Modelling of biochemical reaction networks

Masters thesis

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Acknowledgements

This report is the culmination of my masters thesis in cybernetics/control engineering at the university of Stavanger. My learning curve has been very steep in this project as this is a mixture of control engineering and biochemistry, which is a whole new field for me. I found it difficult to comprehend at first and it was a challenge to make sense of it all. It has been very interesting and giving experience however.

I would like to thank my family and friends for their support throughout the past five years, especially my childhood friends Kristian Løken and Kenneth Bjørnevåg for being there whenever I needed it. My mother and father Liv Berit and Geir for being inspiring and supportive and last but not least my brothers Ole Marius and Bjørn Emil, you are the best. Whenever things got too stressful I can always go back home and charge my batteries. Thank you.

I would like to thank my supervisor, Tormod Drengstig we have had regular meetings on a weekly basis and he has contributed greatly to my understanding of this interesting subject. I would also like to thank my co-supervisor Peter Ruoff for making sense of the biochemical part.

Arne Gunnar Gloppen Jørgensen
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Abstract

This report investigates signalling in reaction kinetic networks. The main topic is signalling between a substance being controlled by another substance and how this can be related to control theory.

Different types of so-called natural controllers are compared and certain properties are investigated. Natural controllers are models on how a catalyst enzyme controls, for example the concentration, of a substance. There are sixteen different combinations of signalling between these substances, however it is focused on the eight different controllers with negative feedback.

These building blocks have been shown to be accurate models of several systems in nature including, but not limited to, blood glucose, calcium uptake in the human body and nitrate concentrations in plants.

Among the properties that are investigated is pulsation, oscillation and the addition of dynamical variables. This project is a supplement to the research of Peter Ruoff, Tormod Drengstig and others at the University of Stavanger.

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Chapter 1

Introduction

1.1 Report outline

Chapter 1 is an introduction to the subject of biochemistry and Hamiltonian systems and gives a brief introduction of the relevance towards control systems.
Chapter 2 is an experimental chapter describing a model made for simulating a system with Hamiltonian oscillations and utilising it to investigate properties of the system.
Chapter 3 is an experimental chapter comparing natural controllers to each other using Simulink (www.mathworks.com).
Chapter 4 consists of discussion and conclusion.

1.2 Motivation

Several systems in nature have been found to use negative feedback and integral control. These include, but are not limited to:

- Calcium homeostasis in the human body[10].
- Temperature compensation in circadian clocks, more commonly known as biological clocks[13].
- Temperature compensation in yeast[14].
- Hormone secretion in humans[7].

- Bacterial chemotaxis[15].

However, there is not much knowledge about what the systems are made up of. The negative feedback loops in biology are suggested to consist of the building blocks of natural controllers[5], explained in 1.5.

1.3 Introduction to physical chemistry

1.3.1 Substances, catalysts, substrates and enzymes

The work in this report focuses on a substance A which is controlled by a catalyst, the enzyme E_{adapt}. An example substance can be the amount of calcium in the body or blood glucose. A catalyst is a substance that increases the reaction rate while not being consumed in the process[2]. An example of a catalyst is insulin which regulates carbohydrate and fat metabolism in the human body. The enzyme E_{adapt}, which is central in this report, controls the flow into or out of A. This is what is called enzyme kinetics in biochemistry. The initial substance is called the substrate, the catalyst is the enzyme, the end substance is called the product and the intermediate binding between the substrate and the enzyme is called the substrate-enzyme complex. See figure 1.1. Note that concentrations are not written in brackets in this report, in order to simplify the notation.

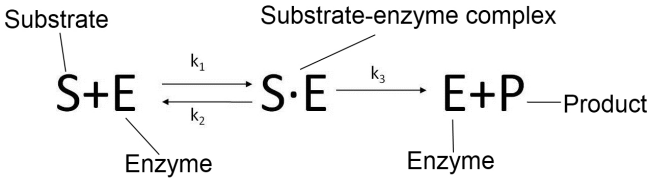


Figure 1.1: Enzyme kinetics model

Here k_1, k_2 and k_3 are the reaction velocities between the different stages. The reaction velocity rate constant here named k_2 is often called k_{-1} in biochemistry. The flow is called flux, often labelled 'J'. It is measured as the amount flowing through an area per time unit.

1.3.2 Substrate and enzyme saturation

A theory of the binding between enzyme and substrate is the "lock and key" concept. The enzyme has one slot in which the substrate fits and it is then locked into place, forming the enzyme-substrate complex [2]. The enzyme can only bind a finite amount of the substance simultaneously.

1.3.3 Inhibition

An inhibitor reduces the efficiency of an enzyme catalyst[11]. Depending on which type of inhibitor is used, the inhibition substance is either;

- Competing with the substrate forming an enzyme-inhibitor complex.
- Binding to the enzyme-substrate complex preventing the product from being formed.
- A combination of the two above.

These types of inhibition are called competitive, uncompetitive and noncompetitive, respectively, and result in a reduction of the amount of product being formed, by lowering the overall reaction velocity[2]. The inhibition-enzyme complex is inert and like the catalyst is not consumed in the reaction.

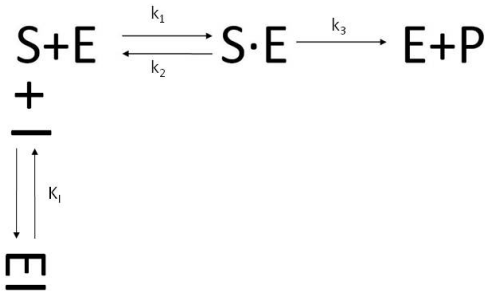


Figure 1.2: Model of competitive inhibition

Figure 1.2 shows an example of inhibition. In this case, the enzyme also binds with the inhibitor and competes with the substrate-enzyme complex. The enzyme and inhibitor form a enzyme-inhibitor complex in a reversible process, meaning that the complex returns to the separate states.

1.3.4 Reaction orders

The order of a reaction is determined by how the reaction rates are dependent of the substance concentration. The reaction order from equation (1.1)

$$S + E \longrightarrow Products \tag{1.1}$$

can be extracted from the rate law as in Eq. (1.2).

$$\frac{-dS}{dt} = k \cdot S^{\alpha} \cdot E^{\beta} \tag{1.2}$$

where S and E are the concentrations of the substances and k is the reaction rate. The exponents α and β are the reaction orders and their sum determines the reaction order [11].

A zero order reaction is the most basic form, where the reaction velocity is independent of the concentration of the substance. In other words, the sum of α and β is 0.

$$J = k \tag{1.3}$$

where J is the flux and k is the rate flow constant. In the case of a zero order reaction as in Eq. 1.3, they are equal. A first order reaction is a reaction where the flux is proportional to either of the substances.

$$J = S \cdot k \tag{1.4}$$
$$J = E \cdot k \tag{1.5}$$

where S and E are the concentrations of the substances. Second order reactions can be either of the substances squared or both multiplied by each other.

$$J = S^2 \cdot k \tag{1.6}$$
$$J = E^2 \cdot k \tag{1.7}$$
$$J = S \cdot E \cdot k \tag{1.8}$$

1.3.5 Max reaction velocity

If the amount of enzyme is kept constant while the substrate concentration is gradually increased, the first order reaction velocity will increase until it reaches a maximum, V_{MAX}. This is the point where every enzyme molecule is used to bind substrate. Beyond this point, an increase in the substrate concentration will not increase the reaction velocity[2]. This is shown in figure 1.3. The maximum velocity can be calculated with equation (1.9).

$$V_{MAX} = k_{cat} \cdot E_0 \tag{1.9}$$

where k_{cat} is the catalyst reaction velocity and E_0 is the initial enzyme concentration.

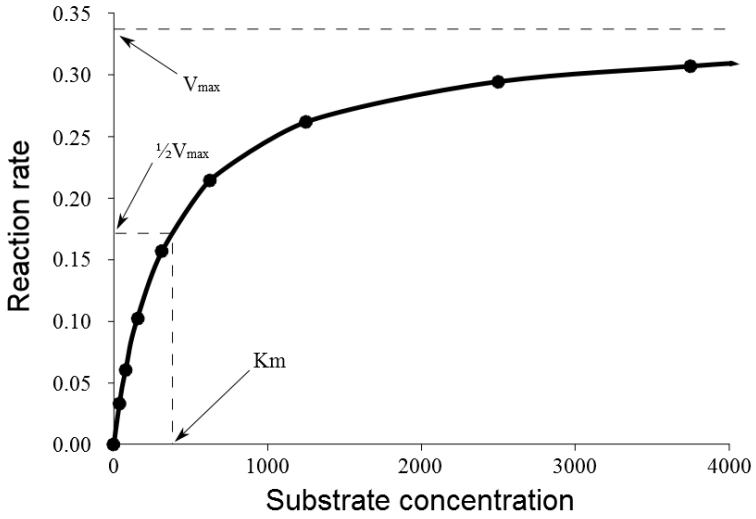


Figure 1.3: Reaction velocity graph, from wikipedia article on Michaelis Menten kinetics(http://en.wikipedia.org/wiki/Michaelis-Menten)

1.3.6 Chemical equilibrium and steady state in chemistry

Chemical equilibrium is a balanced state where usually the forward reaction proceeds at the same rate as the reverse reaction. There is no net change in the reaction rates. Steady state on the other hand is when the state variables are constant while there is a flow through the system. In other words, when

there is no change in the output with time. Unlike the equilibrium state, the net reaction rate can be different from zero.

1.4 Michaelis-Menten Kinetics

The Michaelis-Menten constant is defined as the substrate concentration at half the maximum velocity, see Fig. 1.3. Leonor Michaelis and Maud Menten developed the expression seen in equation (1.10) for the reaction velocity in terms of this constant and the substrate concentration [2].

$$V = \frac{V_{MAX} \cdot S}{K_M + S} \tag{1.10}$$

where V is the velocity, S is the substrate concentration, V_{MAX} is the maximum reaction velocity and K_M is the Michaelis-Menten constant for the enzyme in question.

The numerical value of the Michaelis-Menten constant provides information about the enzyme; a small K_M indicates that the enzyme only requires a small amount of substrate to become saturated (see section 1.3.2), while a large K_M indicates the need for a high concentration of the substrate in order to achieve the maximum reaction velocity.

A reaction can be approximated to be of order zero if the Michaelis-Menten constant is assumed to be equal to zero. In this case, the substance S in the denominator is cancelled by the numerator as seen in equation (1.11).

$$V = \frac{V_{MAX} \cdot S}{S} \tag{1.11}$$

The reaction velocity is in this case equal to V_{MAX}.

The following part is an excerpt from an assignment in "Reaction kinetic modelling" which is a doctoral course in reaction kinetics at the University of Stavanger taught by Peter Ruoff. There are two main approximations in Michaelis-Menten kinetics, one is the steady state assumption and the other is the rapid equilibrium assumption. The general model for Michaelis-Menten kinetics is shown in figure 1.4.

Figure 1.4: Catalyst system

where S is the substrate, E is the catalyst and P is the product. As one can tell from the rate equation, there is a reversible process between the substance and the catalyst and the binding between the two. There is also an irreversible process resulting in product and frees the enzyme to bind again.

The rate equations of the model are as shown in Eqs. (1.12) through (1.15)

$$\frac{dS}{dt} = -k_1 \cdot S \cdot E + k_2 \cdot SE \tag{1.12}$$

$$\frac{dP}{dt} = k_3 \cdot SE \tag{1.13}$$

$$\frac{dSE}{dt} = k_1 \cdot E \cdot S - (k_2 + k_3) \cdot SE \tag{1.14}$$

$$\frac{dE}{dt} = -\frac{dSE}{dt} \tag{1.15}$$

The numerical value of the velocity of the product can be seen in Eq. (1.16).

$$V_{num} = k_3 \cdot SE \tag{1.16}$$

if it is approximated with MM, it becomes as in Eq. (1.17).

$$V_M = \frac{Vmax \cdot S}{K_M + S} \tag{1.17}$$

where the maximum velocity is shown in equation (1.18).

$$V_{max} = k_3 \cdot (SE + P) \tag{1.18}$$

The K_M is then replaced by the chosen approximation, either rapid equilibrium or steady state.

1.4.1 Rapid equilibrium assumption on K_M

If rapid equilibrium is assumed the value of the Michaelis-Menten constant is based on the assumption that the first part of the bonding of the substrate and the catalyst happens very quickly, while the making of the product takes a long time and can be neglected. See figure 1.5.

Figure 1.5: Rapid equilibrium assumption

Resulting in the following equation (1.19) for the Michaelis-Menten constant.

$$K_{Mre} = \frac{k_2}{k_1} \tag{1.19}$$

1.4.2 Steady state assumption on K_M

The steady state assumption calculates the Michaelis-Menten constant based on the assumption that the making of the product happens rapidly, while the change in the substrate-enzyme complex [SE] is close to zero [9].

Figure 1.6: Steady state assumption

Resulting in equation (1.20) for the Michaelis-Menten constant.

$$K_{Mss} = \frac{k_2 + k_3}{k_1} \tag{1.20}$$

1.4.3 Recommended reading

Much of the introductory material in reaction chemistry is derived from the book Physical Chemistry for the Biosciences [Chang,2005], chapter 9 and 10

is recommended reading for procuring a basic understanding of the concepts for someone new to reaction kinetics.

1.5 Introduction to natural controllers

The natural controllers consist of sixteen types of combinations of signals between a substrate and an enzyme. Eight of these are positive feedback loops and therefore not interesting in this situation.

The remaining negative feedback controllers can be divided into two groups based on the signalling between the substrate and enzyme. These are used to explain the negative feedback systems that are found in nature. The controllers are shown in figure 1.7.

