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A THEORETIC CONTROL APPROACH IN SIGNAL-CONTROLLED METABOLIC PATHWAYS

Ramesh Garimella[1], Uma Garimella[2], and Weijiu Liu[1]

[1]Department of Mathematics
[2]Department of Biology
University of Central Arkansas
201 Donaghey Avenue, Conway, AR 72035, USA

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Abstract. Cells use a signal transduction mechanism to regulate certain metabolic pathways. In this paper, the regulatory mechanism is analyzed mathematically. For this analysis, a mathematical model for the pathways is first established using a system of differential equations. Then the linear stability, controllability, and observability of the system are investigated. We show that the linearized system is controllable and observable, and that the real parts of all eigenvalues of the linearized system are nonpositive using Routh's stability criterion. Controllability and observability are structural properties of a dynamical system. Thus our results may explain why the metabolic pathways can be controlled and regulated. Finally observer-based and proportional output feedback controllers are designed to regulate the end product to its desired level. Applications to the regulation of blood glucose levels are discussed.

1. **Introduction.** Biochemical reactions occurring in cells can be grouped into metabolic pathways containing sequences of chemical reactions in which each reaction is catalyzed by specific enzymes, and the product of one reaction is the substrate for the next one. The compounds formed at each step are the metabolic intermediates (or metabolites) that lead ultimately to the formation of an end product. Figure 1 shows a generic metabolic pathway.

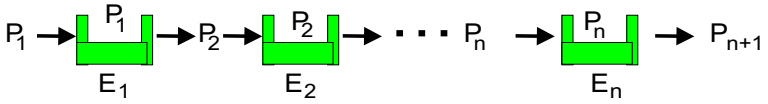

Figure 1. A generic metabolic pathway.

Cells are always in a homeostatic condition, and therefore the amount of product present or produced is always within certain range of concentrations. Homeostasis is maintained by metabolic regulation primarily by feedback inhibition. In feedback inhibition, the enzyme catalyzing the first committed step in a metabolic pathway is temporarily inactivated when the end product binds to allosteric sites of that enzyme. However there are other ways of regulating the metabolic pathways.

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One such pathway is activation or inactivation of enzymes by phosphorylation by kinases and dephosphorylation by phosphatases through cell signaling and signal transduction mechanisms.

An example of such a system of cell signaling can be seen in the regulation of blood glucose levels (Figure 2). Our bodies spend considerable effort maintaining blood glucose levels within a narrow range. The levels of glucose in circulation are monitored by the pancreas. In response to low blood glucose levels, the α cells of the pancreas produce the hormone glucagon. The glucagon binds to its specific receptors, which are on the outer surface of the plasma membrane of target cells. Binding of the hormone initiates a series of reactions that leads to the activation of the enzyme glycogen phosphorylase, which catalyze the breakdown of glycogen into glucose 1-phosphate, and then glucose 6-phosphate, and finally glucose. In addition, the binding of the hormone leads to the inhibition of the enzyme glycogen synthase, which catalyses the opposing reaction in which glucose is converted to glycogen.

In response to high blood glucose levels, the β cells of the pancreas secrets insulin. The insulin functions as an extracellular messenger molecule triggering a cascade of reactions to transport glucose into the cells and subsequently convert it into glycogen. In the presence of insulin, a signal is generated through the IRS-PI3K-PKB pathway that generates the transfer of glucose transporter 4 (GLUT4) onto the membrane. This IRS-PI3K-PKB pathway also leads to the decrease in glycogen synthase kinase-3 (GSK-3) activity, resulting in an increase in glycogen synthase activity [3, 13].

Motivated by this example, we consider a generic metabolic pathway, as shown in Figure 3. In this figure, P_1 stands for a substrate such as glycogen, P_n stands for an end product such as glucose, and P_i $(i = 2, \cdots, n-1)$ are other metabolites such as glucose 1-phosphate. E_i $(i = 1, \cdots, n)$ are enzymes such as the glycogen phosphorylase and glycogen synthase, and u_1 and u_2 are extracellular control signals such as glucagon and insulin. These control signals are usually expressed mathematically as input rates. We assume that the activity of the enzymes E_1 and E_n is controlled, like the glycogen degradation pathway, by the extracellular signals u_1 and u_2 so that the end product P_n reaches its desired level.

The aim of this paper is to use mathematical control theory [14, 21, 24] to design an output feedback controller $u_1 = u_1(p_1, p_n)$ and $u_2 = u_2(p_1, p_n)$ as a function of p_1 and p_n to regulate P_n to a desired level P_n^d, where, as usual, the lower case p_1 and p_n denote the concentrations of P_1 and P_n, respectively. We note that these controllers do not fit in the problem of regulation of blood glucose levels, because the state variable p_1 (glycogen) may not be available for feedback, but there may be biological systems in which they do fit.

Theoretical control approaches have successfully aided the research on cell signaling and signal transduction [1, 7, 10, 11, 17, 20, 28, 32, 33, 34]. Tyson, Chen, and Novak [31] pointed out that recent advances by theoretical biologists have demonstrated that molecular regulatory networks can be accurately modeled in mathematical terms. These models shed light on the design principles of biological control systems and make predictions that have been verified experimentally. Such success has been seen in the control of gene expression. The transcriptional factor NF-κB (nuclear factor κB) regulates many genes that play important roles in intra- and extracellular signaling [17]. Hoffmann, Levchenko, Scott, and Baltimore [11] presented a computational model that describes the temporal negative feedback control of NF-κB activation by the coordinated degradation and synthesis of IκB

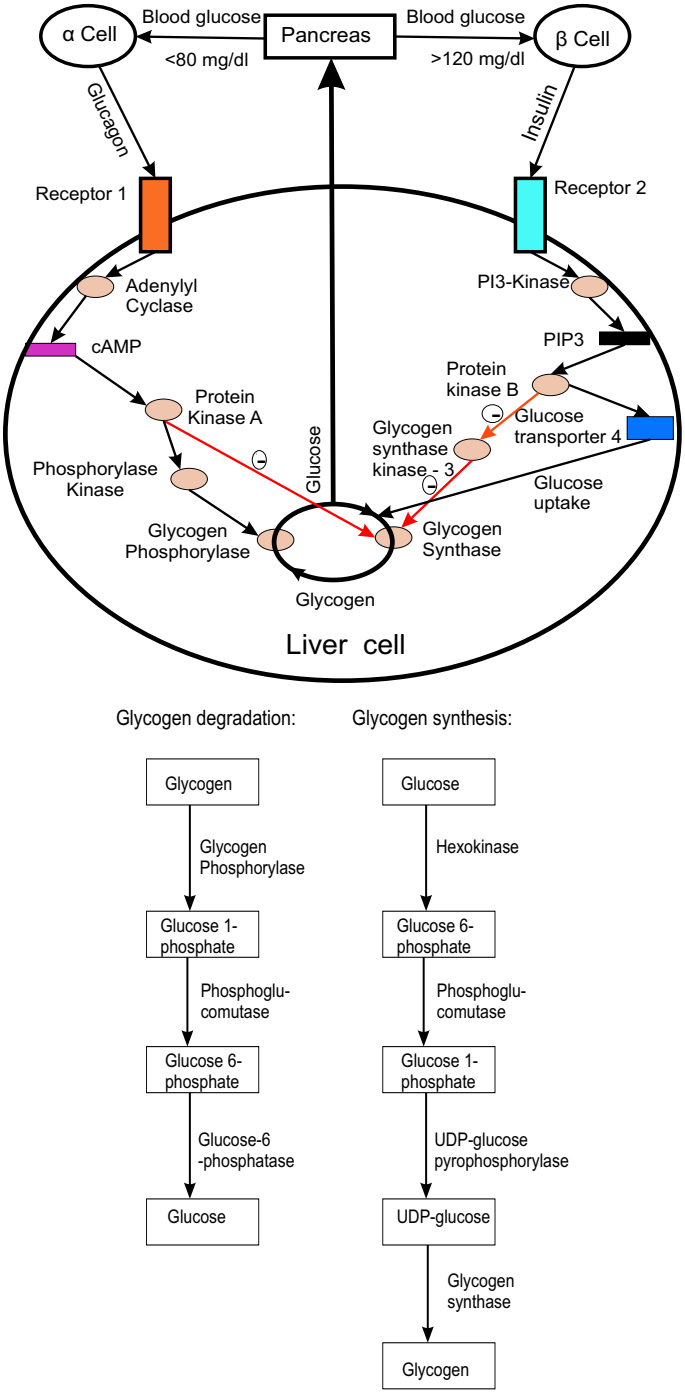

FIGURE 2. Regulation of blood glucose levels.

proteins. Using ordinary differential equations, Lipniacki, Paszek, Brasier, Luxon,

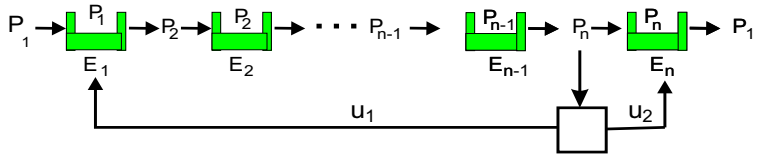

FIGURE 3. A generic signal-controlled metabolic pathway.

and Kimmel [19] modeled the two-feedback-loop regulatory module of NF-κB signaling pathway. Their model allows detailed analysis of the kinetics of the involved proteins and their complexes and gives the predictions of the possible responses of whole kinetics to the change in the level of a given activator or inhibitor. Zak, Pearson, Vadigepalli, Gonye, Schwaber, and Doyle [35] developed a continuous-time approach to identify gene expression models based on ordinary differential equations to overcome limit applicability of discrete-time expression models to common biological data sets.

