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Mathematical Modelling of Quorum Sensing and Bioluminescence in Bacteria

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ABSTRACT

Different types of Quorum Sensing in Bacteria, both intra-species and inter-species, have been analyzed over the last decade. A number of Mathematical Models has been proposed to explain the process of Quorum Sensing which depends on a threshold concentration of autoinducers (or QSM) reflecting high bacterial density. Stability of the solution of the differential equations of such an intra-species model for Vibrio fischeri is discussed in this paper.

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1. INTRODUCTION

Nature is full of amazing facts and one such fact is the existence of bacterial cells in human body. There are trillions of human cells that make each of us but surprising reality is that there are ten trillions bacterial cells residing in and on a human body! So not more than 10% human cells are present compared to the huge bacterial cells. It would not be foolish if we introduce ourselves as *only 10% human but 90% bacteria*! (Bassler et. al. 2001). Though studies of cell-to-cell bacterial communication were initiated almost half a century ago, it was not earlier than by a decade, we have been introduced with the phrase ***Quorum Sensing***, which has potential strength to answer such questions (Dunn et. al. 2007). Bacteria can communicate to one another, using a *chemical language.* Since a single bacterium can do nothing against the host body, they wait and keep sending and receiving such chemical signals until they become numerous enough to crush the host's immune system. Individual bacterium secretes signaling molecules called auto-inducers into their surroundings, and as the density of bacteria increases, so does the concentration of the signaling molecule. Each bacterium also has a receptor for the auto-inducer. When the auto-inducer binds to the receptor, it activates the transcription of certain genes, including those responsible for the synthesis of the inducer itself. As the bacterial population grows, more inducer molecules are synthesized. This forms a positive feedback system and the concentration of the molecule keeps rising. This process continues till a critical mass, or quorum, of bacteria and auto inducers are achieved. Then specific behaviors initiate on a global scale and the bacteria act like enormous multi-cellular organism. Quorum sensing thus enables bacteria to organize and respond quickly to environmental changes, such as the availability of nutrients, other microbes or toxins in their environment. While some auto-inducers are species-specific, many bacteria also produce a universal auto-inducer, known as AI-2, used across different bacterial species. AI-2 was first

discovered in a bioluminescent species of marine bacteria capable of giving off visible light, but it has since been identified in hundreds of other species.

The types of behaviors initiated by quorum sensing are typically those that are beneficial only when performed as a group, such as the release of toxins or the formation of aggregates called biofilms. Theoretically, blocking quorum sensing would prevent the bacteria from turning pathogenic and producing the toxins that are an immediate cause of disease in bacterial infections. Here are some examples of Quorum Sensing that take place in diverse species of bacteria.