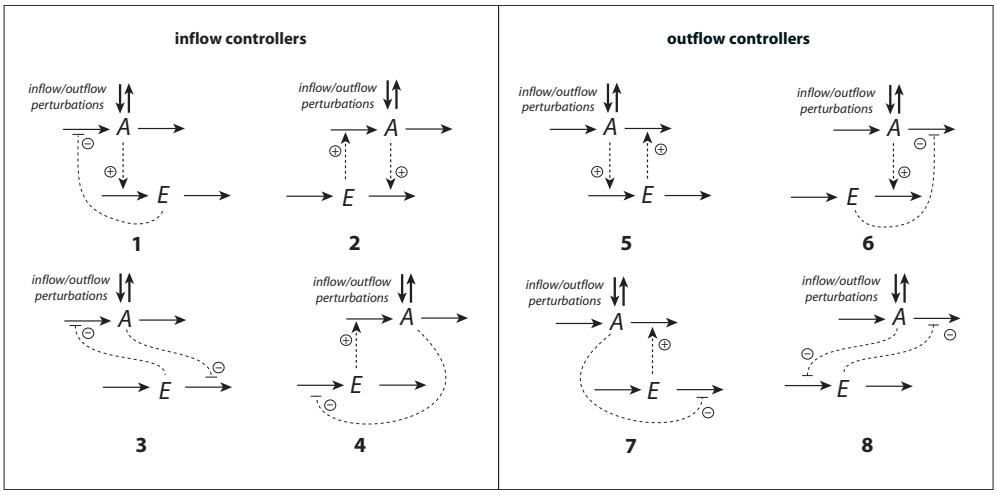


Figure 1.7: Natural controller overview chart

The enzyme in the inflow controllers either activate or inhibits the inflow of the substrate. In controller 1 in figure 1.7, an increased amount of substrate causes an increase in the inflow of the enzyme. This is because the inflow of the enzyme is activated by the substrate. The increase in enzyme causes the inflow of the substrate to decrease, because the enzyme inhibits the inflow of the substrate. In this report, the inflow controllers are named upstream controllers. Figure 1.8 shows an upstream activation controller type 1 with a step in the substrate, while the enzyme is at a steady state. An increase in the enzyme is seen before the substrate enters the steady state.

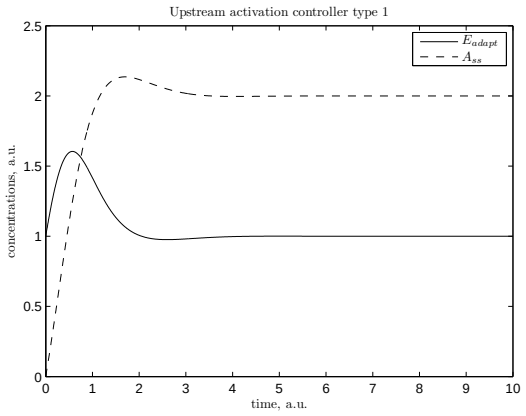


Figure 1.8: Upstream controller example

In the outflow controllers, the enzyme either inhibits or activates the outflow of the substrate. Controller 5 in figure 1.7 has the substrate activating the inflow of the enzyme. An increase in the inflow of the substrate, also causes an increase in the inflow of the enzyme. The increase in the inflow of the enzyme causes an increase in the outflow of the substrate, because the enzyme activates the outflow of the substrate. The outflow controllers are named downstream controllers in this report. Figure 1.9 shows a downstream activation type 1 controller with a step in the substrate, while the enzyme is at a steady state. A decrease in the enzyme is seen before the substrate enters the steady state.

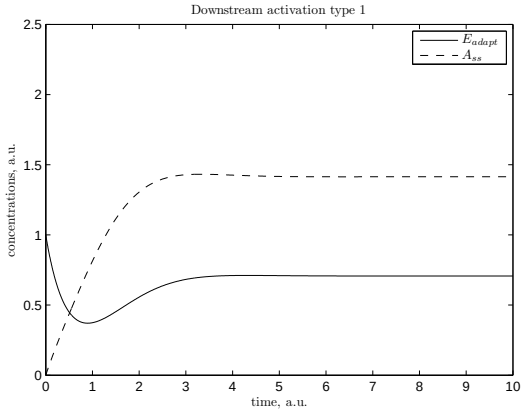


Figure 1.9: Downstream controller example

1.6 A control theory approximation for natural

The control theory version of the kinetic reaction model describes the process of the natural controller as a negative-feedback integral controller with a set-point as the reference[15][10].

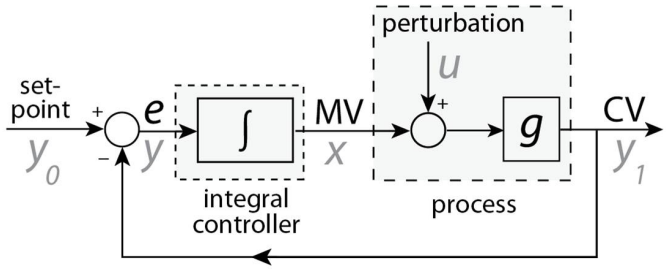


Figure 1.10: Integral controller with negative feedback [Ni et al. (2009)]

In figure 1.10, MV and CV stands for manipulated and controlled variable. The set-point is y_0, with error e and disturbance, or perturbation, u.

If the enzyme controls the flow rate of the substrate, the model can be described as a liquid storage tank system controlled by valves. The substrate concentration is then the amount of liquid in the tank and the enzyme is controlling the flow into or out of the tank by controlling a valve either in the inflow(upstream), or outflow (downstream)[3]. The perturbations , k^{in}_{pert} and k^{out}_{pert} are disturbances in and out of the tank respectively. The reason the outflow disturbance has a valve in it in Fig. 1.11 is because it is a first order flow whereas the inflow disturbance is a zero order flow.

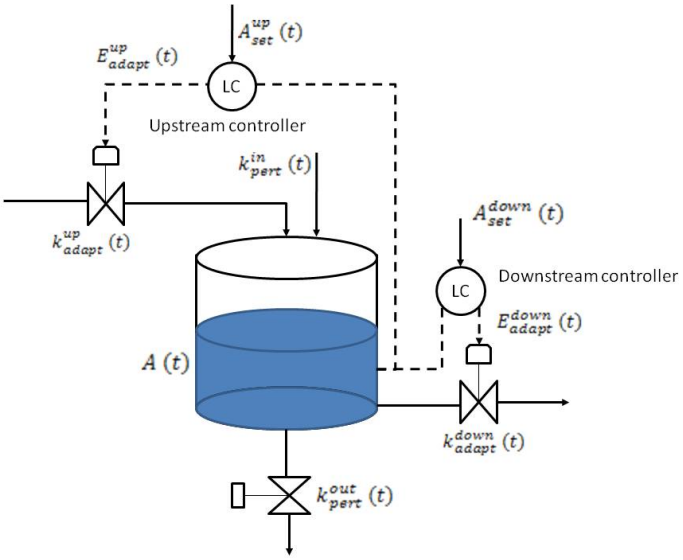


Figure 1.11: Natural controller tank equivalent

A zero order flow can be compared to a pump that pumps at a constant rate independent of the substrate or enzyme. A first order flow is comparable to a proportional valve. The max reaction velocity, V_{max} is the value where the valve is fully opened. Finally, a first order flow with Michaelis-Menten kinetics can be compared to a controlled screw pump.

1.7 Introduction to Hamiltonian systems

Conservative non-linear oscillations

Oscillations are considered conservative when the total energy of the system remains constant. Usually systems like the earths orbit around the sun or other systems can be approximated to be conservative if the period of the investigation is sufficiently short. The simplest form of a conservative system is the motion of a material point on a straight line under the action of a force depending on the distance only [1]. Formulated by Eq. (1.21).

$$x = f(x) \tag{1.21}$$

where f(x) is a force and is the mechanical state of the system. One can split equation(1.21) into two differential equations of the first order as seen in Eqs. (1.22) and (1.23).

$$\frac{dx}{dt} = y \tag{1.22}$$

$$\frac{dy}{dt} = f(x) \tag{1.23}$$

Related to enzyme-substrate kinetics, $\frac{dx}{dt}$ is the change in concentration of the substance A and $\frac{dy}{dt}$ is the change in concentration of the catalyst E_{adapt}.

The change in the phase plane is the change in y-position based on the change in x-position and can therefore be written as in Eq. (1.24).

$$\frac{dy}{dx} = \frac{f(x)}{y} \tag{1.24}$$

and the velocity of the motion in this case is then as in Eq. (1.26).

$$\frac{ds}{dt} = \sqrt{\left(\frac{dx}{dt}\right)^2 + \left(\frac{dy}{dt}\right)^2} \tag{1.25}$$

$$= \sqrt{y^2 + f(x)^2} \tag{1.26}$$

The change in the phase plane in equation (1.24) can be integrated in the form seen in Eq. (1.27).

$$\frac{y^2}{2} + V(x) = h \tag{1.27}$$

where $V(x)$ is such that $V(x) = -f(x)$ and h is a constant of integration. In this case, the equation describes the law of conservation of energy with $\frac{y^2}{2} = \frac{mx^2}{2}$ being the kinetic energy, $V(x)$ being the work by the forces in the system and h is the so-called energy constant or the total energy of the system. If h is assigned, then to each value of h there is a whole curve, $y = \Phi(x)$, containing an infinitive amount of states (x, y). This is called the equi-energy curve. One of the properties of this integral curve is that equation (1.27) is not altered if y is replaced by $-y$, therefore all curves of this family is symmetric with respect to the x axis. Varying h a little will result in another curve slightly displaced on the phase plane[1].

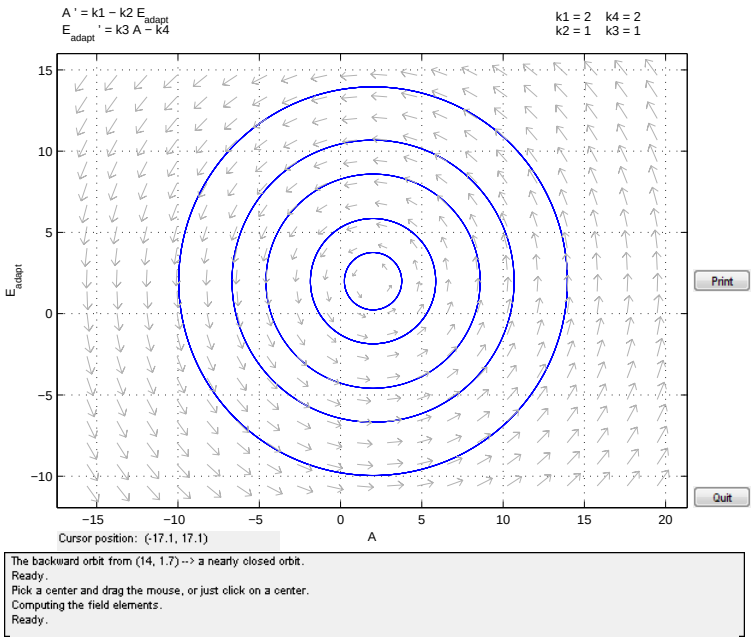


Figure 1.12: Displacement of curves in Hamiltonian system

This displacement can be seen in figure 1.12 which has been made in pplane7, a Matlab(www.Mathworks.com) program by John C. Polking[12] using the equations for the downstream activation type 1 controller.

Hamiltonian system

A Hamiltonian system is described dependent on energy only, not momentum. It is used to describe more complicated conservative systems. The value for the Hamiltonian, H, is the total energy of a closed system. Hamilton's *function* can be seen in Eq. (1.28).

$$H(p,q) = \dot{q} \cdot p - L \tag{1.28}$$

where q is the position, p is the moment, or impulse $p = \frac{\partial L}{\partial \dot{q}}$ and L is the Lagrangian of the system. The Lagrangian is defined as the potential energy of the system minus the kinetic energy, see Eq.(1.29). In an electrical system, this can be the difference between magnetic and electrical energy[1].

$$L = T - V \tag{1.29}$$

where T is the kinetic energy and V is the potential energy. Equation (1.28) can be broken down into two first order differential equations, called Hamilton's *equations*. A Hamiltonian system is a system that can be described by Hamilton's equations[8]. These are seen in Eqs. (1.30) and (1.31).

$$\frac{dq}{dt} = \frac{\partial H}{\partial p} \tag{1.30}$$

$$\frac{dp}{dt} = -\frac{\partial H}{\partial q} \tag{1.31}$$

Hamilton's equations are invariant to transformations of the variables.

For the system in section 2.3, if Michaelis-Menten kinetics is assumed in the outflow of the enzyme and the substrate it is possible to find a function $H(A, E_{adapt})$ such that Eqs. (1.32) and (1.33) can be derived. Where A is the substrate and E_{adapt} is the enzyme. The downstream activation controller type 1 should therefore behave like a Hamiltonian system with harmonic oscillations.

$$\frac{dA}{dt} = -\frac{\partial H}{\partial E_{adapt}} \tag{1.32}$$

$$\frac{dE_{adapt}}{dt} = \frac{\partial H}{\partial A} \tag{1.33}$$

Chapter 2

Investigating oscillations in downstream activation controller type 1

2.1 Implementation

A program is made in Matlab, in order to simulate the natural controllers in figure 1.7 in section 1.5. This report focuses on outflow controller (5), here named downstream activation type 1. In the program, it is possible to select which controller(s) to simulate. The code below is used to set simulation time,step length and absolute and relative tolerance for the solver.

```
7  %% Numerical solver variables, both Simulink and Matlab
8  int.SluttTid = 300;
9  int.MaxSteglengde = 0.001;
10 int.RelTol = 1e-12;
11 int.AbsTol = 1e-16;
```

It is also possible to set the initial conditions for the substrate and enzyme, the values for the different rate flows $k_1 \dots k_6$, the Michaelis constants and choose whether the outflows should be of order zero, one or use the Michaelis-Menten approximation. Furthermore it is possible to toggle dynamic variables on one or both of the substances and choose the initial conditions of these, see the following Matlab code.

```
42 %% Downstream activation controller, type 1 (Inflow controller 1)
43 %Flow rates
44 v.k1_DownAct1 = 1.0;
45 v.k2_DownAct1 = 1.0;
46 v.k3_DownAct1 = 1.0;
47 v.k4_DownAct1 = 2.0;
48
49 %Toggle additional variables
50 o.a.DownAct1 = 0;  % 'a' variable
51 o.e.DownAct1 = 0;  % 'e' variable
52
53 %Flow rates, added variables
54 v.k5_DownAct1 =1.0; %k_a
55 v.k6_DownAct1 = 2.0; %k_e5
56
57 %Order of outstreams
58 o.DownAct1_Order_k2 = 1; %0 = zero order,1 = first order, 2 = MM
59 o.DownAct1_Order_k4 = 1; %0 = zero order,1 = first order, 2 = MM
```

There are several ODE (ordinary differential equation) solvers in Matlab. Most commonly used is the ode45, which is the general purpose solver. If the system is stiff however, this solver can take a lot of time finishing or even crash. In this case ode15s can be used which is a ODE designed specifically to solve stiff systems[12]. In this implementation, ode45 crashes if the dynamic variable a is involved in the system and therefore ode15s is used.

```
213 %ODE solver
214 [tidsvec Ysim] = ode15s(@(t,y) Hamiltonian_Diff_Eq(y,v,o),int.Tspan,int.IC,int.options);
```

In the code above, Hamiltonian_Diff_Eq is the Matlab function containing the differential equations for the ODE solver. The input of the function contains the values for the different variables in the structure v and the options in the o structure, the start and end time, the values for the variables used in the differential equations and options enables the user to customise, for example, relative and absolute tolerance of the solver.

