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Optimal steady-state design of bioreactors in series with Monod growth kinetics

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ABSTRACT

Optimal steady-state design of bioreactors in series with Monod growth kinetics

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Bioreactors are used to carry out bioprocesses and are commonly used in e.g. biogas production and wastewater treatment. Two common hydraulic models of bioreactors are the continuous stirred tank reactor (CSTR) and the plug-flow reactor (PFR). In this paper, a differential equation system that describes the substrate, biomass and inert biomass in the bioreactors is presented. It is used in a steady-state analysis and design of CSTRs in series. Monod kinetics were used to describe the specific growth rate and the decay of biomass was included. Using the derived systems of differential equations, two optimization problems were formulated and solved for both CSTRs in series and for a CSTR+PFR. The first optimization problem was to minimize the effluent substrate level given a total volume, and the second was to minimize the total volume needed to obtain a certain substrate conversion.

Results show that the system of differential equations presented can be used to find optimal volume distributions that solves the optimization problems. The optimal volume for N CSTRs in series decreases as N increases, converging towards a configuration of a CSTR followed by a PFR. Analyzing how the decay rate affects the results showed that when the total volume was kept constant, increasing the decay rate caused less difference between the configurations. When the total volume was minimized, increasing the decay rate caused the configurations to diverge from each other. The presented model can be used to optimally divide reactors into smaller zones and thereby increasing the substrate conversion, something that could be of interest in e.g. existing wastewater treatment plants with restricted space. A fairly accurate approximation to the optimal design of N CSTRs in series is to use the optimal volume for the CSTR in the configuration with a CSTR+PFR and equally distribute the remaining volumes.

Keywords: Bioreactor, CSTR, PFR, optimization, modelling, Monod kinetics, decay rate

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REFERAT

Optimal design av bioreaktorer i serie vid steady-state med tillväxt som följer Monod-kinetik

Hanna Molin

Bioreaktorer används för att utföra olika biologiska processer och används vanligen inom biogasproduktion eller för rening av avloppsvatten. Två vanliga hydrauliska modeller som används vid modellering av bioreaktorer är helomblandad bioreaktor (på engelska continuous stirred tank reactor, CSTR) eller pluggflödesreaktor (på engelska plug-flow reactor, PFR). I den här rapporten presenteras ett system av differentialekvationer som används för att beskriva koncentrationerna av substrat, biomassa och inert biomassa i både CSTR och PFR. Ekvationssystemet används för analys och design av en serie CSTRs vid steady-state. Tillväxten av biomassa beskrivs av Monod-kinetik. Avdödning av biomassa är inkluderat i studien. Från ekvationssystemet formulerades två optimeringsproblem som löstes för N CSTRs i serie och för CSTR+PFR. Det första optimerinsproblemet var att minimera substrathalten i utflödet givet en total volym. I det andra minimerades den totala volymen som krävs för att nå en viss substrathalt i utflödet.

Resultaten visade att ekvationssystemet kan användas för att hitta den optimala volymsfördelningen som löser optimeringsproblemen. Den optimala volymen för N CSTRs i serie minskade när antalet CSTRs ökade. När N ökade konvergerade resultaten mot de för en CSTR sammankopplad med en PFR. En analys av hur avdödningshastigheten påverkade resultaten visade att en ökad avdödningshastighet gav mindre skillnad mellan de två olika konfigurationerna när den totala volymen hölls konstant. När den totala volymen istället minimerades ledde en ökad avdödningshastighet till att de två konfigurationerna divergerade från varandra. Modellen som presenteras i studien kan användas för att fördela en total reaktorvolym i mindre zoner på ett optimalt sätt och på så vis öka substratomvandlingen, något som kan vara av intresse i exempelvis befintliga avloppsreningsverk där utrymmet är begränsat. En relativt bra approximation till den optimala designen av N CSTRs i serie är att optimera volymerna för en CSTR+PFR, använda volymen för CSTR som första volym i konfigurationen med N CSTR i serie, och sedan fördela den kvarvarande volymen lika mellan de övriga zonerna.

Nyckelord: Bioreaktorer, CSTR, PFR, optimering, modellering, Monod-kinetik, avdödningshastighet

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PREFACE

With this master thesis, I will finally have finished my studies at the Master Programme in Environmental and Aquatic Engineering at Uppsala University and the Swedish University of Agricultural Science. The degree project has been carried out in collaboration with Mälardalen University with supervision and guidance from Jesús Zambrano, postdoctoral research fellow in Future Energy at Mälardalen University. My subject reviewer was Bengt Carlsson, professor at the Department of Information Technology, Division of Systems and Control at Uppsala University and examiner was senior lecturer Björn Claremar at the Departement of Earth Sciences at Uppsala University

I would like to thank Bengt and Jesús for giving me the opportunity and trusting me to carry out this project, and for sharing their expertise and providing valuable feedback on the report. I would like to thank Jesús for patiently helping me with Matlab misprints and answering somewhat stupid questions (although stupid questions do not exist, as we have been taught throughout the years at the university).

Finally, I would like to thank everyone that has been supporting me throughout my years of studying. To Patrik, Rasmus, Solveig, Moa, Erik, Olov and Emil, thank you for all the hours we've spent studying and laughing together. I thank my family for all the love and support, especially my mother and grandmother for curiously asking and honestly trying to understand what I've have been studying these last years. And to Johan, thank you for bearing with me and for supporting me no matter what I've wanted to do, and for fixing my bike, doing my dishes, and helping me out whenever needed.

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Bioreaktorer kan användas inom många olika områden, till exempel för att producera biogas eller rena avloppsvatten. Det är ofta önskvärt att minska halten av ett visst substrat i en bioreaktor. I avloppsvattenrening är substratet organisk kol som bryts ned av mikroorganismer. Mikroorganismerna använder kolet i sin metabolism. Genom att bryta ned substratet får således mikroorganismerna energi och deras biomassa ökar. Det finns olika modeller som beskriver tillväxten av mikroorganismer. I den här studien har Monodkinetik använts. Nettotillväxten påverkas också av att mikroorganismer förr eller senare kommer dö. Tidigare studier har gjorts inom samma område som den här studien, men då har det antagits att mikroorganismerna inte dör. Till skillnad från dem har avdödningshastigheten inkluderats i den här studien.

Det finns olika modeller för att beskriva hydrauliken i en bioreaktor där två vanliga modeller är för en helomblandad bioreaktor (på engelska continuous stirred tank reactor, CSTR) eller pluggflödesreaktor (på engelska plug-flow reactor, PFR). Ingen av dessa modeller återspeglar dock vad som normalt återfinns i praktiken. Oftast är bioreaktorer någonstans emellan dessa två idealfall. En mer realistisk modell är att använda flera CSTRs i serie. I den här studien har två olika sammansättningar av bioreaktorer undersökts, nämligen ett antal (N) CSTRs i serie och en CSTR efterföljd av en PFR (CSTR+PFR). Den sistnämnda sammansättningen har tidigare visat sig vara mer effektiv än CSTRs i serie. Det har också bevisats att en PFR kan liknas vid oändligt många, oändligt små CSTRs.

I studien presenteras en uppsättning ekvationer som beskriver hur substrat- och biomassakoncentrationerna förändras i de två olika typerna av bioreaktorer (CSTR och PFR). Analyser har gjorts vid så kallat ”steady-state”, dvs. att ingen förändring sker över tid, och utifrån det har samband tagits fram för att simulera hur halterna förändras från inflödet till utflödet av bioreaktorerna. Två olika optimeringsproblem har studerats. I det första var den totala volymen given och försök gjordes för att fördela den totala volymen mellan N CSTR eller mellan en CSTR och en PFR för att få så låg substrathalt som möjligt i utflödet. I det andra optimeringsproblemet minimerades den totala volymen för att nå en viss given substrathalt i utflödet. Både sammansättningen med N CSTR i serie och CSTR+PFR undersöktes.

