

Various mathematical models on the basis of Patlak-Keller-Segel model have been successfully used as important tools to study the mechanisms of chemotaxis [3]. A comprehensive review on the mathematical modeling of chemotaxis has been presented by Hillen and Painter [4].

Bacterial species including *E. coli* have been observed to form various patterns under various environmental conditions [5], [6], [7]. Populations of bacteria are capable of self-organization into states exhibiting strong inhomogeneities in density [8]. Recently, the spatiotemporal patterns in the fluid cultures of *E. coli* have been observed by employing lux-gene engineered cells and a bioluminescence imaging technique [9], [10]. However, the mechanisms governing the formation of bioluminescence patterns still remain unclear.

Over the last two decades, lux-gene engineered bacteria have been successfully used to develop whole cell-based biosensors [11]. A whole-cell biosensor is an analyte probe consisting of a biological element, such as a genetically engineered bacteria, integrated with an electronic component to yield a measurable signal. Whole-cell biosensors have been successfully used for the detection of environmental pollutant bioavailability, various stressors, including dioxins, endocrine-disrupting chemicals, and ionizing radiation [12]. To solve the problems currently limiting the practical use of whole-cell biosensors, the bacterial self-organization within the biosensors have to be comprehensively investigated.

This paper investigates the bacterial self-organization in a small circular container near the three phase contact line as detected by quasi-one-dimensional bioluminescence imaging. The aim of this work was to develop a computational model for simulating the spatiotemporal pattern formation of bioluminescence in the fluid cultures of *E. coli* [9], [10], [13]. The pattern formation in a luminous *E. coli* colony was modeled by the nonlinear reaction-diffusion-chemotaxis equations assuming two kinds of the chemotactic sensitivity, the signal-dependent sensitivity and the density-dependent sensitivity. The model was formulated on a one-dimensional domain. The numerical simulation at transition conditions was carried out using the finite difference technique [14]. The computational model was validated by experimental data. By varying the input parameters the output results were analyzed with a special emphasis on the influence of the chemotactic sensitivity on the spatiotemporal pattern formation in the luminous *E. coli* colony. Regular oscillations as well as chaotic fluctuations similar to experimental ones were computationally simulated.

The rest of the paper is organized as follows. In Section II, the mathematical model is described. Section III discusses the computational modeling of a physical experiment. Section IV is devoted to present results of the numerical simulation. Finally, the main conclusions are summarized in Section V.

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## II. MATHEMATICAL MODELING

Various mathematical models based of advection-reaction-diffusion equations have been developed for modeling of pattern formation in bacterial colonies [5], [6], [15], [16], [17]. The system of coupled partial differential equations introduced by Keller and Segel are among the most widely used [3], [4].

### A. Governing Equations

According to the Keller and Segel approach, the main biological processes can be described by a system of two conservation equations ($x \in \Omega,\ t > 0$),

$$\begin{aligned} \frac{\partial n}{\partial t} &= \nabla \left( D_n \nabla n - h(n,c) n \nabla c \right) + f(n,c), \\ \frac{\partial c}{\partial t} &= \nabla \left( D_c \nabla c \right) + g_p(n,c) n - g_d(n,c) c, \end{aligned} \tag{1}$$

where $x$ and $t$ stand for space and time, $n(x,t)$ is the cell density, $c(x,t)$ is the chemoattractant concentration, $D_n$ and $D_c$ are the diffusion coefficients usually assumed to be constant, $f(n,c)$ stands for cell growth and death, $h(n,c)$ stands the chemotactic sensitivity, $g_p$ and $g_d$ describe the production and degradation of the chemoattractant [3], [17].

The cell growth $f(n,c)$ is usually assumed to be logistic function, i.e., $f(n,c) = k_1 n(1 - n/n_0)$, where $k_1$ is the constant growth rate of the cell population, and $n_0$ is the "carrying capacity" of the cell population [5].

A number of chemoattractant production functions have been employed in chemotactic models [4]. Usually, a saturating function of the cell density is used indicating that, as the cell density increases, the chemoattractant production decreases. The Michaelis-Menten function is widely used to express the production rate, $g_p(n,c) = k_2/(k_3 + n)$ [3], [13], [16], [18]. The degradation or consumption of the chemoattractant is typically constant, $g_d(n,c) = k_4$. Values of $k_2$, $k_3$ and $k_4$ are not exactly known [17].