2.2 Modelling

The system described in this section is a substrate A, which is controlled by the enzyme E_{adapt} in turn controlled by another enzyme E_{set}. The outflow of

the substrate is activated by the enzyme, while the inflow of enzyme is activated by the substrate. This is therefore a downstream activation controller of type 1. It has been shown that these types of systems can show harmonic oscillations[6]. This chapter investigates these oscillations in the downstream activation controller type 1.

The simulations are divided into 4 cases: case 1a, which is the core oscillator with no added dynamics, case 1b where the substrate has added dynamics, case 1c where the enzyme has added dynamics and finally case 1d where dynamics are added to both the substrate and the enzyme.

For the simulations, the flow rates are chosen to give a set-point at (2,2) in the phase plane for A and E_{adapt} by using these values for the rate constants; $(k_1 \dots k_6) = (2.0, 1.0, 1.0, 2.0, 1.0, 5.0)$. Initial conditions are chosen to be: $(A, E_{adapt}, a, e) = (1.9, 1.51, 1.1, 1.8)$. The simulation time is set to 100 with step length 0.001 and finally the Michaelis-Menten constants are set to: $K_{MA} = K_{ME} = K_{Ma} = 10^{-8}$. This setup was used consistently unless otherwise is specified.

2.3 Case 1a - Core oscillator

Case 1a is the core oscillator. Figure 2.1 shows a sketch of the system with A being the substrate and E_{adapt} being the enzyme. Note that the naming convention in the figures is from the programming shown in section 2.1. These variable names were used because it was desired to have the same notation for the inflows and outflows in the program independent on the type of controller. This way, k_1 and k_2 are always the inflow and outflow of the substrate, while k_3 and k_4 are always the inflow and outflow of the enzyme. In the equations, the descriptive names were used in order to be more specific.

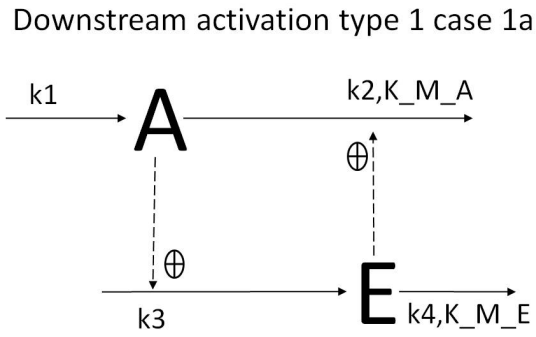


Figure 2.1: Model of case 1a, core oscillator

Using the principles of mass balance and assuming Michaelis-Menten kinetics, case 1a can be described with equations (2.1) and (2.2).

$$\frac{dA}{dt} = k_{pert}^{Down} - k_{cat}^{E_{adapt}^{Down}} \cdot E_{adapt}^{Down} \cdot \frac{A}{K_M^{E_{adapt}^{Down}} + A} \tag{2.1}$$

$$\frac{dE_{adapt}^{Down}}{dt} = k_{adapt} \cdot A - V_{max}^{E_{set}} \cdot \frac{E_{adapt}^{Down}}{K_M^{E_{set}} + E_{adapt}^{Down}} \tag{2.2}$$

where:

- A is the concentration of the substrate.
- k_{pert}^{Down} is the perturbation rate flow of A, named k_1 in the figure.
- $k_{cat}^{E_{adapt}^{Down}}$ is the outflow of A, named k_2 in the figure.
- $K_M^{E_{adapt}^{Down}}$ is the Michaelis-Menten constant of A.
- E_{adapt}^{Down} is the enzyme concentration.
- k_{adapt} is the inflow of E_{adapt}^{Down}, named k_3 in the figure.
- $V_{max}^{E_{set}}$ is the outflow of E_{adapt}^{Down}, named k_4 in the figure.

- $K_M^{E_{set}}$ is the Michaelis-Menten constant of E_{adapt}^{Down}.

When $K_M^{E_{set}} << E_{adapt}^{Down}$, the flow out of A is very close to V_{max} and the steady state value for A can be calculated by setting Eq. (2.2) equal to zero, as shown in equation (2.3). This is comparable to the set-point in control theory and is therefore named A_{set}. This is the desired concentration of A, similar to the reference of a negative feedback loop.

$$A_{set} = \frac{V_{max}^{E_{set}}}{k_{adapt}} \tag{2.3}$$

If the Michaelis-Menten constant of A can be neglected as well ($K_M^{E_{adapt}^{Down}} << A$), equation (2.1) can be written as:

$$\dot{A} = k_{pert}^{Down} - k_{cat}^{E_{adapt}^{Down}} \cdot E_{adapt}^{Down} \tag{2.4}$$

Finding the derivative of equation (2.4) is shown in Eq. (2.5).

$$\ddot{A} = -k_{cat}^{E_{adapt}^{Down}} \cdot \dot{E_{adapt}^{Down}} \tag{2.5}$$

Inserting equation (2.2) while $K_M^{E_{set}} << E_{adapt}^{Down}$ into equation (2.5) gives:

$$\ddot{A} = -k_{cat}^{E_{adapt}^{Down}} \cdot \left(k_{adapt} \cdot A - V_{max}^{E_{set}}\right) \tag{2.6}$$

which becomes as in Eq. (2.7).

$$\ddot{A} + k_{cat}^{E_{adapt}^{Down}} \cdot k_{adapt} \cdot A = k_{cat}^{E_{adapt}^{Down}} \cdot V_{max}^{E_{set}} \tag{2.7}$$

dividing by $k_{cat}^{E_{adapt}^{Down}} \cdot k_{adapt}$ gives Eq. (2.8).

$$\frac{\ddot{A}}{k_{cat}^{E_{adapt}^{Down}} \cdot k_{adapt}} + A = \frac{V_{max}^{E_{set}}}{k_{adapt}} \tag{2.8}$$

which is the same as the set-point for A found in equation (2.3). This shows that if the degradation of A and E_{adapt} is of zero order, the concentration will have undamped oscillations around A_{set} with period as shown in Eq. (2.9)[6].

$$P = \frac{2 \cdot \pi}{\sqrt{k_{adapt} \cdot k_{cat}^{E_{adapt}^{Down}}}} \tag{2.9}$$

2.3.1 Control theory equivalents

In order to show the resemblance to control theory, Eq. (2.2) is rewritten to a form similar to that of an integral controller. Using the assumption that $K_M^{E_{set}} << E_{adapt}^{Down}$, Eq. (2.10) can be derived.

$$\frac{dE_{adapt}^{Down}}{dt} = -k_{adapt} \cdot \left(\frac{V_{max}^{E_{set}}}{k_{adapt}} - A\right) \tag{2.10}$$

where $\frac{V_{max}^{E_{set}}}{k_{adapt}}$ can be said to be the set-point of A, which is the output. The system can hence be described as a integral control system with negative feedback. Starting with the equation for a PI controller[4];

$$u(t) = K_p \cdot e(t) - \frac{K_p}{T_i} \int_o^t e(t)dt \tag{2.11}$$

where $u(t)$ is the manipulated variable, K_p is the gain, T_i is the integral time and $e(t)$ is the error between the reference and the output. Finding the derivative of Eq. (2.11) results in Eq. (2.12).

$$\dot{u(t)} = K_p \cdot \dot{e(t)} - \frac{K_p}{T_i} \cdot e(t) \tag{2.12}$$

Assuming the proportional term equal to zero and calculating the error, $e(t) = r - y(t)$, where r is the reference and y is the output, results in Eq. (2.13).

$$\frac{du}{dt} = -\frac{K_p}{T_i} \cdot (r - y(t)) \tag{2.13}$$

The names of the controller variables and their respective biochemical equivalents are listed below.

- $u = E_{adapt}$
- $\frac{K_p}{T_i} = k_{adapt}$
- $r = \frac{V_{max}^{E_{set}}}{k_{adapt}}$
- $y = A$

2.3.2 Harmonic oscillator simulation

For this simulation, the flow rates are chosen to give a set-point at (2,2) in the phase plane for A and E_{adapt} by using these values for the rate constants; $(k_1 \dots k_4) = (2.0, 1.0, 1.0, 2.0,)$. Initial conditions are chosen to be: $(A, E_{adapt}) = (1.9, 1.51)$. The simulation time is set to 100 with step length 0.001 and finally the Michaelis-Menten constants are set to: $K_{MA} = K_{ME} = 10^{-8}$. In order to investigate whether the model was implemented correctly, the order is set to zero using the Michaelis-Menten approximation. As expected, this resulted in oscillations as shown in figure 2.2, suggesting that the implementation is correct.

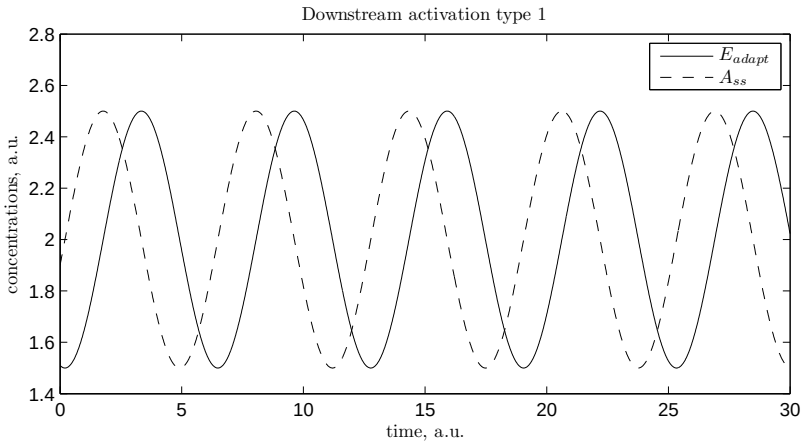


Figure 2.2: Case 1a Substrate plotted vs enzyme

By plotting A versus E_{adapt} the system can be observed in the phase plane. One can then observe that the system has the same properties as a Hamiltonian system. If observed in the phase plane shown in figure 2.3, case 1a gives a similar response to figure 1.12, in section 1.7.

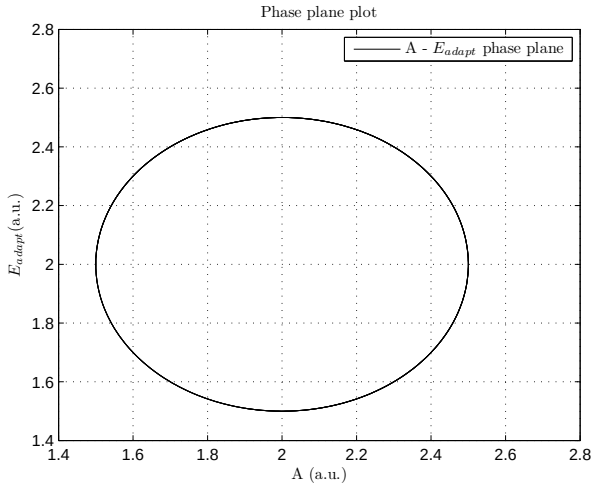
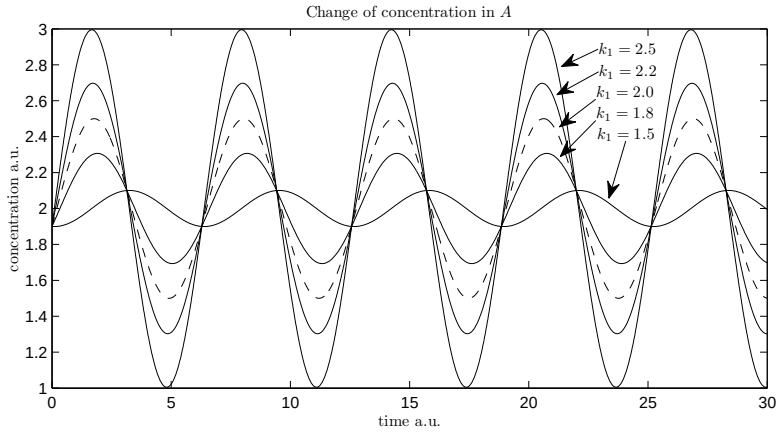


Figure 2.3: Case 1a phase plane plot

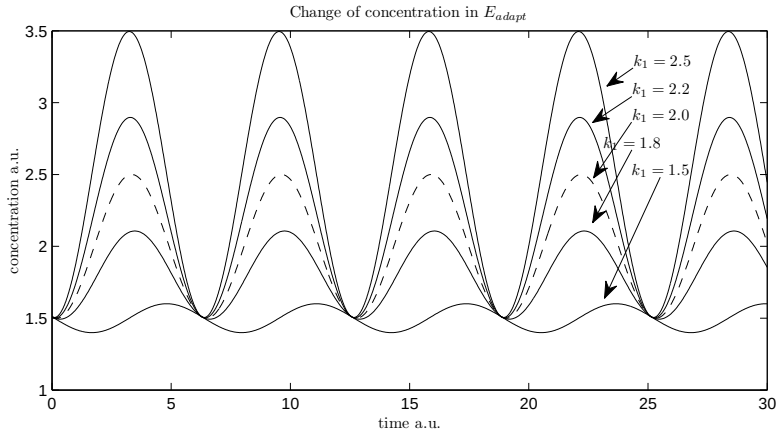
As figure 2.3 shows, the result for the system described by Eqs.(2.1) and (2.2) when $K_{MA} << A$ and $K_{ME} << E_{adapt}$ is undamped oscillations.

