Population regulation in microbial consortia using dual feedback control

Xinying Ren  Ania-Ariadna Baetica  Anandh Swaminathan  Richard M. Murray

Abstract—An ongoing area of study in synthetic biology has been the design and construction of synthetic circuits that maintain homeostasis at the population level. Here, we are interested in designing a synthetic control circuit that regulates the total cell population and the relative ratio between cell strains in a culture containing two different cell strains. We have developed a dual feedback control strategy that uses two separate control loops to achieve the two functions respectively. By combining both of these control loops, we have created a population regulation circuit where both the total population size and relative cell type ratio can be set by reference signals. The dynamics of the regulation circuit show robustness and adaptation to perturbations in cell growth rate and changes in cell numbers. The control architecture is general and could apply to any organism for which synthetic biology tools for quorum sensing, comparison between outputs, and growth control are available.

I. INTRODUCTION

A primary area of study in synthetic biology has been the implementation of synthetic gene circuits with novel functionality in single cells. The first synthetic gene circuits included oscillators [1], [2] and toggle switches [3], [4]. In oscillator circuits, the expression of a gene of interest oscillates repeatedly over time. In toggle switch circuits, a gene’s expression can be switched between two stable steady state levels. In both cases, the circuit is implemented at the single cell level. Recent applications include the engineering of metabolic pathways in single cells to produce fuels [5] or drugs [6] and the manipulation of a cell’s DNA to implement state machines in single cells [7], [8].

However, there are challenges associated with implementing biological circuits in single cells. One challenge is that when composing genetic circuit parts into larger circuits, loading effects from a downstream module can negatively impact the performance of an upstream module. This phenomenon, termed retroactivity, can interfere with circuit behavior when attempting to build complex synthetic gene circuits [9]. Moreover, single cells have limited pools of resources. Complex circuits with many parts use more cellular resources, and hidden interactions that arise through resource competition can also negatively impact circuit performance [10], [11].

Implementing genetic circuits in multiple cells alleviates these two challenges. With different circuit components in different cells, the components have separate resource pools and thus cannot compete for resources. In addition, the communication between cells is solely mediated by small molecules that typically exist at very large copy numbers, which mitigates loading effects from retroactivity. This approach is illustrated in [12], where the authors use different combinations of yeast strains to implement different logical functions.

Previously developed synthetic gene circuits that function at the population level and involve feedback include a population control circuit that regulates the number of cells in a culture [13], a predator prey system with two cell strains [14], a two strain system for programmed pattern formation [15], and a two strain population level oscillator [16]. However, there are challenges associated with implementing circuits at the population level across multiple cell strains. One of these challenges is maintaining a stable population fraction of all cell strains. When implementing a two strain system, the ratio between the two cell types might require tuning for the best performance. In addition, there might also be an optimal total population size as too many cells would deplete the resources of the consortium.

Here, we present a control strategy for tuning the cell type ratio as well as the total population size in a two strain system. By using two separate control loops to control the total cell number and the ratio between the two cell types, we demonstrate that both the total population size and the cell type ratio are independently tunable. We show that our control architecture implements a lag compensator. Furthermore, we show that the total population size and cell type ratio are robust to perturbations in the number of cells of either strain and are also robust to perturbations in the growth rate of either strain.

The organization of the paper is as follows. In Section 2, we give an overview of the biological background for the problem and we introduce our design strategy of using two separate loops to control the total population size and the cell type ratio. In Section 3, we provide a model of the control loop for maintaining the total population size, and we demonstrate its effectiveness. We also show that the global population size control loop implements a lag compensator. In Section 4, we introduce a model for the control loop that maintains cell type ratio and again show that the controller implements a lag compensator. We demonstrate that the cell type ratio is robust to perturbations in cell growth rate. In Section 5, we combine the two loops into one model and show that total population size and cell type ratio are independently tunable. We summarize the main findings of the paper and discuss future work in the Discussion section.
II. THE DESIGN STRATEGY

Our proposed population regulation circuit in microbial consortia consists of two feedback control loops. The global regulation and the co-regulation both involve a controller regulating either cell growth or death processes. By coupling the two loops, we can achieve separate functions that simultaneously regulate the absolute population count and the relative ratio between the two cell strains.