The function $h(n,c)$ stands for the chemotactic sensitivity. The signal-dependent sensitivity and the density-dependent sensitivity are two main kinds of the chemotactic sensitivity [4]. Two commonly used forms of the signal-dependent sensitivity function $h(n,c)$ are the "receptor" $(h(n,c) = k_5/(k_6 + c)^2)$ and the "logistic" $(h(n,c) = k_5/(k_6 + c))$ forms [4], [15], [17]. Assuming that cells carry a certain finite volume, a density-dependent chemotactic sensitivity function as well as volume-filling model were derived, $h(n,c) = k_5(1 - n/n_0)$, where $n_0$ denotes the maximal cell density [4]. Another form for the density-dependent chemotactic sensitivity $(h(n,c) = k_5/(k_6 + n))$ has been introduced by Velazquez [19].

In the simplest form, the chemotactic sensitivity is assumed to be independent of the chemoattractant concentration $c$ as well as the cell density $n$, i.e., $h(n,c)$ is constant, $h(n,c) = k_5$. Since the proper form of the chemotactic sensitivity function $h(n,c)$ to be used for the simulation of the spatiotemporal pattern formation in the fluid cultures of lux-gene engineered *E. coli* is unknown, all these four forms of $h(n,c)$ were used to find out the most useful form.

When modeling the bacterial self-organization in a circular container along the contact line [9], [10], [13], the mathematical model can be defined on a one dimensional domain - the circumference of the vessel. Replacing $f$, $g_p$ and $g_d$ with the concrete expressions above, the governing equations (1) reduce to a cell kinetics model with nonlinear signal kinetics as well as the chemotactic sensitivity,

$$\begin{aligned} \frac{\partial n}{\partial t} &= D_n \Delta n - \nabla \left( h(n,c) n \nabla c \right) + k_1 n \left( 1 - \frac{n}{n_0} \right), \\ \frac{\partial c}{\partial t} &= D_c \Delta c + \frac{k_2 n}{k_3 + n} - k_4 c, \quad x \in (0,l), \quad t > 0, \end{aligned} \tag{2}$$

where $\Delta$ is the Laplace operator formulated in the one-dimensional Cartesian coordinate system, and $l$ is the length of the contact line, i.e., the circumference of the vessel. Assuming $R$ as the vessel radius, $l = 2\pi R$, $x \in (0, 2\pi R)$.

### B. Initial and Boundary Conditions

A non-uniform initial distribution of cells and zero concentration of the chemoattractant are assumed,

$$n(x,0) = n_{0x}(x), \quad c(x,0) = 0, \quad x \in [0,l], \tag{3}$$

where $n_{0x}(x)$ stands for the initial ($t = 0$) cell density.

For the bacterial simulation on a continuous circle of the length $l$ of the circumference, the matching conditions are applied ($t > 0$):

$$\begin{aligned} &n(0,t) = n(l,t), \qquad c(0,t) = c(l,t), \\ &\left.\frac{\partial n}{\partial x}\right|_{x=0} = \left.\frac{\partial n}{\partial x}\right|_{x=l}, \qquad \left.\frac{\partial c}{\partial x}\right|_{x=0} = \left.\frac{\partial c}{\partial x}\right|_{x=l}. \end{aligned} \tag{4}$$

### C. Dimensionless Model

In order to define the main governing parameters of the mathematical model (2)-(4) [4], [7], [18], a dimensionless mathematical model has been derived by setting

$$\begin{aligned} &u = \frac{n}{n_0}, \quad v = \frac{k_3 k_4 c}{k_2 n_0}, \quad t^* = \frac{k_4 t}{s}, \quad x^* = \sqrt{\frac{k_4}{D_c s}}\, x, \\ &D = \frac{D_n}{D_c}, \quad r = \frac{k_1}{k_4}, \quad \phi = \frac{n_0}{k_3}, \\ &\chi(u,v) = \frac{k_2 n_0}{k_3 k_4 D_c} h(n_0 u, k_2 n_0 c/(k_3 k_4)). \end{aligned} \tag{5}$$

Dropping the asterisks, the dimensionless governing equations then become ($t > 0$)

$$\begin{aligned} \frac{\partial u}{\partial t} &= D \frac{\partial^2 u}{\partial x^2} - \frac{\partial}{\partial x} \left( \chi(u,v) u \frac{\partial v}{\partial x} \right) + s r u (1 - u), \\ \frac{\partial v}{\partial t} &= \frac{\partial^2 v}{\partial x^2} + s \left( \frac{u}{1 + \phi u} - v \right), \quad x \in (0,1), \end{aligned} \tag{6}$$

where $x$ and $t$ stand for the dimensionless space and time, respectively, $u$ is the dimensionless cell density, $v$ is the dimensionless chemoattractant concentration, $r$ is

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the dimensionless growth rate of the cell population, $\phi$ stands for saturating of the signal production, $\chi(u,v)$ is the dimensionless chemotactic sensitivity, and $s$ stands for the spatial and temporal scale.