In this paper, employing the law of mass action, we first model the signal-controlled metabolic pathways by a system of differential equations in Section 2. Since the system is not mathematically stable for negative initial data, we consider only its local linear stability, controllability, and observability in Section 3. Finally in Section 4, we first design a pancreas-like proportional output feedback controller and then dynamical observer-based controller.

2. **Mathematical Models.** The series of enzymatic reactions in the signal-controlled metabolic pathway in Figure 3 can be described by the following reaction diagram:

$$\begin{aligned}
u_1 \longrightarrow P_1 + E_1 \quad &\underset{k_{1,2}}{\overset{k_{1,1}}{\rightleftharpoons}} \quad C_1 \xrightarrow{k_{1,3}} P_2 + E_1 \\
P_2 + E_2 \quad &\underset{k_{2,2}}{\overset{k_{2,1}}{\rightleftharpoons}} \quad C_2 \xrightarrow{k_{2,3}} P_3 + E_2 \\
&\vdots \\
P_{n-1} + E_{n-1} \quad &\underset{k_{n-1,2}}{\overset{k_{n-1,1}}{\rightleftharpoons}} \quad C_{n-1} \xrightarrow{k_{n-1,3}} P_n + E_{n-1}, \\
u_2 \longrightarrow P_n + E_n \quad &\underset{k_{n,2}}{\overset{k_{n,1}}{\rightleftharpoons}} \quad C_n \xrightarrow{k_{n,3}} P_1 + E_n,
\end{aligned} \tag{1}$$

where k_{ij}'s denote the reaction constants, u_1, u_2 the coming control signals to activate or inactivate the enzymes E_1 and E_n, and C_i the complexes.

In real biological situations, concentrations of molecules in a cell may vary in different locations and so may not be homogeneous. However, for simplicity, we focus here on a particular tissue, and hence we can assume that the concentrations are uniform. Therefore, by the law of mass action [22, 32], the dynamics of the signal-controlled metabolic pathway (1) can be modeled by the following system of

nonlinear ordinary differential equations:

$$\begin{aligned}
\frac{dp_1}{dt} &= -k_{1,1}e_1p_1 + k_{1,2}c_1 + k_{n,3}c_n, && (2)\\
\frac{de_1}{dt} &= -k_{1,1}e_1p_1 + (k_{1,2}+k_{1,3})c_1 + u_1, && (3)\\
\frac{de_1^i}{dt} &= -u_1, && (4)\\
\frac{de_j}{dt} &= -k_{j,1}e_jp_j + (k_{j,2}+k_{j,3})c_j, \quad j = 2,\cdots,n-1, && (5)\\
\frac{dc_j}{dt} &= k_{j,1}e_jp_j - (k_{j,2}+k_{j,3})c_j, \quad j = 1,\cdots,n, && (6)\\
\frac{dp_j}{dt} &= -k_{j,1}p_je_j + k_{j-1,3}c_{j-1} + k_{j,2}c_j, \quad j = 2,\cdots,n-1, && (7)\\
\frac{dp_n}{dt} &= -k_{n,1}p_ne_n + k_{n-1,3}c_{n-1} + k_{n,2}c_n, && (8)\\
\frac{de_n}{dt} &= -k_{n,1}p_ne_n + (k_{n,2}+k_{n,3})c_n + u_2, && (9)\\
\frac{de_n^i}{dt} &= -u_2, && (10)\\
p_1(0) &= P_1^0,\ p_n(0) = P_n^0,\ e_1(0) = E_1^0,\ e_1^i(0) = E_1^{i,0},\ e_n(0) = E_n^0,\ e_n^i(0) = E_n^{i,0},\\
p_j(0) &= c_j(0) = 0,\ e_j(0) = E_j^0,\ c_1(0) = c_n(0) = 0 \quad j = 2,\cdots,n-1 && (11)
\end{aligned}$$

where, as usual, the lower case letters denote the concentrations of corresponding biological species and P_1^0, P_n^0, E_j^0 the initial concentrations of p_1, p_n, e_j, respectively. Here we have introduced the inactive form e_j^i $(j = 1, n)$ of the enzymes E_1 and E_n (the superscript i stands for inactive). Since the intermediate metabolites are difficult to measure, the observable outputs are

$$\mathbf{y} = \begin{pmatrix} p_1 \\ p_n \end{pmatrix}.$$

From the above system, we can readily derive that

$$\begin{aligned}
\frac{d}{dt}(e_j + e_j^i + c_j) &= 0, \quad j = 1, n,\\
\frac{d}{dt}(e_j + c_j) &= 0, \quad j = 2,\cdots,n-1,\\
\frac{d}{dt}\left(\sum_{j=1}^n p_j + \sum_{j=1}^n c_j\right) &= 0,
\end{aligned}$$

and then

$$\begin{aligned}
e_j + e_j^i + c_j &= E_j^0 + E_j^{i,0}, \quad j = 1, n, && (12)\\
c_j + e_j &= E_j^0, \quad j = 2,\cdots,n-1, && (13)\\
\sum_{j=1}^n p_j + \sum_{j=1}^n c_j &= P_1^0. && (14)
\end{aligned}$$

These equations reflect the conservation of enzymes and substrates. Hence the system (2)-(10) can be reduced to

$$\begin{aligned}
\frac{dp_1}{dt} &= -k_{1,1}e_1p_1 + k_{1,2}c_1 + k_{n,3}c_n, && (15)\\
\frac{de_1}{dt} &= -k_{1,1}e_1p_1 + (k_{1,2}+k_{1,3})c_1 + u_1, && (16)\\
\frac{de_1^i}{dt} &= -u_1, && (17)\\
\frac{de_j}{dt} &= -k_{j,1}e_jp_j + (k_{j,2}+k_{j,3})c_j, \quad j = 2,\cdots,n-1, && (18)\\
\frac{dp_j}{dt} &= -k_{j,1}p_je_j + k_{j-1,3}c_{j-1} + k_{j,2}c_j, \quad j = 2,\cdots,n-1, && (19)\\
\frac{dp_n}{dt} &= -k_{n,1}p_ne_n + k_{n-1,3}c_{n-1} + k_{n,2}c_n, && (20)\\
\frac{de_n}{dt} &= -k_{n,1}p_ne_n + (k_{n,2}+k_{n,3})c_n + u_2, && (21)\\
p_1(0) &= P_1^0,\ p_n(0) = P_n^0,\ e_1(0) = E_1^0,\ e_1^i(0) = E_1^{i,0},\ e_n(0) = E_n^0,\\
p_j(0) &= 0,\ e_j(0) = E_j^0, \quad j = 2,\cdots,n-1 && (22)
\end{aligned}$$

with the output

$$\mathbf{y} = \begin{pmatrix} p_1 \\ p_n \end{pmatrix}. \tag{23}$$

3. **Linear Stability and Controllability.** It is clear that the system (15)-(23) is not stable for mathematical negative initial data. Hence we study only its linear stability and controllability before we design a control law.

Throughout this paper, we consider only nonnegative solutions in accord with the biological situations.