Quorum sensing was originally discovered in the luminescent bacterium Vibrio fischeri. Vibrio fischeri is a marine bacterium which can be found both as free-living organism and as a symbiont in the light-producing organ of an animal host, such as the Hawaiian bobtail squid. The host provides a nutrient-rich environment for the bacterium and the bacterium provides light for the host. It was observed that liquid cultures of Vibrio fischeri produced light only when large numbers of bacteria were present. As a free-living organism, Vibrio fisheri exists at low densities and appears to be non-luminescent. At high cell concentrations, the level of the auto-inducer becomes sufficient to induce transcription of the genes that produce the enzyme luciferase, leading to bioluminescence. A single cell is not capable of producing enough luciferase to cause visible luminescence. Using quorum sensing, the cell can save its effort for the time when sufficient similar cells are around, so that their combined action produces a visible glow. The bacteria thus behave differently in the free-living and symbiotic states. It is important for pathogens to co-ordinate their virulence to escape the immune response of the host and establish a successful infection. The luminescence in Vibrio fischeri is controlled by the transcriptional activation of the lux genes. Most quorum sensing signals are small organic molecules or peptides. For example, gram-negative bacteria employ N-acyl homoserine lactones (AHLs), alkyl quinolones (AQs) and fatty acid methyl esters. Gram-positive bacteria use peptides like the autoinducing peptides (AIPs). The streptomycetes synthesize butyrolactones such asA-factor. AHL-mediated quorum sensing is one of the best characterized cell-to-cell communication mechanisms. More than 70 bacterial species are known to produce AHL-type quorum-sensing signals, with many producing multiple AHLs. Another example of quorum sensing is the creation of bio-films. Quorum sensing is required for full virulence of pathogens like S. aureus and Vibrio cholerae. Also, bacteria sometimes group together to form an organized ‘bio film’ covered by a polymer. Bio films are resistant to UV-radiation, desiccation and antibiotics. In several bacteria, disrupting quorum sensing adversely affects bio film formation. The pathogen Pseudomonas aeruginosa uses quorum sensing to coordinate behaviours such as bio film formation, swarming motility, and aggregation. These bacteria grow inside a host organism without harming it, until they reach a threshold concentration. Then, having detected that their number is sufficient to overcome the host’s immune system, they become aggressive and form a bio film, causing disease. This pathogen uses AHL-mediated quorum sensing to regulate the production of many factors needed for virulence. The last example that we state is the Quorum Sensing in Prokaryote–Eukaryote Interactions. Although quorum sensing signal molecules have largely been considered effectors of prokaryotic gene expression, they can also affect the behaviour of eukaryotic cells. AHLs are known to have immuno modulatory effects. They also induce relaxation of blood vessels. Apparently some bacteria have the power to influence the host’s immune responses to their benefit, and stimulate the delivery of nutrients for their survival by increasing the blood supply. But signal molecules may also benefit the host. 'Probiotic' bacteria are thought to be beneficial to the host organism and are added as dietary supplements in health-promoting food. Cultures of Bacillus subtilis, for example, have been used to treat dysentery and other intestinal problems. Recently, itwas revealed that B. subtilis produces a quorum sensing signal molecule, the competence-and-sporulation-stimulating factor, which induces the synthesis of the heat shock protein Hsp27 in the intestine. This protects intestinal cells against oxidative damage and loss of barrier function. The marine alga Ulva releases zoospores into the water. These attach to a suitable surface and differentiate into new algae. The zoospores are known to settle preferentially on to sites of concentrated AHL biosynthesis. The discovery that several bacteria make identical signal molecules prompted the idea that these signals may be exploited as a cross-talk mechanism between distinct species. Another luminescent bacterium, Vibrio harveyi, produces two auto-inducers. The first (AI-1) is an AHL used for communication only among V. harveyi bacteria. The other, AI-2 is synthesized from S-adenosyl methionine. The enzyme which catalyzes the final step in this synthesis is called LuxS. The gene for LuxS is found in many different bacteria, all of which make and respond to AI-2. This suggests that perhaps AI-2 allows bacteria to sense and react to not only members of their own species, but also to all other species that produce AI-2.

In this paper we study the mathematical model of quorum sensing in Vibrio fischeri. The model was originally proposed by J. P. Ward (Ward et al, 2001). We have tried to modify it and instead of solving it for different particular cases, we have solved it more generally with much flexibility to explain those special cases. Finally, we analyzed the stability of the above general solution.

2. MATHEMATICAL MODELING

In our model we assume a well mixed population of up-regulated and down-regulated cells. The complete process of up-regulation and down-regulation and QSM production rate are described as a system of ordinary differential equations. Bacterial population growth, which was studied in batch culture, is modeled in such a way that the time scale of QSM concentration to achieve a local maximum, corresponding to maximal cell densities, lies between 8-14 hours (Ward et al 2001). This model is based on a set of preliminary assumptions, compatible to the biological conditions, as follows:

Assumption-1. The population consists of up-regulated (density N_u , viewed as the number of cells per unit volume) and down-regulated (with density N_d) sub-populations of cells, corresponding to bacteria with a complex-bound or empty lux-box, respectively.

Assumption-2. Down-regulated cells are up-regulated by QSMs (concentration A) with rate constant α .

Assumption-3. The QSM is produced by both up-regulated and down-regulated cells , at rates k_u and k_d respectively with $k_d << k_u$.

Assumption-4. Down-regulated occurs spontaneously, due to breakdown of lux-box bound QSM-QSP complex at a rate β.

Assumption-5. QSMs can be broken down by the medium, and hence lost to the system, at a rate λ.

Assumption-6. Cell division of one down-regulated cell produces two down -regulated cells.