For the dimensionless simulation of the spatiotemporal pattern formation in a luminous *E. coli* colony, four forms of the chemotactic sensitivity function $\chi(u,v)$ were used to find out the best fitting pattern for the experimental data [9], [10], [13],

$$\chi(u,v) = \frac{\chi_0}{(1+\alpha v)^2}, \tag{7a}$$

$$\chi(u,v) = \chi_0 \frac{1+\beta}{v+\beta}, \tag{7b}$$

$$\chi(u,v) = \chi_0 \left(1 - \frac{u}{\gamma}\right), \tag{7c}$$

$$\chi(u,v) = \frac{\chi_0}{1+\epsilon u}. \tag{7d}$$

The first two forms (7a) and (7b) of the function $\chi(u,v)$ correspond to the signal-dependent sensitivity, while the other two (7c) and (7d) - for the density-dependent sensitivity [4]. Accepting $\alpha = 0$, $\beta \to \infty$, $\gamma \to \infty$ or $\epsilon = 0$ leads to a constant form of the chemotactic sensitivity, $\chi(u,v) = \chi_0$.

The initial conditions (3) take the following dimensionless form:

$$u(x,0) = 1 + \varepsilon(x), \quad v(x,0) = 0, \quad x \in [0,1], \tag{8}$$

where $\varepsilon(x)$ was a $20\%$ random uniform spatial perturbation.

The boundary conditions (4) transform to the following dimensionless equations ($t > 0$):

$$\begin{aligned} &u(0,t) = u(1,t), \qquad v(0,t) = c(1,t), \\ &\frac{\partial u}{\partial x}\Big|_{x=0} = \frac{\partial u}{\partial x}\Big|_{x=1}, \qquad \frac{\partial v}{\partial x}\Big|_{x=0} = \frac{\partial v}{\partial x}\Big|_{x=1}. \end{aligned} \tag{9}$$

According to the classification of chemotaxis models, the dimensionless model of the pattern formation is a combination of the signal-dependent sensitivity (M2), the density-dependent sensitivity (M3), the saturating signal production (M6) and the cell kinetics (M8) models [4].

## III. Numerical Simulation

The mathematical model (2)-(4), as well as the corresponding dimensionless model (6), (8), (9), has been defined as an initial boundary value problem based on a system of nonlinear partial differential equations. No analytical solution is possible because of the nonlinearity of the governing equations of the model [7]. Hence the bacterial self-organization was simulated numerically.

The numerical simulation was carried out using the finite difference technique [14]. To find a numerical solution of the problem a uniform discrete grid with 200 points and the dimensionless step size $0.005$ (dimensionless units) in the space direction was introduced, $250 \times 0.004 = 1$. A constant dimensionless step size $10^{-6}$ was also used in the time direction. An explicit finite difference scheme has been built as a result of the difference approximation [14], [20]. The digital simulator has been programmed by the author in JAVA language [21].

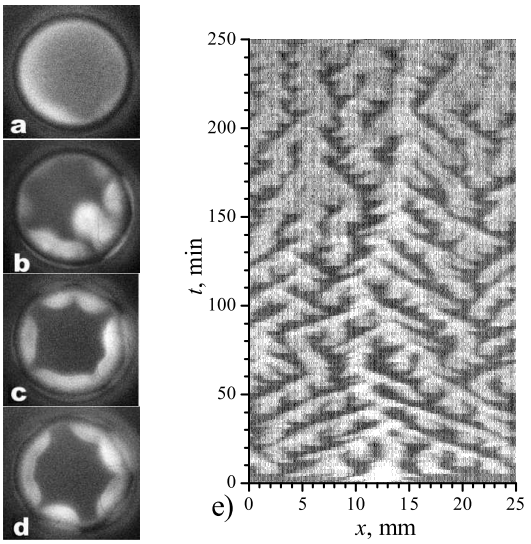


Figure 1. Top view bioluminescence images of the bacterial cultures in the cylindrical vessel at 5 (a), 20 (b), 40 (c), 60 (d) min and space-time plot along the contact line (e) [10].