A. Biological background

In order to control growth of different cells strains, we require biological sensors, comparators, and actuators [17]. The sensors need to sense the population size, the comparators need to compare the population size to a reference signal, and the actuators need to use the output from the comparators to drive cell growth such that the error between the reference population size and the actual population size is reduced. Here, we briefly describe synthetic biological systems that can implement each of these three crucial functions.

Quorum sensing systems in bacteria can be used as sensors for population size. In quorum sensing systems, each cell constitutively produces and secretes a small signal molecule, so the concentration of signaling molecules in solution is proportional to the population size [18]. Downstream gene expression machinery responds to the concentration of the signaling molecules in a graded fashion. While quorum sensing systems are most commonly used in bacteria, similar tools exist in yeast [12] and in mammalian systems [19].

To compare the sensed population size to the reference population size, we need gene circuits that can subtract the two quantities in a chemical manner. This can be achieved by using two proteins, where one protein sequesters the other and inhibits its function. This type of system can be constructed using engineered protein scaffolds [20] or it can be leveraged from a natural system that already exists [21]. Systems that inhibit gene expression at the RNA level can also provide similar functionality [22].

Finally, the difference between the measured and the reference signals must be used to actuate cell growth in order to modulate the population size. Typically, cell growth actuation strategies depend on modulating the expression of a gene that is essential for cell growth. When expression of the essential gene is decreased, the cells grow more slowly. The gp2 phage protein stops bacterial growth by inhibiting bacterial RNA polymerase [23]. Similarly, using an inducible RNA polymerase allows control of cell growth by controlling RNA polymerase expression [24]. Another method that allows for cell growth control is toxin-antitoxin systems. In these systems, a toxin protein slows down cell growth or kills the cell, while an antitoxin protein sequesters the toxin and inhibits its toxicity [21]. Toxin-antitoxin systems are especially useful for building growth controllers, as they can be employed as comparators and actuators.

In this paper, we present a general control design that should be applicable to any synthetic biology organism where the appropriate tools for sensing, comparison, and actuation are available. However, our specific inspiration is to achieve growth control in E. coli using quorum sensing [18] for sensing, the ccdB/ccdA toxin-antitoxin system [21] and RNA antisense technology [22] for comparison, and the ccdB toxin and the gp2 protein [23] for actuation.

B. The global regulation loop for total population control

The global regulation system controls the total population of all strains in the culture and consists of three modules. The cell dynamics module includes the growth and division processes of the cell. The communication module relies on a global quorum sensing system where all cell strains
produce and sense a common signal molecule. The feedback controller module is designed to ensure homeostasis of the total cell population by comparing the output and the reference and by actuating the corresponding cell growth process to decrease the error.

The biological design of the global regulation loop is illustrated in Figure 1a. The reference is set by the induction rate of biochemical species $G$, which activates the cell growth and division processes. Species $G$ can be strongly sequestered and inactivated by species $D$ to decrease cell growth rate. All cell strains release and sense a common signal molecule $S_g$ in a global quorum sensing system. When the total population of all cells increases, more signal molecules $S_g$ are synthesized and released into the environment. These signals diffuse across membranes into cells and activate reactions that produce species $D$. Therefore, more species $D$ molecules bind with species $G$ molecules and inhibit the cell growth. This negative feedback enables the total cell population to maintain a steady state that tracks the reference signal, which can be set by tuning the basal induction of $G$.

C. The co-regulation loop for relative population ratio control

To regulate the relative population ratio between two cell strains, we design a co-regulation loop consisting of a cell dynamics module that regulates cell death, a communication module of two orthogonal quorum sensing systems, and a feedback controller module, which compares the difference between populations of two strains and actuates the antitoxin production in the feedback.