Assumption-7. Cell division of up-regulated cells produces on average γ up- regulated and $(2 - \gamma)$ down-regulated cells (where $0 \leq \gamma \leq 2$) assuming that only a population of replicated chromosomes contain occupied lux-boxes. We anticipate that $\gamma \approx 1$, which indicates that division of one up-regulated cell produces one up-regulated and one down-regulated cell.

Assumption-8. Cell-division rate of up-regulated and down-regulated cells are equal, being determined by the parameter r, where the doubling rate is at low densities.

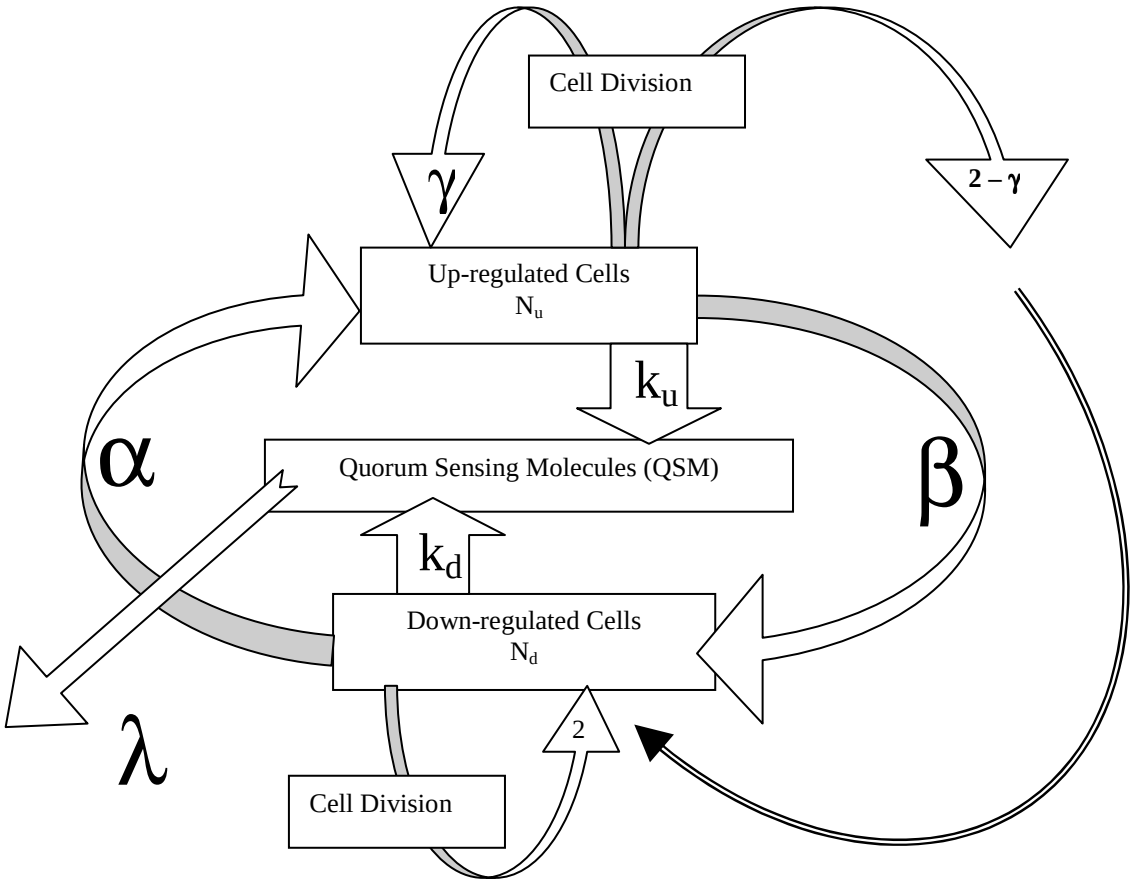


Fig 1. Diagram showing the process of up-regulation and down-regulation of cells.