The computational model was applied to the simulation of bioluminescence patterns observed in a small circular containers made of glass [10], [13]. Figures 1a-1d show typical top view bioluminescence images of bacterial cultures illustrating an accumulation of luminous bacteria near the contact line. In general, the dynamic processes in unstirred cultures are rather complicated and need to be modeled in three dimensional space [1], [9], [10]. Since luminous cells concentrate near the contact line, the three-dimensional processes were simulated in one dimension (quasi-one dimensional rings in Figures 1a-1d). Figure 1e shows the corresponding space-time plot of quasi-one-dimensional bioluminescence intensity.

By varying the model parameters the simulation results were analyzed with a special emphasis to achieving a spatiotemporal pattern similar to the experimentally obtained pattern shown in Figure 1e. Figure 2 shows the results of the informal pattern fitting, where Figures 2a and 2b present simulated space-time plots of the dimensionless cell density $u$ and the chemoattractant concentration $v$, respectively. The corresponding values $\bar{u}$ and $\bar{v}$ averaged on circumference of the vessel are depicted in Figure 2c. Regular oscillations as well as chaotic fluctuations similar to experimental ones were computationally simulated. Accepting the constant form of the chemotactic sensitivity, $\chi(u,v) = \chi_0$, the dynamics of the bacterial population was simulated at the

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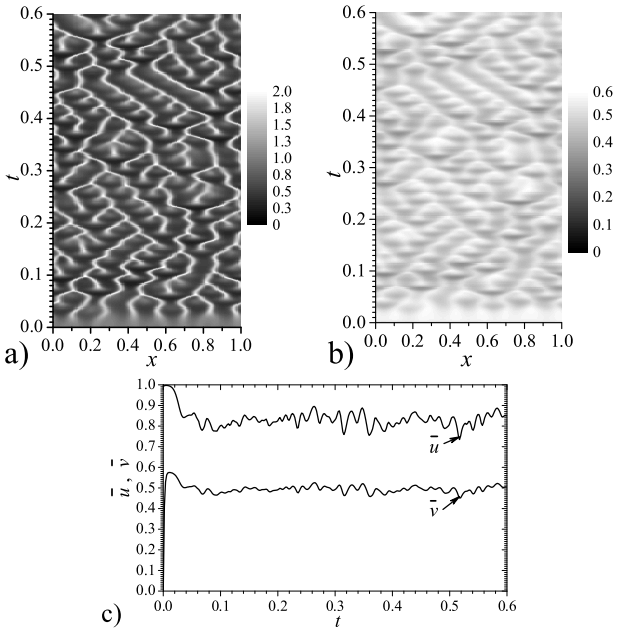


Figure 2. Simulated space-time plots of the dimensionless cell density $u$ (a) as well as the chemoattractant concentration $v$ (b) and the corresponding averaged values $\bar{u}$ and $\bar{v}$ (c). Values of the parameters are as defined in (10).

following values of the model parameters [13]:

$$D = 0.1,\ \chi_0 = 6.2,\ r = 1,\ \phi = 0.73,\ s = 625\,. \qquad (10)$$

Due to a relatively great number of model parameters, there is no guarantee that the values (10) mostly approach the pattern shown in Figure 1e. Similar patterns were achieved at different values of the model parameters. An increase in one parameter can be often compensated by decreasing or increasing another one [4], [17], [22] .

## IV. Results and Discussion

By varying the input parameters the output results were analyzed with a special emphasis on the influence of the chemotactic sensitivity on the spatiotemporal pattern formation in the luminous *E. coli* colony. Figure 2a shows the spatiotemporal pattern for the constant form of the chemotactic sensitivity, $\chi(u, v) = \chi_0$.

### A. The Effect of the Signal-Dependent Sensitivity

The signal-dependent sensitivity was modeled by two forms of the chemotactic sensitivity function $\chi$: (7a) and (7b). The spatiotemporal patterns of the dimensionless cell density $u$ were simulated at very different values of $\alpha$ and $\beta$. Figure 3 shows signal-dependency of the chemotactic sensitivity.

