A continuous-time decision-making model for bacterial growth via quorum sensing: theory and evidence

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ABSTRACT

Quorum sensing is a communication mechanism used by bacteria to coordinate population density dependent collective behavior. In the particular case of bacterial growth, quorum sensing acts as a control mechanism that optimizes the trade-off between intrinsic growth rate and the maximum population size that the environment can sustain. Herein, a model for controlled bacterial growth via quorum sensing in continuous time is proposed. One of the main innovations of the proposed model relative to existing ones is to assume that Nature seeks to optimize the population level over time. The growth curve optimization is formulated as the maximization of a discounted objective function where the variable is the activation threshold for the quorum sensing mechanism. Numerical evidence suggests that this discounted objective function based on the growth curves is unimodal in the activation threshold. Growth curves obtained from analyzing experimental data match those predicted by the model.

CCS CONCEPTS

• Applied computing → Systems biology.

KEYWORDS

Decision-making, bacterial networks, biological communications, systems Biology, public goods

ACM Reference Format:

Marcos M. Vasconcelos, Urbashi Mitra, Odilon Câmara, Manasi S. Gangan, and James Boedicker. 2019. A continuous-time decision-making model for bacterial growth via quorum sensing: theory and evidence. In *The Sixth Annual ACM International Conference on Nanoscale Computing and Communication (NANOCOM '19)*, September 25–27, 2019, Dublin, Ireland. ACM, New York, NY, USA, 6 pages. <https://doi.org/10.1145/3345312.3345499>

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NANOCOM '19, September 25–27, 2019, Dublin, Ireland
© 2019 Association for Computing Machinery.
ACM ISBN 978-1-4503-6897-1/19/09...\$15.00
<https://doi.org/10.1145/3345312.3345499>

1 INTRODUCTION

Microbial communities play a significant role in bioremediation, plant growth promotion, human and animal digestion, drive elemental cycles, the carbon-cycle and the production of clean water. They are also posed to be the engines of renewable energy via microbial fuel cells which can reverse the process of electrosynthesis. Many of these operations are enabled via collective behavior. Despite being simple unicellular organisms, complex collaborative tasks can be achieved by a large population via the mechanism of *quorum sensing* (QS). The conventional wisdom is that bacteria secrete compounds (*auto-inducers*) which can also be sensed from the environment enabling microbial population estimation. If the colony is sufficiently large, cells start producing *exofactors* responsible for certain tasks such as: degrading antibiotics, metabolizing food, luminescence and biofilm formation.

Herein, we pose quorum sensing as a mechanism that regulates the production enzymes which facilitate the metabolization of food in the environment by the bacterial colony. Our theoretical model assumes that Nature, when optimally designing the activation policy, faces a fundamental tradeoff: activating imposes an immediate cost (reduction of the intrinsic growth rate) but delivers a longer run benefit (higher carrying capacity). We deem the enzyme concentration as our *public good* and the net gain in steady state population size as our *public benefit*. This framework echoes those considered in economics for human population growth [15]. However, the mathematical model for bacterial population growth is unique [4], and classical results from economics are not applicable to our problem. Our goal is to formulate a continuous-time sequential optimization problem where each cell in the colony decides at each time instant whether to engage or not in the production of a costly exofactor that leads to the long term increase in the maximum achievable population.

1.1 Literature review and summary of contributions

Quorum sensing was discovered in 1970 in the context of growth of the luminous bacteria *Aliivibrio fischeri* [11]. Quorum sensing has since been found to be a collective behavior mechanism in many bacterial species [19]; a recent quorum sensing survey summarizes classical and recent findings [1].

Classical mathematical models for QS are based on coupled deterministic differential equations to describe key concentrations (enzymes, population size, etc.) [2]. A recent stochastic alternative based on queuing models is provided in [10] and yields excellent match with experimental data; however the decision making question is not addressed, in contrast to [3]. Within this new paradigm, the optimization of certain parameters of the QS system have been studied under different performance metrics.

The optimization of the “sensing potential” was studied in [13], where the overall fitness of the cells in a colony is defined as the benefit gained minus the cost incurred from producing exofactors. A similar approach was used in [18] where QS was modeled as a networked decision system, where each cell is an agent whose actions have local costs but global impact on “fitness” of the colony. In [18] it was proposed a model that considers dynamics and sequential optimization; and provided a structural result on the form of the optimal policy under the assumption of perfect population size knowledge.

In contrast to prior related optimization problems [5, 12] which only considered instantaneous population size with dynamics, we argue that the reward function of interest must consider current and future payoffs thus suggesting control theoretic approaches [8]. Under the assumption of perfect observation of the state of the system, the problem introduced admits an analytical solution through optimal control theory. The optimal growth curve is attained by employing a threshold policy on the population, where the optimal thresholding variable is computed in closed form as a function of parameters of the system [17].