As illustrated in Figure 1b, we consider two different cell strains, Cell$_1$ and Cell$_2$, in mixed culture. Cell$_1$ produces signal molecule $S_1$ and Cell$_2$ produces signal molecule $S_2$. In each cell, toxin $T$ is produced by the activation of signal molecules released by cells of its own type. The antitoxin $A$ is actively produced by signal molecules released by cells of the other type. The antitoxin $A$ sequesters the toxin $T$ and forms a stable complex $TA^*$ to repress the death process.

We set the relative population between Cell$_1$ and Cell$_2$ to unity 1 for demonstration. When cell strain Cell$_1$ has a larger population than Cell$_2$, more $S_1$ than $S_2$ will be synthesized and released into the environment. Signal molecules $S_1$ will then diffuse into cells of both strains. In Cell$_1$, toxin $T$ will be produced in higher amount than antitoxin $A$, so the population of Cell$_1$ will decrease. The opposite occurs in Cell$_2$ since there is a higher amount of antitoxin $A$ than toxin $T$. This stops cells in strain Cell$_2$ from dying. As a result, the population of Cell$_1$ decreases and the population of Cell$_2$ increases until they are equal. This feedback control loop using two orthogonal quorum sensing systems ensures mutual population tracking and enforces the relative ratio between Cell$_1$ and Cell$_2$ to be one at steady state.

D. The dual loop control strategy

The dual loop control strategy is illustrated in Figure 1c. The total population size and the relative ratio are independently set by two reference signals. It is necessary that the three quorum sensing molecules $S_g$, $S_1$, and $S_2$ are mutually orthogonal to avoid crosstalk.

We introduce the feedback control for the two controller modules in the dual loop, which requires species that act to effectively annihilate or stabilize each other in biochemical reactions at either RNA or protein level. For example, $D$ sequesters $G$ and $A$ sequesters $T$ to form functionless complexes.

III. THE GLOBAL REGULATION LOOP

A. The biochemical reactions model

The deterministic model for global regulation corresponding to the biochemical reactions in Table I is derived accord-

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**TABLE I**

**SPECIES, PARAMETERS AND BIOCHEMICAL REACTIONS IN THE GLOBAL REGULATION**

<table>
<thead>
<tr>
<th>Species</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G$</td>
<td>Cell population</td>
</tr>
<tr>
<td>$G$</td>
<td>Species deciding the rate of cell growth and division</td>
</tr>
<tr>
<td>$D$</td>
<td>Species strongly binding with and annihilating $G$</td>
</tr>
<tr>
<td>$S_g$</td>
<td>Global quorum sensing signal molecules</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_C$</td>
<td>Cell growth rate constant</td>
<td>0.01 µM$^{-1}$min$^{-1}$</td>
</tr>
<tr>
<td>$c_{max}$</td>
<td>Carrying capacity for cell growth</td>
<td>10$^7$ mL$^{-1}$</td>
</tr>
<tr>
<td>$\gamma_c$</td>
<td>Cell division rate constant</td>
<td>0.01 min$^{-1}$</td>
</tr>
<tr>
<td>$k_g$</td>
<td>Dissociation constant for $S_g$</td>
<td>2 µM</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Hill function coefficient</td>
<td>2</td>
</tr>
<tr>
<td>$r^*$</td>
<td>Binding rate of effective annihilation</td>
<td>0.02 µM$^{-1}$min$^{-1}$</td>
</tr>
<tr>
<td>$r_G$</td>
<td>Basal production rate of $G$</td>
<td>0.3 µMmin$^{-1}$</td>
</tr>
<tr>
<td>$r_D$</td>
<td>Basal production rate of $D$</td>
<td>0.01 µMmin$^{-1}$</td>
</tr>
<tr>
<td>$k_D$</td>
<td>Maximal production rate of $D$</td>
<td>0.2 µMmin$^{-1}$</td>
</tr>
<tr>
<td>$d_G$</td>
<td>Dilation rate of $G$</td>
<td>0.01 min$^{-1}$</td>
</tr>
<tr>
<td>$d_D$</td>
<td>Dilation rate of $D$</td>
<td>0.01 min$^{-1}$</td>
</tr>
<tr>
<td>$c_S$</td>
<td>Synthesis rate of $S_g$</td>
<td>10000 µMmin$^{-1}$</td>
</tr>
<tr>
<td>$d_S$</td>
<td>Degradation rate of $S_g$</td>
<td>0.25 min$^{-1}$</td>
</tr>
<tr>
<td>$\gamma_S$</td>
<td>Dilation rate of $S_g$</td>
<td>0.01 min$^{-1}$</td>
</tr>
</tbody>
</table>