Accepting $\alpha = 0$ or $\beta \to \infty$ leads to a constant form of the chemotactic sensitivity, $\chi(u, v) = \chi_0$. Results of multiple simulations showed that the simulated patterns

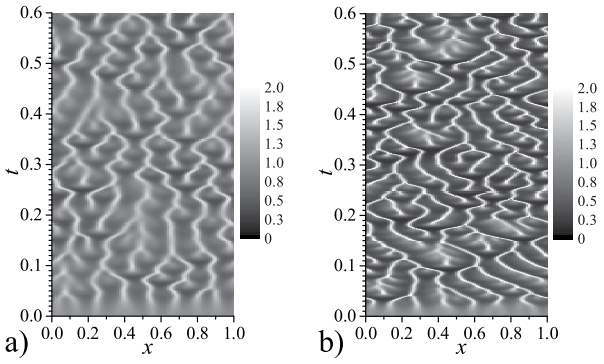


Figure 3. Spatiotemporal plots of the dimensionless cell density $u$ for two forms of the signal-dependent chemotactic sensitivity $\chi(u, v)$: (7a) ($\alpha$ = 0.05) (a) and (7b) ($\beta$ = 10) (b). Values of other parameters are as defined in (10).

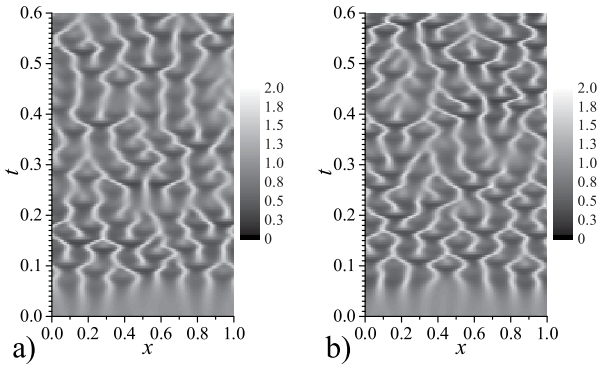


Figure 4. Spatiotemporal plots of the dimensionless cell density $u$ for two forms of the density-dependent chemotactic sensitivity $\chi(u, v)$: (7c) ($\gamma$ = 10) (a) and (7d) ($\epsilon$ = 0.1) (b). Values of other parameters are as defined in (10).

distinguish from the experimental ones (Figure 1e) when increasing $\alpha$-parameter (Figure 3a) or decreasing $\beta$-parameter (Figure 3b). Because of this, there is no practical reason for application of a non-constant form of the signal-dependent sensitivity to modeling the formation of the bioluminescence patterns in a colony of luminous *E. coli.*

### B. The Effect of the Density-Dependent Sensitivity

Two forms, (7c) and (7d), of the function $\chi$ were employed for modeling the density-dependent chemotactic sensitivity. The spatiotemporal patterns of the cell density $u$ were simulated at various values of $\gamma$ and $\epsilon$. Figure 4 shows how the density-dependency affects the pattern formation.

Accepting $\gamma \to \infty$ or $\gamma = 0$ leads to a constant form of the chemotactic sensitivity, $\chi(u, v) = \chi_0$. Multiple simulation showed that the simulated patterns distinguish from the experimental ones (Figure 1e) when decreasing $\gamma$-parameter (Figure 4a) or increasing $\epsilon$-parameter (Figure 4b). Because of this, similarly to the signal-dependent chemotactic sensitivity, there is no practical reason for application of a non-

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constant form also of the density-dependent sensitivity when modeling the pattern formation in a colony of luminous *E. coli.*

A simple constant form ($\chi(u,v) = \chi_0$) of the chemotactic sensitivity can be successfully applied to modeling the formation of the bioluminescence patterns in a colony of luminous *E. coli.* Oscillations and fluctuations similar to experimental ones can be computationally simulated ignoring the signal-dependence as well as the density-dependence of the chemotactic sensitivity.

## V. Conclusions

The quasi-one dimensional spatiotemporal pattern formation along the three phase contact line in the fluid cultures of lux-gene engineered *Escherichia coli* can be simulated and studied on the basis of the Patlak-Keller-Segel model.

The mathematical model (2)-(4) and the corresponding dimensionless model (6), (8), (9) of the bacterial self-organization in a circular container as detected by bioluminescence imaging may be successfully used to investigate the pattern formation in a colony of luminous *E. coli.*

A constant function ($\chi(u,v)$ as well as $h(n,c)$) of the chemotactic sensitivity can be used for modeling the formation of the bioluminescence patterns in a colony of luminous *E. coli.* Oscillations and fluctuations similar to experimental ones can be computationally simulated ignoring the signal-dependence as well as the density-dependence of the chemotactic sensitivity.

The more precise and sophisticated two- and three-dimensional computational models implying the formation of structures observed on bioluminescence images are now under development.