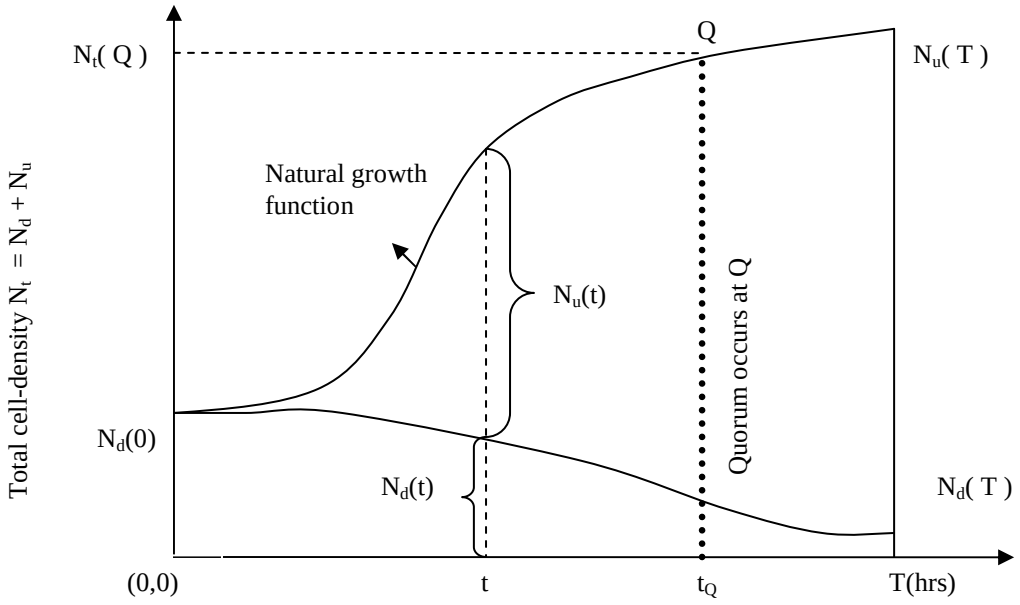


Fig 2. Diagram showing the quorum at attaining a threshold concentration (Q).

3. SOLUTION AND STABILITY ANALYSIS

In biological systems, it is extremely difficult to find analytically the exact solution of such system of differential equations. However numerical solutions are quite easy to find on obtaining estimated values of the parameters involved. We have tried to solve the system analytically.

Stability of the Linear System: Points of equilibrium (N_d^*, N_u^*, A^*) i.e., where $f_i(N_d^*, N_u^*, A^*) = 0$ for all **i = 1, 2, 3**, are not always stable. Since stable and unstable equilibria bear different characteristics in the dynamics of a system, it will be wise to classify equilibrium points based on their stability (Wang et. al. 2005).

Equilibria are found by determining the values of the variables that cause all of the variables to remain constant, i.e.

$$f_i(N_d^*, N_u^*, A^*) = 0 \text{ for all } i = 1, 2, 3 \quad (1)$$

Again, there may be multiple equilibria, and finding them may be difficult.

Suppose that $\mathbf{x}^*$ is an equilibrium point. By definition, $\mathbf{f}(\mathbf{x}^*) = \mathbf{0}$.

The partial derivative in the above equation is to be interpreted as the **Jacobian matrix**. If the components of the state vector **x** are $x_1, x_2, \ldots, x_n$ and the components of the rate vector **f** are $f_1, f_2, \ldots, f_n$, then the Jacobian is

$$\mathbf{J} = \begin{bmatrix} \frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial x_2} & \cdots & \frac{\partial f_1}{\partial x_n} \\ \frac{\partial f_2}{\partial x_1} & \frac{\partial f_2}{\partial x_2} & \cdots & \frac{\partial f_2}{\partial x_n} \\ \vdots & \vdots & & \vdots \\ \frac{\partial f_n}{\partial x_1} & \frac{\partial f_n}{\partial x_2} & \cdots & \frac{\partial f_n}{\partial x_n} \end{bmatrix}.$$

If $\delta\mathbf{x}$ is small, then only the first term in equation (6) is significant since the higher terms involve powers of our small displacement from equilibrium. If we want to know how trajectories behave *near* the equilibrium point, e.g. whether they move toward or away from the equilibrium point, it should therefore be good enough to keep just this term. The eigen values of the Jacobian are, in general, complex numbers. Let $\lambda_j = \mu_j + i\nu_j$, where μ_j and ν_j are, respectively, the real and imaginary parts of the eigen value. Each of the exponential terms in the expansion can therefore be written as

$$e^{\lambda_j t} = e^{\mu_j t} e^{i\nu_j t}.$$

The complex exponential in turn can be written as

$$e^{i\nu_j t} = \cos(\nu_j t) + i\sin(\nu_j t).$$

The complex part of the eigen value therefore only contributes an oscillatory component to the solution. It's the real part that matters: If $\mu_j > 0$ for any *j*, $e^{\mu_j t}$ grows with time, which means that trajectories will tend to move away from the equilibrium point.