**Reactions**

| $\emptyset \xrightarrow{k_{GC}(1 - \frac{C}{M_{max}})} C$ | Logistic growth of cell |
| $\emptyset \xrightarrow{\gamma_G} G$ | Production of species $G$ by basal induction |
| $\emptyset \xrightarrow{g_{min}kD\text{Hill}(S_g)} D$ | Production of species $D$ by basal induction and activation by $S_g$ |
| $G + D \xrightarrow{c_D} GD^*$ | Annihilating binding of $G$ and $D$ |
| $G \xrightarrow{a_{S_g}} S_g, D \xrightarrow{a_{S_g}} S_g, S_g \xrightarrow{d_S} \emptyset$ | Dilation of $G$, $D$ and degradation of $S_g$ |
| $C \xrightarrow{a_S} S_g, S_g \xrightarrow{d_S} \emptyset$ | Dilation of $C$ and $S_g$ in chemostat |
ing to mass-action and Michaelis-Menten kinetics. We make the following assumptions:

- Every cell in the population contains an identical negative feedback loop.
- Cell growth follows logistic kinetics with growth rate constant $k_G$ and carrying capacity $C_{\text{max}}$, and the growth rate is proportional to the concentration of the growth regulating species $G$.
- There is dilution of the cell population and signal molecules, because the entire experiment is assumed to take place in a chemostat.
- The production of a species $x$ is characterized by its basal and maximal rates $g_x,k_x$.
- Activation by regulator $x$ is governed by a Hill function with dissociation constant $K_x$ and Hill coefficient $\beta_x$.
- Effective annihilation is achieved under the assumption that the binding reaction is much faster than the unbinding reaction and the complex is difficult to degrade.
- All species are assumed to decay with first-order kinetics.
- The synthesis of signal molecules $S_g$ occurs at a constant rate and $S_g$ reaches quasi-steady state by fast diffusion and degradation. Fast degradation can be implemented enzymatically as in [25].

We obtain the following model:

\[
\frac{dC}{dt} = k_G G \left( 1 - \frac{C}{C_{\text{max}}} \right) C - \gamma C, \\
\frac{dG}{dt} = g_G - k^+ G D - d_G G, \\
\frac{dD}{dt} = g_D + k_D S_g^{\beta} \frac{S_g^{\beta}}{K_g + S_g^{\beta}} - k^+ G D - d_D G, \\
\frac{dS_g}{dt} = c_S C - (d_S + \gamma_S) S_g.
\]  

(1)

**B. The lag compensator**

Let $C_0$ be the total population reference. It is set by tuning the basal induction rates $g_G$ and $g_D$ of $G$ and $D$, according to the equation:

\[
g_G - g_D = \frac{k_D c_S C_0^{\beta}}{K_g (d_S + \gamma_S)^{\beta} + c_S^{\beta} C_0^{\beta}}.
\]

(2)

We remark that the conditions $0 < C_0 < C_{\text{max}}$ and $g_D < g_G - g_D + k_D$ must hold for the reference $C_0$ to exist. In other words, it is not possible to tune the feedback controller to an arbitrary reference signal [26].

