Examensarbete

Mathematical models of bacteria population growth in bioreactors: formulation, phase space pictures, optimisation and control.

Per Erik Strandberg

LiTH - MAI - EX - - 04 / 04 - - SE

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Examensarbete: **20 p**

Level: **D**

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Linköping: **March 2004**

LINKÖPINGS UNIVERSITET

Avdelning, Institution
Division, Department

Matematiska Institutionen
581 83 LINKÖPING
SWEDEN

Datum
Date

march 2004

Språk
Language

☐ Svenska/Swedish
☒ Engelska/English
☐ _____

Rapporttyp
Report category

☐ Licentiatavhandling
☐ Examensarbete
☐ C-uppsats
☒ D-uppsats
☐ Övrig rapport
☐ _____

ISBN

ISRN
LiTH - MAT - EX - - 04 / 04 - - SE

Serietitel och serienummer **ISSN**
Title of series, numbering 0348-2960

URL för elektronisk version

http://www.ep.liu.se/exjobb/mai/2004/tm/004/

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Författare
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Sammanfattning
Abstract

There are many types of bioreactors used for producing bacteria populations in commercial, medical and research applications. This report presents a systematic discussion of some of the most important models corresponding to the well known reproduction kinetics such as the Michaelis-Menten kinetics, competitive substrate inhibition and competitive product inhibition.
We propose a modification of a known model, analyze it in the same manner as known models and discuss the most popular types of bioreactors and ways of controlling them. This work summarises much of the known results and may serve as an aid in attempts to design new models.

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We propose a modification of a known model, analyze it in the same manner as known models and discuss the most popular types of bioreactors and ways of controlling them. This work summarises much of the known results and may serve as an aid in attempts to design new models.

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Abstract in Swedish: Sammanfattning

Det finns många typer av bioreaktorer som tillämpas kommersiellt, och inom medicin och forskning. Denna rapport presenterar en systematisk redovisning av några av de viktigaste modellerna som motsvarar de kända kinetiska formerna Michaelis-Menten kinetik, kompetitiv substratinhibering och kompetitiv produktinhibiering.

Vi föreslår en modifiering av en känd modell, analyserar den på samma sätt som kända modeller och redogör för de populäraste bioreaktortyperna och hur de kan kontrolleras. Detta verk sammanfattar mycket av idag kända resultat och kan användas som hjälp i design av nya modeller.

Acknowledgements

I would like to thank my supervisor and examiner Stefan Rauch, at the division of applied mathematics for his excellent guidance, our rewarding discussions and his constant feedback.

I also have to thank Berkant Savas for helping to mature mathematically, for introducing me to the wonderful world of LaTeX, and for his efforts in proofreading this material.

My opponent Andréas Andersson also deserves my thanks for his constructive feedback and help in proofreading.

Nomenclature

Most of the reoccurring abbreviations and symbols are described here.

Symbols

Y_0	The amount of the variable Y inserted into a system.
$\hat{Y}$	The unit-dimension of the variable Y, for example $\hat{t} = 1s$.
$\bar{Y}_i$	A steady state (number i) value of Y.
K_i	Constants used in kinetic expressions, for example K_I.
$\mathbf{A}$	The system matrix.
a, b	Growth- and non growth-associated production yield coefficients.
c, φ, δ	Fraction of X, F or S_0. $0 < c < 1$, $0 < \varphi < 1$, $0 < \delta < 1$.
$\mathbb{C}$, $\mathbb{R}$	The set of complex and real numbers.
D	Dilution coefficient; fraction of V replaced per timeunit.
E	Enzyme concentration in a system.
F	The flow of a media in or out of a system.
P	Product concentration in a system.
S	Substrate concentration in a system.
V	The volume of a system.
X	Biomass concentration in a system (kilogram/litre, etc.)
$\mathbf{X}$	Vector containing S, X and P.
α_1	Dimensionless maximal reproduction rate.
α_2	Dimensionless nutrition feed concentration.
α, β	Unitconsumtion of S needed to produce one unit of X or P.
μ	The general rate expression. $\mu = \mu(S, X, P, \dots)$

Abbreviations

CPI	Competitive Product Inhibition (or Inhibited)
CSI	Competitive Substrate Inhibition (or Inhibited)
CSTR	Continuous Stirred Tank (bio)Reactor
MMI	Michaelis-Menten Inhibition (or Inhibited)

Contents

Chapter 1

Introduction

This text is written as a master of science final thesis[1] at the University of Linköping by Per Erik Strandberg with Stefan Rauch as supervisor and examiner, with Emacs in LaTeX in 2003/2004.

This first chapter will define what is a Continuous stirred tank bioreactor and a chemostat. We give some background and formulate some main questions we wish to answer, and describe what topics are covered.

1.1 Background

Modeling of biological growth in reactors by dynamical systems started in the 1950's, when Monod, and Novick and Szilard developed the concept of *Continuous Stirred Tank (bio)Reactors*(CSTRs) that differed from traditional batchreactors.

In a CSTR kinetics of the cellgrowth is sometimes referred to as a *blackbox model*, since all of the intra- and extra-cellular reactions are lumped into one overall reaction. Typical equations describing blackbox models are very similar to enzymatic kinetics (where there is often one substrate and one product in one reaction), such as the Michaelis-Menten kinetics used in the ideal CSTR (the chemostat), often referred to as Monods model.

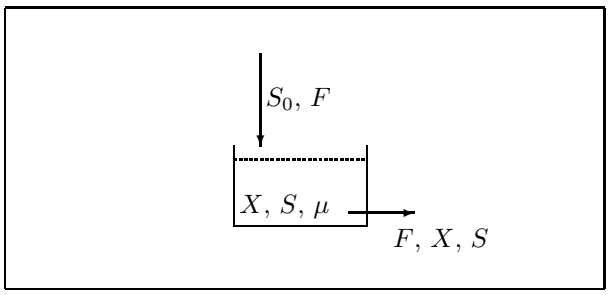

Figure 1.1: The ideal CSTR: the chemostat, its inflow of nutrition, its growth (μ), and its outflow.

[1] Swedish: Filosofie magister examensarbete.

A chemostat is typically made of a reactor, containing a bacteria-population, of density $X(t)$, and a substrate of density $S(t)$ feeding the bacteria. The reactor is supplied with stocknutrient of concentration S_0 from some kind of nutrient reservoir with the flow F that is constant over time. To maintain a constant volume the inflow equals the outflow in which bacteria and/or products are harvested.

1.2 Problems we want to solve

This report provides a summary of already existing knowledge of bioreactors and a detailed analysis of differential equations modelling bioreactors that one usually encounters in textbooks. Some modifications of existing models are proposed and studied in detail.

This text aims to answer questions like "What types of bioreactors are there?", "If my bacteria population reproduces according to one of the kinetic forms, how will it behave?", "If my model is not really correct, how could I change it?", "What do I need to consider before fixing my model?", and some more.

1.3 Topics covered

There are five chapters (and this introduction) and two appendixes. Main topics dealt with are:

Chapter 2: We explain the chemostat equations with the standard Michaelis-Menten kinetics by starting with two simple models of biological growth. We also present some tools useful for studying dynamical systems in two dimensions.

Chapter 3: A new kinetic form is proposed and applied to an ideal CSTR.

Chapter 4: We leave the ideal CSTR and introduce, motivate and examine some modified CSTRs.

Chapter 5: We look at controlled CSTRs.

Chapter 6: Summary and conclusion.

Appendix A: An appendix dealing with tools used to simplify the analysis of linearized stability in steady states.

Appendix B: A Matlab M-file is appended, showing how a program like Matlab can be used to visualize and experiment with a given mathematical model.

Chapter 2

The ideal CSTR: the chemostat

In this chapter we study exponential growth, the logistic equation and the batchreactors. We introduce the terminology, and explain how to think and how to look at models and their describing differential equations.

We will derive and carefully analyze the chemostat equations. These equations will constitute a reference model for the remaining parts of this text.

2.1 Some simple models of biological growth

2.1.1 Exponential growth

An extremely simple model could be $\frac{dX}{dt} = \mu \cdot X$ where μ is the birth coefficient and X stands for bacteria density. If $\mu =$ constant > 0, we get $X(t) = X_0 e^{\mu t}$.

This is too simple a model. To limit the production of organisms we introduce a variable S describing concentration of the nutrient into the dynamic equations.

2.1.2 The logistic equation

Let us assume that $\frac{dX}{dt} = \mu \cdot X$, with $\mu = \mu(S) = k \cdot S$, and that $\frac{dS}{dt} = -\alpha k S X$, meaning that each unit of bacteria density produces kS units of offspring per time unit. With $\alpha =$ constant > 0 we could mean that each produced unit of offspring requires α units of nutrition. This model corresponds to our intuition: the term SX says how often bacteria and food meet, giving the bacteria an opportunity to consume nutrient particles from the inflow S_0 and to reproduce.

We get a system of ordinary differential equations:

$$\begin{cases} \frac{dX}{dt} = kSX & (a) \\ \frac{dS}{dt} = -\alpha k S X & (b) \end{cases}$$

Multiplying (a) by α and adding (b) we get: $\frac{d}{dt}(S + \alpha X) = 0$, thus $(S + \alpha X)(t) = S_0 + \alpha X_0 =$ constant. In particular with $t = 0$ and $X(0) = X_0 \approx 0$ (or at least small in comparison to a normal $X(t)$) we have $S(0) + \alpha X(0) \approx S_0$,

since X_0 small implies $S(t) = S_0 - \alpha X(t)$, giving us a reason to eliminate (b), and rewrite (a) as:

$$\frac{dX}{dt} = k(\underbrace{S_0 - \alpha X}_{\approx S(t)})X = \underbrace{kS_0}_{r>0}(1 - \frac{X}{\underbrace{S_0/\alpha}_{B>0}})X = r(1 - \frac{X}{B})X$$

By changing some factors we have reduced our system of ordinary differential equations to a single equation, called the logistic equation:

$$\frac{dX}{dt} = r(1 - \frac{X}{B})X \tag{2.1}$$

The factor $r(1-\frac{X}{B})$, that corresponds to our old μ, is called an intrinsic[1] growth-speed, and B the carrying capacity. By eliminating X instead of S, we quickly find $\frac{dS}{dt} = -\alpha r(1 - \frac{S}{\alpha B})S$, an equation describing time change of the nutrient concentration.

When analyzing (2.1) we discover that for small values of X, when $0 < X \ll B$ we can approximate (2.1) by an exponential term and our model gives exponential growth for very small initial population densities.

The factor $\frac{-XX}{B}$ in the equation corresponds to a crowding effect, inhibiting the reproduction rate.

The sign of $(1 - \frac{X}{B})$ is important to analyze. Let us assume that $X \geq 0$. We also know that $r > 0$. Meaning that $(1 - \frac{X}{B})$ is what determines the sign of $\frac{dX}{dt}$.

$X = 0 \Rightarrow \frac{dX}{dt} = 0$, a trivial solution, there is no population;

$0 < X < B \Rightarrow (1 - \frac{X}{B}) > 0$, the population grows;

$X = B \Rightarrow \frac{dX}{dt} = 0$, constant population; and

$X > B \Rightarrow \frac{dX}{dt} < 0$, decreasing population.

We now know that the model corresponds to our intuition. A small population initially grows exponentially, later the term $(1 - \frac{X}{B})$ starts to play role. If ever $X = B$ we will get a constant population. Finally: if $X > B$, N will eventually diminish towards $X = B$. $X = 0$ and $X = B$ are equilibrium solutions, or steady states.

