CLASSICAL CONTROL THEORY APPROACH TO ENZYMATIC REACTIONS

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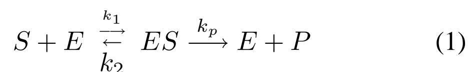
Abstract

In this paper we present some models of various enzymatic reaction systems amenable to analytic techniques of classical control theory. These models extend from simple and most abundant cases to more involved cases where (negative) feedback plays a major role at the level of a single enzyme reaction (including the so called 'feedback inhibition'). Using the above models we point at the specific feedback loop at the molecular level, if it exists, of a given reaction scheme. A simple numerical analysis of an enzyme reaction system demonstrates the applicability of our approach.

1 Introduction

All life forms on Planet Earth, with no exception, are organic by nature and are being transformed, developed and evolved through a series of remarkably complicated biochemical pathways. These pathways, whether they occur in micro-organisms such as bacteria or in higher organism such as the animal kingdom or plants, are composed of cascades of enzymatic reactions where typically, each step in the reaction pathway is carried out via a single enzyme. A typical biochemical pathway may include several enzymes and is highly organized in both space and time (i.e in certain compartments of the cell interior and in synchronization with other pathways). Most biochemical pathways are branched and some are circular (see for example the citric acid cycle, [1] which is a principle pathway in both the anabolic and catabolic phases of the cell metabolism).

Excluding recent discoveries [2], all known enzymes are proteins which are encoded by a specific gene in the cell genome. The role and function of these remarkable molecules, still mysterious to some extent, were studied extensively during the second half of the last century along with the advances made in protein chemistry and later on in molecular biology. In principle, the function of a single enzyme is exerted by increasing the rate by which a certain (and highly specific) reaction system is driven from a non-equilibrium initial state to a final chemical (dynamical) equilibrium state. Once an equilibrium is reached the presence of the enzyme (say in a test tube) can not change the ratios of the reaction components (i.e reactants to products), [1], [3]. Remarkably, during the entire course of the chemical enzymatic reaction, the enzyme molecule possesses a series of transitory states however, at the final stage of the overall reaction the enzyme molecule is freely released, ready to begin a second cycle. The study of the chemical kinetics of enzymatic reaction was greatly advanced since the pioneering works of the first half of the last century [3]. The reaction scheme for the simplest case is described by the following



$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_p} E + P \qquad (1)$$

where E is the enzyme, S is the substrate, ES is the enzyme-substrate complex and where k_1 and k_2, k_p are second-order and first-order rate constant, respectively. Note that the left hand step is reversible whereas the sec-

ond step is irreversible (the latter can be easily relaxed).

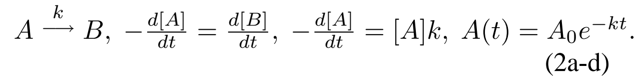
The above reaction scheme accounts for a fraction of the large variety of enzyme reactions. Typically, key enzymes in a given biochemical pathway are allosteric, most of them are composed of several sub-units with multiple sites that regulate the enzyme kinetic and thermodynamic features. The latter more involved cases include enzymes that act simultaneously on two or more substrate molecules to produce two or more self products. An interesting case is the case where a self product of a specific enzyme reaction (driven by a single enzyme) acts also as an inhibitor of the same enzyme. Clearly, as seen through the eyes of control engineers (or control theoreticians) the ' hidden technology' of control engineering plays a significant role in these elementary hardware of life itself (how could it not be ?).

We intend to explore, in this work, mainly by modeling and as a starting point, enzymatic reactions as processes that are amenable to the analytical techniques used in classical control theory. An attempt in this direction has been made by [4], however the arguments there were heuristic by nature and no quantitative aspects were considered. In the present work we start by modeling simple enzymatic systems, (with and without inhibitory mechanism) and we proceed by considering more involved systems, where the presence of feedback loop may be of a major functional significance. In order to clarify the derivations made here, we present some basic principles of chemical kinetics. We also demonstrate via a simple example, the applicability of our models.