2.3.3 Effect of rate constants

This part investigates how a change in the different rate constants effect the system. In the figures, the stapled line represents the value of the substrate and enzyme with the default reaction constants and the solid lines represents the substrate or enzyme with either a decrease or increase in the rate flows. Figure 2.4 compares the change in concentrations with a variation in $k_1 = [1.5, 1.8, 2.0, 2.2, 2.5]$.



(a) Case 1a, variation in k_1, response in concentration of A



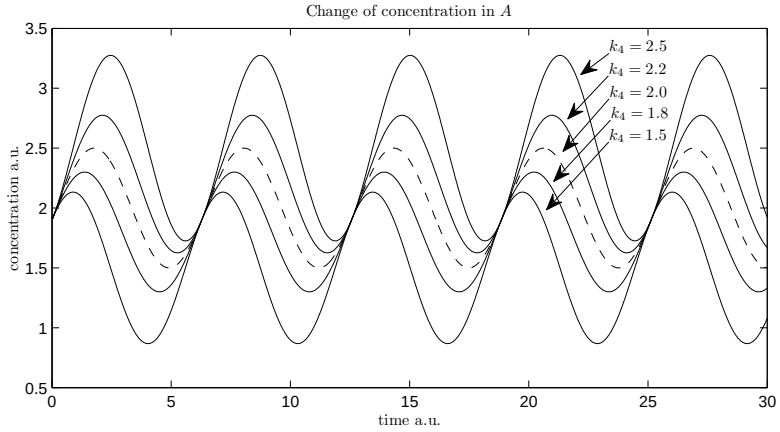
(b) Case 1a variation in k_1, response in E_{adapt}

Figure 2.4: Response of concentrations on variations of k_1

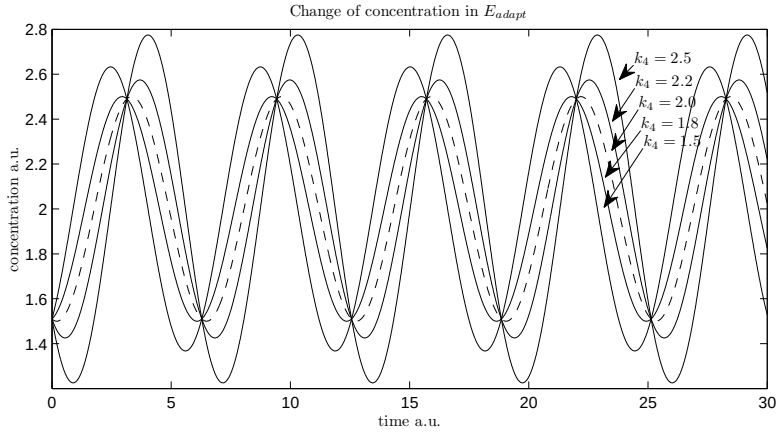
Figure 2.4 shows that the amplitude in A is increased proportionally to the increase in k_1 which is expected since the flow into A is increased, though the set-point of A is unchanged. In the response of E_{adapt} on the other hand, the state value the concentration is oscillating around is moved according to the increase in k_1, suggesting that the calculations were correct. The increase in inflow increases the amplitude of E_{adapt} which is expected considering this is an inflow compensation controller.

In another simulation, the flow out of E_{adapt} is set to; $k_4 = [1.5, 1.8, 2.0, 2.2, 2.5]$, while the inflow of A remains constant, $k_1 = 2$. In order to observe how the

system reacts to an increase in the outflow of the enzyme This can be seen in figure 2.5.



(a) Case 1a, variation in k_4, response of concentration in A



(b) Case 1a, variation in k_4, response of concentration in E_{adapt}

Figure 2.5: Case 1a, variation in k_4, response of concentrations

As shown in figure 2.5, an increase in k_4 changes the set point of A accordingly. Using Eq. (2.15), the set-points can be found.

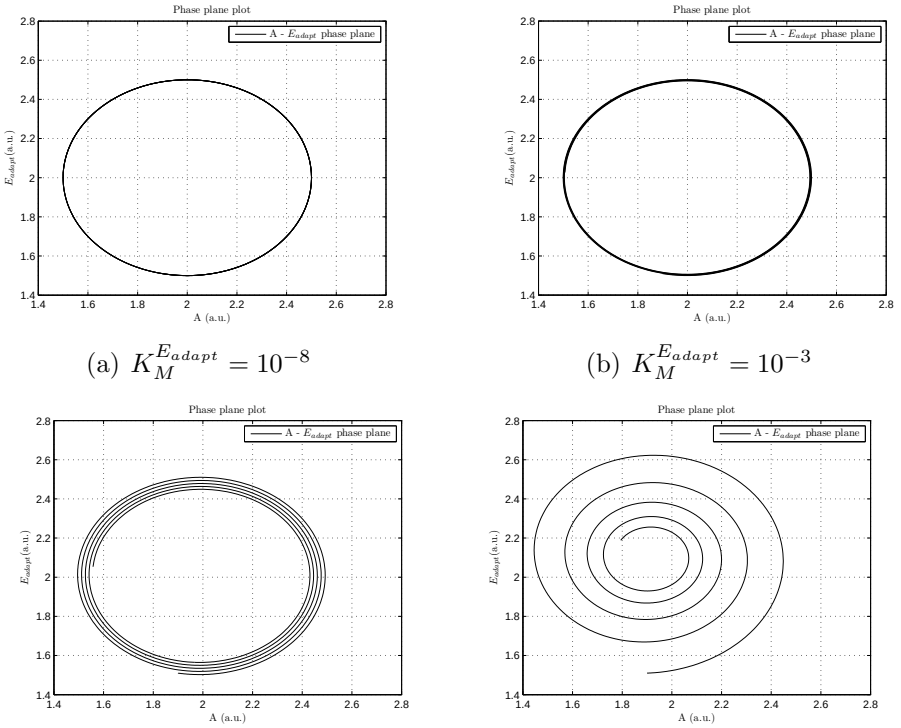
$$A_{set} = \frac{V_{max}^{E_{set}}}{k_{adapt}} \tag{2.14}$$

$$= \frac{k_4}{k_3} \tag{2.15}$$

The set-points for the substrate with $k_3 = 1$ are then: $A_{set} = [2.5, 2.2, 2.0, 1.8, 1.5]$ for $k_4 = [2.5, 2.2, 2.0, 1.8, 1.5]$.

2.3.4 Michaelis-Menten effect on damping

In order to investigate the effect of an increase in Michaelis-Menten constant on the damping of the system. The constant, $K_M^{E_{adapt}}$, is set to different values without changing any other variables. The effect of this is best seen in the phase plane, but can also be observed in the time domain, as oscillations being damped and the system eventually reaching homeostasis or steady state.



(a) $K_M^{E_{adapt}} = 10^{-8}$

(b) $K_M^{E_{adapt}} = 10^{-3}$

(c) $K_M^{E_{adapt}} = 10^{-2}$

(d) $K_M^{E_{adapt}} = 10^{-1}$

Figure 2.6: Phase plane plots for case 1a with varying MM

The results presented in figure 2.6 shows that the oscillations are damped as the Michaelis-Menten constant increases.

2.3.5 Michaelis-Menten effect on negative concentration

With a large initial value for the enzyme, in this case $E_{adapt} = 4.51$ the phase plane curve would cross the y-axis, meaning that the concentration of the substrate would have been negative. Figure 2.7 shows that the concentration of the substrate is reduced no further than the value of the MM constant. The Michaelis-Menten constant is set to 10^{-8}.

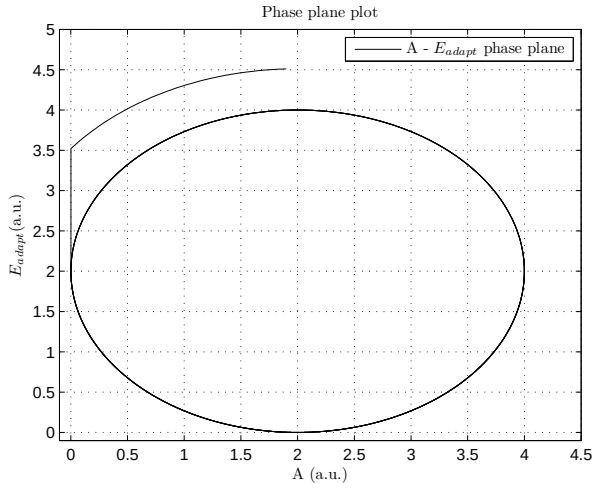


Figure 2.7: Case 1a phase plane plot, MM prevents negative concentration

2.4 Case 1b - Added substrate dynamics

Case 1b is a system based on the downstream activation controller type 1, with added dynamics to the substrate. This is achieved by introducing the variable a between the substrate and the activation of the enzyme. A sketch of the system can be seen in figure 2.8. The enzyme now controls the outflow of the dynamic variable a instead of the substrate directly. Case 1b is introduced in order to investigate whether the homeostatic properties remain.

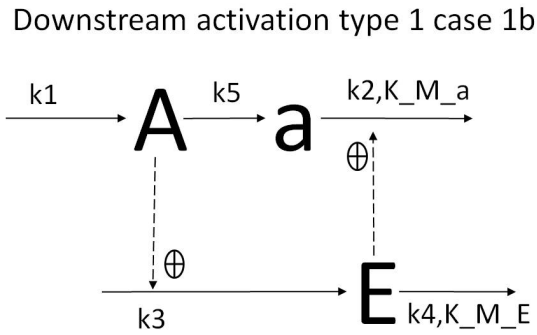


Figure 2.8: Model of case 1b

Case 1b can be described by Eqs. (2.16), (2.17) and (2.18).

$$\frac{dA}{dt} = k_{pert}^{Down} - k_5 \cdot A \tag{2.16}$$

$$\frac{da}{dt} = k_5 \cdot A - k_{cat}^{E_{adapt}^{Down}} \cdot E_{adapt}^{Down} \cdot \frac{a}{K_M^{E_{adapt}^{Down}} + a} \tag{2.17}$$

$$\frac{dE_{adapt}^{Down}}{dt} = k_{adapt} \cdot A - V_{max}^{E_{set}} \cdot \frac{E_{adapt}^{Down}}{K_M^{E_{adapt}^{Down}} + E_{set}} \tag{2.18}$$

where:

- A is the concentration of the substrate.
- $k_{pert}^{Down} = k_1$ and k_5 are the flow rates in and out of the substrate A.
- E_{adapt}^{Down} is the enzyme which is regulating the concentration of A.
- $k_{adapt} = k_3$ and $V_{max}^{E_{set}} = k_4$ are the in- and outflow rates of E_{adapt}^{Down}.
- $K_M^{E_{set}}$ is the Michaelis-Menten constant of E_{adapt}^{Down}.
- a is a dynamic variable.
- $K_M^{E_{adapt}^{Down}}$ is the Michaelis-Menten constant of a.

When the Michaelis-Menten constant ($K_M^{E_{adapt}^{Down}}$) is very low compared to the enzyme concentration, E_{adapt}^{Down}, the set-point for A can still be calculated by setting $\frac{dE_{adapt}^{Down}}{dt} = 0$:

$$A_{set} = \frac{k_4}{k_3} \tag{2.19}$$

however, the introduction of the dynamic variable changes the properties of the enzyme.

The results with added dynamics on the substrate are shown in figure 2.9. It can be seen that the set-point of the substrate remains the same.

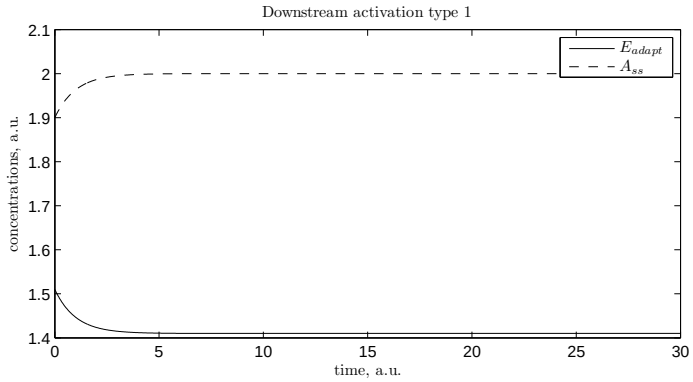


Figure 2.9: Case 1b - added dynamics in substrate

Both the enzyme and the substrate reach homeostasis, and there are no oscillations as shown in the phase plane plot in figure 2.10.