An explicit solution to (2.1) is: $X(t) = \frac{X_0 B}{X_0 + (B - X_0)e^{-rt}}$, if $0 < X_0 < B$. It can be found by separating variables in equation (2.1)

2.1.3 A general batch reactor

Before we introduce the chemostat let us make a comment on a more general case that is similar to logistic growth: the batch reactor.

Into a vessel (reactor) we insert a nutrition solution with concentration S_0 and a small population of bacteria X_0 and close the lid. Assume we have some kind of kinetic expression μ. We then get the equations:

$$\begin{cases} \frac{dX}{dt} = \mu X & (a) \\ \frac{dS}{dt} = -\alpha \mu X & (b) \end{cases} \tag{2.2}$$

Again, as with the logistic growth we sum up $\alpha(a) + (b)$ and, for the same reasons as above, find: $S = S_0 + \alpha(X_0 - X)$. If we can assume $X_0 \ll X$ we get

[1] Swedish: "inre" or "inneboende"

the nice, and general expression: $S = S_0 - \alpha X$. Again we can eliminate S and, if $\mu = \mu(X)$, end up with:

$$\frac{dX}{dt} = \mu(X) \cdot X \tag{2.3}$$

We can now insert various kinetics instead of μ and investigate what happens.

2.2 The chemostat

A chemostat is made of two main parts; a nutrient reservoir, and a growth-chamber, reactor, in which the bacteria reproduces. Via an inflow from the reservoir fresh nutrition is added and from an outflow bacteria are harvested.

The purpose of the chemostat is to have a quasi-constant X and S, allowing us to harvest at a constant rate. The name chemostat stands for "the *chem*ical environment is *stat*ic".

We must adjust our earlier models by introducing an inflow and in doing so, we will also define units used in our growth-chamber.

We will also reduce the number of parameters of the chemostat by reducing it to the dimensionless form.

2.2.1 The Continuous flow

We let $F = F_{in} = F_{out}$ having dimensions of volume/time. With F_{in} comes S_0: "mass"/volume[2]. In the reactor we have a population of bacteria of density X: mass/volume, and S: mass/volume.

We modify equation (2.1) by adding terms describing the inflow of the nutrition-solution and the outflow of bacteria, and assume $\mu = \mu(S)$. Thus:

$$\begin{cases} \frac{dX}{dt} = \mu(S)X \overbrace{-X\frac{F}{V}}^{\text{new}} \\ \frac{dS}{dt} = -\alpha\mu(S)X \underbrace{-S\frac{F}{V} + S_0\frac{F}{V}}_{\text{new}} \end{cases} \tag{2.4}$$

As we can see, F appears together with V in the form of F/V, so we set $F/V = D$ - a dilution coefficient. It describes the fraction of volume being replaced in a unit of time.

The term X/V describes the density of bacteria, and by multiplying it with F we get the amount of bacteria being flushed away in a unit of time. Similarly $-DS$ corresponds to the outflow of the nutrient, and $+DS_0$ to the inflow of the nutrient.

2.2.2 The Michaelis-Menten-kinetics

We will now discuss the reproduction coefficient, $\mu(S)$, by relating it to the Michaelis-Menten kinetics. This is the main "black box" we will see, and it is often also referred to as Monods kinetics.

Experimental data and similar cases in enzyme kinetics motivate the need of the reproduction-constant to be almost linear for small positive values for S,

[2] "Mass" could be moles, kilograms, molecules, etc.

but we also require an upper limit for μ so that: $\mu(S) \overset{S\to\infty}{\longrightarrow} \mu_{max}$. We write down the Michaelis-Menten-kinetics:

$$\mu(S) = \frac{\mu_{max} S}{K_N + S}$$

The new constant K_N has an interesting meaning: $\frac{K_N}{K_N+K_N} = \frac{1}{2}\mu_{max}$. So K_N corresponds to the concentration at which $\mu = \frac{1}{2}\mu_{max}$

Let us see how $\mu(S)$ depends on S:

Small S's give us $\mu(S) \approx S\frac{\mu_{max}}{K_N}$, if we can assume $S \ll K_N$, thus $\mu(S)$ is almost linear. The maximum μ, μ_{max}, is never reached, no matter how great S gets, we have $\mu(S) < \mu_{max}$.

The limit $\mu(S) \overset{S\to\infty}{\longrightarrow} \mu_{max}$ is what we wanted. So μ_{max} is the maximal reproduction rate, achieved when the nutrient is unlimited.

With this $\mu(S)$, the model becomes:

$$\begin{cases} \frac{dX}{dt} = \mu_{max}\frac{S}{K_N+S}X - DX \\ \frac{dS}{dt} = -\alpha\mu_{max}\frac{S}{K_N+S}X - DS + DS_0 \end{cases} \tag{2.5}$$

A more general CSTR, where Ω stands for other possible variables (X, (bi-) products, pH, ...) that may influence the reproduction rate, and X_0 stands for bacteria density inserted via the in-flow, could be described by:

$$\begin{cases} \frac{dX}{dt} = \quad \mu(S,\Omega)X - DX + DX_0 \\ \frac{dS}{dt} = -\alpha\mu(S,\Omega)X - DS + DS_0 \end{cases} \tag{2.6}$$

It is important to understand the structure of (2.6). We will look at others CSTR's, where this form is the common origin.

2.2.3 The dimensionless form of the chemostat

A quick glance at our equations show that we have a number of variable parameters: μ_{max}, K_N, D, α, and S_0. Each one of them is important and necessary, but is there a way for us to reduce the number of parameters somehow? The answer is yes, and we will see that it is possible to eliminate three of our constants (we eliminate five of them and introduce two new ones, this is possible since we have five parameters, and three dimensions).

As the title of this subsection suggests, we will actually eliminate the dimensions/units of the equations, but first we must investigate the units of our constants. The first relation:

$\dim[\frac{dX}{dt}] = \frac{number}{volume\cdot time}$ (here we assume $\dim[X] = number/volume$ and $\dim[t] = time$), thus: $\dim[\frac{\mu_{max}XS}{K_N+S}] = \frac{number}{volume\cdot time}$. With $\dim[S] = \frac{mass}{volume}$ we get $\dim[K_N] = \frac{mass}{volume}$ in order to keep $K_N + S$ meaningful, only allowing $\dim[\mu_{max}] = \frac{1}{time}$.

Our second relation: $\dim[\frac{dS}{dt}] = \frac{mass}{volume\cdot time}$, and since we know that $\dim[S] = \frac{mass}{volume}$, we get $\dim[S_0] = \frac{mass}{volume}$. So for α we get $\dim[\alpha] = \frac{mass/volume}{number/volume} = 1$.

And now, to simplify, we replace S, X, and t with $S\cdot\hat{S}$, $X\cdot\hat{X}$, and $t\cdot\hat{t}$. Where $\hat{S}$, $\hat{X}$ and $\hat{t}$ corresponds to the unit-dimension, whatever they may be.[3]

[3]Note that $\hat{t}$ could be a second, a year, 3.25489677 minutes, etc, $\hat{X}$ could be one bacteria, twelve bacterias or 2.553 kg of dry cellweight per litre, gallon or tankvolume, and $\hat{S}$ could typically be: mol/l, molecules/mm^3 or 3.333 lb/gallon.

Our elegant equation (2.5) will now for a moment be replaced with:

$$\begin{cases} \frac{dX}{dt} \cdot \frac{\hat{X}}{\hat{t}} = \mu_{max} \frac{S\hat{S}}{K_N + S\hat{S}} X\hat{X} - DX\hat{X} \\ \frac{dS}{dt} \cdot \frac{\hat{S}}{\hat{t}} = -\alpha\mu_{max} \frac{S\hat{S}}{K_N + S\hat{S}} X\hat{X} - DS\hat{S} + DS_0 \end{cases}$$

Now, to get a dimensionless form, we multiply the dX-row with $\hat{t}/\hat{X}$, and the dS-row with $\hat{t}/\hat{S}$, allowing us to fix $\hat{t} = \frac{1}{D}$, $\hat{S} = K_N$, $\hat{X} = \frac{\hat{S}}{\alpha\mu_{max}\hat{t}} = \frac{K_N D}{\alpha\mu_{max}}$, and replace $\alpha_1 = \hat{t}\mu_{max} = \frac{1}{D}\mu_{max}$ and $\alpha_2 = \frac{S_0}{\hat{S}} = \frac{S_0}{K_N} = \frac{\hat{t}DS_0}{\hat{S}}$. These replacings give:

$$\begin{cases} \frac{dX}{dt} = \alpha_1 \frac{S}{1+S} X - X \\ \frac{dS}{dt} = -\frac{S}{1+S} X - S + \alpha_2 \end{cases} \tag{2.7}$$

Showing that the chemostat effectively depends on two parameters α_1 and α_2.

2.3 Analyzing the chemostat equations

For the chemostat equations we find equilibrium solutions, null-clines, investigate interesting values of the parameters α_1 and α_2, linearize equations (2.7) around equilibrium points and find an invariant line.

2.3.1 Equilibrium solutions of the chemostat

To find equilibrium solutions we solve:

$$\begin{cases} \frac{dX}{dt} = \alpha_1(\frac{S}{1+S})X - X = 0 \\ \frac{dS}{dt} = -(\frac{S}{1+S})X - S + \alpha_2 = 0 \end{cases} \Rightarrow \begin{cases} 0 = \alpha_1(\frac{S}{1+S})X - X \\ 0 = -(\frac{S}{1+S})X - S + \alpha_2 \end{cases}$$

One trivial solution is $X = 0$. Inserting this into the second relation gives us $S = \alpha_2$ thus one equilibrium solution is: $(\bar{X}_0, \bar{S}_0) = (0, \alpha_2)$. This is our first hint indicating that α_2 has a meaning of a dimensionless stock-nutrient-concentration.

The other (non-trivial) solution is more interesting. We get: $X = \alpha_1(\frac{S}{1+S})X$ and for $X \neq 0$, $S = \frac{1}{\alpha_1 - 1}$.

Combining this with the second equation, we get: $X = \alpha_1(\alpha_2 - \frac{1}{\alpha_1 - 1})$. So we have two equilibrium points:

$$\begin{aligned} (\bar{X}_0, \bar{S}_0) &= (0, \alpha_2) \\ (\bar{X}_1, \bar{S}_1) &= \left(\alpha_1(\alpha_2 - \tfrac{1}{\alpha_1 - 1}), \tfrac{1}{\alpha_1 - 1}\right) \end{aligned} \tag{2.8}$$

2.3.2 Parameters α_1 and α_2

Let us discuss when α_1 and α_2 give us an equilibrium having biological meaning.

We saw that $\alpha_1 = \frac{\mu_{max}}{D}$. Giving us the hint that α_1 could be interpreted as a kind of dimensionless reproduction-rate, or even more interestingly: as a maximum reproduction-rate multiplied by the inverse of the flushing factor.

Combining our intuition with the solutions we found above, we notice: $\bar{S}_1 = \frac{1}{\alpha_1 - 1}$, this must be positive for the population to exist, giving us: $\alpha_1 > 1$.

Meaning that we must limit the dilution rate to be smaller than the maximal reproduction rate.