Notation: Throughout the paper the sign '[ ]' stands for chemical concentration (usually in Molar units, but note that enzyme concentration is typically around micro-Molar units and that of the substrate around mili-Molar). Derivatives of reactants or products are chemical velocities. A positive velocity represents rate of formation and a negative one represent the rate of degradation (note that no distinction is made here between rate and velocity). Small 'k's' represent chemical rate constant, where large 'K's' are chemical equilibrium constants.

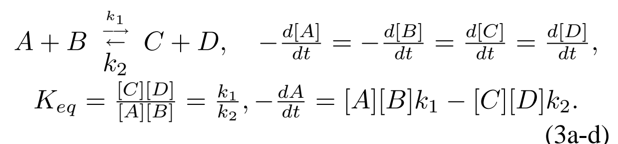
2 Some Basic Facts from Chemical Kinetics

Since the subject material of this research topic is not usually within the scope of knowledge of control scientists, we bring some necessary basic facts from the field of chemical kinetics. Consider the following chemical reaction and it's related equations:



$$A \xrightarrow{k} B,\ -\frac{d[A]}{dt} = \frac{d[B]}{dt},\ -\frac{d[A]}{dt} = [A]k,\ A(t) = A_0 e^{-kt}. \quad \text{(2a-d)}$$

The reaction of (2a) is not reversible and in principle can not reach chemical equilibrium. The mole ratio of the reaction components dictates the velocities relation as in (2b) where (2c) is a consequence of the law of mass action [5] applied to experimental data. The solution of latter simple first order equation is given in (2d). Note that taking Laplace transform of both sided of (2c), a single integrator system is obtained. However, in contrast to, say, electrical systems the amount (i.e the number of molecules) of B produced is related, at any given time, to the amount of A consumed. The latter is a basic principle that is considered in all the derivations made in this paper. Note also that kinetic differential equations are not necessarily related to the formal ratios of the reaction components. However, in the case where a chemical reaction depicts the dynamics at the molecular level (i.e the number and identity of the colliding molecules [5]), the reaction which is called an **elementary reaction** has unique significance. Elementary reactions constitute, **at the molecular level** a given chemical mechanism and are formulated relying on some experimental data and a proposed model for the reaction mechanism. Once an elementary reaction is formulated (say (2a)), the differential rate equation is immediately determined (say (2c)). We bring this fact here in order to emphasize that all the enzymatic reactions we deal with are composed of elementary steps (which were, usually, proven using a variety of physico-chemical techniques). Consider next the following reversible **elementary** reaction and it's related equations:



$$A + B \underset{k_2}{\overset{k_1}{\rightleftarrows}} C + D, \quad -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = \frac{d[C]}{dt} = \frac{d[D]}{dt},$$
$$K_{eq} = \frac{[C][D]}{[A][B]} = \frac{k_1}{k_2}, -\frac{dA}{dt} = [A][B]k_1 - [C][D]k_2. \quad \text{(3a-d)}$$

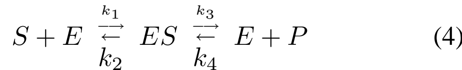
The above reaction is a second order reaction characterized by the equilibrium constant K_{eq}, which can proceed to a chemical equilibrium given the initial concentrations,

at time t_0 of $[A(t_0)], [B(t_0)]$ and $[C(t_0)], [D(t_0)]$,. Note that the reaction can proceed from a non equilibrium state to an equilibrium (where all concentrations are constant) by a net reaction of either the leftward or the rightward direction, depending on the initial concentrations of the system components. In the context of this research, assuming that (3a) depicts some biological reaction, the addition of a specific enzyme for this reaction will enhance the tendency of the reaction towards equilibrium (in some cases by six orders of magnitude !!!) but it will never change the equilibrium constant [5], [3]. Finally, as is traditionally assigned in enzymology, the left hand components of (3a) are called the substrates (in the simplest case only one substrate) and the right hand components are called the products. In the sequel we take, without loss of generality, $t_0 = 0$.