Assuming a feasible reference signal $C_0$, let $S_{g_0}$ and $S_g$ be the corresponding quasi-steady states of the signal molecules. Then we can define the tracking error $e_{glo}$ in global regulation as

\[
e_{glo} := C - C_0.
\]

(3)

Thus, we obtain that

\[
e_{glo} = \frac{d_S + \gamma_S}{c_S} (S_g - S_{g_0}).
\]

(5)

To emphasize the input term in our controller, we define

\[
\Delta G := D - G.
\]

(6)

By subtracting the corresponding equations that describe the dynamics of $G$ and $D$ in equation (1), we can obtain that

\[
\frac{d\Delta G}{dt} = k_D \left( \frac{(d_S + \gamma_S)^{\beta} + c_S^{\beta} C_0^{\beta}}{K_g + S_g^{\beta}} - \frac{S_g^{\beta}}{K_g + S_g^{\beta}} \right) (d_D - d_G D) - (d_D D - d_G G).
\]

(7)

where for simplicity, we have assumed $\beta = 2$.

Then equations (1) and (7) set up the following dynamical system:

\[
\frac{dC}{dt} = k_G G \left( 1 - \frac{C}{C_{\text{max}}} \right) C - \gamma C, \\
\frac{d\Delta G}{dt} = F_{glo}(C) - (d_D D - d_G G),
\]

(8)

where $F_{glo}(C) = k_D c_S C_0^{\beta} \left( \frac{S_g + S_{g_0}}{(K_g + S_g^{\beta}) (K_g + S_{g_0}^{\beta})} \right) e_{glo}$.

In order to achieve perfect adaptation and be an integral controller, the control input dynamics should only be a function of the state $C$ [26]. However, as Ang and McMillen note, this is not realistic for biological systems when protein degradation and dilution are present. Here, the rates $d_D$ and $d_G$ encompass both the degradation and the dilution processes. While we may assume that the degradation of $G$ and $D$ takes place at a low rate and can be approximated to 0, their dilution rate must equal the cell growth rate $k_C G (1 - \frac{C}{C_{\text{max}}})$. Thus, equation (8) is equivalent to

\[
\frac{d\Delta G}{dt} = F_{glo}(C) - k_G (1 - \frac{C}{C_{\text{max}}}) \Delta G.
\]

(9)

The feedback implemented in our system will be a lag compensator. It can be tuned to become closer to integral control by decreasing the cell growth rate $k_C G$. When cells divide slowly, the error will decrease. For a cell division time of 60 minutes, $k_C G \approx 0.01 \text{ min}^{-1}$. We remark that the controller will have $e_{glo} \approx 0$ at steady state given $\frac{d\Delta G}{dt} = 0$. 


C. Local stability of the global regulation loop

We convert the ODE model of global regulation into a linearized state space model to better understand the proposed lag compensator and to examine the local stability of the closed-loop system. We assume that the regulated cell population is much smaller than carrying capacity, so the cell population is only regulated by the proposed controller, i.e. \( C \ll C_{\text{max}} \). Also, the dilution rates of \( G \) and \( D \) are \( d = d_G = d_D = kCG \), so the linearized state space model is in the following form:

\[
\dot{x} = Jx + Br, \\
\dot{y} = Hx,
\]

where the state \( x \), input \( r \), output \( y \), Jacobian matrix \( J \), input matrix \( B \) and output matrix \( H \) are given by

\[
x = (C G D S_j)^T, \quad r = (g_G g_D)^T, \quad y = C,
\]

\[
J = \begin{pmatrix} kCG - yC & kCG & 0 & 0 \\ -k^+D - d_G & -k^+G & 0 & 0 \\ 0 & -k^+D & -k^+G - d_D & \frac{2k_DkCS_j}{(k_S + S_j)^2} \\ 0 & 0 & 0 & -(d_S + yS) \end{pmatrix},
\]

\[
B = \begin{pmatrix} 0 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & 0 \end{pmatrix}, \quad H = \begin{pmatrix} 1 & 0 & 0 & 0 \end{pmatrix}. \quad (10)
\]

To determine the local stability of the global regulation, we can derive eigenvalues from the Jacobian matrix. They are computed as \(-0.076, -0.120, -0.065 + 0.065j, -0.065 - 0.065j\), which all lie in the left half space. Hence, the global regulation loop is locally stable.