However this theorem cannot enlighten what happens if some of the eigen values have zero real parts while the others are all negative. This case can't be decided based on linear stability analysis. The nonlinear terms in fact determine the stability in this case, which requires a detailed nonlinear theory. On the other hand linear stability analysis tells us how a system behaves near an equilibrium point. It cannot however tell us anything about what happens farther away from equilibrium. Phase-plane analysis combined with linear stability analysis can generally give us a full picture of the dynamics, but things become much more difficult in higher-dimensional spaces. Now we consider a technique due to Liapunov, which can be used to determine the stability of an equilibrium point both near and far from the equilibrium point.
Liapunov's method is based on a simple idea. Suppose that $V(\mathbf{x})$ is a function of our state variables which has a minimum at an equilibrium point and which has no local minima. Now suppose that we can show that the dynamics of our system results in a steady decrease in V in some neighborhood of the equilibrium point. This necessarily means that we are tending toward the minimum of V, which is just the equilibrium point. Having shown this, we can conclude that the equilibrium point is stable over the entire neighborhood of $\mathbf{X}^*$ over which V decreases. A function V with these properties is called a **Liapunov function**.

Definition *Let U be a region of phase space containing the equilibrium point* $\mathbf{X}^*$. *Let* $V : U \to \mathsf{R}$ *be a continuous and differentiable function. V is a* ***positive definite function*** *for the point* $\mathbf{X}^*$ *if it satisfies the following two conditions:*

1. $V(\mathbf{x}^*) = 0$, *and*
2. $V(\mathbf{x}) > 0$ *for* $\mathbf{x} \in U - \{\mathbf{x}^*\}$

In order to use this theorem, we have to obtain a Liapunov function. Unfortunately, it's often really difficult to come up with a Liapunov function for a given system, except in some special cases where the physics of the problem suggests a particular choice.
So we use the following algorithm to analyze the stability of any system, linear or non-linear: First of all, we find all equilibria. Then we determine whether and when they are biologically meaningful. If we get so, we calculate the Jacobian J and its value J^* at an equilibrium of interest. To find out numerically, the eigen values of the matrix J^*, we solve the characteristic equation $\det(J^* - \lambda I) = 0$. The equilibrium is locally stable is the real parts of all eigen values are negative. Equivalently, the real part of the leading eigen value (i.e. the eigen value with the largest real part) must be negative. If the real part of the leading eigen value is exactly zero, the analysis is inconclusive. If the eigenvalues are purely imaginary, the system will spiral around the equilibrium along some axes. Instead of actually calculating the eigen values, one can use the Routh- Hurwitz criteria. For a model with two variables and a Jacobian $J^* = (a,b,c,d)$, the equilibrium is stable if $\det(J^*)$

= ad – bc > 0 and tr(J^*) = a + d < 0. Furthermore, the eigen values are imaginary if $[\text{tr}(J^*)]^2 < 4 \det(J^*)$. We repeat previous five steps for all equilibria of interest. It will be better to present the whole procedure as a flow-chart.