The linearized system of the nonlinear system (15)-(21) at any equilibrium point

$$e_1^i = \bar{E}_1^i, \quad p_j = \bar{P}_j, \quad e_j = \bar{E}_j, \quad j = 1,\cdots,n$$

is given by

$$\frac{d\mathbf{x}}{dt} = \mathbf{A}\mathbf{x} + \mathbf{B}\mathbf{u}, \quad \mathbf{y} = \mathbf{C}\mathbf{x}, \tag{24}$$

where

$$\begin{aligned}
\mathbf{x} &= (p_1 - \bar{P}_1, e_1 - \bar{E}_1, e_1^i - \bar{E}_1^i, p_2 - \bar{P}_2, e_2 - \bar{E}_2, \cdots,\\
&\quad p_{n-1} - \bar{P}_{n-1}, e_{n-1} - \bar{E}_{n-1}, p_n - \bar{P}_n, e_n - \bar{E}_n)^T,\\
\mathbf{B} &= \begin{pmatrix} 0 & 1 & -1 & 0 & \cdots & 0 & 0 \\ 0 & 0 & 0 & 0 & \cdots & 0 & 1 \end{pmatrix}^T,\\
\mathbf{C} &= \begin{pmatrix} 1 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \cdots & 0 & 1 & 0 \end{pmatrix},
\end{aligned}$$

and $\mathbf{A}$ is the linearized Jacobian matrix given by

$$\mathbf{A} = \begin{pmatrix} \mathbf{A}_1 & \mathbf{F} & \mathbf{F} & \cdots & \mathbf{F} & \mathbf{F} & \mathbf{Q} \\ \mathbf{D}_1 & \mathbf{A}_2 & 0 & \cdots & 0 & 0 & 0 \\ 0 & \mathbf{D}_2 & \mathbf{A}_3 & \cdots & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & \mathbf{A}_{n-2} & 0 & 0 \\ 0 & 0 & 0 & \cdots & \mathbf{D}_{n-2} & \mathbf{A}_{n-1} & 0 \\ \mathbf{R} & \mathbf{U} & \mathbf{U} & \cdots & \mathbf{U} & \mathbf{D}_{n-1} & \mathbf{A}_n \end{pmatrix}, \tag{25}$$

$$\mathbf{A}_1 = \begin{pmatrix} -k_{1,1}\bar{E}_1 - k_{n,3} & -k_{1,1}\bar{P}_1 - k_{1,2} + k_{n,3} & -k_{1,2} + k_{n,3} \\ -k_{1,1}\bar{E}_1 & -k_{1,1}\bar{P}_1 - k_{1,2} - k_{1,3} & -k_{1,2} - k_{1,3} \\ 0 & 0 & 0 \end{pmatrix}, \tag{26}$$

$$\mathbf{A}_i = \begin{pmatrix} -k_{i,1}\bar{E}_i & -k_{i,1}\bar{P}_i - k_{i,2} \\ -k_{i,1}\bar{E}_i & -k_{i,1}\bar{P}_i - k_{i,2} - k_{i,3} \end{pmatrix}, \quad i = 2, \cdots, n-1, \tag{27}$$

$$\mathbf{A}_n = \begin{pmatrix} -k_{n,1}\bar{E}_n - k_{n,1} & -k_{n,1}\bar{P}_n \\ -k_{n,1}\bar{E}_n - k_{n,2} - k_{n,3} & -k_{n,1}\bar{P}_n \end{pmatrix}, \tag{28}$$

$$\mathbf{D}_1 = \begin{pmatrix} 0 & -k_{1,3} & -k_{1,3} \\ 0 & 0 & 0 \end{pmatrix}, \tag{29}$$

$$\mathbf{D}_i = \begin{pmatrix} 0 & -k_{i,3} \\ 0 & 0 \end{pmatrix}, \quad i = 2, \cdots, n-2, \tag{30}$$

$$\mathbf{D}_{n-1} = \begin{pmatrix} -k_{n,2} & -k_{n-1,3} + k_{n,2} \\ -k_{n,2} - k_{n,3} & k_{n,2} + k_{n,3} \end{pmatrix}, \tag{31}$$

$$\mathbf{F} = \begin{pmatrix} -k_{n,3} & k_{n,3} \\ 0 & 0 \\ 0 & 0 \end{pmatrix}, \tag{32}$$

$$\mathbf{Q} = \begin{pmatrix} -k_{n,3} & 0 \\ 0 & 0 \\ 0 & 0 \end{pmatrix}, \tag{33}$$

$$\mathbf{R} = \begin{pmatrix} -k_{n,2} & k_{n,2} & k_{n,2} \\ -k_{n,2} - k_{n,3} & k_{n,2} + k_{n,3} & k_{n,2} + k_{n,3} \end{pmatrix}, \tag{34}$$

$$\mathbf{U} = \begin{pmatrix} -k_{n,2} & k_{n,2} \\ -k_{n,2} - k_{n,3} & k_{n,2} + k_{n,3} \end{pmatrix}. \tag{35}$$

For a large n, the above system is difficult to analyze. So, in what follows, we consider only the case where $n = 2$. This simple case is of biological interest. For instance, in the regulatory network of blood glucose, only two regulatory enzymes, glycogen synthase and glycogen phosphorylase, are important.

In the case of $n = 2$, the Jacobian matrix is equal to

$$\mathbf{A} = \begin{pmatrix} -k_{1,1}\bar{E}_1 - k_{2,3} & -k_{1,1}\bar{P}_1 - k_{1,2} + k_{2,3} & -k_{1,2} + k_{2,3} \\ -k_{1,1}\bar{E}_1 & -k_{1,1}\bar{P}_1 - k_{1,2} - k_{1,3} & -k_{1,2} - k_{1,3} \\ 0 & 0 & 0 \\ -k_{2,2} & -k_{1,3} + k_{2,2} & -k_{1,3} + k_{2,2} \\ -k_{2,2} - k_{2,3} & k_{2,2} + k_{2,3} & k_{2,2} + k_{2,3} \end{pmatrix}$$

$$\left.\begin{array}{cc} -k_{2,3} & 0 \\ 0 & 0 \\ 0 & 0 \\ -k_{2,1}\bar{E}_2 - k_{2,2} & -k_{2,1}\bar{P}_2 \\ -k_{2,1}E_2 - k_{2,2} - k_{2,3} & -k_{2,1}\bar{P}_2 \end{array}\right).$$

THEOREM 3.1. *Assume that $n = 2$.*

(i) *If $\bar{E}_1 = \bar{E}_2 = 0$, then the characteristic polynomial of the Jacobian matrix $\mathbf{A}$ is equal to*

$$\det(\lambda\mathbf{I} - \mathbf{A}) = \left(\lambda + k_{1,1}\bar{P}_1 + k_{1,2} + k_{1,3}\right)\left(\lambda + k_{2,1}\bar{P}_2 + k_{2,2} + k_{2,3}\right)\lambda^3. \tag{36}$$

(ii) *If either $\bar{E}_1$ or $\bar{E}_2$ is not equal to zero, then the characteristic polynomial of the Jacobian matrix $\mathbf{A}$ is equal to*

$$\det(\lambda\mathbf{I} - \mathbf{A}) = \lambda^2\left(a_1\lambda^3 + a_2\lambda^2 + a_3\lambda + a_4\right), \tag{37}$$

and the real parts of all roots of the polynomial are non-positive, where

$$\begin{aligned}
a_1 &= 1, \\
a_2 &= k_{2,1}\bar{E}_2 + k_{2,1}\bar{P}_2 + k_{2,2} + k_{1,1}\bar{P}_1 + k_{1,3} + k_{1,2} + k_{1,1}\bar{E}_1 + k_{2,3}, \\
a_3 &= k_{1,2}k_{2,1}\bar{E}_2 + k_{2,2}k_{1,2} + k_{1,2}k_{2,3} + k_{1,3}k_{2,1}\bar{P}_2 + k_{1,2}k_{2,1}\bar{P}_2 + k_{1,1}\bar{E}_1k_{2,1}\bar{P}_2 \\
&\quad + k_{1,3}k_{2,2} + k_{1,1}\bar{E}_1k_{2,3} + k_{1,1}\bar{P}_1k_{2,1}\bar{E}_2 + k_{1,3}k_{2,3} + k_{2,3}k_{2,1}\bar{E}_2 + k_{1,1}\bar{E}_1k_{1,3} \\
&\quad + k_{1,1}\bar{P}_1k_{2,3} + k_{1,1}\bar{P}_1k_{2,1}P_2 + k_{1,1}\bar{E}_1k_{2,1}\bar{E}_2 + k_{1,3}k_{2,1}\bar{E}_2 + k_{2,2}k_{1,1}\bar{P}_1 \\
&\quad + k_{1,1}\bar{E}_1k_{2,2}, \\
a_4 &= k_{1,1}\bar{E}_1k_{1,3}k_{2,2} + k_{2,1}\bar{E}_2k_{1,1}\bar{E}_1k_{2,3} + k_{1,1}\bar{E}_1k_{1,3}k_{2,1}\bar{P}_2 + k_{2,3}k_{1,2}k_{2,1}\bar{E}_2 \\
&\quad + k_{1,3}k_{2,3}k_{2,1}\bar{E}_2 + k_{1,1}\bar{E}_1k_{1,3}k_{2,1}\bar{E}_2 + k_{2,3}k_{1,1}\bar{E}_1k_{1,3} + k_{2,3}k_{1,1}\bar{P}_1k_{2,1}\bar{E}_2.
\end{aligned}$$