D. The tracking function performance of the controller

To demonstrate that the global regulation loop maintains the total population of cells, we simulate the dynamics of total population \( C \) with different induction rates \( g_G \) of \( G \), as illustrated in Figure 2a. Furthermore, we perturb the cell growth rate of one of the strains at time \( t = 1500 \) min and show robustness and adaptation in the closed loop system. We compare this with the performance of the open loop system in Figure 2b. Here, the steady state is only bounded by the carrying capacity of the consortium. The global regulation demonstrates set-point tracking of the reference as well as adaptation to a perturbation in the cell growth rate.

IV. THE CO-REGULATION LOOP

A. The biochemical reaction model

We consider cell strains Cell1 and Cell2 in mixed culture. Species, parameters and biochemical reactions are listed in Table II. We list the additional assumptions of the co-regulation model:

- There is an identical negative feedback loop in individual cells of the same strain, Cell1 or Cell2.

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>SPECIES, PARAMETERS AND BIOCHEMICAL REACTIONS IN CO-REGULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Description</td>
</tr>
<tr>
<td>( C_i )</td>
<td>Cell population of strain ( i )</td>
</tr>
<tr>
<td>( T^{(i)} )</td>
<td>Toxin in cell strain ( i )</td>
</tr>
<tr>
<td>( A^{(i)} )</td>
<td>Antitoxin in cell strain ( i )</td>
</tr>
<tr>
<td>( S_j )</td>
<td>Quorum sensing signal molecules ( j )</td>
</tr>
<tr>
<td>Parameters</td>
<td>Description</td>
</tr>
<tr>
<td>( k_{C_i} )</td>
<td>Cell growth rate constant for both strains</td>
</tr>
<tr>
<td>( d_C )</td>
<td>Cell death rate constant</td>
</tr>
<tr>
<td>( g_T )</td>
<td>Basal production rate of ( T )</td>
</tr>
<tr>
<td>( k_T )</td>
<td>Maximal production rate of ( T )</td>
</tr>
<tr>
<td>( g_A )</td>
<td>Basal production rate of ( A )</td>
</tr>
<tr>
<td>( k_A )</td>
<td>Maximal production rate of ( A )</td>
</tr>
<tr>
<td>( d_T )</td>
<td>Dilution rate of ( T )</td>
</tr>
<tr>
<td>( d_A )</td>
<td>Dilution rate of ( A )</td>
</tr>
<tr>
<td>Reactions</td>
<td>Description</td>
</tr>
<tr>
<td>( C_i \rightarrow \frac{1}{C_{\text{max}}} )</td>
<td>Logistic growth of cell strain ( i )</td>
</tr>
<tr>
<td>( C_i \rightarrow d_C T^{(i)} )</td>
<td>Death of cell strain ( i ) by toxin killing</td>
</tr>
<tr>
<td>( A^{(i)} \rightarrow k_T H(T^{(i)} + T) )</td>
<td>Production of toxin ( T^{(i)} ) by basal induction and activation by ( S_j )</td>
</tr>
<tr>
<td>( A^{(i)} \rightarrow k_A H(A^{(i)} + A^{(i)}) )</td>
<td>Production of antitoxin ( A^{(i)} ) by basal induction and activation by ( S_j )</td>
</tr>
<tr>
<td>( T^{(i)} \rightarrow A^{(i)} )</td>
<td>Annihilating binding of ( T^{(i)} ) and ( A^{(i)} )</td>
</tr>
<tr>
<td>( T^{(i)} \rightarrow d_T T^{(i)} )</td>
<td>Dilution of ( T^{(i)}, A^{(i)} ) and degradation of ( S_j )</td>
</tr>
<tr>
<td>( S_j \rightarrow d_S S_j )</td>
<td>Dilution of ( C_i ) and ( S_j ) in chemostat</td>
</tr>
</tbody>
</table>
The cell death rate is proportional to the concentration of the toxin \( T \) in the cell with constant \( d_C \).