From the trivial solution we notice that $\bar{S}_0 = \alpha_2 = \frac{S_0}{K_N}$ characterises nutrient. But as we will see, this is one way of dealing with α_2. Another way of looking at this parameter is to interpret it as an inverse number of K_N, or as the amount of S we have, measured in units of K_N. We saw that a small K_N means that we are closer to μ_{max} for lower values of S. So small K_N means that α_2 is large and vice versa.

Looking at our non-trivial solution again we notice that $\bar{X}_1 = \alpha_2 - \frac{1}{\alpha_1 - 1}$ must be positive for the population to exist. Requiring $\alpha_2 > \frac{1}{\alpha_1 - 1}$. (Thus $\alpha_2 > 0$.) Here we can see that for small $(\alpha_1 - 1)$ (large flushing or weak reproduction) we need α_2 to be large (strong flushing or weak reproduction, requires more food in order to reproduce faster), and a large α_1 allows α_2 to be small. This is how the model tells us that if it easy to reproduce we have a lower need of nutrition.

2.3.3 Nullclines of the chemostat

A useful tool for investigating this kind of dynamic systems are nullclines, the lines where $\frac{dX}{dt} = 0$, or $\frac{dS}{dt} = 0$. We also understand that at the crossing of two different kind of nullclines there is an equilibrium point.

Let us begin with the X-relation: $0 = \frac{dX}{dt} = \alpha_1(\frac{S}{1+S})X - X$, we notice that one nullcline is: $X = 0$, and the other ones is: $\alpha_1(\frac{S}{1+S}) - 1 = 0$ so $S = \frac{1}{\alpha_1 - 1}$.

Continuing with the nullclines for S: $0 = \frac{dS}{dt} = -(\frac{S}{1+S})X - S + \alpha_2 = 0$, after some elementary work, we get: $X = \frac{(\alpha_2 - S)(1+S)}{S}$. Our nullclines are given by:

$$\begin{cases} \dot{X} = 0 & \Rightarrow \quad X = 0 \text{ or } S = \frac{1}{\alpha_1 - 1} \\ \dot{S} = 0 & \Rightarrow \quad X = \frac{(\alpha_2 - S)(1+S)}{S} \end{cases}$$

The nullclines are easy to draw, and by drawing some vectors in the first quadrant, we can get a pretty good idea of how a state $(X(t), S(t))$ moves around in the (X,S)-plane over time.

2.3.4 Linearization around the equilibrium points

We have found two equilibrium solutions in the positive quadrant of the (X,S)-plane: $(\bar{X}_0, \bar{S}_0) = (0, \alpha_2)$ and $(\bar{X}_1, \bar{S}_1) = \left(\alpha_1(\alpha_2 - \frac{1}{\alpha_1 - 1}), \frac{1}{\alpha_1 - 1}\right)$. We want to study in the neighbourhood of these points how the chemostat responds to small disturbances. Does it wander away from an equilibrium or does it fall back onto it? The question we want to answer is: is the solution stable?

In order to analyze the chemostat's behaviour at the equilibrium we will use the stability conditions described in Appendix A. The stability conditions are $tr(\mathbf{A}) < 0$ and $det(\mathbf{A}) > 0$. For eigenvalues of the linearization matrix $\mathbf{A}$ to be real we need $-4\, det(\mathbf{A}) + tr(\mathbf{A})^2 > 0$.

We find (see Appendix A):

$$\mathbf{A} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix} = \begin{pmatrix} \alpha_1 \frac{S}{1+S} - 1 & \frac{\alpha_1 X}{(1+S)^2} \\ -\frac{S}{1+S} & -\frac{X}{(1+S)^2} - 1 \end{pmatrix}$$

Evaluating this at the trivial equilibrium point $(\bar{X}_0, \bar{S}_0) = (0, \alpha_2)$ we get:

$$\mathbf{A} = \begin{pmatrix} \frac{\alpha_1\alpha_2}{1+\alpha_2} - 1 & 0 \\ -\frac{\alpha_2}{1+\alpha_2} & -1 \end{pmatrix} \quad \Rightarrow \quad \begin{cases} tr(\mathbf{A}) = \frac{\alpha_1\alpha_2}{1+\alpha_2} - 2 \\ det(\mathbf{A}) = -\frac{\alpha_1\alpha_2}{1+\alpha_2} + 1 \end{cases}$$

We know that we have two conditions for stability around an equilibrium point: $tr(\mathbf{A}) < 0$ and $det(\mathbf{A}) > 0$. We will notice that our second condition: $-\frac{\alpha_1\alpha_2}{1+\alpha_2} + 1 > 0 \quad \Leftrightarrow \quad 1 > \frac{\alpha_1\alpha_2}{1+\alpha_2}$ is in conflict with the earlier condition for physical meaning, $\alpha_2 > \frac{1}{\alpha_1 - 1} \quad \Leftrightarrow \quad 1 < \frac{\alpha_1\alpha_2}{1+\alpha_2}$. So this steady state is not stable.

At the other equilibrium point, $(\bar{X}_1, \bar{S}_1) = \left(\alpha_1(\alpha_2 - \frac{1}{\alpha_1 - 1}), \frac{1}{\alpha_1 - 1}\right)$, by renaming the term $\frac{\bar{X}_1}{(\bar{S}_1+1)^2} = \sigma$ and remember that $\sigma > 0$, we get:

$$\mathbf{A} = \begin{pmatrix} 0 & \sigma\alpha_1 \\ -\frac{1}{\alpha_1} & -\sigma - 1 \end{pmatrix} \quad \Rightarrow \quad \begin{cases} tr(\mathbf{A}) = -\sigma - 1 & < 0. \\ det(\mathbf{A}) = \sigma & > 0. \end{cases}$$

The non-trivial equilibrium point is thus stable. We also note that:
$-4\, det(\mathbf{A}) + tr(\mathbf{A})^2 = -4\sigma + \sigma^2 + 2\sigma + 1 = \sigma^2 - 2\sigma + 1 = (\sigma - 1)^2 > 0$. So trajectories of a state near this point are not spiraling since their eigenvectors are real. They go more straight-forward towards $(\bar{X}_1, \bar{S}_1)$.

2.3.5 The invariant line

In this subsection we study the line $X = -\alpha_1 S + \alpha_1\alpha_2$.

All solutions asymptotically approaches the invariant line

From (2.7): $\begin{cases} \frac{dX}{dt} = \alpha_1(\frac{S}{1+S})X - X & (a) \\ \frac{dS}{dt} = -(\frac{S}{1+S})X - S + \alpha_2 & (b) \end{cases}$ we perform $(a) + \alpha_1(b)$ and get: $\frac{d}{dt}(X + \alpha_1 S)(t) = \alpha_1\alpha_2 - (X + \alpha_1 S)(t)$. We can look at this relation as an ordinary differential equation with one function $(X + \alpha_1 S)(t)$. One solution is: $(X + \alpha_1 S)(t) = Ke^{-t} + \alpha_1\alpha_2$. One nice property of $(X + \alpha_1 S)(t)$ is that $(X + \alpha_1 S)(t) \overset{t\to\infty}{\longrightarrow} \alpha_1\alpha_2$.

Thus when $t \to \infty$ we have $X - \alpha_1 S = \alpha_1\alpha_2 \Leftrightarrow X = -\alpha_1 S + \alpha_1\alpha_2$, all solutions wandering in the positive quadrant of the (X,S)-plane asymptotically approaches this line.

We also notice that putting $X = 0$ will give $S = \alpha_2$, the trivial equilibrium point, and $S = 0$ give us $X = \alpha_1\alpha_2$. You can also easily verify that this line passes through the non-trivial equilibrium point as well.

The invariant line has the direction of one of the eigenvectors

We remember that $\mathbf{A} = \begin{pmatrix} 0 & \sigma\alpha_1 \\ -\frac{1}{\alpha_1} & -\sigma - 1 \end{pmatrix}$, $\sigma = \frac{\bar{X}_1}{(\bar{S}_1+1)^2} > 0$, now assume that a solution has the form: $\begin{pmatrix} X(t) \\ S(t) \end{pmatrix} = \mathbf{X}(t) = \mathbf{v}e^{\lambda t}$. When differentiating this and looking at our linearization we get: $\mathbf{A}\mathbf{v}e^{\lambda t} = \lambda\mathbf{v}e^{\lambda t}$, and when using the classic secular equation to find the eigenvalues and eigenvectors we get:

$$(\mathbf{A} - \mathbf{I}\lambda)\mathbf{v} = 0 \quad \Rightarrow \quad 0 = det(\mathbf{A} - \mathbf{I}\lambda) = det\begin{pmatrix} -\lambda & \alpha_1\sigma \\ -\frac{1}{\alpha_1} & -\sigma - 1 - \lambda \end{pmatrix}$$

$$\Rightarrow \quad \lambda(\sigma + 1 + \lambda) + \sigma = 0 \quad \Rightarrow \quad \lambda_1 = -1, \text{and } \lambda_2 = -\sigma.$$

Since we saw $-4\, det(\mathbf{A}) + tr(\mathbf{A})^2 > 0$ we had expected real eigenvalues and the stability conditions are fulfilled, so we had expected them to be negative. Now we must find the eigenvectors. We insert our eigenvalues in $(\mathbf{A} - \mathbf{I}\lambda)\mathbf{v} = 0$:

$$(\mathbf{A} - \mathbf{I}\lambda_1)\mathbf{v_1} = \begin{pmatrix} 1 & \alpha_1\sigma \\ -\frac{1}{\alpha_1} & -\sigma \end{pmatrix} \begin{pmatrix} v_{11} \\ v_{12} \end{pmatrix} = 0 \quad \Rightarrow \quad \mathbf{v_1} = \begin{pmatrix} \alpha_1\sigma \\ -1 \end{pmatrix}$$

$$(\mathbf{A} - \mathbf{I}\lambda_2)\mathbf{v_2} = \begin{pmatrix} \sigma & \alpha_1\sigma \\ -\frac{1}{\alpha_1} & -1 \end{pmatrix} \begin{pmatrix} v_{21} \\ v_{22} \end{pmatrix} = 0 \quad \Rightarrow \quad \mathbf{v_2} = \begin{pmatrix} \alpha_1 \\ -1 \end{pmatrix}$$

By elongating the eigenvector $\mathbf{v_2}$ in positive and negative directions from $(\bar{X}_1, \bar{S}_1)$ we can easily verify that this is the same line as $X = -\alpha_1 S + \alpha_1\alpha_2$. Thus a state starting on the invariant line can not move away from it.

An observation

By the theorem of existence and uniqueness of solutions of ordinary differential equations two trajectories cannot cross each other. Thus the invariant line (consisting of trajectories) cannot be crossed. This helps in understanding how trajectories behave in the neighbourhood of the invariant line (see figure 2.1).

The invariant line: conclusions

We can now conclude that the invariance line can be seen as a barrier for the solutions. No solution above it can get below it and vice versa. This gives us a guidance in drawing a phase portrait, and we can see in the Matlab simulation that, indeed, the solutions near the non-trivial equilibrium solution can not move around in spirals.

2.3.6 Looking at the phaseportrait

When looking at the phaseportrait we will plot trajectories using Eulers method: $\mathbf{X_{n+1}} = \mathbf{X_n} + \Delta t \cdot \frac{d}{dt}\mathbf{X_n}$. We can call this kind of trajectory an Euler path.