3 Problem Formulation and Results

3.1 Simple Michaelis-Menten enzymatic mechanism- I

We consider the following Michaelis-Menten enzymatic reaction system [3]:



$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \underset{k_4}{\overset{k_3}{\rightleftarrows}} E + P \tag{4}$$

where ES is the enzyme-substrate complex and where we note that the reaction of $E + P \overset{k_4}{\longrightarrow} ES$ is abolished. The rate of the product P formation is described by the following kinetic equation:

$$v = \frac{d[P]}{dt} = \frac{[S]V_{max}}{[S] + K_M}. \tag{5}$$

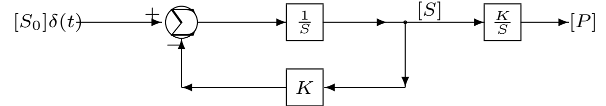
The above equation refers to the velocity of the product formation $v = \frac{d[P]}{dt}$ as a function of the substrate concentration where the total concentration of the enzyme is conserved (ie $[E_{total}] = [E_{free}] + [ES] = constant$). The derivation of (5) can be found in most introductory books of General Biochemistry (see [1], page 214) Traditionally three cases of substrate concentrations are considered by the experimental biochemist:

Case-1: low substrate concentration

In the case where $[S] << K_M$ we obtain

$$v = \frac{d[P]}{dt} = \frac{[S]V_{max}}{K_M} = K[S], \quad K = \frac{V_{max}}{K_M}. \tag{6}$$

Applying Laplace transform on the latter equation we obtain $P(s) = \frac{KS(s)}{s}$, where $P(s)$ and $S(s)$ are the Laplace transforms of $[P]$ and $[S]$, respectively. In the case where the amount of S (i.e number of moles) at a given concentration $[S]$ is practically unlimited (say a constant supply of S) one obtains a single integrator behavior of the reaction system, where S is taken as a step function input signal. In the (usual) case where the amount of S is limited (i.e $[S]$ decreases as the reaction proceeds) we obtain the following block diagram:



Case-2: mid substrate concentration

In the case where $[S] \cong K_M$, the initial velocity of the product concentration can be derived around a given set point, say $[S_0]$, for example $[S_0] = K_M$ for which $v = \frac{V_{max}}{2}$. The following linearization procedure is applied :

Defining

$$F([S]) \triangleq v = \frac{d[P]}{dt},$$

we obtain, taking a Taylor series around $[S_0]$ the following linear approximation:

$$F([S]) = F([S_0]) + \frac{\partial F}{\partial [S]}|_{[S_0]}([S] - [S_0]).$$

Defining $\bar{S} \triangleq [S] - [S_0]$ we obtain:

$$\tilde{F}(S) = F([S_0]) + \frac{\partial F}{\partial [S]}|_{[S_0]}\bar{S}.$$

Considering $[S_0] = K_M$ and performing the partial derivative we have:

$$\frac{\partial \tilde{F}}{\partial [S]}|_{[S_0]} = \frac{V_{max}[[S_0] + K_M] - V_{max}[S_0]}{([S_0] + K_M)^2} = \frac{V_{max}}{4K_M}.$$

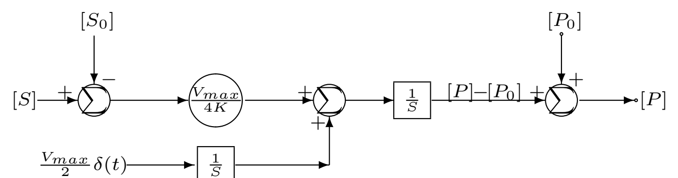
We arrive at the following kinetic equation around the chosen set point:

$$v = \frac{dP}{dt} = 0.5V_{max} + \frac{V_{max}}{4K_M}\bar{S}. \tag{7}$$

Applying Laplace transform of (7) we obtain the following :

$$\bar{P}(s) \triangleq P(s) - P_0(s) = \frac{\bar{S}(s)V_{max}}{4K_M}\frac{1}{s} + \frac{0.5V_{max}}{s^2}. \tag{8}$$

The block diagram of this case is the following:

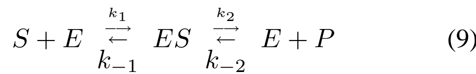


Case-3: high substrate concentration
In this case $[S] >> K_M$ therefore the enzyme reaction works at the maximal velocity of V_{max}.