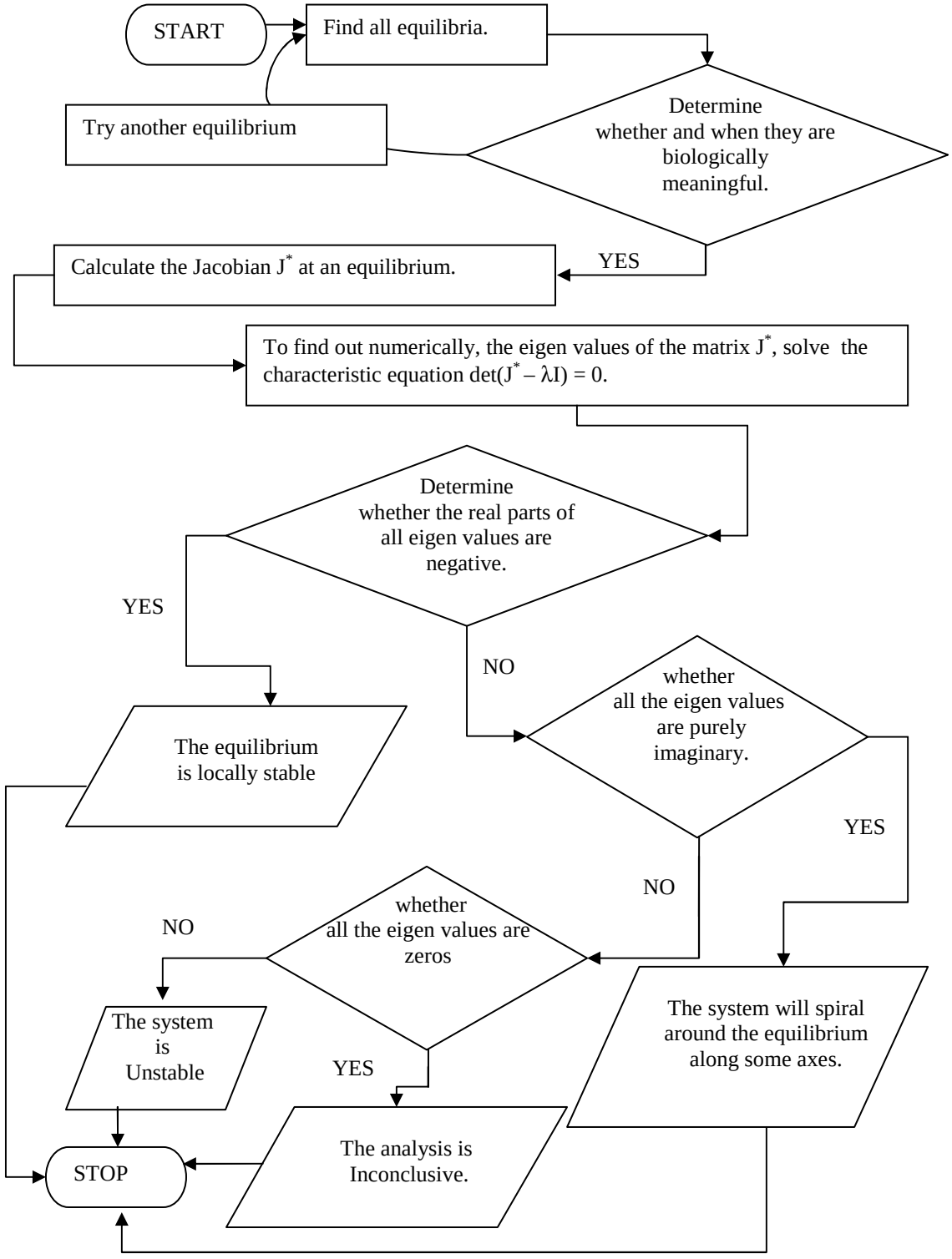
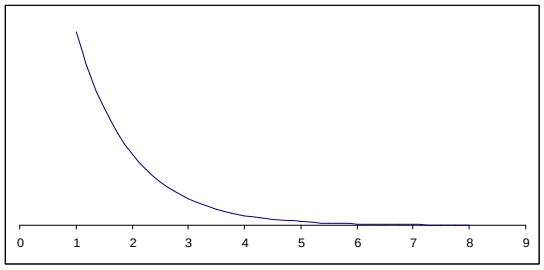
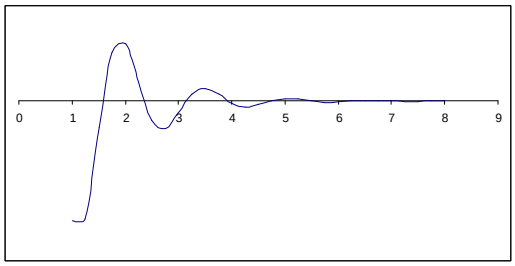