Studien har visat att de ekvationssystem som sattes upp går att använda för att lösa de två optimeringsproblemen. Den har också visat att om antalet CSTR ökar (dvs. $N \to \infty$) så närmar sig lösningen för N CSTR i serie den för CSTR+PFR. Hur många CSTR i serie som krävs för att nå detta beror på vilka parameterar som väljs, främst hur stor avdödningshastigheten är. I studien undersöktes därför hur avdödningshastigheten påverkade resultaten. Två intressanta resultat är att (1) om avdödningshastigheten är tillräckligt stor kommer det inte vara någon skillnad mellan att använda N CSTRs i serie eller att använda en CSTR+PFR om den totala volymen är given, samt att (2) om volymen istället ska minimeras blir det större skillnad mellan de två sammansättningarna när avdödningshastigheten ökar.

Resultaten från studien visar att den metod som har använts här kan användas i exempelvis befintliga avloppsreningsverk där det inte finns möjlighet att bygga ut reningsbassängerna. Genom att använda den befintliga volymen och dela upp den i zoner kan man öka reningsgraden. Studien har också visat att man inte behöver optimera alla volymer för att få bättre reningsgrad. Det räcker att optimera den första zonen och sedan fördela den kvarvarande volymen jämnt mellan de efterföljande zonerna.

NOMENCLATURE

Bioreactor	Apparatus to carry out bioprocesses
Steady-state	No change with time
Substrate	Reactant consumed during a catalytic or enzymatic reaction
Microorganisms	Microscopic organisms including bacteria, protozoa and archaea amongst others.
Biomass	Another word here used for microorganisms. Refers to the total mass that the microorganisms make up.

Abbreviations

CSTR	Continuous stirred tank reactor
PFR	Plug-flow reactor
ASP	Activated sludge process

Parameters and variables

A	Area [m^2]
b	Decay rate [d^{-1}]
f_p	Fraction between inert biomass and substrate [-]
h	Position in the PFR [m]
K_S	Half-saturation constant [kgm^{-3}]
N	Number of CSTRs [-]
Q	Flow rate [m^3d^{-1}]
S	Substrate [kgm^{-3}]
S_e	Substrate level in the effluent [kgm^{-3}]
S_i	Substrate level in the *i*-th reactor [kgm^{-3}]
S_{in}	Substrate level in the influent [kgm^{-3}]
S_{min}	The minimum substrate level that can be obtained in the reactors [kgm^{-3}]
$V(N)$	The optimal total volume for N CSTRs in series [m^3]
V_1^*	The optimal volume of the CSTR in the configuration of a CSTR+PFR [m^3]
V_1^{min}	Wash-out volume, the minimum volume the first CSTR must have [m^3]
V_1^{opt}	The optimal volume of the first CSTR [m^3]
V_i	The volume of the *i*-th CSTR [m^3]
V_{opt}	The optimal total volume for the CSTR+PFR [m^3]
V_{tot}	Total volume [m^3]
X	Biomass [kgm^{-3}]
X_e	Biomass level in the effluent [kgm^{-3}]
X_i	Biomass level in the *i*-th CSTR [kgm^{-3}]
X_{in}	Biomass level in the influent [kgm^{-3}]
Y	Yield factor [-]
Z	Inert biomass [kgm^{-3}]
Z_e	Inert biomass level in the effluent [kgm^{-3}]
Z_i	Inert biomass level in the *i*-th CSTR [kgm^{-3}]
Z_{in}	Inert biomass level in the influent [kgm^{-3}]
$\mu(S)$	Specific growth rate [d^{-1}]
μ_{max}	Maximum specific growth rate [d^{-1}]

Contents

1 INTRODUCTION

A bioreactor, in a broad definition, is an apparatus used to carry out bioprocesses. Bioreactors are frequently used in various industrial processes. They can be used for biogas production where organic material is fermented to produce biogas (see e.g. Bouallagui et al., 2005) or in pharmaceutical production (see e.g. Miao et al., 2008). Furthermore are bioreactors vastly used in wastewater treatment (see Lee et al., 2006; Radjenovic et al., 2009, amongst others).

1.1 BACKGROUND

The optimal design of bioreactors has been of interest for the last decades in order to e.g. minimize costs, increase performance, or minimize the required space (Harmand and Dochain, 2005). In wastewater treatment, bioreactors are used to reduce the substrate concentration of the incoming wastewater. This can be done by passing the flow through one or several bioreactors in series. The bioreactors are typically modelled as complete stirred tank reactors (CSTRs) where microorganisms (biomass) consume the substrate, i.e. the biomass increases as the substrate is reduced (von Sperling, 2007). When the number of CSTRs is large enough, one can model the several CSTRs as only one CSTR connected to a plug flow reactor (PFR) (Zambrano et al., 2015). Mathematically, the process in the bioreactors can be described using dynamic models consisting of ordinary differential equations (ODEs) that account for growth and decay of the biomass, as well as properties of the reactors and the treated wastewater.

Analytical and numerical results on optimizing bioreactors can be found in early work by e.g. Bischoff (1966), to more recent work by e.g. Gómez-Pérez and Espinosa (2017). Bischoff (1966) studied the total residence time for two CSTRs in series and showed that for many cases, combining a CSTR and a PFR gives the lowest residence time to achieve a certain substrate conversion. Gómez-Pérez and Espinosa (2017) analyzed the design of continuous bioreactors in series by representing them as a system of linear equations and found non-trivial solutions by using singular value decomposition as an analysis tool. The singular value decomposition analysis made it possible to characterize the solutions to the equation system, and thereby improve the design of bioreactors in series.

Zambrano et al. (2015) recently presented a new approach to the optimal design of zone volumes of bioreactors using Monod kinetics. They studied the optimal design of CSTRs in series when the number of CSTRs is large (2-10 CSTRs in series). Assumptions that were made include that the process followed Monod growth kinetics, the decay rate was zero, and there were only two main components included in the model (one particulate biomass and one soluble substrate). Since the study did not include the decay of biomass, an interesting way to continue this study is to incorporate and analyze the effect of a decay term.

1.2 OBJECTIVE

The objective of this study is to extend the analysis by Zambrano et al. (2015) by adding the biomass decay rate and one more ODE which represents the inert biomass. The study will include two optimization problems:

- Minimize the effluent substrate level by optimally distribute the volumes, given a certain total volume
- Minimize the total volume needed to obtain a certain substrate conversion

that will be solved numerically for several CSTRs in series as well as of one CSTR connected to a PFR. If possible, an analytical solution of the process is to be found by analyzing a large number of CSTRs in series as one CSTR connected to a PFR. In this case, a comparison between the behavior of optimally designed CSTRs in series and optimally designed CSTR+PFR would be interesting to obtain.

1.3 ASSUMPTIONS AND DELIMITATIONS

Some assumptions were made to simplify the analysis. It was assumed that the growth follow Monod kinetics (Monod, 1949). The Monod equation is an empirical formula that was developed for a single organism metabolizing a single substrate (see ch. 2.1.1). Thus, it must be assumed that there is one main biomass which consumes one main dissolved substrate, although in wastewater treatment, this assumption is usually not valid (von Sperling, 2007). The Monod formula has however been proven to give a fair approximation and has been widely used in many mathematical models for wastewater treatment.