The phaseportrait of this kind of a system gives us a lot of information. In this example we can see the invariant line (dotted), the nullclines, and an Euler path (point-dotted) starting at a low amounts of both bacteria and nutrition rates. In this example we understand that the state at first wanders towards higher amounts of nutrition (the bacteria are slowly reproducing). When there is sufficient amounts of nutrition the state wanders off towards more bacteria slowing the increase of nutrition, bending it off to the right then getting almost parallel to the invariant line, and finally asymptotically reaching the steady state.

In figure 2.1 (and the following figures in later sections) we can see α_2 where the invariant line crosses the S-axis (in the trivial steady state there are no bacteria and stock-concentration of nutrient). We can also find $\alpha_1\alpha_2$ at the invariant lines crossing of the X-axis. From this information we can find the dimensionless units of X and S if we would like.

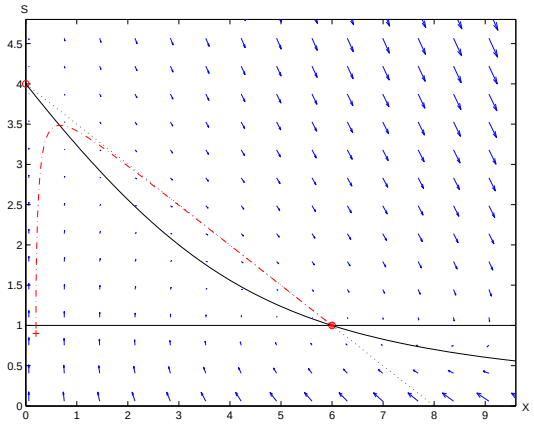

Figure 2.1: A phaseportrait of a chemostat. The dotted line is the invariant line, the point-dotted line an Euler path and the Continuous lines are nullclines (the S-axis is an X-nullcline). The arrows represent the vector-field $(\frac{dX}{dt}, \frac{dS}{dt})$. Notice that the Euler path approaches the invariant line but never crosses it.

2.3.7 Optimisation of the chemostat

Now we will leave the dimensionless form and return to the general model, (2.6). We will adapt it to fit the chemostat, and express the steady states in a different way. We will do so since many of the parameters we changed in finding the dimensionless form includes the dilution coefficient D, and we need to compute $\frac{d}{dD}(D\bar{X})$ in order to find an optimal value for D. The model is now:

$$\begin{cases} \frac{dX}{dt} = \quad \mu X - DX \\ \frac{dS}{dt} = -\alpha\mu X - DS + DS_0 \end{cases}$$

If we focus on the nontrivial steady state (ignoring the indexes): $(\bar{X}, \bar{S})$, we find $D = \mu$ if we let $\frac{dX}{dt} = 0$, simplifying the relation $\frac{dS}{dt} = 0 = -\alpha D\bar{X} - D\bar{S} + DS_0 \quad \Leftrightarrow \quad \bar{X} = \frac{1}{\alpha}(S_0 - \bar{S})$.

For Monods kinetic expression, we let $D = \mu = \mu_{max}\frac{\bar{S}}{K_S+\bar{S}} \quad \Leftrightarrow \quad \bar{S} = \frac{DK_S}{\mu_{max}-D}$, and now we can find an expression for $\bar{X}$: $\bar{X} = \frac{1}{\alpha}(S_0 - \frac{DK_S}{\mu_{max}-D})$. We are easily convinced that this expression multiplied with D is one way to measure the amount of productivity, if we assume that productivity is growth-associated, or if we are interested in the bacteria in itself (as in the yeast-production).

Now we have $X_{out} = \bar{X}D$ and we want to maximize this, and find an optimum. We calculate $\frac{d}{dD}(\bar{X}D)$ and find $D = \mu_{max}(1 \pm \sqrt{\frac{K_S}{S_0+K_S}})$, but (as we saw earlier) $D = \mu$, so there is no way D could be greater than μ_{max}, leaving us with the only possible solution: $D_{max} = \mu_{max}(1 - \sqrt{\frac{K_S}{S_0+K_S}}) \quad \Rightarrow$
$X_{out,max} = \frac{1}{\alpha}\Big(S_0 + K_S - \sqrt{K_S(S_0+K_S)}\Big)$.

2.3.8 Something about the products

So far, we have only considered a system where biomass and substrate concentration were described. Biomass, as for yeast production is usually the variable of interest. Sometimes, as for waste treatment, the substrate is what is interesting. But there is also the possibility that another variable P, the product, is what we want to know more about.

A common way to describe the change in product density is to assume that there are both growth- and non-growth-associated production. The growth-associated production is proportional to the ammount of growth by the constant a, and the non-growth-associated production to the bacteria density by the constant b. We get the us the system:

$$\begin{cases} \frac{dX}{dt} = \mu X - DX \\ \frac{dS}{dt} = D(S_0 - S) - \alpha\mu X - \beta(a\mu X + bX) \\ \frac{dP}{dt} = a\mu X + bX - DP \end{cases} \tag{2.9}$$

We recognize α as the unit-consumption of S required to produce one unit of X, and β as the unit-consumption of S required to produce one unit of P The "new" constants a and b describe the difference between growth-associated production and non-growth-associated production respectively.

Looking for a nontrivial steady state we let $\frac{dX}{dt} = 0$ and find $\mu = D$, from this we can usually find $\bar{S}$ if we have $\bar{X}$, and $\bar{P}$. $\bar{P}$ is found by taking $\mu = D$ and $\frac{dP}{dt} = 0 \quad \Rightarrow \quad \bar{P} = a\bar{X} + \frac{b\bar{X}}{D}$. Finding $\bar{X}$ requires a little work, and we calculate: $\frac{dS}{dt} = 0 \quad \Leftrightarrow \quad \bar{X}(\alpha D + \beta a D + \beta b) = D(S_0 - \bar{S}) \quad \Leftrightarrow \quad \bar{X} = \frac{S_0 - \bar{S}}{\alpha + \beta(a + \frac{b}{D})}$, so the (non-trivial) steady state for a general μ is:

$$\begin{cases} \bar{X} = \frac{S_0 - \bar{S}}{\alpha + \beta(a + \frac{b}{D})} \\ \bar{P} = a\bar{X} + \frac{b\bar{X}}{D} \\ \mu = D \quad \Rightarrow \quad \bar{S} \end{cases}$$

If we are interested in $\bar{S}$ we can often easily find it from the above relations. If we use the only kinetics we have encountered so far, the Monod kinetics, we again find: $\bar{S} = \frac{DK_S}{\mu_{max} - D}$ and get:

$$\begin{cases} \bar{S} = \frac{DK_S}{\mu_{max} - D} \\ \bar{X} = \frac{S_0 - \bar{S}}{\alpha + \beta(a + \frac{b}{D})} \\ \bar{P} = a\bar{X} + \frac{b\bar{X}}{D} \end{cases}$$

As in Optimising X_{out} the Optimisation of P requires computing $\frac{d(D\bar{P})}{dt} = 0$.

Chapter 3

A Chemostat with modified kinetics

We have considered so far a chemostat with Monods kinetics. In this chapter we will investigate our modification of this model.

Similarities with Monods equations will aid us in performing an analysis of the equations in a similar way as in Chapter 2.

3.1 The MMI-CSTR

We call this model the Michaelis-Menten inhibited Continuous stirred tank reactor (MMI-CSTR) since we use a kind of Michaelis-Menten kinetics to lower the influence of growing values of X in the kinetic expression. We take the term $\mu = \mu_{max}\frac{S}{K_S+S}\frac{X}{K_X+X}$ in our equations instead of $\mu_{max}\frac{S}{K_S+S}X$, as in Monods chemostat. This kinetic form describes the situation when a high density, X, of bacteria inhibits the growth in the chemostat.

By introducing the proposed inhibition we get the MMI-CSTR:

$$\begin{cases} \frac{dX}{dt} = \mu_{max}\frac{S}{K_S+S}\frac{X}{K_X+X} - XD \\ \frac{dS}{dt} = -\alpha\mu_{max}\frac{S}{K_S+S}\frac{X}{K_X+X} - SD + S_0D \end{cases} \tag{3.1}$$

To obtain a nondimensional form we replace S, X and t with $S \cdot \hat{S}$, $X \cdot \hat{X}$ and $t \cdot \hat{t}$. We start by multiplying the relations with $\hat{t}/\hat{X}$ and $\hat{t}/\hat{S}$ respectively then replace $\hat{t} = \frac{1}{D}$, $\hat{S} = K_S$, $\hat{X} = \frac{\hat{S}}{\alpha\frac{\mu_{max}}{K_X}\hat{t}}$, and replace $\alpha_1 = \frac{\mu_{max}}{K_X D}$ and $\alpha_2 = \frac{S_0}{S}$. Finally, we let $K_X = X_C\hat{X}$, to write the equations as:

$$\begin{cases} \frac{dX}{dt} = \alpha_1\frac{S}{1+S}\frac{X}{1+\frac{X}{X_C}} - X \\ \frac{dS}{dt} = -\frac{S}{1+S}\frac{X}{1+\frac{X}{X_C}} - S + \alpha_2 \end{cases} \tag{3.2}$$

3.2 Invariant Line

As previously, we can find that $X = \alpha_1(\alpha_2 - S)$ in an invariant line.

3.3 Nullclines

We let $\frac{dX}{dt} = 0$ and find the line $X = 0$, but also $S = \frac{1+\frac{X}{X_C}}{\alpha_1-(1+\frac{X}{X_C})}$. Now, if $\frac{dS}{dt} = 0$ we get: $X = \frac{(\alpha_2-S)(1+S)}{S+\frac{1}{X_C}(\alpha_2-S)(1+S)}$. So the nullclines are:

$$\begin{cases} \frac{dX}{dt} = 0 & \Rightarrow \quad X = 0 \text{ or } S = \frac{1+\frac{X}{X_C}}{\alpha_1-(1+\frac{X}{X_C})} \\ \frac{dS}{dt} = 0 & \Rightarrow \quad X = \frac{(\alpha_2-S)(1+S)}{S+\frac{1}{X_C}(\alpha_2-S)(1+S)} \end{cases}$$

3.4 Steady states

Using the first of the $\frac{dX}{dt} = 0$ nullclines we find the same trivial steady state: $(\bar{X}_0, \bar{S}_0) = (0, \alpha_2)$. If we use the second relation in combination with the invariant line to eliminate X we end up with the second degree equation:
$S^2 + S\underbrace{(X_C + 1 - \frac{X_C}{\alpha_1} - \alpha_2)}_{p-q} + \underbrace{(-\frac{X_C}{\alpha_1} - \alpha_2)}_{-q} = 0$, with the solutions
$S = \frac{q-p}{2} \pm \sqrt{(\frac{q-p}{2})^2 + q}$. One can quickly find that the solution with a minus is negative, so we reject it. The other solution however must be smaller than α_2 in order for the X associated to it to be positive, so the following assumptions are needed: $\alpha_2 > -\frac{p-q}{2} + \sqrt{(\frac{p-q}{2})^2 + q} \quad \Leftrightarrow \quad \alpha_2 > \frac{1}{\alpha_1-1}$, for any non-trivial steady state to exist. We also understand that $\alpha_1 > 1$. These conditions will be used later.