Proof. All the above polynomials are computed by the Maple software. It suffices to prove that the real parts of all roots of the cubic polynomial are nonpositive. We use the Routh's stability criterion to prove it. The Routh's array for the polynomial is as follows:

$$\begin{array}{lll} \lambda^3: & a_1 & a_3 \\ \lambda^2: & a_2 & a_4 \\ \lambda^1: & b_1 & 0 \\ \lambda^0: & a_4 & \end{array}$$

where

$$b_1 = a_2a_3 - a_4.$$

We can easily check that every term in a_4 is contained in a_2a_3. So the first column of Routh's array is all positive, and then all the real parts of the roots of the cubic polynomial are negative. □

The system (24) is controllable if for any initial state $\mathbf{x}_0$ and any desired state $\mathbf{x}_f$, there exists a control $\mathbf{u}$ such that $\mathbf{x}(T) = \mathbf{x}_f$ for some $T > 0$. The system (24) is observable if any initial state can be uniquely determined by the output $\mathbf{y}(t)$ over $(0, T)$ for some $T > 0$.

THEOREM 3.2. *Assume that $n = 2$.*

(i) *If $\bar{P}_2 \neq \frac{k_{2,3}+k_{2,2}+k_{2,1}\bar{E}_2}{k_{2,1}}$, then the linear system (24) is controllable.*

(ii) *If $\bar{P}_1 \neq \frac{k_{2,3}-k_{1,2}}{k_{1,1}}$, then the linear system (24) is observable.*

Proof. It is well known [21] that it suffices to show that the Kalman controllability matrices

$$\begin{aligned}\mathcal{C}_1 &= [\mathbf{B}|\ \mathbf{AB}|\ \mathbf{A}^2\mathbf{B}|\ \mathbf{A}^3\mathbf{B}|\ \mathbf{A}^4\mathbf{B}],\\ \mathcal{C}_2 &= [\mathbf{C}^T|\ \mathbf{A}^T\mathbf{C}^T|\ (\mathbf{A}^T)^2\mathbf{C}^T|\ (\mathbf{A}^T)^3\mathbf{C}^T|\ (\mathbf{A}^T)^4\mathbf{C}^T]\end{aligned}$$

have rank 5. For this, we used the Maple software to compute the determinant of the matrix $\mathbf{M}$ consisting of the first five columns of $\mathcal{C}_1$ and obtained

$$\det(\mathbf{M}) = 2\,{k_{1,1}}^2\bar{P}_1^2 k_{1,3}(k_{2,3}+k_{2,2}-k_{2,1}(\bar{P}_2-\bar{E}_2)) \neq 0.$$

In the same way, the determinant of the matrix $\mathbf{M}$ consisting of the first five columns of $\mathcal{C}_2$ is equal to

$$\det(\mathbf{M}) = k_{1,1}k_{2,1}\bar{P}_1^2\bar{P}_2(k_{1,3}+k_{2,3})(k_{2,3}-k_{1,2}-k_{1,1}\bar{P}_1) \neq 0.$$

So they have rank 5, respectively. □

We recall that $\bar{P}_1, \bar{P}_2$, and $\bar{E}_2$ in the above theorem denote the equilibrium of the system (15)-(21), which has infinite equilibria. The equilibrium $\bar{P}_2$ of the end product is the target of regulation. Hence the controllability condition $\bar{P}_2 \neq \frac{k_{2,3}+k_{2,2}+k_{2,1}\bar{E}_2}{k_{2,1}}$ implies that if the desired level of the end product is equal to $\frac{k_{2,3}+k_{2,2}+k_{2,1}\bar{E}_2}{k_{2,1}}$, then a controller may not exist to regulate the end product to $\bar{P}_2$. The observability condition $\bar{P}_1 \neq \frac{k_{2,3}-k_{1,2}}{k_{1,1}}$ implies that if the substrate is equal to $\frac{k_{2,3}-k_{1,2}}{k_{1,1}}$ at equilibrium, then the system may not be observable. We could not find an example of biological systems that exhibit such controllability and observability phenomena; but it may be an interesting problem to study and we will continue to pursue in the future.

If we use only the end product p_n as the output $y = p_n$, then $\mathbf{C} = (0,0,0,1,0)$. Using the Maple software, we compute that the rank of

$$\mathcal{C} = [\mathbf{C}^T|\ \mathbf{A}^T\mathbf{C}^T|\ (\mathbf{A}^T)^2\mathbf{C}^T|\ (\mathbf{A}^T)^3\mathbf{C}^T|\ (\mathbf{A}^T)^4\mathbf{C}^T]$$

is equal to 4. So the linear system (24) is not observable.

Controllability and observability are structural properties of a dynamical system. Thus Theorem 3.2 may explain why the metabolic pathways can be controlled and regulated under certain circumstances.

4. Output Feedback Controllers.

For a desired level P_n^d of the end product, we now design a number of controllers to regulate it to the desired level. We start with the well-known proportional controllers.

4.1. Proportional Controllers.

At an equilibrium, we wish that the end product reaches the desired level P_n^d, that is, $\bar{p}_n = P_n^d$, where the bar $\bar{}$ denotes the steady state. It is clear that the system (15)-(21) has infinite equilibria, but the equilibrium that makes sense biologically is

$$\bar{p}_1 = P_1^0 + P_n^d - P_n^d,\ \bar{p}_j = 0\ (j = 2,\cdots,n-1),\ \bar{p}_n = P_n^d, \tag{38}$$

$$\bar{e}_1 = 0,\ \bar{e}_j = E_j^0\ (j = 2,\cdots,n-1),\ \bar{e}_n = 0, \tag{39}$$

$$\bar{e}_1^i = E_0^1,\ \bar{e}_2^i = E_n^0. \tag{40}$$

We first propose the following proportional feedback controller

$$u_1 = -K_1(p_n - P_n^d), \quad u_2 = K_2(p_n - P_n^d), \tag{41}$$

where the feedback gains K_1, K_2 are nonnegative constants.

THEOREM 4.1. *Assume that $n = 2$. Then the characteristic polynomial of the Jacobian matrix of the nonlinear system (15)-(20) with the proportional controller (41) at the equilibrium point*

$$\bar{p}_j = \bar{P}_j, \quad \bar{e}_j = 0, \quad j = 1, 2$$

is given by

$$\det(\lambda \mathbf{I} - \mathbf{A}) \quad = \quad \lambda \left(a_1 \lambda^4 + a_2 \lambda^3 + a_3 \lambda^2 + a_4 \lambda + a_5 \right), \tag{42}$$

where

$$\begin{aligned}
a_1 &= 1, \\
a_2 &= k_{2,2} + k_{1,2} + k_{1,3} + k_{2,1}\bar{P}_2 + k_{1,1}\bar{P}_1 + k_{2,3}, \\
a_3 &= k_{1,1}\bar{P}_1 k_{2,1}\bar{P}_2 + k_{2,1}\bar{P}_2 K_{\mathit{2}} + k_{1,3}k_{2,2} + k_{2,2}k_{1,2} + k_{1,2}k_{2,1}\bar{P}_2 \\
&\quad + k_{1,2}k_{2,3} + k_{2,2}k_{1,1}\bar{P}_1 + k_{1,3}k_{2,1}\bar{P}_2 + k_{1,3}k_{2,3} + k_{1,1}\bar{P}_1 k_{2,3}, \\
a_4 &= k_{1,1}\bar{P}_1 k_{1,3} K_{\mathit{1}} + k_{1,3}k_{2,1}\bar{P}_2 K_{\mathit{2}} + k_{1,1}\bar{P}_1 k_{2,1}\bar{P}_2 K_{\mathit{2}} \\
&\quad + k_{2,3}k_{2,1}\bar{P}_2 K_{\mathit{2}} + k_{1,2}k_{2,1}\bar{P}_2 K_{\mathit{2}}, \\
a_5 &= K_{\mathit{2}}\, k_{1,3}k_{2,3}k_{2,1}\bar{P}_2 + k_{1,3}K_{\mathit{1}}\, k_{2,1}\bar{P}_2 k_{1,1}\bar{P}_1 + K_{\mathit{2}}\, k_{1,2}k_{2,3}k_{2,1}\bar{P}_2 \\
&\quad + k_{2,3}k_{1,1}\bar{P}_1 k_{1,3}K_{\mathit{1}} + k_{2,2}k_{1,1}\bar{P}_1 k_{1,3}K_{\mathit{1}} + k_{1,1}\bar{P}_1 k_{2,3}k_{2,1}\bar{P}_2 K_{\mathit{2}}.
\end{aligned}$$

Moreover, if $K_1, K_2 \geq 0$ and K_1 is sufficiently smaller than K_2, then the real parts of all roots of the quartic polynomial in the above expression are negative.