The key contributions of this work are to:

- Provide a continuous time version of the model in [17] and investigate the case in which the concentration of auto-inducers and enzymes accumulate over time.
- Provide preliminary experimental results which confirm there is a trade-off between intrinsic growth rate and carrying capacity, which is the maximal size reached by a population cells in a given environment, controlled via quorum sensing.

2 MATHEMATICAL MODEL

In this paper we will focus on the scenario in which the population of a bacterial colony grows over time. In our model, we assume that Nature plays the role of a *social planner*, which selects a certain parameter of the model such as to optimize an objective function.

We will consider a dynamical system in continuous time, where the population of a colony at time t is denoted by $N(t)$. The evolution of the population over time occurs according to a Logistic Population Growth (LPG) model [9, 20]:

$$\dot{N}(t) = \lambda(t)N(t) \left(1 - \frac{N(t)}{\kappa(t)}\right), \quad (1)$$

where $\lambda(t)$ and $\kappa(t)$ denote *intrinsic growth rate* and *carrying capacity*, respectively. Equation (1) is widely used to describe population growth in resource constrained environments [7, 16]. Figure 1 illustrates the growth curves, which are solutions of the differential equation in Eq. (1), for two pairs of static parameters (λ, κ) .

The main idea in our model is that the colony’s enzyme production affects the intrinsic growth rate and the carrying capacity. The

Figure 1: Two growth curves generated by the LPG model with two different sets of parameters.

fundamental trade-off that exists in bacterial population growth is that producing enzymes to metabolize food in the environment is energetically costly, therefore, it results in less energy available for reproduction, but it leads to larger future populations. This means that expressing the gene which leads to enzymes will correspond to slowing down the intrinsic growth rate and increasing carrying capacity.

The control signal is denoted by $u(t)$ and represents the fraction of the population that is active at time t . An active cell is one which produces enzymes that will metabolize food in the environment. The active population is denoted by $N_a(t)$, and it is given by:

$$N_a(t) \triangleq N(t)u(t), \quad (2)$$

where $u(t) \in [0, 1]$ for all $t \geq 0$.

When the entire colony is inactive, $u(t) = 0$, the rate of growth is a constant denoted by λ_0 . Similarly, when the colony is fully active, $u(t) = 1$, the rate of growth decreases and is a constant $\lambda_1 < \lambda_0$. This relationship is captured by the following expression:

$$\lambda(t) = \lambda_0 + (\lambda_1 - \lambda_0)u(t), \quad (3)$$

We refer to the enzymes produced by the cells as a *public good*. As the number of enzymes in the environment increase, the carrying capacity of the system will also increase. The gain in carrying capacity, which is the maximum population size that the environment can sustain, is what we refer to as *public benefit*. However, the public benefit does not increase indefinitely as the quantity of public goods increase. We assume that the public benefit is a soft-threshold function of the quantity of public good e , indexed by two parameters: the nominal *threshold*, τ , and the *responsiveness*, l . This function is given by Eq. (4):

$$f(e, \tau, l) \triangleq \frac{(e/\tau)^l}{1 + (e/\tau)^l}, \quad (4)$$

and is illustrated in Fig. 2. This choice of public benefit function captures the following desirable properties [5]:

- The public benefit should be an increasing function of the quantity of public goods in the environment.
- The public benefit should be equal to zero in the absence of public goods in the environment.
- The public benefit should not increase indefinitely with the quantity of public goods in the environment.

Figure 2: Soft-threshold benefit function. The threshold parameter τ is equal to 50 across all curves.

The controlled carrying capacity is given by the following formula:

$$\kappa(t) = \kappa_0 + (\kappa_1 - \kappa_0)f(e(t), \tau, l), \quad (5)$$

where $\kappa_1 > \kappa_0$.

The quorum sensing mechanism is determined by a pair of differential equations that describe the evolution of the concentrations of auto-inducer molecules and enzymes in the environment, and an activation policy. Let $s(t)$ and $e(t)$, denote the concentration of auto-inducer signal and enzymes, respectively. The aforementioned variables evolve according to:

$$\dot{s}(t) = -\gamma_s s(t) + \sigma_s N(t) + m\sigma_s N_a(t) \quad (6)$$

$$\dot{e}(t) = -\gamma_e e(t) + \sigma_e N_a(t), \quad (7)$$

where the constants γ_s and γ_e correspond to the degradation rates of auto-inducer signal and enzyme in the environment. The constants σ_s and σ_e correspond to the emission rates of auto-inducer signal and enzymes and m corresponds to the additional amount of auto-inducer signal injected in the environment by the active population.