3.5 Linearization around the equilibrium points

For the MMI-CSTR the system matrix $\mathbf{A}$ is:

$$\mathbf{A} = \begin{pmatrix} \alpha_1 \frac{S}{1+S} \frac{1}{(1+\frac{X}{X_C})^2} - 1 & \alpha_1 \frac{1}{(1+S)^2} \frac{X}{1+\frac{X}{X_C}} \\ -\frac{S}{1+S} \frac{1}{(1+\frac{X}{X_C})^2} & -\frac{1}{(1+S)^2} \frac{X}{1+\frac{X}{X_C}} - 1 \end{pmatrix}.$$

When we investigate the trace and the determinant at the steady states we may use the relation: $\frac{dX}{dt} = 0 \quad \Leftrightarrow \quad \alpha_1 XS = X(1+S)(1+\frac{X}{X_C})$, to simplify the matrix $\mathbf{A}$ (for $X \neq 0$):

$$\mathbf{A} = \begin{pmatrix} \frac{1}{1+\frac{X}{X_C}} - 1 & \frac{X}{S(1+S)} \\ -\frac{1/\alpha_1}{1+\frac{X}{X_C}} & -\frac{X/\alpha_1}{S(1+S)} - 1 \end{pmatrix} = \begin{pmatrix} \frac{-\frac{X}{X_C}}{1+\frac{X}{X_C}} & \frac{X}{S(1+S)} \\ -\frac{1/\alpha_1}{1+\frac{X}{X_C}} & -\frac{X/\alpha_1}{S(1+S)} - 1 \end{pmatrix}.$$

As we can see: $tr\mathbf{A} < 0$, but how about $det(\mathbf{A})$, is it positive? We eliminate some of our X by using the invariant line:

$$\mathbf{A} = \begin{pmatrix} \frac{-\frac{X}{X_C}}{1+\frac{X}{X_C}} & \frac{\alpha_1(\alpha_2-S)}{S(1+S)} \\ -\frac{1/\alpha_1}{1+\frac{X}{X_C}} & -\frac{(\alpha_2-S)}{S(1+S)} - 1 \end{pmatrix} \quad \Rightarrow$$

$$det(\mathbf{A}) = \frac{\frac{X}{X_C}}{1+\frac{X}{X_C}}\left(\frac{\alpha_2 - S}{S(1+S)} + 1\right) + \frac{\alpha_2 - S}{(1+\frac{X}{X_C})(1+S)} > 0$$

Thus, the nontrivial steady state is stable.

If we now study the trivial steady state, we have $X = 0$, so $\mathbf{A}$ is:

$$\mathbf{A} = \begin{pmatrix} \frac{\alpha_1\alpha_2}{1+\alpha_2} - 1 & 0 \\ -\frac{\alpha_2}{1+\alpha_2} & -1 \end{pmatrix} \quad \Rightarrow$$

$$\begin{cases} tr(\mathbf{A}) = \frac{\alpha_1\alpha_2}{1+\alpha_2} - 2 & \Rightarrow \quad \frac{\alpha_1\alpha_2}{1+\alpha_2} < 2, \text{ for stability.} \\ det(\mathbf{A}) = -(\frac{\alpha_1\alpha_2}{1+\alpha_2} - 1) & \Rightarrow \quad \frac{\alpha_1\alpha_2}{1+\alpha_2} < 1, \text{ for stability.} \end{cases}$$

So, we have to have $\frac{\alpha_1\alpha_2}{1+\alpha_2} < 1 \quad \Leftrightarrow \quad \alpha_2 > \frac{1}{\alpha_1 - 1}$ to have a stable trivial steady state, but we remember the constraint for the non-trivial steady state to exist: $\alpha_2 < \frac{1}{\alpha_1 - 1}$, we can conclude that if we flush too much; no population will exist, like expected.

3.6 Looking at the phaseportrait

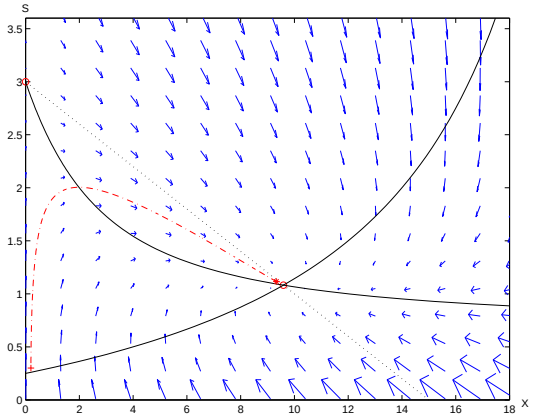

Figure 3.1: Phaseportrait of the MMI-CSTR.

Again, in figure 3.1, we have $\alpha_1\alpha_2$ where the invariant line crosses the X-axis, and α_2 where it crosses the S-axis. We see that the nullclines have similar positions in the phaseplane and that the Euler path has a similar behaviour. This phase-portrait is very similar to the chemostat one. But we notice that indeed the MMI-CSTR responds to large values of X by inhibition.

3.7 Optimisation

From the general model (2.6) we quickly get: $\mu = D$ and $\bar{X} = \frac{1}{\alpha}(S_0 \bar{S})$ if we ignore the trivial steady state and drop the indices. From $\mu = D$ we can find $\bar{X}$ in order to eliminate it: $\bar{X} = \frac{\mu_{max}}{S}\frac{\bar{S}}{K_S + \bar{S}} - K_X = \frac{1}{\alpha}(S_0 - \bar{S})$.

This expression give rise to the same kind of second degree equation as earlier: $\bar{S}^2 + \bar{S}\underbrace{(\frac{\alpha\mu_{max}}{D} + K_S - \alpha K_S - S_0)}_{\frac{2w}{D}+2\lambda} + \underbrace{K_S(-\alpha K_X - S_0)}_{-v} = 0$. This equation gives us an $\bar{S}$ with dimensions. We can use this dimensional $\bar{S}$ to find $D\bar{X}$: $D\bar{X} = S_0 + \frac{w}{\alpha} + \frac{D\lambda}{\alpha} - \sqrt{(\frac{w}{\alpha} + \frac{D\lambda}{\alpha})^2 + \frac{D^2 v}{\alpha^2}}$.

This can be differentiated in order to find $\frac{d}{dD}(D\bar{X}) = 0$.

3.8 From MMI-CSTR to chemostat

A quick comparison of the dimensionless μ's of the chemostat and the MMI-CSTR reveals that the new term $\frac{1}{1+\frac{X}{X_C}}$ inhibits both $\frac{dX}{dt}$ and $\frac{dS}{dt}$. This inhibition is stronger the smaller X_C is. Thus in the limit of $X_C \to \infty$ we recover: $\mu_{MMI} = \alpha_1 \frac{S}{S+1}\frac{1}{1+\frac{X}{X_C}} \overset{X_C\to\infty}{\longrightarrow} \alpha_1 \frac{S}{S+1} = \mu_{chemostat}$.

3.9 Summary and comparison of the CSTRs

We have discussed two different chemostatmodels with two different kinetic forms: Monods Kinetics and the Michaelis-Menten Inhibited kinetics. Two other well studied kinetics are Competitive Substrate Inhibition (where the dimensionless $\mu = \frac{S}{1+S+\frac{S^2}{K_I}}$) and Competitive Product Inhibition ($\mu = \frac{S}{1+S+K_I X}$) that we call CSI-CSTR and CPI-CSTR.

In the table below there is a summary of the models (we have excluded the trivial X-nullcline $X = 0$ and abbreviated nullcline 'XNC' and 'SNC' for X-nullcline and S-nullcline).

By comparing the phaseportraits (figure 3.2) it is easy to see obvious similarities in all models. Some common features are the existence of the invariant line and the equilibrium points. The invariant line exist due to the fact that the model produces a constant value of bacteria density per consumed unit of substrate density. The equilibrium points vary with the parameters α_1, α_2 and various constants. By choosing them carefully we may determine the position of the equilibrium points in the phase-plane, or we can force the model to flush the bacteria population out of the chemostat.

Looking at the CSI-CSTR one can see that the steady state value for S is relatively low (compared to the other models), one can reason that this is due to the response of this model to inhibition of S. In this model we can also see that the points on the Euler path never reach large values of S (relative to the other models), this must a problem for real CSI-CSTRs: starting such a reactor must be done carefully. Another feature of this model is that the vectorfield is not "clockwise" around the non-trivial steady state. Instead it seems to rapidly push a state towards the unstable steady state (for small or large values of both S and X) and close to the invariant lines it pushes the state towards the trivial or non-trivial steady state.

Model	Monods Chemostat	CSI-CSTR
μ	$\frac{S}{1+S}$	$\frac{S}{1+S+\frac{S^2}{K_I}}$
$\frac{dX}{dt}$	$\alpha_1 \frac{S}{1+S} X - X$	$\alpha_1 \frac{S}{1+S+\frac{S^2}{K_I}} X - X$
$\frac{dS}{dt}$	$-\frac{S}{1+S} X - S + \alpha_2$	$-\frac{S}{1+S+\frac{S^2}{K_I}} X - S + \alpha_2$
XNC	$S = \frac{1}{\alpha_1 - 1}$	$S = \frac{K_I(\alpha_1-1)}{2} \pm \sqrt{\left(\frac{K_I(\alpha_1-1)}{2}\right)^2 - K_I}$
SNC	$X = \frac{(\alpha_2 - S)(1+S)}{S}$	$X = \frac{(\alpha_2 - S)(1+S+\frac{S^2}{K_I})}{S}$
limit	–	$K_I \to \infty$
Model	MMI-CSTR	CPI-CSTR
μ	$\frac{S}{1+S} \frac{1}{1+\frac{X}{X_C}}$	$\frac{S}{1+S+K_I X}$
$\frac{dX}{dt}$	$\alpha_1 \frac{S}{1+S} \frac{X}{1+\frac{X}{X_C}} - X$	$\alpha_1 \frac{S}{1+S+K_I X} X - X$
$\frac{dS}{dt}$	$-\frac{S}{1+S} \frac{X}{1+\frac{X}{X_C}} - S + \alpha_2$	$-\frac{S}{1+S+K_I X} X - S + \alpha_2$
XNC	$S = \frac{1+\frac{X}{X_C}}{\alpha_1 - (1+\frac{X}{X_C})}$	$S = \frac{XK_I+1}{\alpha_1 - 1}$
SNC	$X = \frac{(\alpha_2 - S)(1+S)}{S + \frac{1}{X_C}(\alpha_2 - S)(1+S)}$	$X = \frac{(\alpha_2 - S)(1+S)}{S - K_I(\alpha_2 - S)}$
limit	$X_C \to \infty$	$K_I \to 0$

The other three models, the chemostat, the MMI-CSTR and the CPI-CSTR are quite similar in comparison to the CSI-CSTR. They have the similar nullclines and steady states in almost the same places and their vectorfields are also similar. The chemostat S-nullcline is parallel to the X-axis and in two other models this nullcline increases with increasing values of X. This can be interpreted as an inhibiting effect of X on μ: the vectorfield changes from pushing a state towards increasing X's to smaller X's when it crosses the S-nullcline (from large values of S). Monods chemostat does not "feel" this inhibition and does not care if the value of X is very large, it is S that determines if X should grow or not.

The striking similarities of the MMI-CSTR and the CPI-CSTR are probably an effect of their very similar μ's, only the denominator differs.