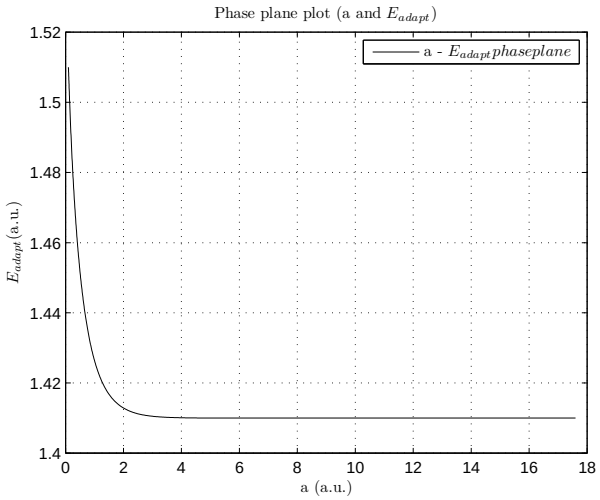


Figure 2.10: Case 1b - Phase plane plot of a and E_{adapt}

The a variable increases linearly, while the enzyme and substrate reach a steady state. The set-point for A is the same as in the general oscillator, while the steady state value of E_{adapt} is lowered. This is because the enzyme is activating the outflow of the substrate, meaning that less enzyme is needed to keep the substrate in homeostasis. Additional figures can be seen in appendix B.1.1.

2.5 Case 1c - Added enzyme dynamics

Case 1c is a system with added enzyme dynamics. This is enabled by introducing a variable, e, between the activation from A and the enzyme. The inflow of this new variable is controlled by the substrate and the inflow of the enzyme is of first order.

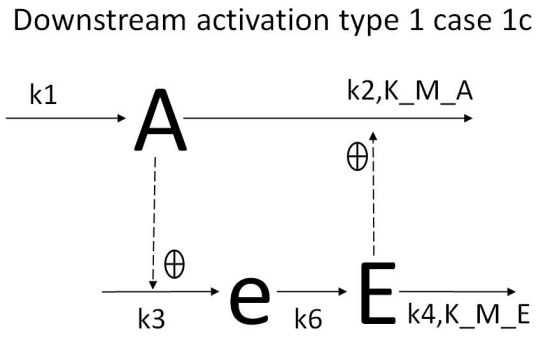


Figure 2.11: Model of case 1c

Case 1c can be described by Eqs. (2.20) through (2.22).

$$\frac{dA}{dt} = k_{pert}^{Down} - k_{cat}^{E_{adapt}^{Down}} \cdot E_{adapt}^{Down} \cdot \frac{A}{K_M^{E_{adapt}^{Down}} + A} \tag{2.20}$$

$$\frac{dE_{adapt}^{Down}}{dt} = k_6 \cdot e - V_{max}^{E_{set}} \cdot \frac{E_{adapt}^{Down}}{K_M^{E_{set}} + E_{adapt}^{Down}} \tag{2.21}$$

$$\frac{de}{dt} = k_{adapt} \cdot A - k_6 \cdot e \tag{2.22}$$

where:

- A is the concentration of the substance which is being controlled.
- $k_{pert}^{Down} = k_1$ and $k_{cat}^{E_{adapt}^{Down}} = k_2$ are the flow rate constants in and out of A.
- $K_M^{E_{adapt}^{Down}}$ is the Michaelis-Menten constant of A.
- E_{adapt}^{Down} is the catalyst which is controlling the concentration A.
- $k_{adapt} = k_6$ and $V_{max}^{E_{set}} = k_4$ is the in- and outflow rate constants of E_{adapt}^{Down}.

- $K_M^{E_{set}}$ is the Michaelis-Menten constant of E_{adapt}^{Down}.
- e is the dynamic variable with inflow rate $k_{adapt} = k_3$ and outflow rate k_6.

The simulations of case 1c are made with added dynamics on the enzyme and the following results are found for the substrate and the enzyme:

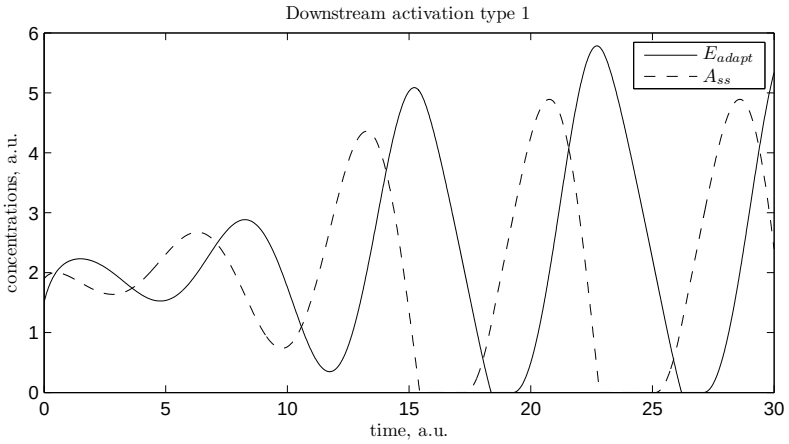


Figure 2.12: Case 1c - Substrate and enzyme vs. time

As shown in figure 2.12, there are oscillations for both the substrate and the enzyme. These oscillations increase in amplitude, but the simulation time is not long enough to observe whether they keep increasing or are eventually damped.

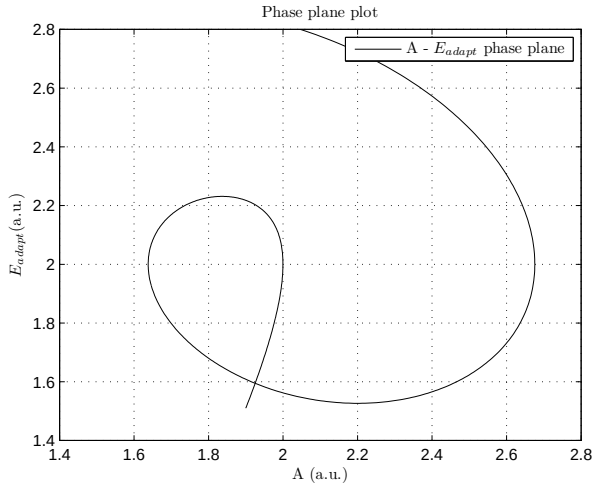


Figure 2.13: Case 1c - Phase plane plot A and E_{adapt}

The phase plane plot provides no new information, however looking at figure 2.12, it can seem like the system enters harmonic oscillations after some time. To investigate if this is the case, the same simulation variables are used, with an increased simulation time (t=300).

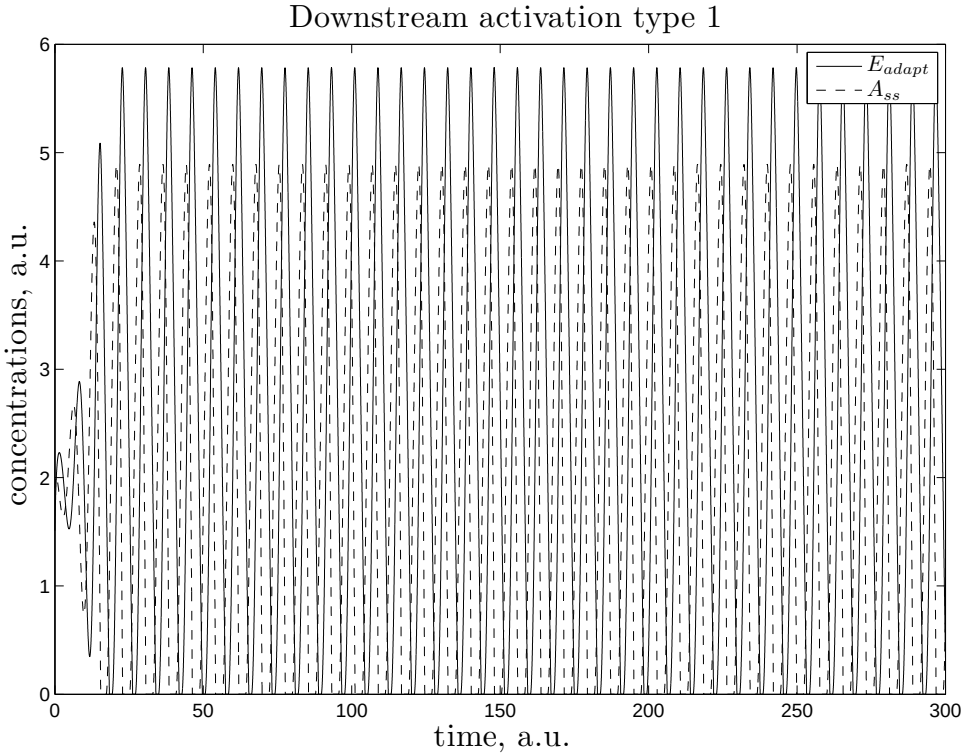


Figure 2.14: Case 1c, t=300 - Concurrent plot A and E_{adapt}

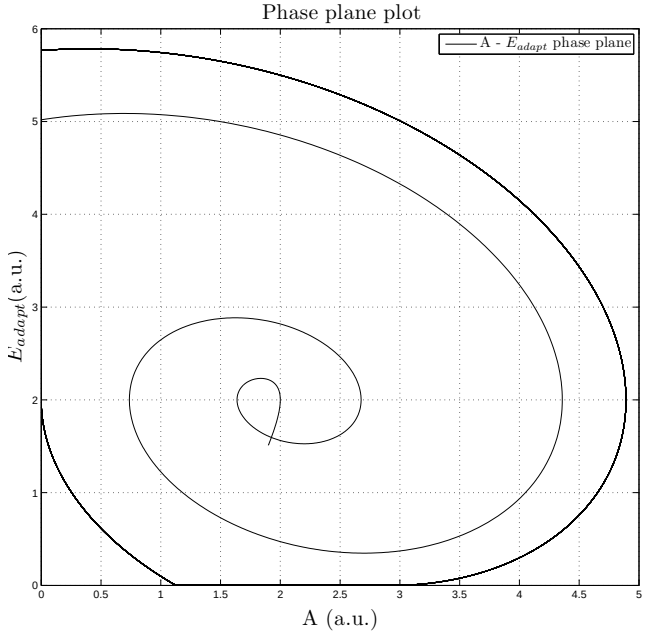


Figure 2.15: Case 1c, t=300 - Phase plane plot A and E_{adapt}

As can be observed from figures 2.14 and 2.15, the system does eventually reach a harmonic oscillating state for both the substrate and the enzyme, so-called limit cycle oscillations.

2.6 Case 1d - Added dynamics to enzyme and substrate

Case 1d is a system with added dynamics to both substrate and enzyme. This is achieved by introducing both the substrate dynamic variable, a, from case 1b and the enzyme dynamic variable,e, from case 1c.

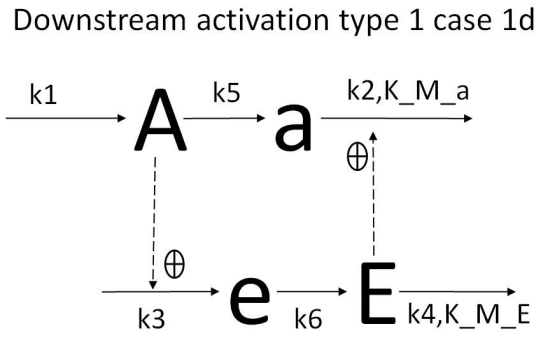


Figure 2.16: Model of case 1d

Case 1d can be described with equations (2.23) through (2.26).

$$\frac{dA}{dt} = k_{pert}^{Down} - k_5 \cdot A \tag{2.23}$$

$$\frac{da}{dt} = k_5 \cdot A - k_{cat}^{E_{adapt}^{Down}} \cdot E_{adapt}^{Down} \cdot \frac{a}{K_M^{E_{adapt}^{Down}} + a} \tag{2.24}$$

$$\frac{dE_{adapt}^{Down}}{dt} = k_6 \cdot e - V_{max}^{E_{set}} \cdot \frac{E_{adapt}^{Down}}{K_M^{E_{set}} + E_{adapt}^{Down}} \tag{2.25}$$

$$\frac{de}{dt} = k_{adapt} \cdot A - k_6 \cdot e \tag{2.26}$$

where:

- A is the concentration of the substrate.
- $k_{pert}^{Down} = k_1$ and k_5 is the flow rate in and out of A.
- E_{adapt}^{Down} is the catalyst which is controlling the concentration A.
- k_6 and $V_{max}^{E_{set}} = k_4$ is the in- and outflow rates of E_{adapt}^{Down}.
- $K_M^{E_{set}}$ is the Michaelis-Menten constant of E_{adapt}^{Down}.
- a and e are dynamic variables.

- k_5 and $k_{cat}^{E_{adapt}^{Down}} = k_2$ are the inflow and outflow rates of a.
- $k_{adapt} = k_3$ and k_6 are the inflow and outflow rates of e.
- $K_M^{E_{adapt}^{Down}}$ is the Michaelis-Menten constant of a.

In the simulation of case 1d, the simulation time was changed as well as the initial values for A and E_{adapt} to match case 1b. The results with added dynamics on the substrate and the enzyme side were as follows in figure 2.17. Oscillations are not expected as the equations are too non-linear which means there is little chance for a zero order outflow in either A or E_{adapt}. It can be seen however, that the set-point for the substrate is not changed.

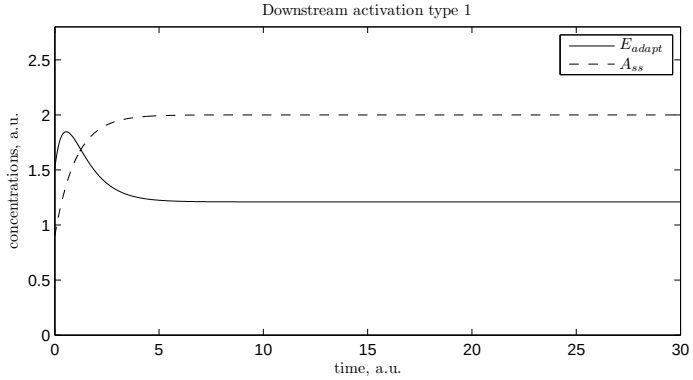


Figure 2.17: Case 1d - Concurrent plot, A and E_{adapt}

Plotting A versus E_{adapt}, the simulation can be observed in the phase plane as shown in figure 2.18.