Proof. The polynomial (42) is obtained by using the Maple software. We now use the Routh's stability criterion to prove that the real parts of all roots of the quartic polynomial are negative.

Routh's array for the quratic polynomial is given by

$$\begin{array}{lllll}
\lambda^4: & a_1, & a_3, & a_5 \\
\lambda^3: & a_2, & a_4 & 0 \\
\lambda^2: & b_1, & a_5 & 0 \\
\lambda^1: & c_1, & 0 \\
\lambda^0: & a_5,
\end{array}$$

where

$$\begin{aligned}
a_2 b_1 &= a_2 a_3 - a_1 a_4 \\
&= k_{2,1}{}^2 \bar{P}_2^2 K_{\mathit{2}} - k_{1,1}\bar{P}_1 k_{1,3} K_{\mathit{1}} + 2\, k_{1,1}\bar{P}_1 k_{1,3}k_{2,2} + k_{1,1}{}^2 \bar{P}_1^2 k_{2,1}\bar{P}_2 \\
&\quad + 2\, k_{1,1}\bar{P}_1 k_{2,2}k_{1,2} + 2\, k_{1,1}P_1 k_{1,3}k_{2,3} + 2\, k_{1,1}\bar{P}_1 k_{1,2}k_{2,3} + 2\, k_{2,1}\bar{P}_2 k_{1,3}k_{2,2} \\
&\quad + 2\, k_{2,1}\bar{P}_2 k_{2,2}k_{1,2} + k_{2,1}{}^2 \bar{P}_2^2 k_{1,1}\bar{P}_1 + 2\, k_{2,3}k_{1,2}k_{2,1}\bar{P}_2 + 2\, k_{2,3}k_{1,3}k_{2,1}\bar{P}_2 \\
&\quad + 2\, k_{2,3}k_{2,2}k_{1,1}\bar{P}_1 + 2\, k_{1,3}k_{1,2}k_{2,1}\bar{P}_2 + k_{1,3}k_{2,3}{}^2 + k_{1,2}k_{2,3}{}^2 + k_{1,3}{}^2 k_{2,2} \\
&\quad + k_{1,3}{}^2 k_{2,3} + k_{1,3}k_{2,2}{}^2 + k_{2,2}{}^2 k_{1,2} + k_{2,2}k_{1,2}{}^2 + k_{1,2}{}^2 k_{2,3} \\
&\quad + 2\, k_{1,3}k_{1,2}k_{2,3} + 2\, k_{1,3}k_{2,2}k_{1,2} + 2\, k_{2,3}k_{2,2}k_{1,2} \\
&\quad + 2\, k_{2,3}k_{1,3}k_{2,2} + k_{1,2}{}^2 k_{2,1}\bar{P}_2 + k_{2,2}{}^2 k_{1,1}\bar{P}_1 + k_{1,3}{}^2 k_{2,1}\bar{P}_2 + k_{2,1}{}^2 \bar{P}_2^2 k_{1,3} \\
&\quad + k_{2,1}{}^2 \bar{P}_2^2 k_{1,2} + k_{1,1}\bar{P}_1 k_{2,3}{}^2 + k_{1,1}{}^2 \bar{P}_1^2 k_{2,2} + k_{1,1}{}^2 \bar{P}_1^2 k_{2,3} + 2\, k_{1,1}\bar{P}_1 k_{1,3}k_{2,1}P_2 \\
&\quad + 2\, k_{1,1}\bar{P}_1 k_{1,2}k_{2,1}\bar{P}_2 + 2\, k_{2,1}\bar{P}_2 k_{2,2}k_{1,1}\bar{P}_1 + 2\, k_{2,3}k_{1,1}\bar{P}_1 k_{2,1}\bar{P}_2 + k_{2,2}k_{2,1}\bar{P}_2 K_{\mathit{2}},
\end{aligned}$$