Two major factors affecting the growth of the microorganisms are oxygen level and temperature. The reaction rate in chemical reactions increase with temperature. The same tendency can be seen in biochemical processes as well, but within certain ranges (Randall et al., 1982; von Sperling, 2007). For this analysis, it was assumed that none of the biological parameters change with the liquid temperature. Furthermore, it was assumed that the oxygen demand was fulfilled throughout the reactors. Microorganisms consume oxygen in their metabolism. Ideally, the oxygen level is sufficient to cover the oxygen demand in the whole reactor volume whereas in reality, hypoxic or anoxic conditions can occur locally.

One key assumption in this study is that the parameters and variables are time-invariant, meaning that steady-state conditions prevail. We assume instant steady-state (no spin up). Assuming steady-state simplifies the analysis, although one drawback is that the dynamic differential equations become static. In reality, both the substrate and biomass levels, and other variables such as flow rate, might change with time. As an example, in wastewater treatment plants there are diurnal variations in both the composition of the incoming wastewater and the flow rate. The presented model will not take such changes into account. It will however provide new insight on the dynamics and the design procedure of bioreactors in series.

2 THEORY

Due to the variety in applications for bioreactors, there are also different types of bioreactors. Thereby, there are several models which can be used to model the hydraulics of the reactors. Two common hydraulic models are the CSTR and the PFR (von Sperling, 2007). Both the CSTR and PFR are idealized reactors where the flow is continuous. In the PFR, the flow stream enters the tank on one end and the particles then pass through the reactor. The particles discharge in the same sequence in which they entered and no longitudinal mixing occurs in the tank. In the CSTR, the particles are immediately identically dispersed in the reactor volume. The composition in the outflow thus reflects the composition in the reactor. However, both total and identical dispersion and complete absence of longitudinal dispersion is hard to obtain in practice. A hydraulic model between the PFR and the CSTR is using several CSTRs in series. As the number of CSTRs goes towards infinity, the system will reproduce a PFR (von Sperling, 2007). This hydraulic model is more realistic since reactors are seldom ideal PFR or CSTR in reality (Tsai and Chen, 2011).

When comparing CSTRs to PFRs, it has been established that PFRs require a smaller volume than CSTRs to obtain a certain conversion rate. However, PFRs suffers from some drawbacks which limits their practical use, e.g.: (i) in multiphase systems, the gaseous phases can affect and increase back mixing which thwart the plug flow, and (ii) in a perfect autocatalytic PFR, the biomass must be continuously inoculated which might be hard to achieve in practice (Harmand and Dochain, 2005).

In the following sections, a short introduction to the microbial processes within bioreactors will be given followed by a review on previous research in the subject field to motivate the importance of the intended study.

2.1 MICROBIAL GROWTH AND DECAY

This section aims to give an insight to the biological processes occurring in the bioreactors to give a better understanding of the following sections were the optimization of bioreactors will be further addressed. Often, especially in wastewater treatment applications, the purpose of a bioreactor is to reduce a certain substrate with the use of microorganisms. A widely used model that describes the biological processes in wastewater treatment systems is the IAWQ (International Association on Water Quality) Activated Sludge Model no. 1 (ASM1; Henze et al., 1987). The bisubstrate model used in ASM1 models the process as presented in Figure 1. Slowly biodegradable matter becomes readily biodegradable through hydrolysis, where long-chained molecules are broken down to smaller molecules. The hydrolysis is assumed to be instantaneous in this study, which simplifies the model (Fig. 1).

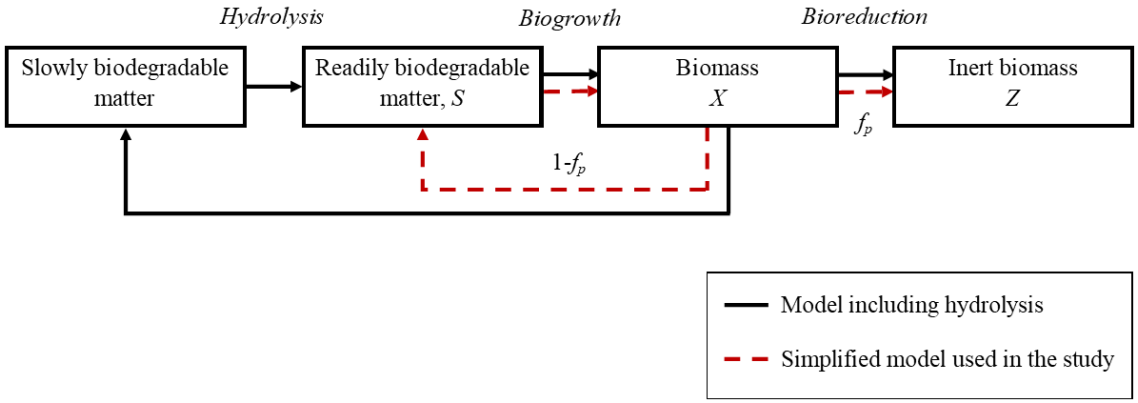

Figure 1. A schematic overview of the biological process. The black arrows show the bisubstrate model in ASM1 (Henze et al., 1987), and the red arrows show the simplified model used in this study where the hydrolysis is assumed to be instantaneous.

The microorganisms consume the substrate in their metabolism, causing a decrease in the substrate level and an increase in biomass (microorganisms). The growth of biomass can be divided into four phases (Fig. 2; Comeau, 2008),

1. Lag phase: cells acclimate to the new situation. Little biomass increase and substrate consumption. Growth rate close to zero.

2. Exponential phase: the substrate is readily available. The growth rate is constant and at its maximum.

3. Stationary phase: little external substrate is available. Growth rate is back to almost zero, thus the biomass concentration is relatively constant.

4. Death phase: the biomass starts to decrease due to shortage of substrate, predation and lysis. Thus, the growth rate is negative.

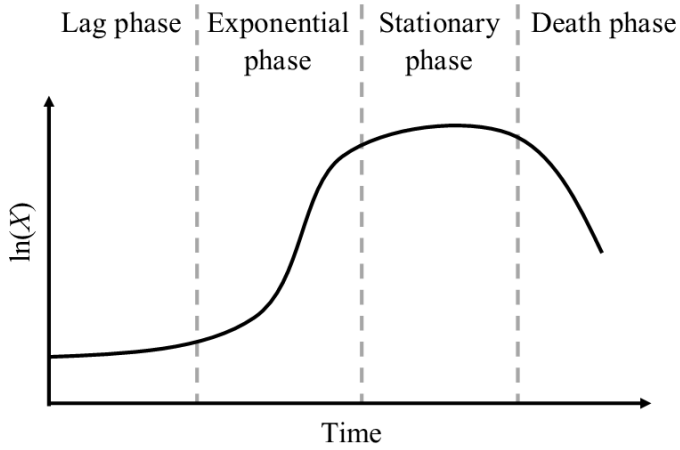

Figure 2. The logarithmic biomass concentration with time, divided into the four phases: lag phase, exponential phase, stationary phase and death phase.

The exponential phase can be regarded as a steady-state where the ratio between the concentration of the substrate and the concentration of the biomass is constant. During the lag phase, there is a gradual build up towards the steady-state. The rate of the build-up is dependent on the specific conditions and properties of the microorganisms (Monod, 1949).

In this section, the exponential growth phase will be considered, starting off by defining cell concentration as the number of individual cells per unit volume of a culture. The cell concentration is denoted $X(t)$ and is a time dependent function. Exponential growth means that after a certain time interval, t_d, the cell concentration will have doubled, or in mathematical terms,

$$X(t) = X_0 2^{(t-t_0)/t_d} \tag{1}$$

where X_0 is the initial concentration (at $t = t_0$). Using logarithms on both sides of the expression results in the following expression

$$\frac{\ln X(t) - \ln X_0}{t - t_0} = \frac{1}{t_d}\ln 2 \tag{2}$$

The growth rate can be found by letting $t \to t_0$ in the derivative of $X(t)$

$$\frac{\mathrm{d}}{\mathrm{d}t}\ln X(t) = \frac{1}{X(t)}\frac{\mathrm{d}X(t)}{\mathrm{d}t} = \frac{1}{t_d}\ln 2 = \mu \tag{3}$$

where μ is the specific growth rate (see section "The specific growth rate").