The quorum sensing systems are orthogonal.

We derive the model for \( \{i, j\} = \{1, 2\} \) as

\[
\frac{dC_i}{dt} = \kappa_{C_i} \left( 1 - \frac{C_i + C_j}{C_{\text{max}}} \right) C_1 - d_C T^{(i)} C_i - \gamma C_i,
\]

\[
\frac{dT^{(i)}}{dt} = g_T + k_T S_i^\beta K_S + S_j^\beta - k_T^{+} T^{(i)} A^{(i)} - d_T T^{(i)},
\]

\[
\frac{dA^{(i)}}{dt} = g_A + k_A S_j^\beta K_S + S_j^\beta - k_T^{+} T^{(i)} A^{(i)} - d_A A^{(i)},
\]

\[
\frac{dS_i}{dt} = c_S C_i - (d_S + \gamma_S) S_i.
\]

**B. The lag compensator**

We first remark that the toxin and antitoxin species \( A^{(i)} \) and \( T^{(i)} \) dilute because of cell division. Hence, \( d = d_T = d_A = \kappa_{C_i} \left( 1 - \frac{C_i + C_j}{C_{\text{max}}} \right) \). The lag compensator occurs when strong sequestration happens between \( T \) and \( A \) in the toxin-antitoxin system.

Let the relative population ratio between Cell 1 and Cell 2 be set to one, which defines a mutual tracking function. Then we can define the tracking error in the co-regulation as

\[
e_{co} := C_2 - C_1.
\]

Consider \( S_1 \) and \( S_2 \) be the corresponding quasi-steady states of the signal molecules and they are then derived as

\[
S_1 = \frac{c_S}{d_S + \gamma_S} C_1, \quad S_2 = \frac{c_S}{d_S + \gamma_S} C_2.
\]

Thus, we obtain that

\[
e_{co} = \frac{d_S + \gamma_S}{c_S} (S_2 - S_1).
\]

To emphasize the controller in Cell 2, we define

\[
\Delta T^{(2)} := T^{(2)} - A^{(2)}.
\]

By subtracting the corresponding equations describing dynamics of \( T^{(2)} \) and \( A^{(2)} \) in equation (13), we can obtain

\[
\frac{d\Delta T^{(2)}}{dt} = \left( g_T - g_A \right) \left( \frac{S_2^\beta K_S + S_2^\beta}{K_S + S_2^\beta} - \frac{S_1^\beta K_S + S_1^\beta}{K_S + S_1^\beta} \right) - d\Delta T^{(2)}
\]

\[
= \left( g_T - g_A \right) \left( \frac{S_2^\beta}{K_S + S_2^\beta} - \frac{S_1^\beta}{K_S + S_1^\beta} \right) - d\Delta T^{(2)}
\]

\[
= k \left( \frac{S_2^\beta}{K_S + S_2^\beta} - \frac{S_1^\beta}{K_S + S_1^\beta} \right) - d\Delta T^{(2)}
\]

\[
= \frac{k K_S c_S}{d_S + \gamma_S} \left( \frac{S_1 + S_2}{K_S + S_1^2} \frac{S_2}{K_S + S_2^2} \right) e_{co} - d\Delta T^{(2)},
\]

where we set \( g_A = g_T = g, k_A = k_T = k \) for the relative population ratio to be one and \( \beta = 2 \).

Equations (12) and (17) set up the dynamical system for Cell 2:

\[
\frac{dC_2}{dt} = k_C \left( 1 - \frac{C_1 + C_2}{C_{\text{max}}} \right) C_2 - d_C T^{(2)} C_2 - \gamma C_2,
\]

\[
\frac{d\Delta T^{(2)}}{dt} = F_{co}(C_2) - d\Delta T^{(2)},
\]

where \( F_{co}(C_2) = \frac{k K_S c_S}{d_S + \gamma_S} \left( \frac{S_1 + S_2}{K_S + S_1^2} \frac{S_2}{K_S + S_2^2} \right) e_{co} \).