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## References

[1] M. Eisenbach, *Chemotaxis*. London: Imperial College Press, 2004.

[2] T. C. Williams, *Chemotaxis: Types, Clinical Significance, and Mathematical Models*. New York: Nova Science, 2011.

[3] E. F. Keller and L. A. Segel, "Model for chemotaxis," *J. Theor. Biol.*, vol. 30, no. 2, pp. 225–234, 1971.

[4] T. Hillen and K. J. Painter, "A users guide to pde models for chemotaxis," *J. Math. Biol.*, vol. 58, no. 1-2, pp. 183–217, 2009.

[5] E. O. Budrene and H. C. Berg, "Dynamics of formation of symmetrical patterns by chemotactic bacteria," *Nature*, vol. 376, no. 6535, pp. 49–53, 1995.

[6] M. P. Brenner, L. S. Levitov, and E. O. Budrene, "Physical mechanisms for chemotactic pattern formation by bacteria," *Biophys. J.*, vol. 74, no. 4, pp. 1677–1693, 1998.

[7] J. D. Murray, *Mathematical Biology: II. Spatial Models and Biomedical Applications*, 3rd ed. Berlin: Springer, 2003.

[8] S. Sasaki *et al.*, "Spatio-temporal control of bacterial-suspension luminescence using a pdms cell," *J. Chem. Engineer. Japan*, vol. 43, no. 11, pp. 960–965, 2010.

[9] R. Šimkus, "Bioluminescent monitoring of turbulent bioconvection," *Luminescence*, vol. 21, no. 2, pp. 77–80, 2006.

[10] R. Šimkus, V. Kirejev, R. Meškienė, and R. Meškys, "Torus generated by *Escherichia coli*," *Exp. Fluids*, vol. 46, no. 2, pp. 365–369, 2009.

[11] S. Daunert *et al.*, "Genetically engineered whole-cell sensing systems: coupling biological recognition with reporter genes," *Chem. Rev.*, vol. 100, no. 7, pp. 2705–2738, 2000.

[12] R. J. M. M. B. Gu and B. C. Kim, "Whole-cell-based biosensors for environmental biomonitoring and application," *Adv. Biochem. Eng. Biotechnol.*, vol. 87, pp. 269–305, 2004.

[13] R. Šimkus and R. Baronas, "Metabolic self-organization of bioluminescent *Escherichia coli*," *Luminescence*, DOI 10.1002/bio.1303. [Online]. Available: http://onlinelibrary.wiley.com/doi/10.1002/bio.1303/full (Accessed Aug. 27, 2011).

[14] A. A. Samarskii, *The Theory of Difference Schemes*. New York-Basel: Marcel Dekker, 2001.

[15] I. R. Lapidus and R. Schiller, "Model for the chemotactic response of a bacterial population," *Biophys. J.*, vol. 16, no. 7, pp. 779–789, 1976.

[16] P. K. Maini, M. R. Myerscough, K. H. Winters, and J. D. Murray, "Bifurcating spatially heterogeneous solutions in a chemotaxis model for biological pattern generation," *Bull. Math. Biol.*, vol. 53, no. 5, pp. 701–719, 1991.

[17] R. Tyson, S. R. Lubkin, and J. D. Murray, "Model and analysis of chemotactic bacterial patterns in a liquid medium," *J. Math. Biol.*, vol. 38, no. 4, pp. 359–375, 1999.

[18] M. R. Myerscough, P. K. Maini, and K. J. Painter, "Pattern formation in a generalized chemotactic model," *Bull. Math. Biol.*, vol. 60, no. 1, pp. 1–26, 1998.

[19] J. J. L. Velazquez, "Point dynamics for a singular limit of the keller-segel model. i. motion of the concentration regions," *SIAM J. Appl. Math.*, vol. 64, no. 4, pp. 1198–1223, 2004.

[20] R. Baronas, F. Ivanauskas, and J. Kulys, *Mathematical Modeling of Biosensors: An Introduction for Chemists and Mathematicians*, ser. Springer Series on Chemical Sensors and Biosensors, G. Urban, Ed. Dordrecht: Springer, 2010.

 ISBN: 978-1-61208-169-4 

[21] J. E. Moreira *et al.*, “Java programming for high-performance numerical computing,” *IBM Syst. J.*, no. 6, pp. 21–56, 2000.

[22] K. J. Painter and T. Hillen, “Spatio-temporal chaos in a chemotactic model,” *Physica D*, vol. 240, no. 4-5, pp. 363–375, 2011.

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