The exponential phase ends when the growth is limited. Limiting factors include exhaustion of nutrients (or substrate), accumulation of toxic metabolic products, and changes in ion equilibrium (Monod, 1949). The biomass will eventually decay. This can be considered by adding a decay term. The specific biomass decay rate, b, is similar to the specific growth rate, although negative. It is defined

$$b = -\frac{\mathrm{d}X(t)}{X\mathrm{d}t} \tag{4}$$

The net growth with decay is $\mu - b$. Introducing a decay rate in the system will cause a lower net growth.

The dead biomass either becomes substrate or inert biomass (Fig. 1). The amount that becomes inert is decided by the parameter f_p, which takes on values in the interval 0.0-1.0. Consequently, the amount that becomes substrate is $1 - f_p$. A low value on f_p will thus cause a higher substrate generation, especially if combined with a high decay rate (Fig. 1).

2.1.1 The specific growth rate

The specific growth rate is the rate of increase in cell concentration per unit cell concentration and it can be modelled in various ways. Monod (1949) presented an empirical relation between the concentration of the growth limiting substrate, S, and the half saturation constant, K_S,

$$\mu(S) = \mu_{max} \frac{S}{(K_S + S)} \tag{5}$$

where μ_{max} is the rate limit for increasing concentrations of S, or the maximum specific growth rate.

Other kinetic models include Contois, Haldane, and Michaelis-Menten, whom all have been used in the modelling of bioreactors. A model similar to the Monod growth model is the Michaelis-Menten equation. It is based on theoretical principles and was derived for enzymatic reactions, as opposed to the Monod equation which was derived for biological reactions (von Sperling, 2007). Haldane kinetics accounts for inhibitory effects at high substrate concentration. With high substrate concentrations, the bioreactors can suffer from overloading. This is not accounted for in the Monod equation. The Contois model, unlike the Monod, depend on the biomass concentration.

Carlsson and Zambrano (2014) presented a study on the optimal design of CSTRs in series where both Monod and Contois kinetics were used. They showed that the optimal design differed depending on the choice of growth kinetics. The optimal volume needed for the first CSTR and the effluent substrate level decrease when the substrate level entering the system increase when using Monod kinetics. The optimal volume needed for the first CSTR is independent on the influent substrate level, and the effluent substrate level is proportional to that of the influent when using Contois kinetics (Carlsson and Zambrano, 2014).

Monod kinetics are frequently used in wastewater treatment modelling and have proven to be suitable for this application (Braha and Hafner, 1984). Thus, Monod kinetics will be used to describe the growth kinetics in this study. The Monod equation was derived for a single substrate metabolized by a single microorganism. This must be acknowledged when applying the Monod equation to processes where the substrate is not homogeneous and several populations of microorganisms are active (von Sperling, 2007). There are ways of extending the Monod equation to also include various substrates and nutrients, or environmental factors such as pH and temperature in the model. This will however not be done in this study.

2.2 OPTIMIZATION OF BIOREACTORS IN SERIES

Finding the optimal design of bioreactors has been extensively studied for the last decades. Optimization of bioreactors has important advantages that can be related to e.g. minimizing costs, increase performance, and minimize the required space (Harmand and Dochain, 2005). When it comes to optimize CSTRs, the general approach has been to find the optimal distribution of volumes for a certain requirement on the substrate concentration in the

effluent. Early studies on the optimal design of bioreactors can be found in e.g. Bischoff (1966). Bischoff (1966) minimized the total residence time for two CSTRs in series fed with a single stream under the assumption that there was no decay of biomass. The study showed that for many cases, combining a CSTR and a PFR (CSTR+PFR) will give the lowest residence time to achieve a certain substrate conversion. This combination of a CSTR followed by a PFR can be regarded as one CSTR followed by an infinite number of infinitesimally small CSTRs. The degree of conversion in a system consisting of N CSTRs in series will converge towards the CSTR+PFR when N becomes large (Bischoff, 1966).

Luyben and Tramper (1982) investigated the behaviour of N CSTRs in series, with N ranging from 1-10, using Michaelis-Menten kinetics. They defined optimal design as finding the minimum mean holding time to perform a specific conversion and studied two cases: optimum volumes and equal-sized volumes. The study included an evaluation of a PFR as well to use as comparison. The study showed that the mean holding time is lowest for a PFR, that the mean holding time of the CSTRs in series decreases when N increase, and that the performance of N CSTRs in series converges towards one CSTR followed by a PFR as N increases. Hill and Robinson (1989) also studied the optimal design of CSTRs in series but with Monod kinetics. They derived an expression to find the minimum possible total residence time to achieve any desired substrate conversion. Findings include that three optimally designed CSTRs in series provide the same required total mean residence time as a PFR (Hill and Robinson, 1989). de Gooijer et al. (1996) derived expressions for the minimum holding time for one and two CSTRs in series for different growth kinetics. They presented an optimization criterion to decide if and when multiple CSTRs in series are more productive than a single CSTR.

Many studies focus on the optimal design of CSTRs in series and the mathematical description of this is well established. There are only a few attempts on finding steady-state mathematical models to design PFRs in the literature (Liotta et al., 2015). Recently, Zambrano et al. (2015) presented a differential equation approach to find the optimal steady-state design of zone volumes. Monod kinetics were used and the decay of biomass was neglected. They derived an analytical expression to find the optimal volume of a CSTR followed by a PFR. The solution was evaluated with some numerical examples and compared to the solution of N CSTRs in series. Two design problems were evaluated: (i) minimize the substrate effluent level given a certain total volume, and (ii) minimize the total volume required to achieve a certain substrate effluent level. The explicit expressions derived for the CSTR+PFR showed that the optimal volume of the CSTR is the same for both design problems (Zambrano et al., 2015).

2.3 APPLICATION OF BIOREACTORS IN WASTEWATER TREATMENT

Bioreactors are widely used in the biological treatment of wastewater. They can be applied for removal of nitrogen, phosphorous, and organic matter. The optimization of volumes in bioreactors are of fundamental importance when it comes to wastewater treatment. The optimization can either be done as a means of minimizing operational or production

costs, or fulfilling law binding restrictions on the effluent substrate levels. In already existing wastewater treatment plants, with given total reactor volumes, there might be an interest in how to find the optimal zone volumes to minimize to steady-state effluent substrate concentration. This was studied by Zambrano and Carlsson (2014) where they used both Contois and Monod kinetics and optimized the zone volumes given $N = 1, \ldots, 5$ zones. The substrate effluent level can be decreased by dividing the total volume in several zones, and the more zones, the lower substrate effluent level (Zambrano and Carlsson, 2014). They also optimized the zone volumes, given a total volume, for more than two bioreactors in an activated sludge process and showed that the optimal zone volumes differ depending on growth kinetics.

The activated sludge process (ASP) is a biological treatment technique used in wastewater treatment. The idea behind it is to maintain a certain part of the sludge suspended in the wastewater. Microorganisms use the organic material in the wastewater as its energy source and degrade it while consuming oxygen. In the ASP, the bioreactor is followed by a settler where the sludge settles and the microorganism concentration is increased. A recycle stream returns a certain amount of the sludge to the bioreactors. A common use of bioreactors in the treatment process is in the ASP.