In quorum-sensing the state of the system is the population of the colony at a given time. However, the state is not directly observed by the individual cells. The state of the system is inferred from the concentration of auto-inducer signal $s(t)$. Therefore, the control signal $u(t)$ is computed according to an *activation policy*, which is a memoryless function of the concentration of auto-inducer signal molecules in the environment:

$$u(t) \triangleq \frac{(s(t)/\alpha)^h}{1 + (s(t)/\alpha)^h}. \quad (8)$$

Notice that this activation policy is a soft-threshold policy, similar to Eq. (4). In this paper, we take the responsiveness parameter h

as given. Our focus is to study how Nature chooses the activation threshold α in the optimization problem that will be described in the next section.

In [17], it was shown that when the state of the system is perfectly observed, the optimal activation policy has a threshold structure. In the current work, we are imposing the structure of the activation policy to be a soft-threshold policy with parameters α and h , and we will show how the performance of the system varies with respect to α .

2.1 Trivial activation policies

In order to illustrate how Eqs. (6) and (7) evolve, we consider them under the trivial policies “always off” ($u = 0$), and “always on” ($u = 1$). Figure 3 shows the concentration of auto-inducer $s(t)$ under the two policies. In this figure, we assumed that $\lambda_0 = 1$, $\lambda_1 = 0.5$, $s(0) = 0$, $\sigma_s = 0.1$, $\gamma_s = 0.01$ and $m = 9$. These values were chosen arbitrarily, and do not correspond to any bacterial species. However, there is empirical evidence that supports the fact that active cells emit auto-inducer molecules at rates 5 to 10 times higher than inactive cells [6].

Figure 3: Concentration of auto-inducer signal over time for the case in which every cell in the colony is either active (“always on”) or inactive (“always off”).

The production of enzymes only happens when $u(t) > 0$. Therefore, for $e(0) = 0$ a colony consisting of inactive cells yields a concentration of enzymes in the environment constant and equal to zero. Assuming $\sigma_e = 0.1$ and $\gamma_e = 0.001$, Fig. 4 displays the concentration of enzymes in the environment for colony where every cell is always active.

3 OPTIMAL THRESHOLDS

Bacteria have evolved through billions of years by selecting strategies that maximize the fitness of the species. There are many ways of defining the fitness of a colony. We argue that fitness is determined by the number of individuals in the colony that can be sustained over time. In other words, the performance of the system is determined by the growth curves $N(t)$, $t \geq 0$. Inspired by the economics literature on dynamic problems with discounted utility functions, we propose as our performance metric the following

Figure 4: Concentration of enzymes over time for the case in which every cell in the colony is active (“always on”).

discounted reward function:

$$\mathcal{J}(\alpha) = \int_0^\infty e^{-\beta t} N(t) dt, \quad (9)$$

where α is the activation threshold chosen by Nature, and $\beta \geq 0$ is a subjective constant used to capture an inter-temporal trade-off. Notice that the activation threshold α determines the growth curve $N(t)$. Therefore, the dependency of the objective function in Eq. (9) on α is implicit.

The discount factor β has the role of weighting the growth curve $N(t)$. The value of β determines how much the decision to activate or not the production of the public good at a time t depends on the future population. For $\beta \approx 0$, the entire future population is heavily taken into consideration. For larger values of β , the decision becomes myopic in the sense that it only the immediate future matters. If Nature is “patient” (captured in the model by a lower β), then it is very concerned about population levels now and in the more distant future. If Nature is “impatient” (captured in the model by a higher β), then it is mostly concerned about population levels in the short-run.

The optimization problem that Nature as a social planner is interested in solving is to select the parameter activation threshold α such as to maximize the objective function $\mathcal{J}(\alpha)$.

3.1 Unimodality

One property of the objective function in Eq. (9) is that it is unimodal with respect to the activation threshold α . Unimodality is a desirable property because it implies that there exists a unique maximizer for the objective function. Figure 5 shows the unimodality of $\mathcal{J}$ corresponding to $\beta = 0.08$ for which the optimal threshold $\alpha^* \approx 1.8$ is found numerically. Here we assume that the responsiveness parameters in Eqs. (4) and (8) are set to $l = h = 5$.

3.2 Optimal thresholds

In this section, we investigate the dependency of the optimal threshold α^* with β . Figure 6 shows that by increasing β , the value of α^* also increases. Therefore, as the value of β increases, the colony will delay its activation since the present and immediate future

Figure 5: Unimodality of the objective function.

is prioritized. The parameter β has important implications in the inference of whether a bacterial colony prioritizes immediate or long term population growth. Our key observation is that a “patient” Nature will optimally choose to activate sooner, while an “impatient” Nature will optimally choose to activate later. Figure 6 illustrates this fact: α^* increases with β (an “impatient” Nature activates later).