$$
\begin{aligned}
c_1 b_1 a_2 &= a_2 a_3 a_4 - a_1 a_4^2 - a_2^2 a_5 \\
&= 2\,{k_{2,1}}^3 \bar{P}_2^3 k_{1,3} k_{1,2} K_2 - {k_{2,1}}^2 \bar{P}_2^2 k_{1,2} k_{1,1} \bar{P}_1 k_{1,3} K_1 \\
&\quad -{k_{1,1}}^2 \bar{P}_1^2 {k_{2,3}}^2 k_{1,3} K_1 + {k_{1,1}}^3 \bar{P}_1^3 k_{2,2} k_{2,1} \bar{P}_2 K_2 \\
&\quad -2\,k_{2,1} \bar{P}_2 k_{2,2} {k_{1,1}}^2 \bar{P}_1^2 k_{1,3} K_1 + 2\,{k_{2,1}}^2 \bar{P}_2^2 k_{2,2} {k_{1,1}}^2 \bar{P}_1^2 K_2 \\
&\quad -2\,k_{2,3} {k_{1,1}}^2 \bar{P}_1^2 k_{2,1} \bar{P}_2 k_{1,3} K_1 + k_{2,2} k_{2,1} \bar{P}_2 K_2\, k_{1,1} \bar{P}_1 k_{1,3} K_1 \\
&\quad +k_{2,2} {k_{2,1}}^2 \bar{P}_2^2 {K_2}^2 k_{1,3} + k_{2,2} {k_{2,1}}^2 \bar{P}_2^2 {K_2}^2 k_{1,1} \bar{P}_1 \\
&\quad +k_{2,2} {k_{2,1}}^2 \bar{P}_2^2 {K_2}^2 k_{2,3} + k_{2,2} {k_{2,1}}^2 \bar{P}_2^2 {K_2}^2 k_{1,2} \\
&\quad -{k_{1,3}}^2 k_{2,1} \bar{P}_2 K_2\, k_{1,1} \bar{P}_1 K_1 - {k_{1,1}}^2 \bar{P}_1^2 k_{2,1} \bar{P}_2 K_2\, k_{1,3} K_1 \\
&\quad -k_{1,2} k_{2,1} \bar{P}_2 K_2\, k_{1,1} \bar{P}_1 k_{1,3} K_1 + {k_{2,1}}^2 \bar{P}_2^2 K_2\, k_{1,1} \bar{P}_1 k_{1,3} K_1 \\
&\quad +{k_{2,1}}^3 \bar{P}_2^3 {K_2}^2 k_{1,1} \bar{P}_1 - k_{2,3} k_{2,1} \bar{P}_2 K_2\, k_{1,1} \bar{P}_1 k_{1,3} K_1 \\
&\quad +3\,k_{1,1} \bar{P}_1 {k_{1,3}}^2 k_{2,2} k_{2,1} \bar{P}_2 K_2 + 3\,{k_{1,1}}^2 \bar{P}_1^2 k_{1,3} k_{2,2} k_{2,1} \bar{P}_2 K_2 \\
&\quad +2\,k_{1,1} \bar{P}_1 k_{1,3} k_{2,2} k_{2,3} k_{2,1} \bar{P}_2 K_2 + 6\,k_{1,1} \bar{P}_1 k_{1,3} k_{2,2} k_{1,2} k_{2,1} \bar{P}_2 K_2 \\
&\quad +3\,{k_{1,1}}^2 \bar{P}_1^2 {k_{2,1}}^2 \bar{P}_2^2 k_{1,3} K_2 + {k_{1,1}}^3 \bar{P}_1^3 {k_{2,1}}^2 \bar{P}_2^2 K_2 \\
&\quad +{k_{1,1}}^2 \bar{P}_1^2 {k_{2,1}}^2 \bar{P}_2^2 k_{2,3} K_2 + 3\,{k_{1,1}}^2 \bar{P}_1^2 {k_{2,1}}^2 {P_2}^2 k_{1,2} K_2 \\
&\quad +{k_{2,1}}^3 \bar{P}_2^3 {K_2}^2 k_{1,3} + {k_{2,1}}^3 \bar{P}_2^3 {K_2}^2 k_{2,3} \\
&\quad +{k_{2,1}}^3 \bar{P}_2^3 {K_2}^2 k_{1,2} + {k_{1,2}}^3 {k_{2,1}}^2 \bar{P}_2^2 K_2 \\
&\quad +{k_{1,3}}^3 {k_{2,1}}^2 {P_2}^2 K_2 + {k_{2,1}}^3 \bar{P}_2^3 {k_{1,3}}^2 K_2 \\
&\quad +{k_{2,1}}^3 \bar{P}_2^3 {k_{1,2}}^2 K_2 - {k_{1,1}}^2 \bar{P}_1^2 {k_{1,3}}^2 {K_1}^2 \\
&\quad +3\,{k_{1,1}}^2 {P_1}^2 k_{2,2} k_{1,2} k_{2,1} \bar{P}_2 K_2 + 2\,k_{1,1} P_1 k_{2,2} k_{1,2} k_{2,3} k_{2,1} \bar{P}_2 K_2 \\
&\quad +3\,k_{1,1} \bar{P}_1 k_{2,2} {k_{1,2}}^2 k_{2,1} \bar{P}_2 K_2 - 2\,k_{2,1} \bar{P}_2 {k_{1,3}}^2 k_{2,2} k_{1,1} \bar{P}_1 K_1 \\
&\quad +2\,{k_{2,1}}^2 \bar{P}_2^2 {k_{1,3}}^2 k_{2,2} K_2 + 4\,{k_{2,1}}^2 \bar{P}_2^2 k_{1,3} k_{2,2} k_{1,1} \bar{P}_1 K_2 \\
&\quad +4\,{k_{2,1}}^2 \bar{P}_2^2 k_{1,3} k_{2,2} k_{1,2} K_2 - 2\,k_{2,1} \bar{P}_2 k_{2,2} k_{1,2} k_{1,1} \bar{P}_1 k_{1,3} K_1 \\
&\quad +4\,{k_{2,1}}^2 \bar{P}_2^2 k_{2,2} k_{1,2} k_{1,1} \bar{P}_1 K_2 + 2\,{k_{2,1}}^2 \bar{P}_2^2 k_{2,2} {k_{1,2}}^2 K_2 \\
&\quad -{k_{2,1}}^2 \bar{P}_2^2 {k_{1,1}}^2 \bar{P}_1^2 k_{1,3} K_1 + 2\,{k_{2,1}}^3 \bar{P}_2^3 k_{1,1} \bar{P}_1 k_{1,3} K_2 \\
&\quad +{k_{2,1}}^3 \bar{P}_2^3 {k_{1,1}}^2 \bar{P}_1^2 K_2 + 2\,{k_{2,1}}^3 \bar{P}_2^3 k_{1,1} \bar{P}_1 k_{1,2} K_2 \\
&\quad -2\,k_{2,3} k_{1,2} k_{2,1} \bar{P}_2 k_{1,1} \bar{P}_1 k_{1,3} K_1 + 2\,k_{2,3} k_{1,2} {k_{2,1}}^2 \bar{P}_2^2 k_{1,3} K_2 \\
&\quad +2\,k_{2,3} k_{1,2} {k_{2,1}}^2 \bar{P}_2^2 k_{1,1} \bar{P}_1 K_2 + k_{2,3} {k_{1,2}}^2 {k_{2,1}}^2 \bar{P}_2^2 K_2 \\
&\quad -2\,k_{2,3} {k_{1,3}}^2 k_{2,1} \bar{P}_2 k_{1,1} \bar{P}_1 K_1 + k_{2,3} {k_{1,3}}^2 {k_{2,1}}^2 \bar{P}_2^2 K_2 \\
&\quad +2\,k_{2,3} k_{1,3} {k_{2,1}}^2 \bar{P}_2^2 k_{1,1} \bar{P}_1 K_2 - 2\,k_{2,3} k_{2,2} {k_{1,1}}^2 \bar{P}_1^2 k_{1,3} K_1 \\
&\quad +k_{2,3} k_{2,2} {k_{1,1}}^2 \bar{P}_1^2 k_{2,1} \bar{P}_2 K_2 + 3\,{k_{1,3}}^2 k_{1,2} {k_{2,1}}^2 \bar{P}_2^2 K_2 \\
&\quad +6\,k_{1,3} k_{1,2} {k_{2,1}}^2 \bar{P}_2^2 k_{1,1} \bar{P}_1 K_2 + 3\,k_{1,3} {k_{1,2}}^2 {k_{2,1}}^2 \bar{P}_2^2 K_2 \\
&\quad -{k_{1,3}}^2 {k_{2,3}}^2 k_{1,1} \bar{P}_1 K_1 - k_{1,2} {k_{2,3}}^2 k_{1,1} \bar{P}_1 k_{1,3} K_1 \\
&\quad +{k_{1,3}}^3 k_{2,2} k_{2,1} \bar{P}_2 K_2 + {k_{1,3}}^2 k_{2,2} k_{2,3} k_{2,1} \bar{P}_2 K_2 \\
&\quad +3\,{k_{1,3}}^2 k_{2,2} k_{1,2} k_{2,1} \bar{P}_2 K_2 - {k_{1,3}}^2 {k_{2,2}}^2 k_{1,1} \bar{P}_1 K_1 \\
&\quad +{k_{1,3}}^2 {k_{2,2}}^2 k_{2,1} \bar{P}_2 K_2 + 2\,k_{1,3} {k_{2,2}}^2 k_{1,1} \bar{P}_1 k_{2,1} \bar{P}_2 K_2 \\
&\quad +2\,k_{1,3} {k_{2,2}}^2 k_{1,2} k_{2,1} \bar{P}_2 K_2 - {k_{2,2}}^2 k_{1,2} k_{1,1} \bar{P}_1 k_{1,3} K_1 \\
&\quad +2\,{k_{2,2}}^2 k_{1,2} k_{1,1} P_1 k_{2,1} \bar{P}_2 K_2 + {k_{2,2}}^2 {k_{1,2}}^2 k_{2,1} \bar{P}_2 K_2
\end{aligned}
$$

$$
\begin{aligned}
&+3\,k_{2,2}{k_{1,2}}^2k_{1,3}k_{2,1}P_2K_{\mathit{2}} + k_{2,2}{k_{1,2}}^2k_{2,3}k_{2,1}\bar{P}_2K_{\mathit{2}}\\
&+k_{2,2}{k_{1,2}}^3k_{2,1}\bar{P}_2K_{\mathit{2}} + 2\,k_{1,3}k_{2,2}k_{1,2}k_{2,3}k_{2,1}\bar{P}_2K_{\mathit{2}}\\
&-2\,k_{2,3}k_{2,2}k_{1,2}k_{1,1}\bar{P}_1k_{1,3}K_{\mathit{1}} - 2\,k_{2,3}{k_{1,3}}^2k_{2,2}k_{1,1}\bar{P}_1K_{\mathit{1}}\\
&+3\,{k_{1,2}}^2{k_{2,1}}^2\bar{P}_2^2k_{1,1}\bar{P}_1K_{\mathit{2}} - {k_{2,2}}^2{k_{1,1}}^2\bar{P}_1^2k_{1,3}K_{\mathit{1}}\\
&+{k_{2,2}}^2{k_{1,1}}^2{P_1}^2k_{2,1}\bar{P}_2K_{\mathit{2}} + 3\,{k_{1,3}}^2{k_{2,1}}^2\bar{P}_2^2k_{1,1}\bar{P}_1K_{\mathit{2}}\\
&-{k_{2,1}}^2\bar{P}_2^2{k_{1,3}}^2k_{1,1}\bar{P}_1K_{\mathit{1}} - 3\,k_{2,3}{k_{2,1}}^2{P_2}^2k_{1,3}K_{\mathit{1}}\;k_{1,1}\bar{P}_1\\
&-3\,{k_{2,3}}^2k_{2,1}\bar{P}_2k_{1,1}\bar{P}_1k_{1,3}K_{\mathit{1}} - 6\,k_{2,3}k_{2,1}\bar{P}_2k_{2,2}k_{1,1}\bar{P}_1k_{1,3}K_{\mathit{1}}\\
&-3\,k_{2,2}{k_{2,1}}^2\bar{P}_2^2k_{1,3}K_{\mathit{1}}\;k_{1,1}\bar{P}_1 - 3\,{k_{2,2}}^2k_{2,1}\bar{P}_2k_{1,1}\bar{P}_1k_{1,3}K_{\mathit{1}}\\
&-{k_{2,1}}^3\bar{P}_2^3k_{1,3}K_{\mathit{1}}\;k_{1,1}\bar{P}_1 - 3\,{k_{2,3}}^2k_{2,2}k_{1,1}\bar{P}_1k_{1,3}K_{\mathit{1}}\\
&-3\,k_{2,3}k_{2,2}{}^2k_{1,1}\bar{P}_1k_{1,3}K_{\mathit{1}} - {k_{2,2}}^3k_{1,1}P_1k_{1,3}K_{\mathit{1}} - {k_{2,3}}^3k_{1,1}\bar{P}_1k_{1,3}K_{\mathit{1}}.
\end{aligned}
$$