The feedback implemented in co-regulation is also a lag compensator, and when cells divide slowly, we will have \( e_{co} = C_2 - C_1 \approx 0 \) at steady state given \( d\Delta T^{(2)} = 0 \).

**C. Cell population dynamics depend on their growth and death rates**

The dynamics of the cell populations depend on the cell growth rate \( k_C \) and the death rate \( d_C \). The steady states of the two cell populations can switch to oscillations from fixed stable points when growth rate increases or death rate decreases. The simulation results of cell populations \( C_1 \) and \( C_2 \) illustrated in Figures 3a and 3b show that cells must grow slowly to prevent oscillations. The Hopf bifurcation diagrams demonstrate that the dynamics switch when \( k_C > 0.07 \mu M^{-1} \text{min}^{-1} \) and \( d_C < 0.025 \mu M^{-1} \text{min}^{-1} \), as in Figures 3c and 3d.

This oscillatory behavior in this two-strain mutual tracking system is caused by the delay one cell strain experiences in following population changes in the other one. When one cell strain grows fast because its growth rate is high or its death rate is low, there is a longer relative delay before the quorum sensing module is fully settled and regulates the production of toxin or antitoxin for tracking current state. This results in oscillations in both strains. Since the tracking is mutual, the oscillation of cell strains Cell 1 and Cell 2 is of same frequency and of a 180 degree phase difference.

**V. THE DUAL CONTROL LOOP**

**A. The controller performance and robustness**

To assess the behavior of the dual control loop system in response to internal set-point references on the total population and the relative population ratio, we define performance metrics of stability, response sensitivity, robustness, and adaptation to disturbances.

The set-point reference of the total population \( C_{\text{tot}} \) is fixed. We start with an initial condition \( (C_1(0), C_2(0), C_{\text{tot}}(0)) \) at time \( t_0 = 0 \). We only consider scenarios when cell population converges to steady state after a time period \( T \). At time \( t_1 \) in the interval \( [t_0, t_0 + T] \), we add perturbations on the growth rate or we change the cell numbers of one cell strain and we observe the resulting dynamics of population. The metrics are defined as

- steady state error \( e_{ss} = \frac{(C_{\text{tot}}(T) - \text{ref})}{\text{ref}} \times 100\% \),
- rise time \( t_{\text{rise}} \): the first time when \( C_{\text{tot}}(t) = \text{ref} \),
- overshoot \( = \frac{\max(C_{\text{tot}}(t)) - C_{\text{tot}}(T)}{C_{\text{tot}}(T)} \times 100\% \).
VI. DISCUSSION

In this paper, we considered a dual lag compensator to separately regulate the total population size and the relative population ratio of two cell strains in a microbial consortium. The general control strategy of dual loop controller design can be applied to any synthetic systems with sensors, comparators, and actuators. We proposed a mathematical

\begin{align}
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\text{In this paper, we considered a dual lag compensator to separately regulate the total population size and the relative population ratio of two cell strains in a microbial consortium. The general control strategy of dual loop controller design can be applied to any synthetic systems with sensors, comparators, and actuators. We proposed a mathematical}
\end{align}
model for the dual loop regulation by considering reactions and parameters from the synthetic biology literature and we implemented the resulting circuit in in-silico experiments. Our simulation results demonstrate that dual lag compensator control enables set-point tracking with adaptation for a range of total population and relative ratio reference signals. We investigated the robustness of the closed-loop system by assessing its adaptation to perturbations on the cell growth rate and also changes in cell number. The introduced response metrics of the system are representative of a realistic environment.

We provide design guidelines and predict experimental results in microbial consortia. We are constructing corresponding biological circuits and measuring preliminary data based on synthetic tools such as the ccdB/ccdA toxin-antitoxin system, gp2/RNA antisense technology and AHL based on synthetic tools such as the ccdB/ccdA toxin-antitoxin system, gp2/RNA antisense technology and AHL based on synthetic tools such as the ccdB/ccdA toxin-antitoxin system, gp2/RNA antisense technology and AHL.

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