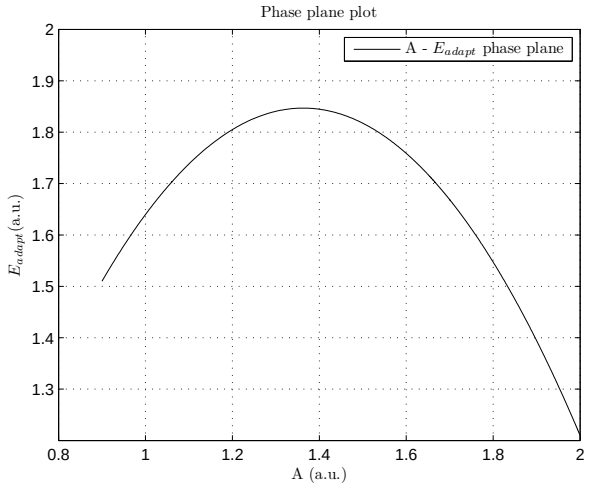


Figure 2.18: Case 1d - Phase plane plot, A vs. E_{adapt}

In this case, all variables reach homeostasis. The set-point for A remains the same while the steady state value of E_{adapt} is decreased.

2.7 Pulsating system with upstream inhibition type 1 controller

This simulation is using the upstream inhibition type 1 controller. This is an outflow compensation controller. A dynamic variable a is also introduced. The inflow of the dynamic variable is inhibited by the enzyme, E_{adapt}. The rate flow constant k_7 is a perturbation, or disturbance in a. The Enzyme concentration is activated by the substrate, A. Michaelis-Menten kinetics is assumed on the outflow of both the substrate and the enzyme. The system is shown in figure 2.19.

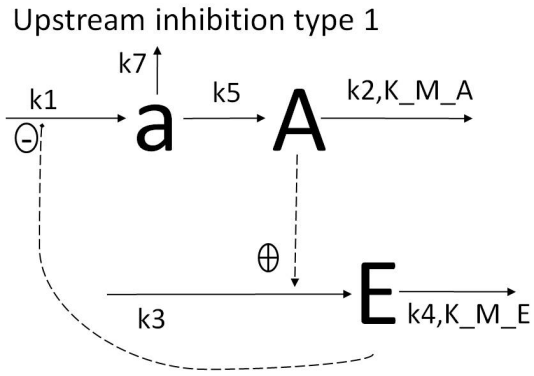


Figure 2.19: Model sketch of upstream pulsation system

Mathematically, the system is described by equations 2.27 through 2.29.

$$\frac{dA}{dt} = k_5 \cdot a - k_2 \cdot A \tag{2.27}$$

$$\frac{da}{dt} = \frac{k_1}{E_{adapt}^{Up}} - a \cdot (k_5 + k_7) \tag{2.28}$$

$$\frac{dE_{adapt}^{Up}}{dt} = k_3 - k_4 \cdot E_{adapt}^{Down} \tag{2.29}$$

where:

- A is the concentration of the substrate.
- k_5 and k_2 are the flow rates in and out of A.
- E_{adapt}^{Up} is the enzyme.
- k_3 and k_4 are the in- and outflow of E_{adapt}^{Up}.
- $K_M^{E_{adapt}^{Up}}$ is the Michaelis-Menten constant of E_{adapt}^{Up}.
- a is a dynamic variable.
- k_1 is the flow in to a and k_7 is the perturbation of a.

2.7 Pulsating system

For the simulations, the flow rates are chosen to give a set-point at (2,2) by in the phase plane for A and E_{adapt}. $(k_1 \ldots k_7) = (1.275, 10.0, 1.0, 1.0, 2.0, 0.0, 1.0,)$. Initial conditions are chosen to be: $(A, E_{adapt}, a, e) = (1.9, 1.51, 1.1, 1.8)$. The Michaelis-Menten constants are set to: $K_{MA} = K_{ME} = K_{Ma} = 10^{-8}$. The simulation time is set to 3000 in order to give the system time to start pulsating. Step length is 0.001

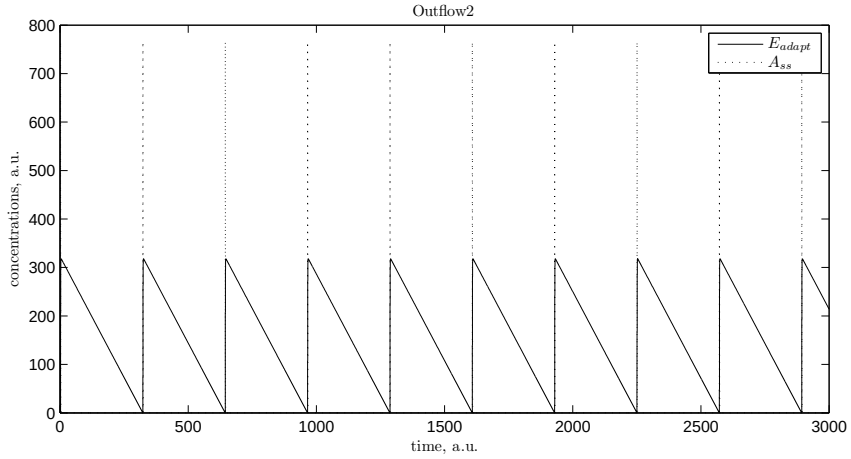


Figure 2.20: Simulation result pulse

It can be seen from the output in figure 2.20 that the substrate concentration is pulsating.

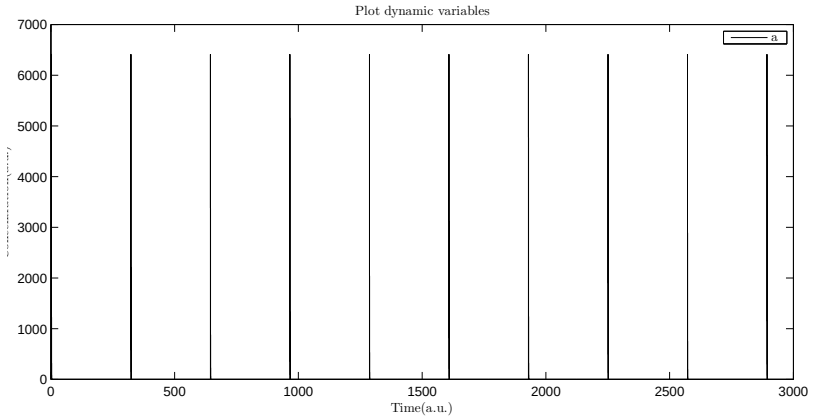


Figure 2.21: Simulation result pulse, dynamic variable

It can also be seen that the concentration of a is pulsating.

Chapter 3

Controller comparison

This chapter compares the different motifs of the controllers in order to investigate whether it is possible to generalise. It is also desirable to find if the different types of controllers have distinct properties in certain situations.

3.1 Upstream controller comparison

A basic system with each of the four upstream controllers has been made using a controller library in Simulink, created by Tormod Drengstig. The first system consists of the upstream controllers. Each controller is connected to, and regulates the concentration of one species or the concentration of a substance.

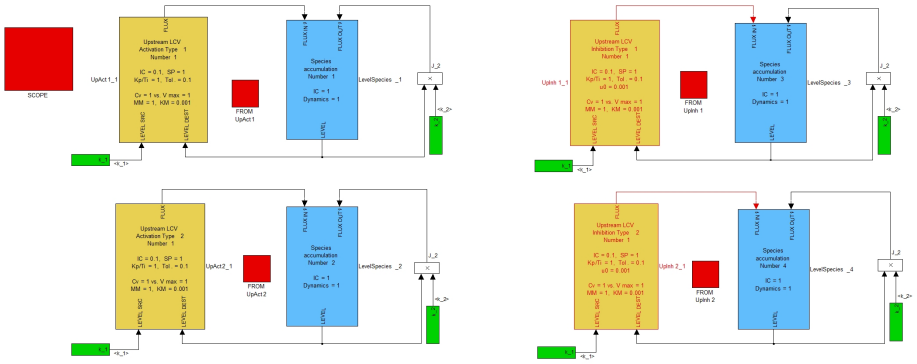


Figure 3.1: Simulink Model of system

The system can be compared to a tank system as seen in figure 3.2.

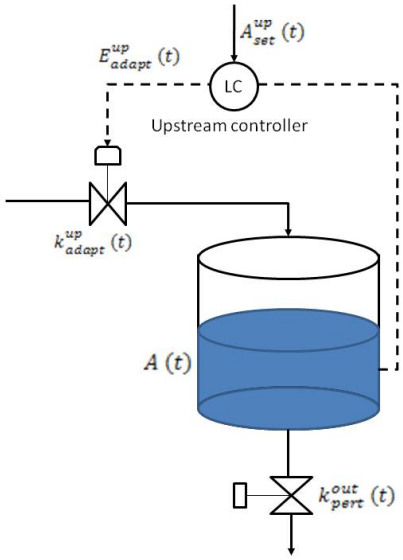


Figure 3.2: Tank sketch, upstream control system

The flux into the system is of order zero so $J_1 = k_1$. The flux out of the system is of first order so $J_2 = k_2 \cdot A$ and the set-point of the controllers is 1. The system is simulated with a simulation time of 300 and a step length of 0.01.

Controller variables upstream inhibition type 1

$k_{adapt} = 1, V^{E_{set}}_{max} = 1, K^{E_{set}}_{M} = 10^{-5}, K^{E_{adapt}}_{I} = 10^{-3}, V^{E_{deg}}_{max} = 1, K^{src}_{M} = 10^{-3}, k^{E_{adapt}}_{cat} = 1.$

Controller variables upstream inhibition type 2

$k_{adapt} = 1, V^{E_{set}}_{max} = 1, K^{E_{set}}_{M} = 10^{-5}, K^{dest}_{I} = 10^{-3}, K^{E_{adapt}}_{I} = 10^{-3}, V^{E_{deg}}_{max} = 1, K^{src}_{M} = 10^{-3}, k^{E_{adapt}}_{cat} = 1.$

Controller variables upstream activation type 1

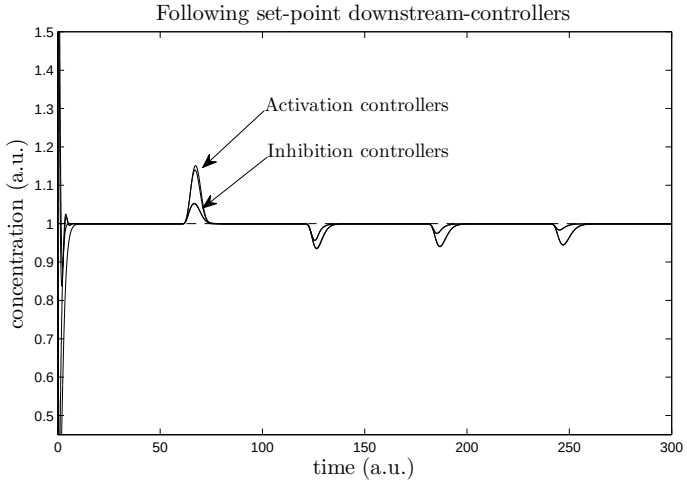
$k_{adapt} = 1, V^{E_{set}}_{max} = 1, K^{E_{set}}_{M} = 10^{-5}, V^{E_{deg}}_{max} = 1, K^{src}_{M} = 10^{-3}, k^{E_{adapt}}_{cat} = 1.$

Controller variables upstream activation type 2

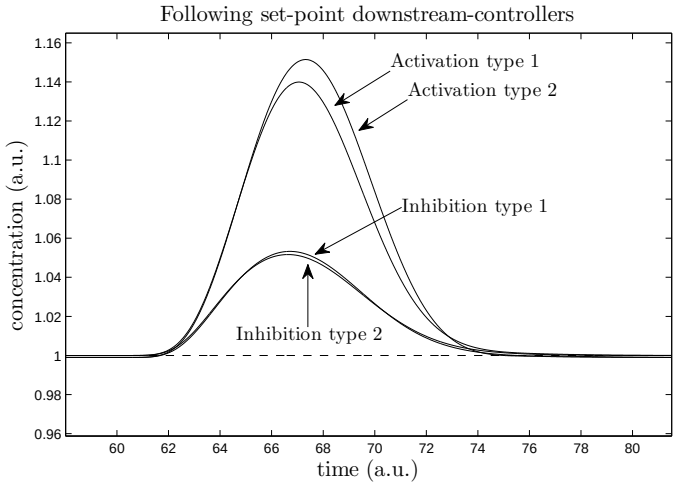
$k_{adapt} = 1, V^{E_{set}}_{max} = 1, K^{E_{set}}_{M} = 10^{-5}, K^{E_{adapt}}_{I} = 10^{-3}, V^{E_{deg}}_{max} = 1, K^{src}_{M} = 10^{-3}, k^{E_{adapt}}_{cat} = 1.$

3.1.1 Outflow compensation, small outflow

The stationary values of the flow rates; $(k_1, k_2) = (1, 2)$. The outflow rate is being varied at the step values $k_2 = [0.8, 1.2, 1.6, 2.0]$ at times $t = [60, 120, 180, 240]$ with initial value $k_2 = 2.0$ at time $t = 0$. Figure 3.3 shows the overall response to the steps of the concentration in the species as well as a more detailed view of one of them.



(a) Initial step response



(b) Overview of response to steps in k_2

Figure 3.3: Detailed view of step response

Figure 3.3 shows that the inhibition controllers respond with less amplitude than the activation controllers. The time it takes for the species concentration to return to the set-point value however, is equal. Figure 3.4 shows the concentration of the enzyme, E_{adapt} in the different upstream controllers.