Remark 1: The case where $S \cong K_M$ was shown to be the basal state (i.e intracellular concentration) at the level of the cell interior (the so called 'in vivo') [1], [3]. In this case, the enzyme works at half of it's catalytic power (recall that $v = 0.5V_{max}$)- an experimental fact that shows the flexibility and adaptability of the system to respond to changing conditions and demands of the cell metabolic need [1], [3]. Once the product P is highly 'needed' by the cell, the formation rate 'jumps' from the basal state to higher levels in relatively short time. Likewise, in the case where P is poorly needed, the rate relaxes to lower rate values (say $0.1V_{max}$), again in relatively short time. We note this fact to emphasize the point that the modeling of Case 2, though restricted to small perturbation fits, in many cases, the normal biochemistry of the cell. Evidently as the substrate concentration deviates strongly from K_M (i.e $\bar{S}$ increases), the non-linear behavior of the system is heavily accentuated therefore the simple linearization procedure of Case 2 is no longer valid.

Remark 2: A question may arise whether the linear approximation of Case 2 is biochemically relevant. Considering both (5) and (7) we note that at the 'natural' setpoint of $[S_0] = K_M$ the substrate concentration may fluctuate by 50%, causing only $\approx 5\%$ error when using the linearized approximation of (7). The latter fluctuation has been shown to cover the significant range of variation, **at a basal operating mode of the cell** [1], [3], in the intracellular concentration of $[S]$.

3.2 Simple Michaelis-Menten enzymatic mechanism-II
We consider the following Michaelis-Menten enzyme system:



$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E + P \qquad (9)$$

where we note that the product can associate with the enzyme via the rate constant k_{-2}. The later fact implies that the net reaction can proceed to either $S \longrightarrow P$ or $P \longrightarrow S$, depending on the initial concentration of both substances. Strictly speaking, all enzyme-catalyzed reactions are reversible, therefore the system of (9) is more realistic then that of Section 3.1. In fact, the system of (9) involves only one central complex (i.e ES) whereas in reality the system involves a second central complex of EP. Restricting our inquiry to (9), the kinetic equation for the later reaction is (see [3], page 29):

$$\dot{y} \triangleq \frac{dP}{dt} = v_{net} = \frac{\frac{V_{max_f}}{K_s}u - \frac{V_{max_r}}{K_p}y}{\Delta}, \; \Delta \triangleq 1 + \frac{u}{K_s} + \frac{u}{K_p},$$

where $u \triangleq [S],\ y \triangleq [P]$ and where $K_s = \frac{k_{-1}+k_2}{k_1},\ K_p = \frac{k_2+k_{-1}}{k_{-2}},\ V_{max_f} = k_2[E]_t$ and $V_{max_r} = k_{-1}[E]_t$ and where $[E]_t$ is the total amount of the enzyme. Applying similar arguments to those of Case 2 we obtain the following linear model: $\tilde{F}(u, y) \triangleq \dot{y}(u, y) =$

$$\tilde{F}(u_0, y_0) + \frac{\partial \tilde{F}}{\partial u}|_{u_0,y_0}(u - u_0) + \frac{\partial \tilde{F}}{\partial y}|_{u_0,y_0}(y - y_0)$$

Defining $\bar{u} \triangleq u - u_0,\ \bar{y} \triangleq y - y_0,\ \bar{\beta} \triangleq \frac{\partial \tilde{F}}{\partial u}|_{u_0,y_0},\ \bar{\alpha} \triangleq \frac{\partial \tilde{F}}{\partial y}|_{u_0,y_0}$, we obtain :

$$\dot{\bar{y}} = \tilde{F}(y_0, u_0) - \bar{\alpha}\bar{y} + \bar{\beta}\bar{u}, \qquad (10)$$