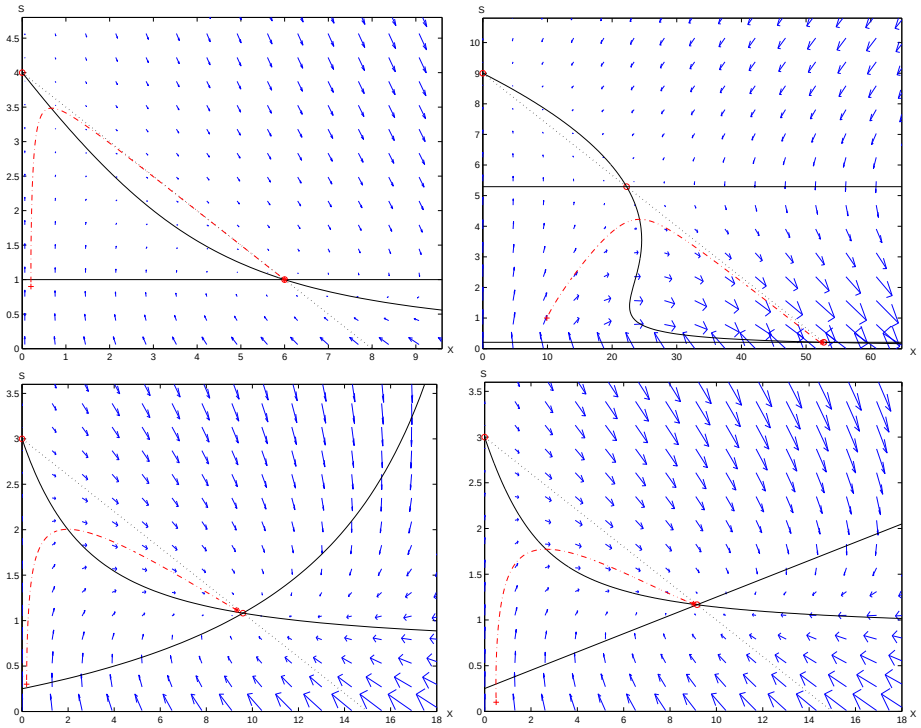

Figure 3.2: Phaseportraits of Monods Chemostat, the CSI-CSTR, the MMI-CSTR and the CPI-CSTR. Eulerpaths leading to their stable non-trivial steady-states are included.

Chapter 4

Other CSTR-types

We shall see that there are other ways of designing CSTRs that differ from the model of a one-tank reactor with constant flow. This chapter will describe chemostats in series, a chemostat with recirculation, and a chemostat-like enzyme reactor.

4.1 Chemostats in series

It is sometimes convenient to use CSTRs in series, if for example the product we want is produced by "mature" bacteria we would like to have a first tank where there is rapid production of bacteria. A second reactor would allow bacteria to maturate in a different chemical environment (pH, temperature, etc.) and to produce our desired product.

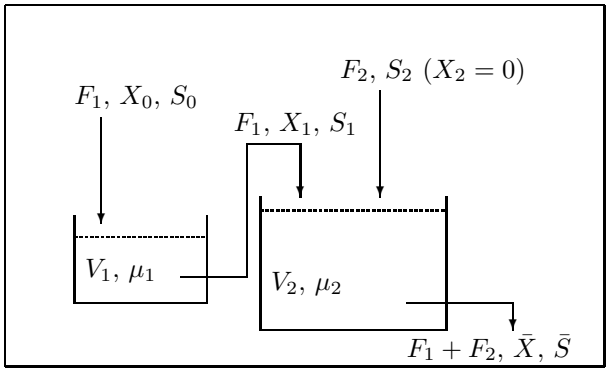

Figure 4.1: Two Chemostats in series with sterile feed in the second.

We will consider two chemostats in series. We assume the first reactor feeds the second chemostat with X_1, S_1 and flow F_1. There is also another inflow to the second chemostat of substrate with the density S_2 and with inflow F_2. The outflow from the second chemostat is thus $F_1 + F_2$. The volumes of the reactors are V_1 and V_2. The equations for the second reactor are:

$$\begin{cases} \frac{dV}{dt} = F_1 + F_2 - (F_1 + F_2) = 0 \\ \frac{dX}{dt} = \frac{F_1}{V_1}X_1 + \mu X - \frac{F_1+F_2}{V_2}X \\ \frac{dS}{dt} = \frac{F_1}{V_1}S_1 - \alpha\mu X - \frac{F_1+F_2}{V_2}S + \frac{F_2}{V_2}S_2 \end{cases} \tag{4.1}$$

We let $D_1 = \frac{F_1}{V_1}$ and $D_2 = \frac{F_1+F_2}{V_2} \quad \Leftrightarrow \quad \frac{F_2}{V_2} = D_2 - \frac{F_1}{V_2} = D_2 - D_1\frac{V_1}{V_2} \quad (> 0)$ gives us the equations describing the equilibrium point in the second chemostat:

$$\begin{cases} \frac{dX}{dt} = D_1X_1 + \mu\bar{X} - D_2\bar{X} = 0 \\ \frac{dS}{dt} = D_1S_1 + (D_2 - D_1\frac{V_1}{V_2})S_2 - \alpha\mu\bar{X} - D_2\bar{S} = 0 \end{cases}$$

We assume that $\mu \neq \mu(X)$ for simplicity. We notice that there is only one solution to the first equation: $D_1X_1 + \mu\bar{X} - D_2\bar{X} = 0 \quad \Leftrightarrow \quad \bar{X} = \frac{D_1X_1}{D_2-\mu}$.

From the second relation and depending on the rate expression, we get different values of $\bar{S}$ and $\bar{X}$.

4.2 Chemostat with recirculation

In some cases it is shown that recirculation of a fraction of the bacteria increases the productivity of the system.

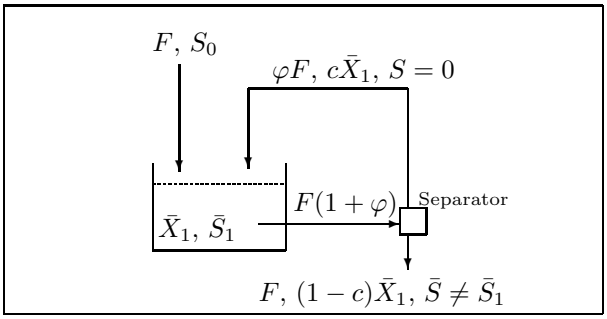

Figure 4.2: The chemostat with recirculation.

Here we let the fraction φ of F recirculate back into the reactor. In this fraction of medium (assumed to be without any S) we let the fraction c of $\bar{X}$ be reinserted into the system. The equations become:

$$\begin{cases} \frac{dX}{dt} = +cX \cdot \varphi D + \mu X - X(1+\varphi)D \\ \frac{dS}{dt} = -\alpha\mu X - D(1+\varphi)S + DS_0 \end{cases} \tag{4.2}$$

When searching for steady states we let $X \neq 0$ and from the first line in (4.2) we get $\frac{dX}{dt} = 0 \quad \Leftrightarrow \quad \mu = (1+\varphi-\varphi c)D$, now we notice that $\varphi(1-c) > 0$, since c is only a small fraction, and thus: $\mu > D$, which is better that the reproduction we had in the earlier chemostats.

Again, different rate-expression give rise to different $\bar{X}$ and $\bar{S}$. With Monods μ we get: $\mu = \frac{\mu_{max}S}{K_S+S} = (1+\varphi-\varphi c)D$ The steady state is thus: $(\bar{X}, \bar{S}) = \left(\frac{S_0-(1+\varphi)\bar{S}}{\alpha(1+\varphi-\varphi c)}, \frac{K_SD(1+\varphi-\varphi c)}{\mu_{max}-D(1+\varphi-\varphi c)}\right)$.

4.3 Chemostat-like enzyme reactors

In this section we consider a CSTR with enzymes instead of bacteria.

If we make two fair assumptions: (1) one substrate molecule spawns one product molecule giving $S_0 - S = P - P_0$ (thus "consumed substrate" = "produced product"), and (2) the enzyme concentration (E) is constant (and equal to one) as time passes, we get the equations:

$$\begin{cases} \frac{dE}{dt} = 0 & = 0 \\ \frac{dS}{dt} = D(S_0 - S) - 1 \cdot E \cdot \mu & = D(S_0 - S) - \mu \\ \frac{dP}{dt} = E\mu - DP & = \mu - DP \end{cases} \tag{4.3}$$

This is sufficient to find $\bar{S}$ if we first specify the kinetics. Let us use the Michaelis-Menten kinetics: $\mu = \frac{\mu_{max} S}{K_S + S} = D(S_0 - S) \quad \Rightarrow$
$S = \frac{S_0 - K_S - \frac{\mu_{max}}{D}}{2} \pm \sqrt{(\frac{S_0 - K_S - \frac{\mu_{max}}{D}}{2})^2 + S_0 K_S}$.

This is one way of finding the steady state S. It is however not always what we are interested in. If we use an alternative approach and introduce the relation $\delta = \frac{S_0 - S}{S_0}$, corresponding to the fraction of S_0 that has been converted into product. We notice $S = S_0(1 - \delta)$ and we are able to search the dilution coefficient as a function of δ and S_0 instead. This is often very convenient. We will now look at D's for different kinetic cases.

Michaelis-Menten kinetics in an enzyme reactor

From (4.3) we get the relation $D = \frac{\mu}{(S_0 - S)}$ and the Michaelis-Menten kinetics is $\mu = \frac{\mu_{max} S}{K_S + S}$ so we get: $D = \frac{\mu}{S_0 - S} = \frac{\mu_{max} S}{(K_S + S)(S_0 - S)} = \frac{\mu_{max}(1-\delta)}{\delta(K_S + S_0(1-\delta))}$.

Competitive Substrate Inhibition in an enzyme reactor

Now we apply Competitive Substrate Inhibition instead and in a similar way we get: $D = \frac{\mu}{S_0 - S} = \frac{\mu_{max} S}{(S_0 - S)(K_M + S + \frac{S^2}{K_I})}$. Again using $\delta = \frac{S_0 - S}{S_0}$ gives us:
$D = \frac{\mu_{max}/\delta S_0}{1 + \frac{K_M}{S_0(1-\delta)} + \frac{S_0(1-\delta)}{K_I}}$.

Competitive Product Inhibition in an enzyme reactor

We remember the stochiometric relation $S_0 - S = P - P_0 \quad \Leftrightarrow \quad P = S_0 - S + P_0$, allowing us to eliminate P in μ.

By the same procedure as above and get:
$D = \frac{\mu_{max} S}{(S_0 - S)(K_M(1 + PK_I) + S)} = \frac{\frac{1-\delta}{\delta}\mu_{max}}{K_M + K_M K_I(\delta S_0 + P_0) + S_0(1-\delta)}$.

Michaelis-Menten Inhibition in an enzyme reactor

This is actually not possible, since the kinetic expression contains X, a variable not present in an enzyme reactor.

The dilutions rates summarised

Kinetics	Kinetic expression	Dilution coefficient
Michaelis-Menten	$\frac{\mu_{max}S}{K_S+S}$	$\frac{\mu_{max}(1-\delta)}{\delta(K_S+S_0(1-\delta))}$
CSI	$\frac{\mu_{max}S}{K_M+S+\frac{S^2}{K_I}}$	$\frac{\mu_{max}/\delta S_0}{1+\frac{K_M}{S_0(1-\delta)}+\frac{S_0(1-\delta)}{K_I}}$
CPI	$\frac{\mu_{max}S}{K_M(1+PK_I)+S}$	$\frac{\frac{1-\delta}{\delta}\mu_{max}}{K_M+K_MK_I(\delta S_0+P_0)+S_0(1-\delta)}$

Chapter 5

Controlled CSTRs and the turbidostat

In this chapter we will discuss controlled CSTRs. By controlling a CSTR we will see that the possibility to choose a steady state increases. Controlling a CSTR can also be wise when the concentration of substrate in the inflow is fluctuating to limit fluctuations in the population density.