Fig 3. Flow-chart of Stability Analysis

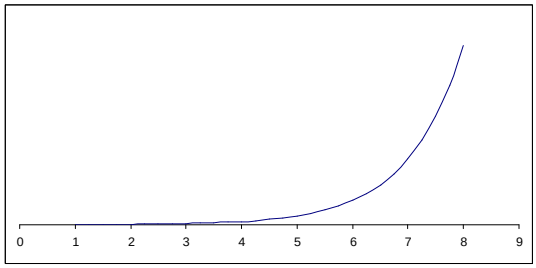
If we make a small perturbation to the point of equilibrium of the above system, the disturbance decays or increases or oscillates according to the nature of its stability. Here are the different possibilities or patterns how the disturbance changes with time:



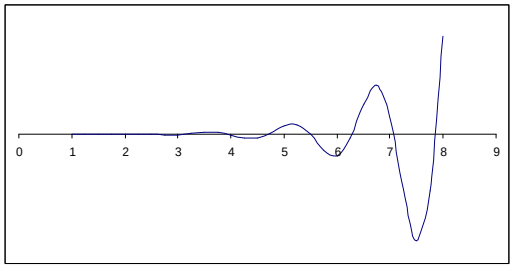
All the eigen values are real and negative causing exponential decay of the disturbance. The equilibrium is stable.



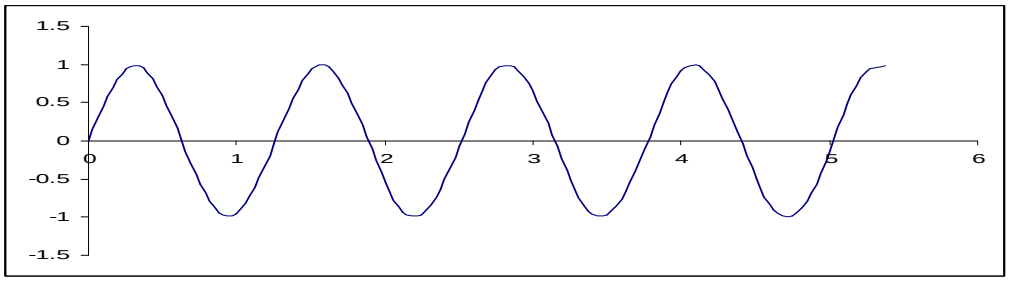
Some eigen values are imaginary and the real part of all such eigen-values ard the real eigen values are negative, the disturbance oscillates and decays rapidly with time. The equilibrium is stable.



All eigen values are real with at least one positive. The disturbance increases exponentially. The equilibrium is unstable.



At least one eigen value is imaginary having positive real part. The disturbance increases with oscillation. The equilibrium is thus unstable.



All eigen values have zero real parts and nonzero imaginary parts, causing stable oscillation.

Fig 4. Different states of stability

Now we come back to our original problem of Quorum Sensing Model. We assume that the biological conditions are compatible to the linearization process of the system.
If λ_1, λ_2, λ_3 are the eigen values of the matrix Q, the general solution of the equation (1) can be written, in terms of the corresponding eigen-vectors Q_1 , Q_2 , Q_3 , as

$$\mathbf{X(t) = C_1 Q_1 e^{\lambda_1 t} + C_2 Q_2 e^{\lambda_2 t} + C_3 Q_3 e^{\lambda_3 t}} \quad (2)$$

where C_1 , C_2 , C_3 are arbitrary constants.

From the experimental data, an equilibrium point is found to be of the form (ε, ε, M), where M >> ε > 0, resulting one of the eigen values of the Quorum Sensing Matrix to be –M. Though other two eigen values are zeros, which are inconclusive about the stability of the system, but the presence of the dominating – M clearly shows that the system is locally stable.

4. CONCLUSION

The proposed mathematical model for quorum sensing in Vibrio fischeri is shown to give rise a stable solution and the general solution is compatible with the real situation. However it is not clear, at least at the present state of analysis, what happens after the quorum is achieved. Time evolution of the up-regulated and down-regulated cells as well as that of the QSM-concentration cannot enlighten the exact duration or fate of the bioluminescence occurred during a local maximum is attained by the QSM-concentration. Not only that, the bacterial communication through diffusion of the QSMs deserves the inevitable analysis about the noise acquired and its consequences. This will be done in subsequent papers.

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