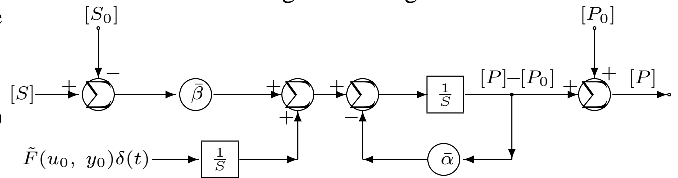
where

$$\bar{\alpha} \triangleq \frac{\frac{V_{max_r}}{K_p} + \frac{2V_{max_f}}{K_s K_p}u_0}{\Delta^2}, \quad \text{and} \bar{\beta} \triangleq \frac{\frac{V_{max_f}}{K_s} + \frac{2V_{max_r}}{K_s K_p}y_0}{\Delta^2}.$$

Choosing, as an example, the special case where $[S_0] = [P_0]$ and where the equilibrium constant of $S \overset{K_1}{\rightleftarrows} P$ is such that the net reaction proceeds in the forward direction of $S \longrightarrow P$, one obtains that for a given chosen pair of $[S_0], [P_0]$ one gets $\tilde{v}_{net} = \frac{\frac{V_{max_f}}{K_s}[S_0] - \frac{V_{max_r}}{K_p}[S_0]}{1 + \frac{[S_0]}{K_s} + \frac{[S_0]}{K_p}}$. Applying Laplace transform to (10) the following is found:

$$\bar{Y}(s) = \frac{\bar{\beta}\bar{U}(s)}{s - \bar{\alpha}} + \frac{\tilde{F}(u_0, y_0)}{(s + \bar{\alpha})s},\ \tilde{F}(u_0, y_0) = v_{net}.$$

We obtain the following block diagram:



3.3 Regulation via Energy Charge - Product Inhibition
An interesting case in enzyme biochemistry pathways is one where the total amount of the substrate and the product is constant (i.e $[S] + [P] = constant$) during the

basal state of the cell normal life [1], [3]. We consider the system of (9) and we obtain (see [3], page 120):

$$\frac{v}{V_{max}} = \frac{[S]}{K_s[1+\frac{[P]}{K_p}]+[S]}$$

Defining $y \triangleq [P],\ u \triangleq [S],\ \hat{F} \triangleq \dot{y}(t),\ \alpha \triangleq \frac{K_s}{K_p},\ C = [S]+[P]$, we obtain the following linearized kinetics: $\hat{F}(y) = \hat{F}(y_0) + \frac{\partial \hat{F}}{\partial y}|_{y_0}(y-y_0)$, where $\bar{K} \triangleq \frac{\partial \hat{F}}{\partial y}|_{y_0} =$

$$\frac{-V_{max}[C+K_s+(\alpha-1)y_0]-(\alpha-1)[C-y_0]V_{max}}{[C+K_s+(\alpha-1)y_0]^2} \tag{11}$$

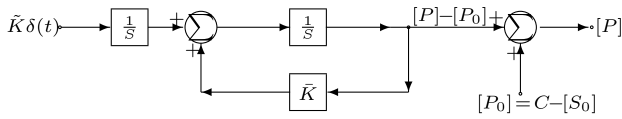
Rearranging the latter equation we have :

$$\dot{y} = \bar{K}y + \tilde{K},\quad \tilde{K} \triangleq -\bar{K}y_0 + \frac{(C-y_0)V_{max}}{C+K_s+(\alpha-1)y_0} \tag{12}$$