The optimal design of bioreactors can be applied and extended to the ASP. San (1989) conducted a study where a recycle loop was incorporated in the optimal design of a PFR. The decay of microorganisms was included and the growth rate was governed by Monod kinetics. A relationship between biomass and substrate concentrations was obtained and compared with numerical solutions. Scuras et al. (2001) optimized the configuration of the activated sludge reactor and studied the kinetics. A procedure to determine optimum reactor configuration for different values of substrate concentrations, half saturation coefficients, and the number of tanks was presented. Results showed that the benefit of staging is greater when the influent substrate concentrations are high and the requirements on the effluent substrate concentration is strict, and that optimizing the volumes give a higher conversion rate than using equal sized tanks. Monod kinetics were used and the decay of biomass was neglected.

Harmand et al. (2003) evaluated and optimized two interconnected step-fed bioreactors, thus providing insight in the optimization of a recirculation loop and/or a distributed feeding system. The total required volume to achieve a certain substrate conversion can be significantly decreased by using a distributed flow and a recirculation loop. Based partly on the study by Harmand et al. (2003), a graphical way to optimally design (here minimum total volume needed to perform a certain conversion) two interconnected reactors, valid for both catalytic and autocatalytic biochemical reactors was later presented by Harmand and Dochain (2005). Sidhu et al. (2015) presented a dimensionless model for both a standard and a step-feed cascade of equal sized reactors. The configuration used is common in the ASP. They used Monod kinetics and included the decay of biomass. The analysis showed that the substrate and biomass concentrations leaving the first reactor of the cascade were the same as in the final reactor in a step-feed reactor. Previously, it has been proposed that the step-feed reactor will improve the biological treatment of wastewater. These results, surprisingly, showed that it is no better to use step-feed reactors if

the feed streams are equally distributed than using only one single reactor (Sidhu et al., 2015).

3 METHODS

In the following sections, the mathematical development will be presented. The problem setup, the attempt on finding an analytical solution to them, and the numerical analysis will be presented.

3.1 MATHEMATICAL DEVELOPMENT

In a completely mixed tank reactor where the influent and effluent flow rates (Q) are equal, i.e. the volume V is constant, the rate of accumulation of biomass can be derived from a simple mass balance (accumulation = input - output + production - consumption). The influent has a substrate concentration S_{in} and a biomass concentration X_{in}. The concentration of biomass in the outflow is equal to the concentration in the tank (X) since the reactor is completely mixed. The change in biomass in the tank is given by

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \mu(S)X + \frac{Q(X_{in}{-}X)}{V} \tag{6}$$

As the biomass increase, the substrate decrease which commonly is expressed as

$$\frac{\mathrm{d}X}{\mathrm{d}t} = -Y\frac{\mathrm{d}S}{\mathrm{d}t} \tag{7}$$

where Y is the yield coefficient. The yield coefficient is defined as the ratio between the mass of cells formed and the mass of the consumed substrate. The yield coefficient can be derived from Eq. 7 and can be expressed as

$$Y = -\frac{\mathrm{d}X}{\mathrm{d}S} \tag{8}$$

Applying a mass balance for the substrate concentration in the tank will give the expression for the change in substrate concentration

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\frac{\mu(S)}{Y}X + \frac{Q(S_{in} - S)}{V} \tag{9}$$

Expressions (6) and (9) do not take the decay of microorganisms into account. Introducing a decay rate will change the net growth rate to $\mu - b$. The dead biomass will either become substrate, S, or inert biomass, Z (Fig. 1). The fractionation between them is determined by f_p. The derivation of an expression for the change in inert biomass follows the same procedure as for Equations (6) and (9), i.e. a simple mass balance over the reactor with a term which takes into account the amount of inert biomass that is created in the reactor.

$$\frac{\mathrm{d}X}{\mathrm{d}t} = (\mu(S) - b)X + \frac{Q(X_{in} - X)}{V}, \tag{10}$$

$$\frac{\mathrm{d}Z}{\mathrm{d}t} = f_p b X + \frac{Q(Z_{in} - Z)}{V}, \tag{11}$$

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\left(\frac{\mu(S)}{Y} - (1 - f_p)b\right) X + \frac{Q(S_{in} - S)}{V}. \tag{12}$$

3.1.1 *N* CSTRs in series

Equations (10)-(12) are valid for a single bioreactor. In this study, several bioreactors in series will be analyzed and the equations must be adjusted to this case. The total volume of the bioreactors, V_{tot}, is divided into N bioreactors, each with volume V_i (i = 1, 2, ..., N; Fig. 3).

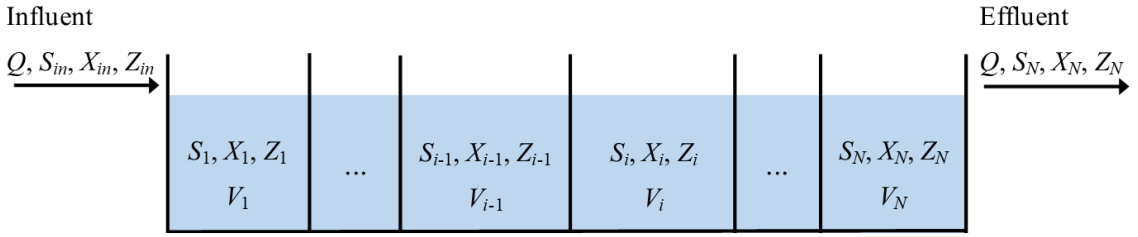

Figure 3. *N* CSTRs in series

In the following we will assume $X_0 = X_{in} = 0$, $Z_0 = Z_{in} = 0$ and $S_0 = S_{in} > 0$. The dynamics of the substrate and biomass concentrations in the i-th CSTR are given by

$$\frac{\mathrm{d}X_i}{\mathrm{d}t} = (\mu(s) - b)X_i - \frac{Q(X_{i-1} - X_i)}{V_i}, \tag{13}$$

$$\frac{\mathrm{d}Z_i}{\mathrm{d}t} = f_p b X_i - \frac{Q(Z_{i-1} - Z_i)}{V_i}, \tag{14}$$

$$\frac{\mathrm{d}S_i}{\mathrm{d}t} = -\left(\frac{\mu(s)}{Y} - (1 - f_p)b\right) X_i + \frac{Q(S_{i-1} - S_i)}{V_i} \tag{15}$$

respectively where X_i, Z_i, S_i and V_i are the biomass and substrate concentrations, and the volume of the i-th CSTR, and f_p is the fraction of the dead biomass that becomes inert.

In this study, we are only interested in the steady-state solutions. At steady-state, $\mathrm{d}X_s/\mathrm{d}t = \mathrm{d}Z/\mathrm{d}t = \mathrm{d}S/\mathrm{d}t = 0$, which yields

$$0 = (\mu(S_i) - b)X_i + \frac{Q(X_{i-1} - X_i)}{V_i}, \tag{16}$$

$$0 = f_p b X_i + \frac{Q(Z_{i-1} - Z_i)}{V_i}, \tag{17}$$

$$0 = -\left(\frac{\mu(S_i)}{Y} - (1 - f_p)b\right) X_i + \frac{Q(S_{i-1} - S_i)}{V_i}. \tag{18}$$

From Equation (18), and expression for S_i can be derived

$$S_i = S_{i-1} - \frac{1}{Y}(X_i - X_{i-1}) - \frac{b}{QY}(1 - (1 - f_p)Y)V_i X_i \tag{19}$$

The recursive expression (19) can be used to derive an expression for N CSTRs in series

$$S_N = S_{in} - \frac{1}{Y}X_N - \frac{b}{QY}(1 - (1 - f_p)Y)\sum_{n=1}^{N} V_n X_n \tag{20}$$