In the expression of a_2b_1, the only negative term is $-k_{1,1}\bar{P}_1k_{1,3}K_{\mathit{1}}$. In the expression of $c_1b_1a_2$, we checked it carefully and found that all negative terms contain K_1. Hence if K_1 is sufficiently smaller than K_2, then the first column of Routh's array is all positive and all the real parts of the roots of the quartic polynomial are negative. □

To estimate how much smaller K_1 is than K_2, we need to estimate the expression of $c_1b_1a_2$.

The mathematical assumption that K_1 is sufficiently smaller than K_2 is of biological meaning. In the regulatory network of blood glucose, u_1 is the glucagon input rate and u_2 is the insulin input rate. This assumption suggests that more insulin than glucagon is found.

Although $\mathbf{A}$ has a zero eigenvalue, its multiplicity is equal to 1 and smaller than the multiplicity 3 of zero eigenvalue of $\mathbf{A}$ without control, as shown in Theorem 3.1. So the proportional controller does promote stability.

We now numerically test whether the proportional controller works in regulating the end product level using the example of the glycogen degradation and synthesis pathway. The Michaelis-Menton constant for glycogen synthase is around 0.5 mM (9 mg/dl) with regard to UDP-glucose [23]. So we select the following reaction rate constants

$$k = \begin{pmatrix} .005 & .000454 & .045 \\ .005 & .000454 & .045 \end{pmatrix}$$

such that Michaelis-Menton constant $K_m = 9$ mg/dl. We assume that the initial concentrations of the active or inactive glycogen phosphorylase are 1.33/4, 1.33 (μg/100 mg), respectively (the proportionality between active and inactive is 20:80), and the initial concentrations of active or inactive glycogen synthase are 1.33, 1.33/4 (μg/100 mg), respectively (the proportionality between active and inactive is 80:20). We also suppose the initial concentrations of the glycogen and glucose are 500 and 150 (mg/100 ml), respectively. The desired glucose level is 100 (mg/100 ml), an average normal level in our bodies. We then use the MATLAB to numerically solve the system (15)-(21) with the controller (41). Figure 4 shows that with $K_1 = 0.00001$ and $K_2 = 0.00005$ the controller works well in regulating the glucose levels. Notice that $K_1 = 0.00001$ is smaller than $K_2 = 0.00005$, as claimed in Theorem 4.1.

The proportional controller can be further modified in accord with the function of the pancreas. In response to low blood glucose levels ($p_n < P_n^d$), the α cells

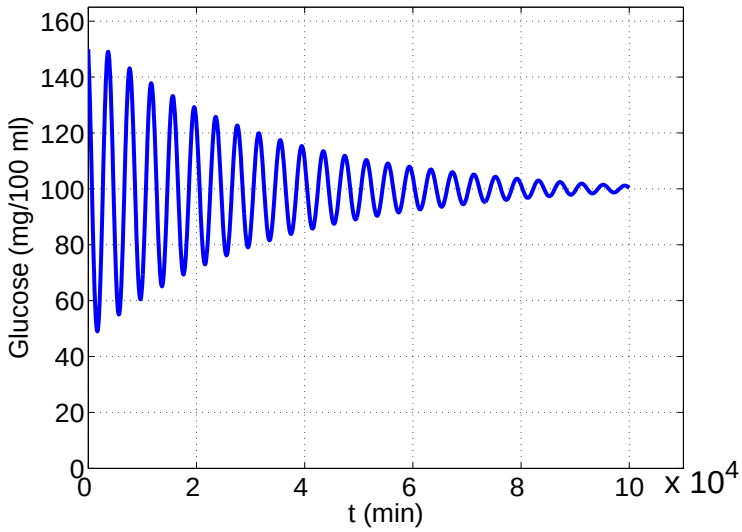

FIGURE 4. The proportional controller (41) with $K_1 = 0.00001$ and $K_2 = 0.00005$ is working in regulating the glucose levels.

of the pancreas produce the hormone glucagon, which increases the activity of the enzyme glycogen phosphorylase and decreases the activity of the enzyme glycogen synthase. In response to high blood glucose levels ($p_n > P_n^d$), the β cells of the pancreas secrete insulin which results in an increase in glycogen synthase activity, but does not impact glycogen phosphorylase. This function of the pancreas can be mathematically translated into

$$u_1 = -K_1 \min(0, p_n - P_n^d), \quad u_2 = K_2 \min(0, p_n - P_n^d) + K_3 \max(0, p_n - P_n^d), \quad (43)$$

where $-K_1 \min(0, p_n - P_n^d)$ denotes the increase of the activity of the enzyme glycogen phosphorylase by the glucagon and no impact from the insulin, $K_2 \min(0, p_n - P_n^d)$ denotes the decrease of the activity of the enzyme glycogen synthase by the glucagon, and $K_3 \max(0, p_n - P_n^d)$ denotes the increase of the activity of the enzyme glycogen synthase by the insulin. Figure 5 shows that this modified controller is also working.

4.2. **Observer-based Dynamic Controllers.** The proportional-integral controller does not exponentially stabilize the systems because the observation on only the error $p_n - P_n^d$ is not enough. Therefore, we assume that the error $p_1 - P_1^d$ is also available for feedback. This assumption is not suitable for the regulation of blood glucose levels, but may be reasonable for other metabolic pathways since the metabolic pathways are substrate-conservative. To ensure that these errors are well processed and synthesized, we propose an observer-based dynamic controller.

By Theorem 3.2, the pair $(\mathbf{A}, \mathbf{B})$ is controllable and the pair $(\mathbf{A}, \mathbf{C})$ is observable. Thus there exist a feedback gain

$$\mathbf{G} = \begin{pmatrix} g_{1,1} & g_{1,2} & \cdots & g_{1,2n+1} \\ g_{2,1} & g_{2,2} & \cdots & g_{2,2n+1} \end{pmatrix}$$

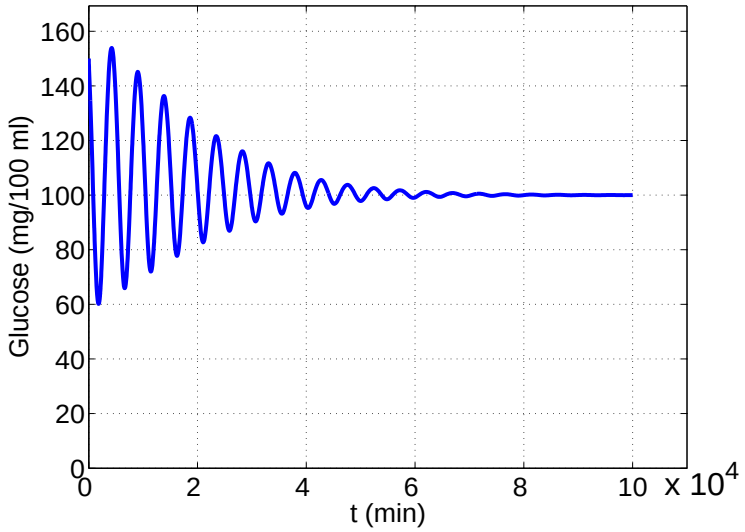

FIGURE 5. The proportional controller (43) with $K_1 = 0.00001, K_2 = 0.00005$, and $K_3 = 0.00003$ is working in regulating the glucose levels.