Applying Laplace transformation to the above equation we obtain the following:

$$\bar{Y}(s) \triangleq Y(s) - Y_0(s) = \frac{\tilde{K}}{s(s-\bar{K})}.$$

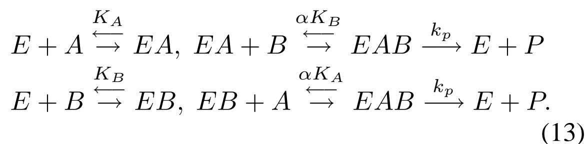
We have the following block diagram:



Note that similarly to Section 3.2 where the product is allowed to produce the substrate via the dynamics of (9), the feedback loop in Fig. 5 is different from that of Fig. 3. Whereas the latter is **inherent to all reversible chemical reactions**, here the accumulation of the product exerts a biochemically significant inhibitory effect which is indicated by the title of this section.

3.4 Random Bireactant Systems

We consider the kinetics of a multireactant enzyme systems where we treat the special case in which the enzyme can bind randomly to either substrates A or B to produce the product P via a complex EAB, as in the following multi-step reaction (see [3], page 274):



$$E+A \overset{K_A}{\leftrightarrows} EA,\ EA+B \overset{\alpha K_B}{\leftrightarrows} EAB \xrightarrow{k_p} E+P$$
$$E+B \overset{K_B}{\leftrightarrows} EB,\ EB+A \overset{\alpha K_A}{\leftrightarrows} EAB \xrightarrow{k_p} E+P. \tag{13}$$

The kinetic analysis of the above reaction is based on the assumption of a rapid equilibrium of all the reversible steps in (13), in comparison with the last catalytic step (the so called ”rate-limiting step”). Note also that the reaction is not sensitive to the order by which both substrates A and B bind to the enzyme. However, once the enzyme binds to one of the substrates say A, the equilibrium constant of say $E+B \overset{K_B}{\leftrightarrows} EB$ is changed by a factor of α. For the reaction of (13) we have [3] :

$$v = \frac{V_{max}\frac{[A][B]}{\alpha K_A K_B}}{1+\frac{[A]}{K_A}+\frac{[B]}{K_B}+\frac{[A][B]}{\alpha K_A K_B}}\ . \tag{14}$$

Rearranging the above and defining $\bar{\alpha} = \alpha K_A K_B,\ \bar{\beta} = \alpha K_B$ and $\bar{\gamma} = \alpha K_A$ the kinetic equation of the above reaction system is $v(t) = \frac{d[P]}{dt}$

$$= \frac{[A][B]V_{max}}{\bar{\Delta}},\quad \bar{\Delta} = \bar{\alpha} + \bar{\beta}[A] + \bar{\gamma}[B] + [A][B].$$

Defining further

$$\tilde{F}(u_1,u_2) \triangleq v,\ u_1 \triangleq [A] \text{ and} u_2 \triangleq [B],$$

we have: $\tilde{F}(u_1,u_2) = \tilde{F}(u_{1_0},u_{2_0})$

$$+\frac{\partial \tilde{F}}{\partial u_1}|_{u_{1_0},u_{2_0}}(u_1-u_{1_0}) + \frac{\partial \tilde{F}}{\partial u_2}|_{u_{1_0},u_{2_0}}(u_2-u_{2_0}).$$

Defining $\bar{u}_1 = u_1 - u_{1_0},\ \bar{u}_2 = u_2 - u_{2_0}$ we obtain the following linearized relation :

$$\hat{F}(u_1,u_2) = \tilde{F}(u_{1_0},u_{2_0}) + \tilde{\alpha}\bar{u}_1 + \tilde{\beta}\bar{u}_2, \tag{15}$$

where

$$\tilde{\alpha} \triangleq \frac{\partial \tilde{F}}{\partial u_1}|_{u_{1_0},u_{2_0}} = \frac{u_{2_0}V_{max}[\bar{\Delta} - u_{1_0}[\bar{\beta}+u_{2_0}]]}{\bar{\Delta}^2},$$

$$\tilde{\beta} \triangleq \frac{\partial \tilde{F}}{\partial u_2}|_{u_{1_0},u_{2_0}} = \frac{u_{1_0}V_{max}[\bar{\Delta} - u_{2_0}[\bar{\gamma}+u_{1_0}]]}{\bar{\Delta}^2}.$$