The same procedure applied on Equation (16) yields the following expression for X_N

$$X_N = Q^N \frac{(S_{in} - S_1)Y}{V_1(\mu(S_1) - (1 - f_p)bY)} \prod_{i=2}^{N} \frac{1}{Q - V_i(\mu(S_i) - b)} \tag{21}$$

Inserting Equation (21) in Equation (20) will give the final expression for S_N

$$\begin{aligned} S_N = S_{in} - Q^N \frac{(S_{in} - S_1)}{V_1(\mu(S_1) - (1 - f_p)bY)} \prod_{i=2}^{N} \frac{1}{Q - V_i(\mu(S_i) - b)} - ... \\ ... - \frac{b}{QY}(1 - (1 - f_p)Y)\sum_{n=1}^{N} V_n X_n \end{aligned} \tag{22}$$

Solving Equation (16) for the first CSTR and assuming no biomass in the influent ($X_{in} = 0$), the solutions are given by $X_1 = 0$ or

$$\mu(S_{in}) = \frac{Q}{V_1} + b \tag{23}$$

The first condition, $X_1 = 0$, is known as wash-out. Wash-out typically occurs if the dilution rate Q/V is too high which causes too much biomass leaving the reactor and the biomass concentration will reach zero as $t \to \infty$. To prevent wash-out, V_1 must be greater than the wash-out volume V_1^{min}, derived from Equation (23)

$$V_1 > V_1^{min} = \frac{Q}{\mu(S_{in}) - b} = \frac{Q}{\mu_{max}\frac{S_{in}}{S_{in}+K_S} - b}. \tag{24}$$

For a single CSTR at steady-state, the substrate and biomass concentrations are given by the following expressions,

$$\mu(S_1) = \frac{Q}{V_1} + b \Rightarrow S_1 = \frac{\left(\frac{Q}{V_1} + b\right)K_S}{\mu_{max} - \frac{Q}{V_1} - b}, \tag{25}$$

$$X_1 = \frac{QY}{Q + V_1 b(1 - (1 - f_p)Y)}(S_{in} - S_1), \tag{26}$$

$$Z_1 = \frac{V_1}{Q} f_p b X_1, \tag{27}$$

3.1.2 The PFR

In this section, the mathematical development of the steady-state equations for the PFR is considered.The derivation follows Zambrano et al. (2015). The PFR can be approximated as an infinite number of infinitesimally small CSTRs in series, each with volume ΔV (Fig. 4).

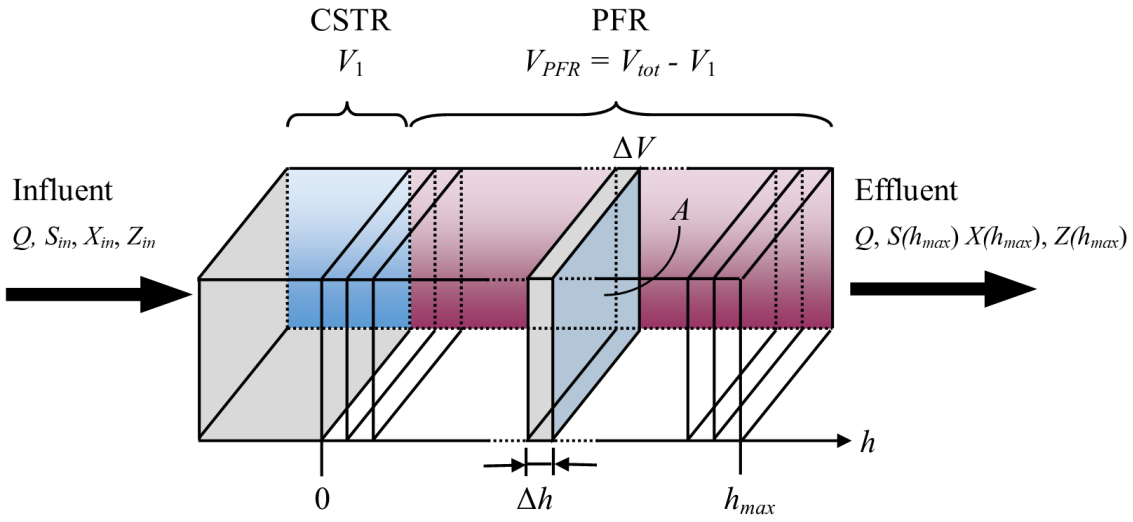

Figure 4. Illustration of a CSTR followed by a PFR. The volume of the PFR is sliced in an infinite number of infinitesimally small CSTRs with volume ΔV.

Consider a large number of CSTRs in series, where the volume of the first CSTR (V_1) is assumed to be large enough to avoid wash-out ($V_1 > V_1^{min}$). The remaining volume, $V - V_1$, is equal to the length of the reactor, h_{max}, times the cross-sectional area, A. Slicing this volume into a large number of volumes ΔV will mimic a PFR (Fig. 4). Assuming A is constant (i.e. not varying along h), the volume of each slice is $\Delta V = A\Delta h$. If considering a small interval $(h, h + \Delta h)$, the conservation of mass for the substrate gives

$$\underbrace{\frac{\mathrm{d}}{\mathrm{d}t}\int_h^{h+\Delta h} AS(x,t)\mathrm{d}x}_{\text{mass increase per time unit}} = \underbrace{QS(h,t)}_{\text{flux in}} - \underbrace{QS(h+\Delta h,t)}_{\text{flux out}} - \ldots \\ \ldots - \underbrace{\int_h^{h+\Delta h} A\left[\frac{\mu(S)}{Y} - (1-f_p)b\right] X\mathrm{d}x}_{\text{consumption per time unit}}. \tag{28}$$

Dividing Equation (28) by $A\Delta h$ and letting $\Delta h \to 0$ results in the following expression for the dissolved substrate

$$\frac{\partial S}{\partial t} + \frac{Q}{A}\frac{\partial S}{\partial h} = -\left(\frac{\mu(S)}{Y} - (1-f_p)b\right) X. \tag{29}$$

The same procedure applied for the active and inert biomass concentrations gives

$$\frac{\partial X}{\partial t} + \frac{Q}{A}\frac{\partial X}{\partial h} = (\mu(S) - b)X, \tag{30}$$

$$\frac{\partial Z}{\partial t} + \frac{Q}{A}\frac{\partial Z}{\partial h} = f_p b X. \tag{31}$$

At steady-state, $\partial X/\partial t = \partial Z/\partial t = \partial S/\partial t = 0$, and Equations (29)-(31) can thus be written as

$$\frac{Q}{A}\frac{\partial X}{\partial h} = (\mu(S) - b)X, \tag{32}$$

$$\frac{Q}{A}\frac{\partial Z}{\partial h} = f_p b X, \tag{33}$$

$$\frac{Q}{A}\frac{\partial S}{\partial h} = -\left(\frac{\mu(S)}{Y} - (1 - f_p)b\right)X, \tag{34}$$

which are the ODEs that will be used to simulate the dynamics in the PFR. Note that $\partial S/\partial h$ can be both positive and negative (Eq. 34). This means that the substrate concentration is not constantly decreasing along the PFR length, and there will be a minimum substrate level. To get a decrease in the substrate concentration we should have

$$\frac{\mu(S)}{Y} - (1 - f_p)b > 0 \tag{35}$$

Inserting Equation (5) in Equation (35) and solving for S gives the following expression,

$$S_{min} = \frac{(1 - f_p)bYK_s}{\mu_{max} - (1 - f_p)bY} \tag{36}$$

which can be used to calculate the minimum substrate level that can be obtained in the reactors.