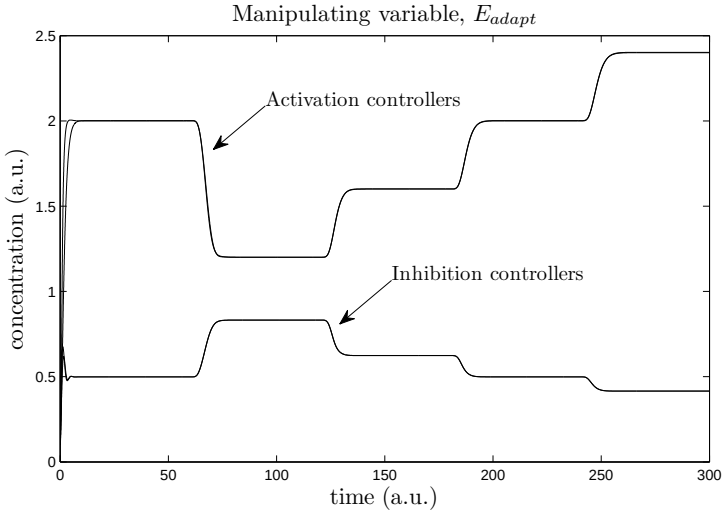


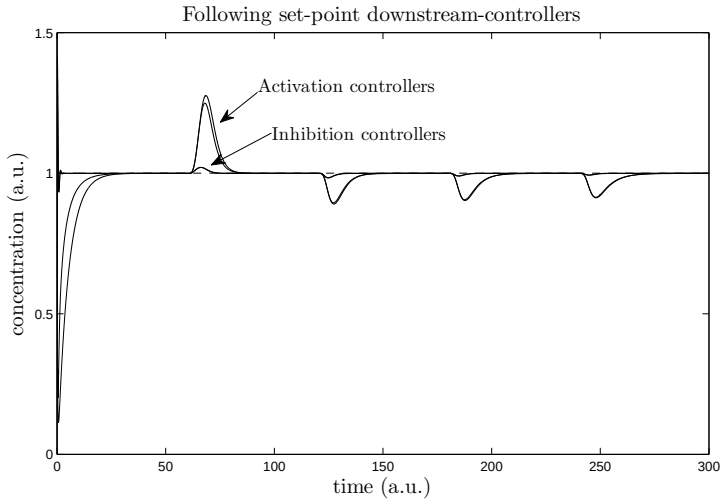
Figure 3.4: Concentration in E_{adapt} during small steps in k_2

Figure 3.4 shows that when the flow rate k_2 increases, the amount of enzyme in the activation controllers decreases while in the inhibition controllers it increases. It can be seen that the inhibition controllers require less enzyme in order to keep the species concentration at the set-point value.

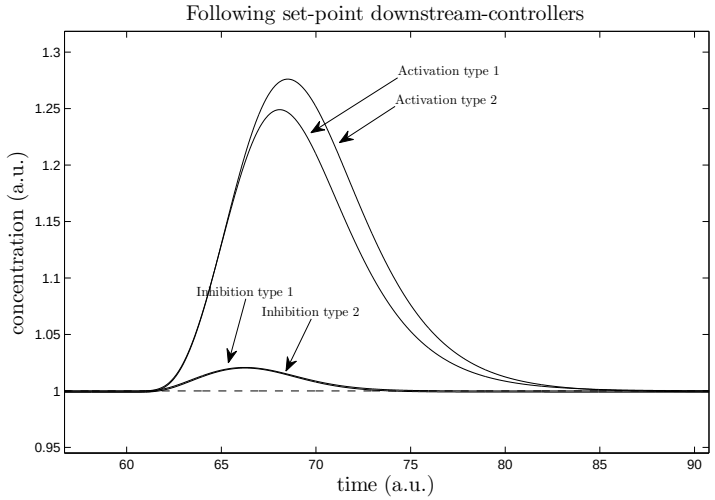
3.1.2 Outflow compensation, large outflow

The stationary values of the flow rates; $(k_1, k_2) = (1, 5)$. The outflow rate is bein varied at the step values $k_2 = [2.0, 3.0, 4.0, 5.0]$ at times $t = [60, 120, 180, 240]$ with initial value $k_2 = 5.0$ at time $t = 0$.

Figure 3.5 shows an overview of the step-response in the species and a more detailed view of one of the steps. It can be seen from the figure that the upstream inhibition controllers react faster and with less amplitude than the activation controllers.



(a) Initial step response



(b) Overview of response to large steps in k_2

Figure 3.5: Detailed view of step response

Figure 3.6 shows the concentration in the enzyme. It can be seen that the inhibition controllers once again require less enzyme in order to regulate the concentration of the species.

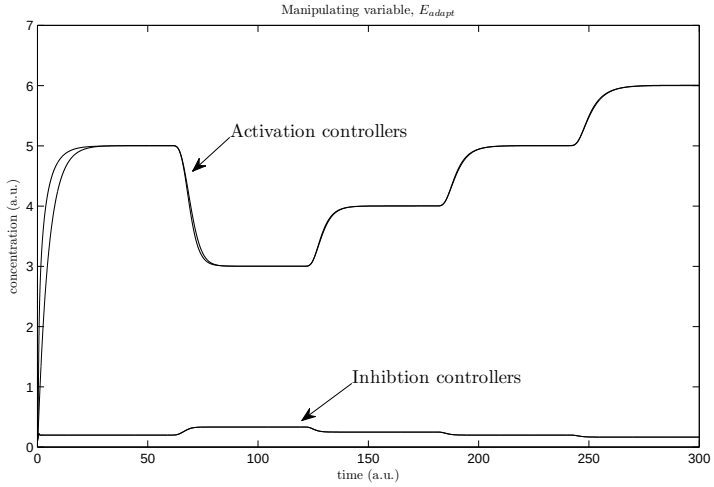


Figure 3.6: Concentration in E_{adapt} during large steps in k_2

The activation controllers can be seen to have a larger concentration than the previous run.

3.2 Downstream controller comparison

Using the same library as mentioned in the previous chapter, a system with the four downstream controllers have also been made. Each downstream controller controls the concentration of one species.

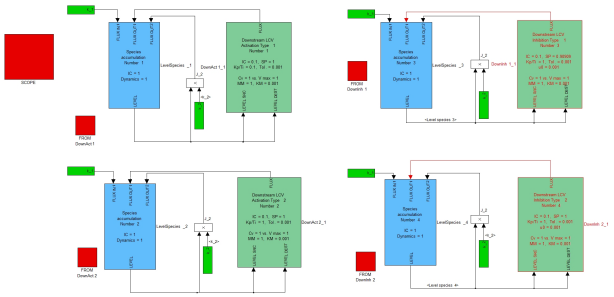


Figure 3.7: Simulink Model of downstream systems

The downstream controller systems can be compared to a tank with a

regulated outflow vent and an inflow disturbance or perturbation. A sketch of the system can be seen in figure 3.8.

The inflow is of zero order so $J_1 = k_1$. The system also has an outflow perturbation of first order, $J_2 = A \cdot k_2$. The enzyme is used to keep the level in the tank, (or the concentration of the species) at the given set-point. The simulation time $t = 300$ and the step length is 0.001.

Controller variables downstream inhibition type 1
$k_{adapt} = 1, V_{max}^{E_{set}} = 1, K_M^{E_{set}} = 10^{-3}, K_I^{E_{adapt}} = 10^{-3}, V_{max}^{E_{deg}} = 1, K_M^{src} = 10^{-3}, k_{cat}^{E_{adapt}} = 1.$
Controller variables downstream inhibition type 2
$k_{adapt} = 1, V_{max}^{E_{set}} = 1, K_M^{E_{set}} = 10^{-3}, K_I^{dest} = 10^{-3}, K_I^{E_{adapt}} = 10^{-3}, V_{max}^{E_{deg}} = 1, K_M^{src} = 10^{-3}, k_{cat}^{E_{adapt}} = 1.$
Controller variables downstream activation type 1
$k_{adapt} = 1, V_{max}^{E_{set}} = 1, K_M^{E_{set}} = 10^{-3}, V_{max}^{E_{deg}} = 1, K_M^{src} = 10^{-3}, k_{cat}^{E_{adapt}} = 1.$
Controller variables downstream activation type 2
$k_{adapt} = 1, V_{max}^{E_{set}} = 1, K_M^{E_{set}} = 10^{-3}, K_I^{dest} = 10^{-3}, V_{max}^{E_{deg}} = 1, K_M^{src} = 10^{-3}, k_{cat}^{E_{adapt}} = 1.$

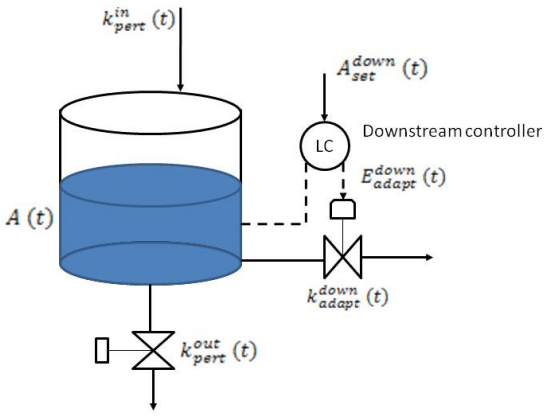


Figure 3.8: Tank sketch, downstream control system

3.2.1 Inflow compensation, small inflow

The system is simulated with a relative low inflow and a set-point of 1, with steps in the inflow, k_1. The step-values are $k_1 = [1.2, 0.8, 2.0, 1.6]$ at times $t = [60, 120, 180, 240]$ with initial value $k_1 = 2.0$ at time $t = 0$. Figure 3.9 show the response in the concentration in the species with the different steps.

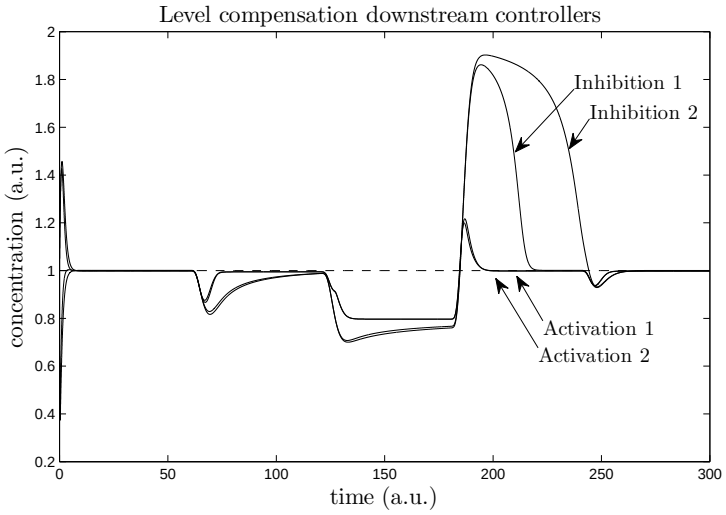


Figure 3.9: Level compensation, small inflow

From figure 3.9, it can be seen that the activation controllers have a fairly good compensation, while the inhibition controllers struggle. Especially at the largest step, from 0.4 to 1.0 shows a large error in both inhibition controllers, especially inhibition type 2. At one time, it can be seen that the controllers break down. This can occur if the outflow is greater than the inflow. As the downstream controllers are inflow compensation controllers, they struggle to maintain the set-point and the concentration in the species is seen to drop.

Figure 3.10 shows the concentration in the enzyme for the different downstream controllers. It can be seen that when the inflow/outflow ratio reaches the breakdown point of the controllers, the concentration of enzyme in the inhibition controllers increases rapidly. In the activation controllers, the concentration of enzyme nears zero. lk

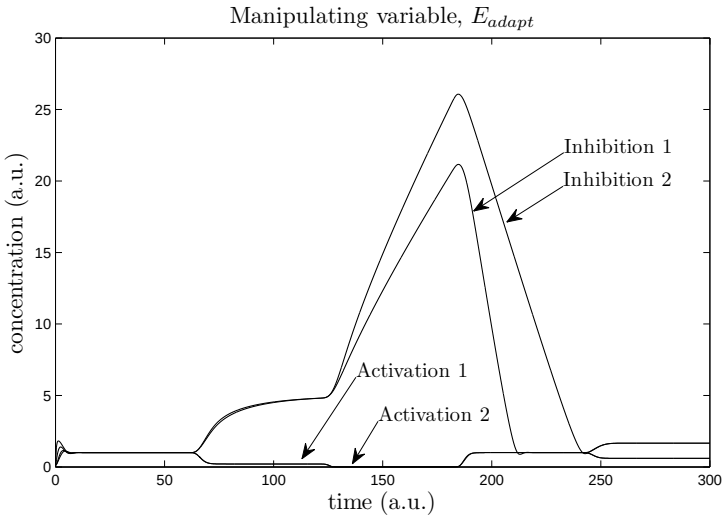


Figure 3.10: Concentration in E_{adapt} with small inflow

3.2.2 Inflow compensation, large inflow

The simulation is repeated with a larger inflow, with the same set-point. The step-values are $k_1 = [2.4, 1.6, 4.0, 3.2]$ at times $t = [60, 120, 180, 240]$ with initial value $k_1 = 4.0$ at time $t = 0$. The plot is divided into several pieces to investigate the step-response. Figure 3.11 is the overview of the response in the species with the different controllers. Figure 3.11 shows an overview of the step-response in the concentration of the species.