Similarly to Case 2 the set point of the above analysis can be chosen as $\tilde{F}(u_{1_0},u_{2_0}) = 0.5V_{max}$. Choosing, for example $[A_0] = [B_0]$, the initial concentration of A can be easily recovered from (14). We have:

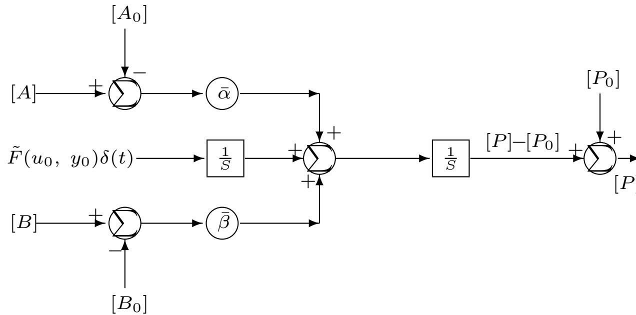
$$\tilde{F}(u_{1_0},u_{2_0}) = 0.5V_{max} = \tilde{F}([A_0],[B_0]V_{max}) =$$

$$\frac{V_{max}[A_0]^2}{\bar{\alpha}+[A_0](\bar{\beta}+\bar{\gamma}+[A_0])}.$$

Applying the Laplace transform to (15) we obtain:

$$\bar{P}(s) \triangleq P(s) - P_0(s) = \frac{\tilde{F}(u_{1_0},u_{2_0})}{s^2} + \frac{\tilde{\alpha}\bar{U}_1(s)}{s} + \frac{\tilde{\beta}\bar{U}_2(s)}{s}.$$

We obtain the following block diagram :



Remark 3: Among the most complex of all enzyme-catalyzed mechanisms, we find a large number of enzymes that are composed of two or more subunits. These enzymes, generally known as **allosteric enzymes** [1],[3], exhibit a complex behavior. In general, these enzymes are composed of one or more catalytic sites (where the substrates are converted into products) and, in addition, one or more regulatory sites (or modulatory sites) which exert an **internal control** on the active catalytic sites. The effect of the regulatory sites is mediated through special and highly specific molecules which may include: metabolites, ATP and related compounds, metal ions, vitamin molecules (sometimes generally known as coenzymes).

4 Conclusions

In this paper we present some models of enzyme-catalyzed chemical reactions. These models, derived mainly around some physiologically sound set-points, serve as building blocks that are amenable to the analytic techniques of classical control theory. We note that the basic **kinetic derivations** that are applied here are based either on the assumption that one or more components of the reaction scheme (usually an enzyme-substrate, enzyme-product complexes [3]) are in steady state, or on the assumption of a rapid equilibrium, as in the case of Section 3.4. These assumptions simplify the kinetics of the adequate enzyme-catalyzed reaction systems and they were shown to be reasonable descriptions of the **measured** kinetic behaviors.

Motivated by the evolution theory we maintain that biochemical reaction schemes which contain components with feedback loops have evolved in such a way that the feedback loops optimize some physiological cost functions and they therefore conform to the principles of feedback control theory. It was thus interesting to isolate these components and to analyze their behavior from the control theory point of view.

Our approach is not confined to the assumption that the pathway as a whole is at steady state, which is the traditional basis of MCA [6], [7]. While the steady state assumption is reasonable in some cases, it is restrictive in many cases (such as transient responses at the onset of rather 'sleepy' biochemical pathway that is turned on by some hormone or some interluekin molecule of immune system origin). It is obvious that under a stress condition, or in cases where the cell has to respond immediately, a steady state type of response is not efficient. In these cases the relevant pathways are controlled by their feedback loops in order to shape the adequate transient response of the system. Using the models of the present work more realistic simulations of the biochemical behavior of the cell can be obtained.

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