5.1 Controlled CSTRs

There are many ways to use automatic control in CSTRs to achieve good results. One general name for a class of controlled CSTR's is *auxostats*. An auxostat is often defined as a continuous culture system in which the concentration of one of the components, for example the pH-level, biomass concentration, or nutrition concentration, is predetermined and the system is controlled to maintain a constant level of this component.

One popular auxostat is the pH-auxostat since the active bacteria produce organic acids as wasteproducts, lowering the pH. Measuring the pH is cheap and simple, and since pH is correlated to the productivity this type of auxostat is easy to control.

Another auxostat is the *turbidostat*. The turbidity of a solution that means how much light it absorbs is simple to measure, and is proportional to the density of biomass X in the vessel. If there is too much density more solution is added, and vice versa.

In general we want to fix our $\mathbf{X}$ at a value $\mathbf{X_P}$, a point in the proximity of the uncontrolled systems steady state $\mathbf{X_0}$. By doing this we assume that the behaviour of the system is the similar to its behaviour in the neighbourhood of $\mathbf{X_0}$, even if this might be at some distance from $\mathbf{X_0}$.

A generalised controlled system can be described as:

$$\frac{d}{dt}\mathbf{X} = \mathbf{AX} + \mathbf{BU}, \qquad \mathbf{Y} = \mathbf{CX}, \tag{5.1}$$

where $\mathbf{U}$ contain variables used to control the system and $\mathbf{B}$ is a constant matrix, for example the values from a linearization around the point of interest. $\mathbf{Y}$ is an output vector letting us observe values of $\mathbf{X}$, and $\mathbf{C}$ describes how $\mathbf{X}$ and $\mathbf{Y}$ are related. The dimension of the vector $\mathbf{BU}$ must be the same as for $\mathbf{AX}$.

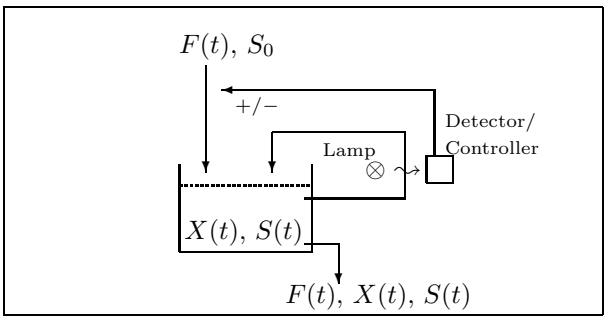

Figure 5.1: The general idea of the Turbidostat. Via a closed loop in the reactor light is passed through the solution. A detector/controller measures the turbidity and sends a signal to regulate the inflow of nutrient.

We will consider the case where $\mathbf{U} = \mathbf{X}$ and the system is controlled by varying D. We let $D = D_0 + D(X, S)$. Returning to (2.6) where D_0 corresponds to our old D and with $X_0 = 0$ we have:

$$\begin{cases} \frac{dX}{dt} = \quad \mu X - D_0 X - D(X,S)X \\ \frac{dS}{dt} = -\alpha\mu X - D_0(S - S_0) - D(X,S)(S - S_0) \end{cases} \tag{5.2}$$

We will not yet describe $D(X, S)$ in detail, but we will require two properties. The first constraint is that we want $D(\mathbf{X_P}) = 0$, we do not want to change anything if we are in the required state $\mathbf{X_P}$. The other constraint is $D_0 + D(X, S) \geq 0$, since we add positive volumes of the nutrition solution to the CSTR.

We linearize (5.2) around $\mathbf{X_0}$: $\frac{d}{dt}\mathbf{X} = \mathbf{AX} + \mathbf{BX}$ where:

$$\mathbf{A} = \begin{pmatrix} \mu'_X X_P + \mu - D_0 & \mu'_S X_P \\ -\alpha\mu'_X X_P - \alpha\mu & -\alpha\mu'_S X_P - D_0 \end{pmatrix}$$

$$\mathbf{B} = \begin{pmatrix} -D'_X X_P & -D'_S X_P \\ D'_X(S_0 - S_P) & D'_S(S_0 - S_P) \end{pmatrix}$$

This expression can now be used to derive convenient forms of controlled CSTRs.

Two important questions about controlled systems is their controllability and stabilisability. A system that is controllable is also stabilisable. By controllability we mean that a system, through a control policy, can be forced to move from an initial state $\mathbf{X_i}$ to a desired state $\mathbf{X_d}$ in finite time. Controllability in this case is guaranteed when $det[\mathbf{B} \ \mathbf{AB} \ \ldots \ \mathbf{A}^{n-1}\mathbf{B}] \neq 0$, and is not obtained for the turbidostat. This means that not all states can be obtained in finite time.

Stabilisability is obtained when all eigenvalues of $\mathbf{A}$ can be made negative by choosing a suitable control, but it may happen that eigenvalues of $\mathbf{A}$ are negative from the beginning. This situation corresponds to our normal stability condition when the trace is negative and the determinant is positive.

5.2 Invariant line for CSTRs controlled with D

As for the uncontrolled systems we can examine $\frac{d}{dt}(S+\alpha X)$. We end up with:

$$\frac{d}{dt}(S+\alpha X) = -(S+\alpha X - S_0)\underbrace{(D_0 + D(X,S))}_{\geq 0}$$

Thus if the equality $\alpha X = S_0 - S$ is fulfilled, we tend to keep it fulfilled: the minus sign in this relation ensures that we return to the invariant line if we for some reason were to move away from it.

So there is an invariant line for CSTRs controlled with D and it is the same as for the uncontrolled CSTRs.

5.3 The turbidostat

We will now look at the case where $D(X,S) = D(X) = K_D(X - X_P)$. X_P is the value of X we want to stabilize our CSTR about. If we apply $\mu = \mu(S)$, we have $\mu'_X = 0$. Also: $D'_S = 0$ and $D'_X = K_D$. The system is then:

$$\begin{cases} \frac{dX}{dt} = & \mu X - D_0 X - K_D(X - X_P)X \\ \frac{dS}{dt} = & -\alpha\mu X - D_0(S - S_0) - K_D(X - X_P)(S - S_0) \end{cases} \tag{5.3}$$

This expression can be analyzed to learn how the controller action acts in this case. If we search for a steady-state value of X we can ignore the trivial steady state and end up with:

$$K_D X = \mu - D_0 + K_D X_P \Leftrightarrow \begin{cases} \mu = D_0, & K_D = 0 \\ X = X_P + \frac{\mu - D_0}{K_D}, & 0 < X_P < \infty \\ X \approx X_P, & K_D \gg \mu - D_0 \end{cases}$$

K_D is the key here: without control action ($K_D = 0$) we return to Monods chemostat, for extremely large values of K_D we end up with $X = X_P$. For reasonable values of K_D we are close to X_P. This middle expression also corresponds to a nullcline.

By linearizing (5.3) we get:

$$\frac{d}{dt}\mathbf{X} = (\mathbf{A}+\mathbf{B})\mathbf{X} = \begin{pmatrix} \mu - D_0 - K_D X_P & \mu'_S X_P \\ -\alpha\mu + K_D(S_0 - S_P) & -\alpha\mu'_S X_P - D_0 \end{pmatrix}\mathbf{X}$$

Now, sufficiently close to the steady state we can approximate $D_0 \approx \mu$, allowing us to do some changes:

$$\frac{d}{dt}\mathbf{X} \approx \begin{pmatrix} -K_D X_P & \mu'_S X_P \\ -\alpha\mu + K_D(S_0 - S_P) & -\alpha\mu'_S X_P - \mu \end{pmatrix}\mathbf{X}$$

As usual we want to know if this controlled system is stable and investigate the trace and the determinant:

$$tr(\mathbf{A}+\mathbf{B}) = -K_D X_P - \alpha\mu'_S X_P - \mu$$

If μ is strictly growing (and its derivative positive) then indeed $tr(\mathbf{A}+\mathbf{B}) < 0$. But if we for example have a CSI-CSTR μ'_S might be negative. A sufficient condition is however $K_D > -\alpha\mu'_S$.

If we assume that we are close to the invariant line and can use $\alpha X_P \approx S_0 - S_P$, we get the determinant:

$$det(\mathbf{A}+\mathbf{B}) = \mu'_S K_D X_P \underbrace{(\alpha X_P - (S_0 - S_P))}_{\approx 0} + K_D X_P \mu + X_P \mu(\alpha \mu'_S + K_D)$$

$$det(\mathbf{A}+\mathbf{B}) \approx K_D X_P \mu + X_P \mu(\alpha \mu'_S + K_D)$$

So we have a positive determinant if $K_D > -\alpha\mu'_S$, or if we have $\mu'_S > 0$. This system is thus stable. But since we do not have controllability we are not really sure where we arrive to in the phase-plane.

5.4 The phaseportrait of a turbidostat

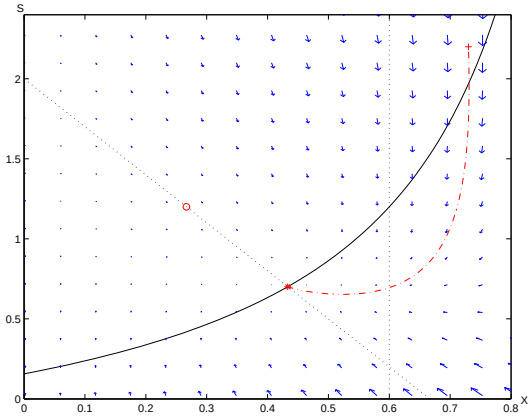

Figure 5.2: A phaseportrait of a Turbidostat. The diagonal dotted line is the invariant line, the vertical dotted line corresponds to $X = X_P$, the point-dotted line is an Euler path starting in (0.73, 2.2) and the full line an X-nullcline. The ring on the invariant line is where the steady state would have been without the controller action. The arrows represent the vector-field composed of $\frac{dX}{dt}$ and $\frac{dS}{dt}$.

Looking at the phaseportrait of a Turbidostat, figure 5.2, the first striking difference is the distance between the normal chemostat steady state and the steady state of the turbidostat. But here there is also an invariant line as before and a nullcline in about the same place as before. This nullcline depends however strongly on K_D.

In figure 5.3 we see how the phase portrait changes with increasing values of K_D: the nullcline tends to approach the line $X = X_P$ and thus the steady state approaches the intersection of the null cline and of the invariant line.