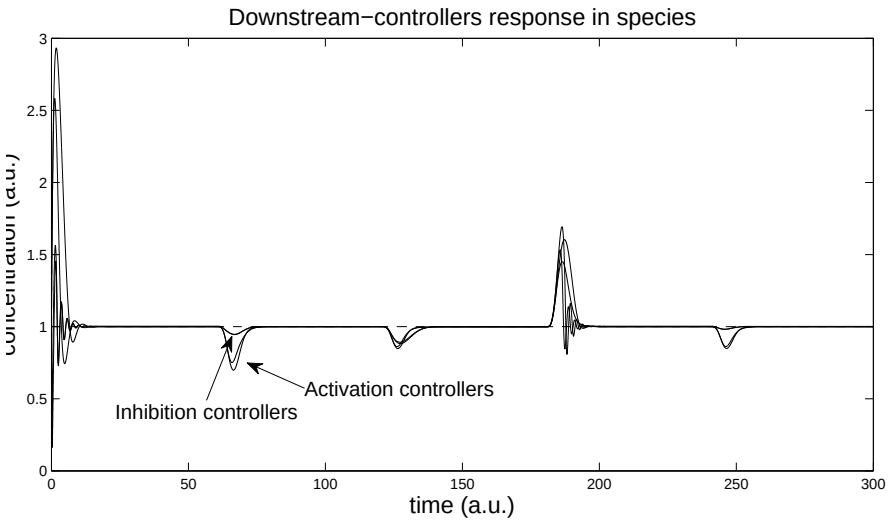
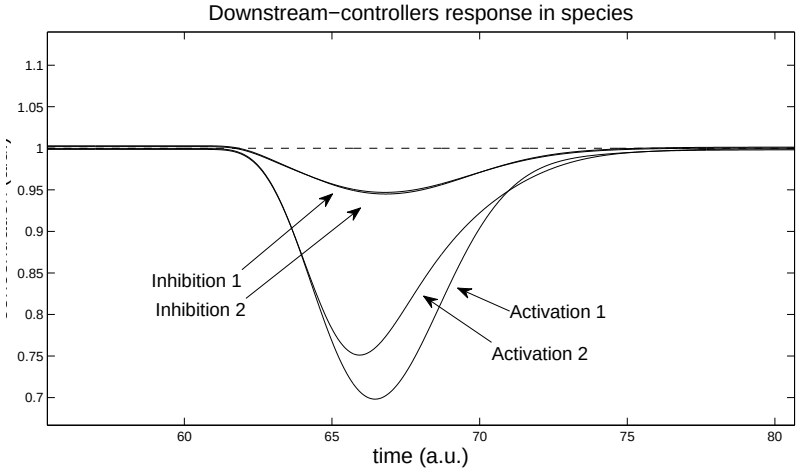
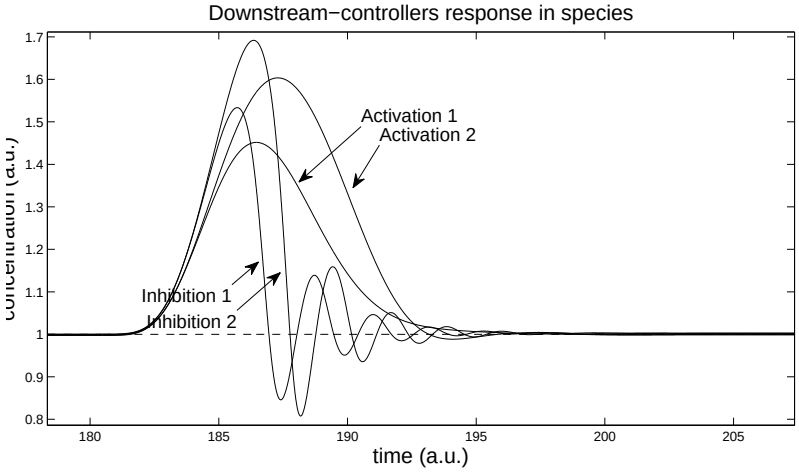


Figure 3.11: Overview of the species level with small inflow

The whole response is divided into smaller parts in order to take a look at the responses to the steps on a more detailed level. This is seen in figure 3.12. Two steps are chosen for the more detailed look.



(a) Response to first step



(b) Response to third step

Figure 3.12: Step response in downstream, small inflow

In figure 3.12, it can be seen that the inhibition controllers have the fastest response when the inflow is large. However as can be seen on the largest step, k_1 step from 1.6 to 4, there is some overshoot in both the inhibition controllers.

The response of the enzyme is seen in figure 3.13.

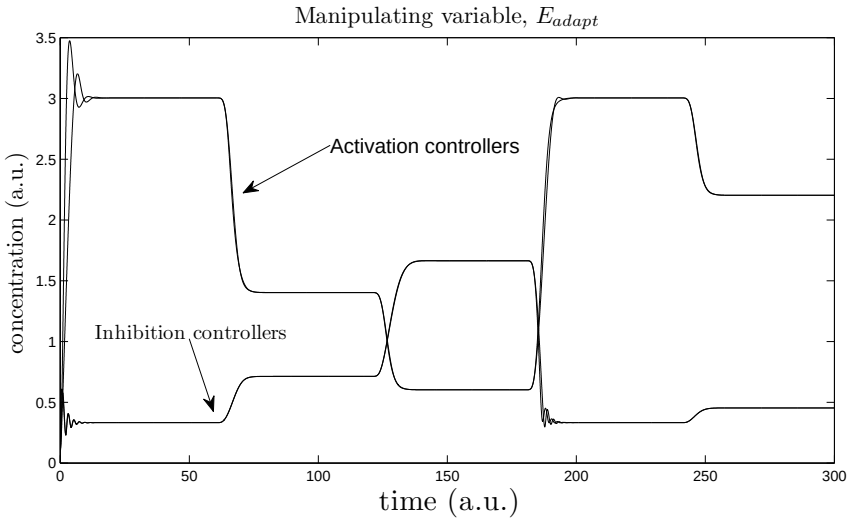


Figure 3.13: Overview of the enzyme level with small inflow

It can be seen that the activation controllers have an increase in the amount of enzyme when the inflow increases, while the inhibition controllers have an increase in the amount of enzyme when the inflow decreases.

Chapter 4

Discussion and conclusion

4.1 Natural controller simulations

4.1.1 Implementation

It has been shown that the upstream activation type 1 system gives harmonic oscillations if Michaelis-Menten kinetics is assumed. This suggests that the implementation has been correct. There are 8 controllers implemented in the model, while only one has been tested in this report. These have not been tested thoroughly and their accuracy should therefore be confirmed.

4.1.2 Rate constants in case 1a

It can be concluded that the rate flow constants control the set-point and the effect of changing these values has been documented.

4.1.3 Michaelis-Menten

It has been shown that if Michaelis-Menten kinetics are assumed, the damping of the system depends on the size of the Michaelis-Menten constant. The larger the Michaelis-Menten constant is compared to its respective outflow, the more damping there is in the system. Additionally it has been shown that if Michaelis-Menten kinetics is assumed, the constant prevents the concentration from becoming negative.

4.1.4 Dynamic variable substrate

It has been shown that if a dynamic variable, a, is introduced in front of the enzyme activation, the system stops oscillating. However the set-point remains the same.

4.1.5 Dynamic variable enzyme

It has been shown that if a dynamic variable, e, is introduced in front of the substrate activation, one gets damped oscillations that converge into standing, harmonic oscillations.

4.1.6 Dynamic variables enzyme and substrate

It has been shown that, like case 1b, is a dynamic variable is involved in the substrate, oscillations are no longer found in the upstream activation type 1 controller. The set-point however, is unaltered.

4.2 Controller comparison

4.2.1 Upstream comparison

From the simulations it can seem like the activation controllers are better at tracking the set-point, while the inhibition controllers settle on a value lower than the set-point. Otherwise, the activation controllers show very similar reactions. The inhibition controllers show different reactions from the activation controllers, but are similar to each other.

4.2.2 Downstream comparison

It can seem from the simulations that inhibition controllers respond faster when the inflow it is compensating is close in value to the outflow of the system. Additionally it has been found that activation controllers respond faster when the inflow is larger than the outflow.

4.3 Further work

As this topic is a vast one, there is plenty of work left to do. All the 8 natural controller types are modelled in Matlab, even though only one has been thoroughly investigated in this report. These should be tested thoroughly and also investigated.

Practically, it could be looked into whether the activation and inhibition controllers show different qualities also in nature with respect to the perturbation size compared to the controlled flow.

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Appendix A

Nomenclature

Nomenclature

Word	Meaning
Activation	When a substance is being catalyzed
Catalyst	A substance which lowers the reaction energy in a process, without being consumed in the said process
Closed system	A system with no exterior input
Downstream controller	A natural controller which affects the outflow of the substrate
Enzyme	A type of catalyst
Homeostasis	A chemical system in steady state
Inhibitor	Substance which increases the reaction energy in a process without being consumed
Natural controller	A model for describing a chemical negative feedback system
Perturbation	An un-regulated disturbance
Product	The final state of the substrate in a enzyme kinetics
Substrate	The initial substance in enzyme kinetics
Substrate-enzyme complex	A conjoined form of the substrate and enzyme after binding takes place
Upstream controller	A natural controller which affects the inflow of the substrate

Controller names

Role	Controller name
Inflow compensation 1	Downstream activation type 1
Inflow compensation 3	Downstream activation type 2
Inflow compensation 6	Downstream inhibition type 1
Inflow compensation 8	Downstream inhibition type 2
Outflow compensation 2	Upstream inhibition type 1
Outflow compensation 4	Upstream inhibition type 2
Outflow compensation 5	Upstream activation type 1
Outflow compensation 6	Upstream activation type 2

Appendix B

Additional plots

B.1 Controller oscillations

B.1.1 Case 1b

In the Phase plane: Figure shows A plotted against E_{adapt}:

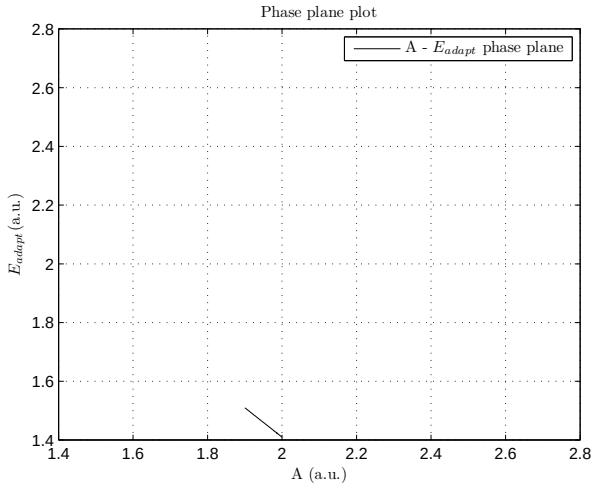

Figure B.1: Phase plane plot

The added variable behaved as shown in figure B.2.

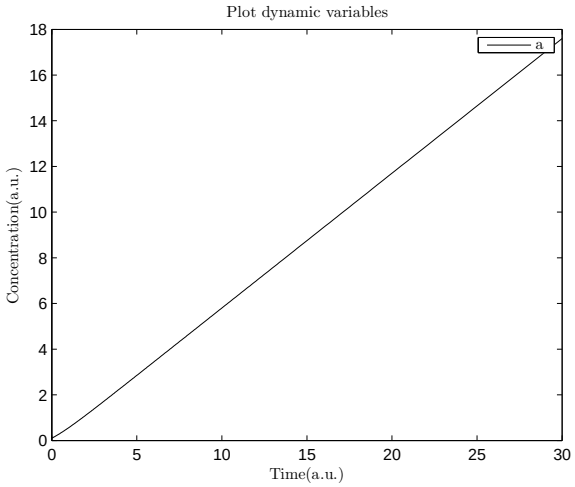

Figure B.2: Case 1b - a vs E_{adapt}

B.1.2 Case 1c

Added variable e:

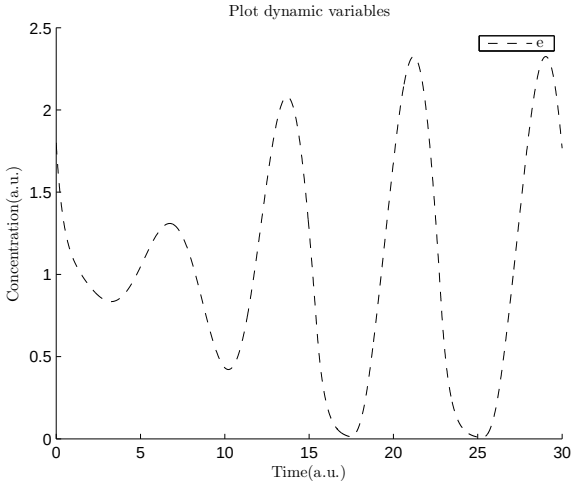

Figure B.3: Case 1c - Plot of e vs. time

B.1.3 Case 1d

The added variables behaved as shown in figure B.4

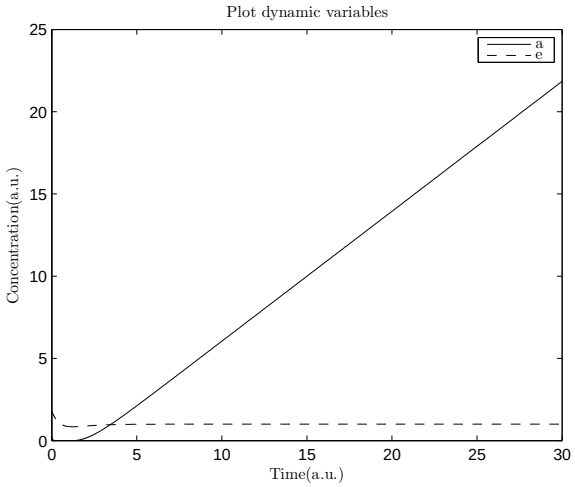

Figure B.4: Case 1d - a and e vs. time

Figure shows a plotted against E_{adapt}:

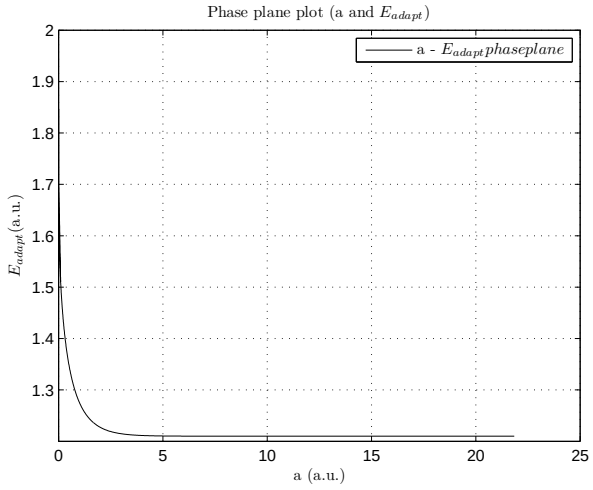

Figure B.5: Case 1d - Phase plane plot, a vs. E_{adapt}