Figure 7 illustrates the system’s performance under 3 different policies: “always on”, “always off” and quorum sensing regulated with an optimal threshold α^* . For an “impatient” Nature (higher β), the policy “always off” is better than the policy “always on”. For a “patient” Nature (lower β), the policy “always on” is better than “always off”. One important observation to be drawn from Fig. 7 is the fact that performing quorum sensing using a soft-threshold strategy with the optimal α^* is always better than the “always on” and “always off” policies.

Figure 6: The optimal threshold is an increasing function of the discount factor.

Figure 7: Optimal performance of the system as a function of the discount factor.

3.3 Optimal growth and activation curves

Fixing the value of the discount factor to $\beta = 0.04$, the optimal threshold is $\alpha^* \approx 1.7$. Using these parameters, our model for bacterial growth using quorum sensing can be used to compute the optimal growth curve $N(t)$, which is shown in Fig. 8. The control signal $u(t)$ is shown in Fig. 9, which behaves approximately as a hard threshold function at $t = 10$.

Figure 8: Growth curves for three different activation policies: “always off”, “always on” and quorum sensing with $\alpha^* = 1.7$.

4 EXPERIMENTAL DATA

We performed experiments in the laboratory following the procedure described in Appendix A. The three policies were programmed into the genetic code of the bacterial cells. The empirical behavior of the growth curves in Fig. 10 is similar to what is predicted by our theoretical model in Fig. 8.

Figure 9: Control signal over time. Nearly the entire colony activates at time $t \approx 10$.

Figure 10: Growth curves obtained in the laboratory.

5 CONCLUSIONS AND FUTURE WORK

In this paper, we use a continuous time framework to model bacterial growth via quorum sensing. As opposed to [17], the social planner does not observe the state of the colony directly. The colony population is observed through the concentration of auto-inducer molecules in the environment, which builds up over time in the environment. The key idea in this paper is that there exists an inherent trade-off between intrinsic growth rate and the carrying capacity, and quorum sensing is the strategy used to activate the production of enzymes.

We provide numerical evidence that the the objective function is unimodal with respect to the activation threshold. The optimal threshold is an increasing function of the discount factor of the discounted reward function used to measure fitness. Our key theoretical result is then to show that a “patient” Nature will optimally choose to activate sooner, while an “impatient” Nature will optimally choose to activate later. Finally, we performed experiments in the laboratory that serve as evidence that the trade-off hypothesized in the model exists. Future work includes calibrating the

model from empirical data, making predictions and estimating the subjective parameter β for different bacterial colonies.

ACKNOWLEDGMENT

The work of M. M. Vasconcelos and U. Mitra was supported by ONR under grant N00014-15-1-2550; NSF under grants CCF-1718560, CCF-1410009, CPS-1446901, CCF-1817200; and ARO No. 74745LSMUR. The work of J. Boedicker and M. Gangan was supported by ARO MURI award No. W911NF1910269.

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A MATERIALS AND METHODS

A.1 Construction of plasmids

Gene encoding for alpha-amylase, amyE, was amplified from the genomic DNA of *B. subtilis* 168. For extra-chromosomal expression of alpha-amylase in *E. coli* MG1655 cells, a plasmid was derived from pTD103luxI_{sf}GFP [14] by replacing luxR gene with amyE, such that bidirectional promoter, plux controls the expression of both amyE and sfGFP. Construct was further modified to make deletion mutant of alpha-amylase by deleting amyE ORF from plasmid.

A.2 Media and growth conditions

Overnight grown culture of amyE⁺ and amyE⁻ cells in M9 + glucose (0.4%) at 37°C and 180rpm were transferred to 5ml fresh media containing M9 salts supplemented with 0.0125% glucose and 0.05% starch at 0.1% inoculum. Constitutive expression of alpha-amylase induced in one of the batches of amyE⁺ cultures by addition of 3μg/ml of N-(3-Oxohexanoyl)-L-homoserine lactone (AHL). Cultures were incubated at 37°C at 180rpm until they enter log phase (OD 0.3) and then inoculated in fresh 5ml media with same respective compositions at 1% inoculum in triplicates, followed by incubation at 37°C and 180rpm. Samples withdrawn at the regular interval of 1 hour from respective cultures were diluted serially and 5μl volume of diluted cultures was plated on LB agar plates. Later were incubated at 37°C for 12 hrs and were analyzed for colony count.