and an observer gain

$$\mathbf{H} = \begin{pmatrix} h_{1,1} & h_{1,2} & \cdots & h_{1,2n+1} \\ h_{2,1} & h_{2,2} & \cdots & h_{2,2n+1} \end{pmatrix}^T$$

such that both $\mathbf{A} - \mathbf{BG}$ and $\mathbf{A} - \mathbf{HC}$ are Hurwitz, that is, the real parts of their eigenvalues are negative. Then we can design an observer-based dynamic feedback controller for the linear system (24) as follows:

$$\frac{d\mathbf{x}}{dt} = \mathbf{Ax} - \mathbf{BGz}, \tag{44}$$

$$\frac{d\mathbf{z}}{dt} = (\mathbf{A} - \mathbf{BG} - \mathbf{HC})\mathbf{z} + \mathbf{HCx}. \tag{45}$$

Since

$$\det\left(\lambda\mathbf{I} - \begin{bmatrix} \mathbf{A} & -\mathbf{BG} \\ \mathbf{HC} & \mathbf{A} - \mathbf{BG} - \mathbf{HC} \end{bmatrix}\right) = \det(\lambda\mathbf{I} - (\mathbf{A} - \mathbf{BG}))\det(\lambda\mathbf{I} - (\mathbf{A} - \mathbf{HC})),$$

we have the following theorem:

THEOREM 4.2. *The feedback control system (44) and (45) is exponentially stable.*

Applying this linear controller to the original nonlinear system, we obtain the regulatory feedback system

$$\begin{aligned}
\frac{dp_1}{dt} &= -k_{1,1}e_1p_1 + k_{1,2}c_1 + k_{n,3}c_n + p_n, && (46)\\
\frac{de_1}{dt} &= -k_{1,1}e_1p_1 + (k_{1,2}+k_{1,3})c_1 - \sum_{j=1}^{2n+1} g_{1,j}z_j, && (47)\\
\frac{de_1^i}{dt} &= \sum_{j=1}^{2n+1} g_{1,j}z_j, && (48)\\
\frac{de_j}{dt} &= -k_{j,1}e_jp_j + (k_{j,2}+k_{j,3})c_j, \quad j=2,\cdots,n-1, && (49)\\
\frac{dp_j}{dt} &= -k_{j,1}p_je_j + k_{j-1,3}c_{j-1} + k_{j,2}c_j, \quad j=2,\cdots,n-1, && (50)\\
\frac{dp_n}{dt} &= -k_{n,1}p_ne_n + k_{n-1,3}c_{n-1} + k_{n,2}c_n - p_n, && (51)\\
\frac{de_n}{dt} &= -k_{n,1}p_ne_n + (k_{n,2}+k_{n,3})c_n - \sum_{j=1}^{2n+1} g_{2,j}z_j, && (52)\\
\frac{d\mathbf{z}}{dt} &= (\mathbf{A}-\mathbf{BG}-\mathbf{HC})\mathbf{z} + \mathbf{H}\begin{pmatrix} p_1-P_1^d \\ p_n-P_n^d \end{pmatrix}, && (53)\\
p_1(0) &= P_1^0,\ p_n(0)=P_n^0,\ e_1(0)=E_1^0,\ \mathbf{z}(0)=0,\ e_1^i(0)=E_1^{i,0},\ e_n(0)=E_n^0,\\
p_j(0) &= 0,\ e_j(0)=E_j^0, \quad j=2,\cdots,n-1, && (54)
\end{aligned}$$

where c_j satisfies (12) –(14).

Since the linearized system of (46)–(54) is the linear system (44)-(45), as a result of Theorem 4.2, we have

COROLLARY 4.1. *The feedback control system (46)–(54) is locally exponentially stable near its equilibrium with* $\bar{p}_1 = P_1^d$ *and* $\bar{p}_n = P_n^d$.

To numerically test the effectiveness of the observer-based controller, we use the same data from subsection 4.1. By trying different feedback gains and observer gains, we find the following gains:

$$\mathbf{G} = \begin{pmatrix} 0.0 & 0.5 & 0.0 & 0.0 & 0.05 \\ 1.9 & 1.5 & 1.6 & 0.0 & 0.08 \end{pmatrix},$$

$$\mathbf{H} = \begin{pmatrix} 0.004 & 0.002 & 0.006 & 0.0 & 0.007 \\ 0.005 & 0.0025 & 0.0 & 0.003 & 0.0002 \end{pmatrix},$$

which make $\mathbf{A}-\mathbf{BG}$ and $\mathbf{A}-\mathbf{HC}$ Hurwitz. In fact, the eigenvalues of $\mathbf{A}-\mathbf{BG}$ are

$$\begin{gathered}
-0.85546552487663,\\
-0.00686816245514 + 0.24183491547608i,\\
-0.00686816245514 - 0.24183491547608i,\\
-0.00005762913352,\\
-0.00010422107958,
\end{gathered}$$

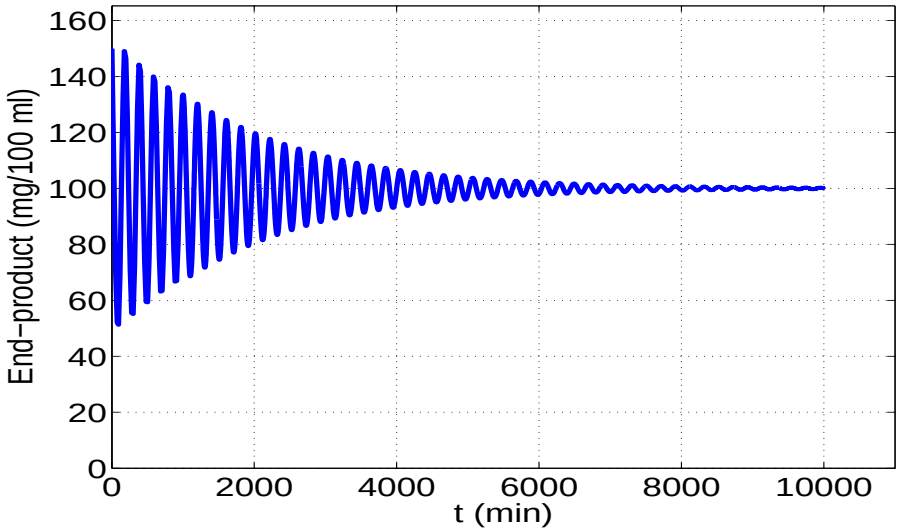

FIGURE 6. The end product is regulated to the desired level by the observer-based control law (53).

and the eigenvalues of $\mathbf{A} - \mathbf{HC}$ are

$$\begin{gathered} -0.28856712327522, \\ -0.00630648446476, \\ -0.00067034886358 + 0.00311076161066i, \\ -0.00067034886358 - 0.00311076161066i, \\ -0.00014939453286. \end{gathered}$$

Figure 6 shows that the end product is regulated to the desired level 100 (mg/100 ml) by the observer-based control law (53).

Notice that the end product in Figures 4, 5, and 6 is oscillating before it reaches its equilibrium. These oscillations may reflect biological phenomena. In fact, numerous studies have established that at least two types of oscillations of glucose and insulin were observed in experiments and simulations: rapid oscillations with periods of 8-15 minutes [8, 9, 12, 15, 16] and ultradian oscillations with periods of 50-200 minutes [4, 5, 18, 25, 27, 26, 29, 30]. In our simulations no external end product like glucose is input. This could suggest that the rapid oscillations are an internal property of a biological system.

Although the observer-based control law (53) does not fit in the regulatory network of blood glucose (because we assumed that the substrate is available for feedback and this is not the case for the regulatory network of blood glucose), the oscillations in Figure 6 may indicate that similar biological systems, in which the observer-based control law (53) fits, also have such oscillation phenomenon.

In our case, after the oscillations, the end product approaches a steady state. This is because the substrate or end product is not input in a pulsatile manner. Such a pulsating input-output problem will be studied in another article.

5. **Conclusions.** We developed a mathematical model for a signal-controlled metabolic pathway using a system of differential equations. We analyzed its linear stability,

controllability, and observability. We showed that the linearized system is controllable and observable, and that the real parts of all eigenvalues of the linearized system are nonpositive using Routh's stability criterion. Controllability and observability are structural properties of a dynamical system. Thus our results may explain why the metabolic pathways can be controlled and regulated. We designed observer-based and proportional output feedback controllers, analyzed their stability, and numerically tested their effectiveness in regulating the end product to a desired level. Our results showed that the designed feedback controls may mimic the control mechanisms of signal-controlled metabolic pathways

For simplicity, we did not consider the molecular diffusion of the end-product. In real biological situations, the end product like glucose diffuses out of a cell through its membrane. Therefore, a more accurate model should take such diffusion into account and then the mathematical models will become a hybrid system of ordinary and partial differential equations. We will consider this more complicated problem in a future work.

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E-mail address: weijiul@uca.edu