3.2 ANALYTICAL SOLUTION

One of the objectives of this study was to find, if possible, an analytical solution to the problems. Due to the complexity in Equation (22), an analytical solution was not possible to find for the CSTR. In accordance with Zambrano et al. (2015), efforts were made to find an analytical expression that could be used to optimize the CSTR+PFR. This was also not possible.

3.3 PROBLEM DESCRIPTION

The remaining objectives of the study was to conduct a numerical analysis of CSTRs in series and a CSTR followed by a PFR, finding the optimal volumes given a certain effluent substrate concentration, and to minimize the total volume needed to maximize the reduction of the substrate concentration. This can be summarized in two minimizing problems applied to two different configurations. The first configuration consists of N CSTRs in series and the other one of one CSTR followed by a PFR. In the first minimizing problem (denoted problem 1N or 1PFR), the total volume V_{tot} was given and the objective was to minimize the effluent substrate level. In the second scenario (denoted 2N or 2PFR), the objective was to minimize the total volume given a set substrate level in the effluent. The mathematical description of these problems are further addressed in the following sections.

3.3.1 Problem 1N

The configuration of problem 1N is N CSTRs in series. The objective was to minimize the substrate level in the effluent of the N-th CSTR, S_N, given a total volume V_{tot}.

$$\underset{(V_1,...,V_N)}{\text{minimize}} \left\{ S_N(V_1, ..., V_N) \right\}, \tag{37}$$

subject to

$$V_1 > V_1^{min},\ V_i > 0,\ i = 2, ..., N,\ \text{and}\ \sum_{i=1}^{N} V_i = V_{tot} \tag{38}$$

3.3.2 Problem 2N

For problem 2N, the same configuration as in problem 1N was used. The objective was to find the optimum volumes which minimize the total volume V_{tot}, given an effluent substrate concentration $S_e < S_{in}$. The problem can be summarized as:

$$\underset{(V_1,...,V_N)}{\text{minimize}} \left\{ V_{tot} = \sum_{i=1}^{N} V_i \right\}, \tag{39}$$

subject to

$$V_1 > V_{min},\ V_i > 0,\ i = 2, ..., N,\ \text{and}\ S_N(V_1, ..., V_N) = S_e \tag{40}$$

Note that the constrains are both linear ($V_1 > V_{min}; V_i > 0$) and nonlinear ($S_N(V_1, ..., V_N) = S_e$).

3.3.3 Problem 1PFR

In this problem, the configuration consist of one CSTR followed by a PFR. The objective was to find the optimal volume V_1 of the CSTR which minimizes the effluent substrate

concentration, S_e, of the PFR, given a total volume V_{tot}. To prevent wash-out, V_1 has to be greater than V_1^{min}.

$$\underset{(V_1)}{\text{minimize}} \left\{ S_e(V_1) \right\}, \tag{41}$$

subject to

$$V_1^{min} < V_1 \leq V_{tot} \tag{42}$$

3.3.4 Problem 2PFR

In problem 2PFR, a configuration of one CSTR followed by a PFR was used. The objective was to find the optimal volumes V_1 of the CSTR and V_{PFR} of the PFR which minimizes the total volume V_{tot}, given an effluent substrate concentration $S_e < S_{in}$, i.e.

$$\underset{(V_1)}{\text{minimize}} \left\{ V_{tot} = V_1 + V_{PFR} = V_1 + Ah \right\}, \tag{43}$$

subject to

$$V_1 > V_{min}, \text{and } S(h) = S_e \tag{44}$$

Note that the constrains are both linear ($V_1 > V_{min}$) and nonlinear ($S(h) = S_e$).

3.4 NUMERICAL ANALYSIS

The solutions to the optimization problems were illustrated with four examples. The examples were selected in accordance with Zambrano et al. (2015) to be able to compare the results. All simulations were carried out in the platform Matlab R2016a. For full codes, see Appendix A.

3.4.1 Matlab commands used

The problems that were to be solved were all minimizing problems and to solve them the Matlab function *fmincon* was used. The function allows the user to set certain constraints, assign initial values, and a function to be minimized (MathWorks, n.d. b).

The system of Equations (16) - (18), describing the dynamics in a CSTR, is a nonlinear system that could be solved by using the Matlab command *fsolve* (MathWorks, n.d. c). The equation system describing the dynamics in the PFR (Eq. 32 - 34), contains three partial differential equations that were evaluated at steady-state, which means that they are time-independent. Thus, they can be seen as ordinary differential equations (ODEs). There are several numerical methods to solve ODEs. For this analysis *ode45* was used. *ode45* is a common and versatile ODE solver. Two drawbacks is that it does not work well for stiff problems or problems where high accuracy is demanded (MathWorks, n.d. a). None of the problems in this study were stiff and the accuracy in *ode45* was sufficient.

3.4.2 Parameter and variable values

The parameter and variable values (Table 1) were kept constant through all simulations, except from f_p and b. The two variables were changed in order to analyze the influence of the decay rate and the amount of the dead biomass that becomes inert.

Table 1. Parameter values used during the simulations.

Parameter	Value
V_{tot}	1.10
A	0.428
Q	1.00
μ_{max}	2.00
Y	0.800
K_S	1.20
S_{in}	10.0
X_{in}	0.00
Z_{in}	0.00
b	0.00-0.87
f_p	0.00-1.00

The parameter values were chosen in accordance with Zambrano et al. (2015). The half saturation constant, K_S, and the maximum specific growth rate, μ_{max}, both affect the specific growth rate (Eq. 5). Higher values of K_S lowers the specific growth rate, while high value of the maximum specific growth rate will have the opposite effect. The yield coefficient, Y, is the ratio between the mass of cells formed and the mass of the consumed substrate. A higher value indicates that more biomass is formed for each unit of substrate consumed.

The parameters b and f_p affect the minimum substrate level that can be obtained in the reactors (S_{min}, Eq. 36). Low values of f_p in combination with high values of b will give a higher S_{min}. Since b also affects the wash-out volume (Eq. 24), this must be considered when evaluating the results. The higher b, the larger volume of the first reactor is required to prevent wash-out. Values of b have been reported in the range 0.09-4.38 d^{-1} (Alex et al., 2008; Henze et al., 1987). With the parameter values as above, the maximum value of the decay rate, b_{max}, is 0.87 d^{-1}, calculated by imposing $V_1^{min} = V_{tot}$ and solving Equation (24) for b.

3.4.3 Evaluating the response for a given V_1

To illustrate how the substrate and biomass concentrations vary along a distance *h* (as defined in Fig. 4) problem 1N and 1PFR were solved for *N* = 3, 5, 10. The volume of the first CSTR, V_1, had to be larger than the wash-out volume, V_1^{min}, calculated using

Equation 24. Note that V_1^{min} varies depending on *b*. In this example b = 0 or $b = 0.1$. The wash-out volume when b = 0.1 is 0.593 and when b = 0 it is 0.560. To prevent wash-out for both values of b, the larger wash-out volume must be used. Therefore V_1 was selected as $1.2V_1^{min}(b = 0.1)$ = 0.712.

For the case with *N* CSTRs in series, the remaining volume was divided into $N-1$ equally sized volumes ($V_2 = ... = V_N = (V_{tot} - V_1)/(N-1)$). The corresponding substrate and biomass levels in the first CSTR (S_1, X_1 and Z_1) were calculated using Equations (25) - (27). These values are the influent to the following CSTR or PFR. The equation systems (16) - (18) and (32) - (34) were solved using *fsolve* and *ode45* respectively.

3.4.4 Optimal design for V_1

The objective of problem 1N and 1PFR was to minimize the effluent substrate level, while optimizing the volume, under the constraints that the total volume V_{tot} = 1.1. In order to compare the solutions of problems 1N and 1PFR, S_e was calculated for different values of V_1, from V_1^{min} to V_{tot}, for both configurations. For problem 1N, V_2, ..., V_N were optimized using *fmincon*. Problem 1PFR has a configuration of one CSTR followed by a PFR. The volume of the PFR was set to $V_{PFR} = V_{tot} - V_1$. This was done for different values of b and f_p.