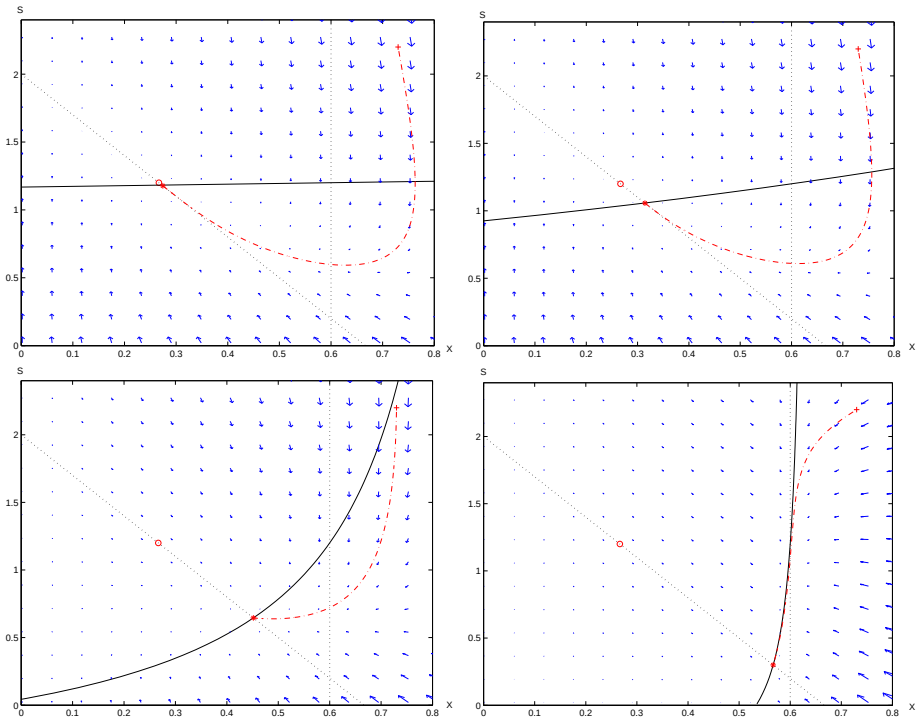

Figure 5.3: Comparing phaseportraits of Turbidostats with values of K_D of 0.03, 0.3, 3 and 30.

Chapter 6

Summary and conclusion

In this report several mathematical models of bacteria population growth in bioreactors were analyzed.

In chapter 2 we explored two simple models of biological growth: exponential growth and by taking into account the substrate concentration, S, the logistic equation. The exponential growth is typically seen when resources are good and the population is very small. The logistic equation explains how a population is restricted by limited resources.

We introduced the in- and outflow of nutrient in our reactor, the Michaelis-Menten kinetics and analyzed Monods Chemostat. Equilibrium solutions, nullclines, the parameters α_1 and α_2 and the phaseportrait are found to be useful characteristics.

Chapter 3 is dedicated to the new model proposed: the Michaelis-Menten inhibited CSTR. The MMI-CSTR exhibits different phaseportrait showing how the MMI-CSTR responds to large values of X by inhibition.

A comparison of this model and Monods chemostat with the competitive substrate and product inhibition has been made.

In chapter 4 we discuss chemostats in series, a chemostat with recirculation and three chemostat-like enzyme reactors. We saw that in the chemostat with recirculation we could achieve $\mu > D$, and that chemostats in series allow us to take into account different ambient conditions for the bacteria.

In chapter 5 we have explained how a controlled CSTR works and investigated the turbidostat: it illustrates a simple way of controlling a CSTR.

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Appendix A

The Linearized stability

A.1 The Linearization

$F(x)$, a one-variable function of x can be Taylor-expanded around a fix X. We get $F(X+x) = F(X) + F'(X)x + O(x^2)$. For small perturbations of x around X we get the linearization: $F(X+x) \approx F(X) + F'(X)x$, containing only the constant and the linear terms.

For functions of two variables $F(X+x, S+s)$ and $G(X+x, S+s)$:

$$\begin{cases} F(X+x,S+s) = F(X,S) + F'_X(X,S)x + F'_S(X,S)s + O((x+s)^2) \\ G(X+x,S+s) = G(X,S) + G'_X(X,S)x + G'_S(X,S)s + O((x+s)^2) \end{cases}$$

If $F(x,s) = \frac{dX}{dt}$, $G(x,s) = \frac{dS}{dt}$ and the point (X,S) is an equilibrium point then the linearization is:

$$\begin{cases} \frac{dX}{dt} = F(X+x,S+s) \approx F'_X(X,S)x + F'_S(X,S)s = a_{11}x + a_{12}s \\ \frac{dS}{dt} = G(X+x,S+s) \approx G'_X(X,S)x + G'_S(X,S)s = a_{21}x + a_{22}s \end{cases}$$

A convenient notation is $\frac{d}{dt}\mathbf{X} \approx \mathbf{AX} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix}\mathbf{X}$, and is often used when looking at the chemostat or other dynamical systems of similar mathematical form.

A.2 The Stability

Now, let us use our linearization and look at some conditions needed for the linearization to be stable. We have $\frac{d}{dt}\mathbf{X} = \mathbf{AX}$. Supposing that $\mathbf{x} = c_1 \cdot \mathbf{v_1} e^{\lambda_1 t} + c_2 \cdot \mathbf{v_2} e^{\lambda_2 t}$ we get, for each λ: $\frac{d}{dt}\mathbf{X} = \lambda \mathbf{A} e^{\lambda t} = \mathbf{A}\mathbf{v}e^{\lambda t}$. Eliminating the exponential in the two last terms, we get the well-known equation: $det(\mathbf{A} - \lambda \mathbf{I}) = 0$ that has two solutions:

$$\lambda = \frac{a_{11}+a_{22}}{2} \pm \frac{\sqrt{4(a_{12}a_{21} - a_{11}a_{22}) + (a_{11}+a_{22})^2}}{2} \Leftrightarrow$$

$$\lambda = \frac{tr(\mathbf{A})}{2} \pm \frac{\sqrt{-4det(\mathbf{A}) + tr(\mathbf{A})^2}}{2}$$

In order to have stable solutions we must have $\mathrm{Re}(\lambda) < 0$ and thus $tr(\mathbf{A}) < 0$. If we assume that $tr(\mathbf{A}) < 0$ and look at the first λ (with a plus), we get $\lambda_1 < 0 \Leftrightarrow$

$$\frac{tr(\mathbf{A})}{2} + \frac{\sqrt{-4det(\mathbf{A}) + tr(\mathbf{A})^2}}{2} < 0 \Leftrightarrow 0 < det(\mathbf{A})$$

Thus, for us to not have a positive real part in our exponentials, we have to have: $tr(\mathbf{A}) < 0$ and $det(\mathbf{A}) > 0$. This allows us to simply look at our linearization to know a lot of our how our system behaves near an equilibrium point.

Worth noting is also if the term $\sqrt{-4det(\mathbf{A}) + tr(\mathbf{A})^2}$ is real or complex. If a λ has a negative real part and no complex part in an equilibrium-point, this point could be considered to be a stable node because the exponentials include only negative real numbers.

If λ contains some complex parts we can easily discover that the pair of λ's must be complex-conjugated and that the exponentials give rise of a spiral-like motion in the (X,S)-plane around the equilibrium-point. We call the point a stable spiral if $tr(\mathbf{A}) < 0$.

Appendix B

A Matlab M-file

This appendix displays a Matlab M-file used to visualize the phase portrait and to control calculations. This example explains the principle, and it is easy to change, for example, the kinetic expression to fit another model.

```
function chemostat_inhibited(alpha1, alpha2, xp0, sp0, xc)
%
%chemostat_inhibited Displays a phaseportrait, nullclines
%   and an Euler-path of an inhibited Chemostat.
%   chemostat_inhibited(alfa1, alfa2, np0, cp0, nc) will run if
%   alpha1 > 1/xc, thus there is a reproduction.
%   alpha2 > 1/(xc*alpha1-1), thus there is sufficient stock-nutrition.
%   xp0 > 0 , you can not have a nonpositive population.
%   sp0 > 0 , you can not have a nonpositive concentration.
%   xc  > 0
%
%   The blue arrows represent the vectorfield.
%   The black lines are two of the three nullclines.
%   The black dotted line is the invariant line (no solution crosses it).
%   The red line is an Eulerpath, starting in + and ending in *.
%
%   Try the following:
%   chemostat_inhibited(5, 3, 0.2, 0.3, 6)
%
%   by Per Erik Strandberg, 2003-2004.
%

% Start-condition:
%------------------
if ((alpha1>1) & (alpha2>0) & (sp0>0) & (xp0>0) & xc>0),

    if (alpha2<1/(alpha1-1)),
        disp(' ')
        disp (' (HINT: Only the trivial steady state, alpha2 is too small...)')
    else
        disp(' ')
        disp (' (HINT: Two steady states, alpha2 is quite large...)')
    end


% The non-trivial equilibrium-solution:
%---------------------------------------
a = (xc+1-alpha2-xc/alpha1);
b = (-alpha2-xc/alpha1);
sbar = -a/2 + sqrt(0.25*a*a-b) ;
xbar = -alpha1*sbar+alpha1*alpha2 ;

hold off
plot(xbar, sbar, 'r0')
hold on

plot(0, alpha2, 'r0')
```

```
% The vector-field:
%--------------------
[xx,ss]=meshgrid(0.01 : (alpha1*alpha2*1.3/15) : alpha1*alpha2*1.3 ,
                 0.01 : (alpha2*1.3/15)        : alpha2*1.3);
dx= (alpha1*xx.*ss./(ss+1))./(1+xx./xc)-xx;
ds= -(xx.*ss./(ss+1))./(1+xx./xc)-ss+alpha2;
quiver(xx,ss,dx,ds, 1)

% One of the x-nullclines:
%---------------------------
x_x_nullcline=[0.001: (- (1-alpha1)*xc)*0.9/100  : - (1-alpha1)*xc*0.999];
c_x_nullcline = (1 + x_x_nullcline./xc) ./ (alpha1 - 1 - x_x_nullcline ./ xc) ;
plot(x_x_nullcline,c_x_nullcline,'k')

% The s-nullcline:
%------------------
s5 = [(-0.5*(xc+1-alpha2) + sqrt(0.25*(-xc-1+alpha2)^2+alpha2))*1.001
      : ((alpha2*0.999)-((-0.5*(xc+1-alpha2) +
          sqrt(0.25*(-xc-1+alpha2)^2+alpha2))*1.001))/100
      : alpha2*0.999];
x5 =  xc*((alpha2-s5).*(1+s5)) ./ (xc*s5 -(alpha2-s5).*(1+s5));
plot(x5,s5,'k')

% The Invariant line:
%-------------------------
s_inv=[0: alpha2/3 :alpha2];
x_inv=alpha1*alpha2 - alpha1*s_inv;

plot(x_inv,s_inv,':k')

% The Euler-path:
%------------------
xp=[1:1:1000];
sp=[1:1:1000];
xp(1)=xp0;
sp(1)=sp0;
i=1;

while i < 1000,
 xp(i+1)=xp(i)+0.005*((alpha1*xp(i)*sp(i)/(sp(i)+1))./(1+xp(i)/xc)-xp(i));
 sp(i+1)=sp(i)+0.005*(((-1)*xp(i)*sp(i)/(sp(i)+1))./(1+xp(i)/xc)-sp(i)+alpha2)
 i=i+1;
end

plot(xp,sp,'r-.')
plot(xp(1), sp(1),'r')

plot(xp(   1), sp(   1), 'r+')
plot(xp(1000), sp(1000), 'r*')

%fixing the axis
%----------------
axis([0 alpha1*alpha2*1.2 0 alpha2*1.2 ])

disp('  chemostat_inhibited.m by Per Erik Strandberg, 2003-2004. Finished OK.')
disp(' ')

% The illegal indata case:
%--------------------------
else
    disp('   chemostat_inhibited.m by Per Erik Strandberg, 2003-2004.')
    disp('   Did not Finish OK. (You used illegal indata.)')
    disp(' ')
    disp('   For syntax help type: help chemostat_inhibited .')
    disp(' ')
end
```