To create comprehensive results, another simulation where all volumes were optimized to minimize S_e was run. The optimal volume of the first CSTR, V_1^{opt}, and the corresponding effluent substrate level, $S_e(V_1^{opt})$, was found for b = [0.00 0.87] and f_p = [0.00 1.00]. The maximum value of the decay rate, b_{max}, is 0.87 to make sure that the wash-out volume does not exceed the total volume.

3.4.5 Optimal and suboptimal design for *N* CSTRs

A numerical analysis of the optimal and suboptimal design for N CSTRs was carried out. Two different optimization procedures were used:

(a) $V_1 = V_1^*$, $V_2 = ... = V_N = (V_{tot} - V_1^*)/(N-1)$

(b) V_1 to V_N were optimized

where V_1^* is the optimal volume of the CSTR found by solving problem 1PFR (i.e. V_1^{opt} for the CSTR+PFR). This was done for different values of b and f_p in order to see the effect of the decay rate. *fmincon* was used to find the optimal volumes in (b), with the objective function and constraints as in Equation (37) and (38).

3.4.6 Optimal design for a given effluent substrate concentration

Problems 2N and 2PFR were evaluated by comparing the results from the two configurations. The total volume required for a CSTR followed by a PFR, V_{opt}, was calculated by solving problem 2PFR. The total volume required for N CSTRs in series, $V(N)$, was calculated by solving problem 2N, with N = 2, 3, 4, 5. This was done for different requirements on the effluent substrate level.

The problems were both solved using different values of b and f_p (b = 0.00, 0.10, 0.25, 0.40 and f_p = 0.00, 0.10, 0.40, 0.80). The requirements on S_e was expressed as a fraction of S_{in}, with values ranging from 1% to 100% of S_{in}.

In this problem, it was important to also evaluate the minimum substrate level, S_{min}, that can be obtained in the reactors. S_{min} was calculated for all values of b and f_p using Equation (36), shown in Table 2.

Table 2. S_{min} for different b and f_p. S_{min} exceeds 1% of S_{in} when (1) b = 0.25, f_p = 0.10, (2) b = 0.40, f_p = 0.10, and (3) b = 0.40, f_p = 0.40

b \ f_p	0.10	0.40	0.80
0.10	0.0448	0.0295	0.00968
0.25	**0.119**	0.0766	0.0245
0.40	**0.202**	**0.127**	0.0397

Note that with S_{in} = 10, 1% of S_{in} is 0.10 and that $S_{min} > 0.10$ when (1) b = 0.25, f_p = 0.10, (2) b = 0.40, f_p = 0.10, and (3) b = 0.40, f_p = 0.40. When $b = 0.4$ and $f_p = 0.1$, $S_{min} > 2\%$ of S_{in} as well. Since S_{min} is the lowest value S_e can be assigned, the requirements on S_e ranged from $1.1S_{min}$-S_{in} for these three cases.

4 RESULTS

4.1 RESPONSE FOR A GIVEN V_1

The analysis of the response for a given V_1 showed the behavior throughout the reactors. The configuration of *N* CSTRs converges towards the configuration of one CSTR followed by a PFR as *N* increase (Fig. 5). The choice of *N* has the biggest impact on the substrate level towards the end of the bioreactors ($h \rightarrow h_{max}$).

When introducing a decay rate, $b > 0$, the biomass concentration decreases and the substrate level increases. The choice of f_p also affects the results. It changes the ratio between the active and inert biomass. The impact on the substrate level is not as obvious, but it is slightly lower when f_p is higher (Fig. 5).

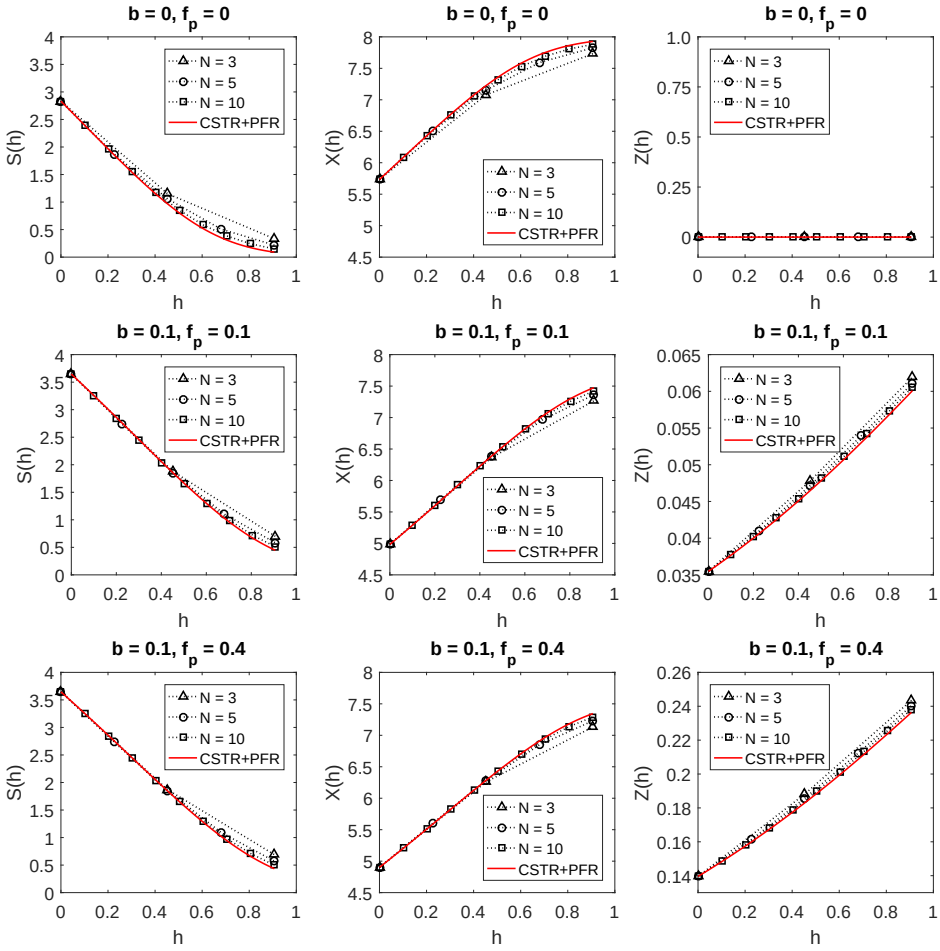

Figure 5. Response for $V_1 = 0.712$. Substrate, S, biomass, X, and inert biomass, Z, in each CSTR (for N = 2, 5, 10, dashed black lines) or as functions of the position, h, in the PFR (for the CSTR+PFR, red line) for different decay rates, b, and fractions between inert biomass and substrate, f_p.

4.2 OPTIMAL DESIGN FOR V_1

The effluent substrate level, S_e, for N CSTRs in series converges towards the one for the CSTR+PFR (red line) as N increase. The optimal volume for V_1 (black dots for N CSTRs and asterisk for CSTR+PFR) is smaller when the number of CSTRs increase (Fig. 6).

The decay rate has quite a large influence on the results, as can be seen in Figure 6. The higher the decay rate, the higher S_e and V_1^{opt}. One can also see that the difference between the configurations is less prominent as b increases. Note also that V_1^{opt} moves closer to the total volume. The influence from f_p is not as obvious as the one from b. It has only a small influence on S_e and V_1^{opt}, barely noticeable in Figure 6.

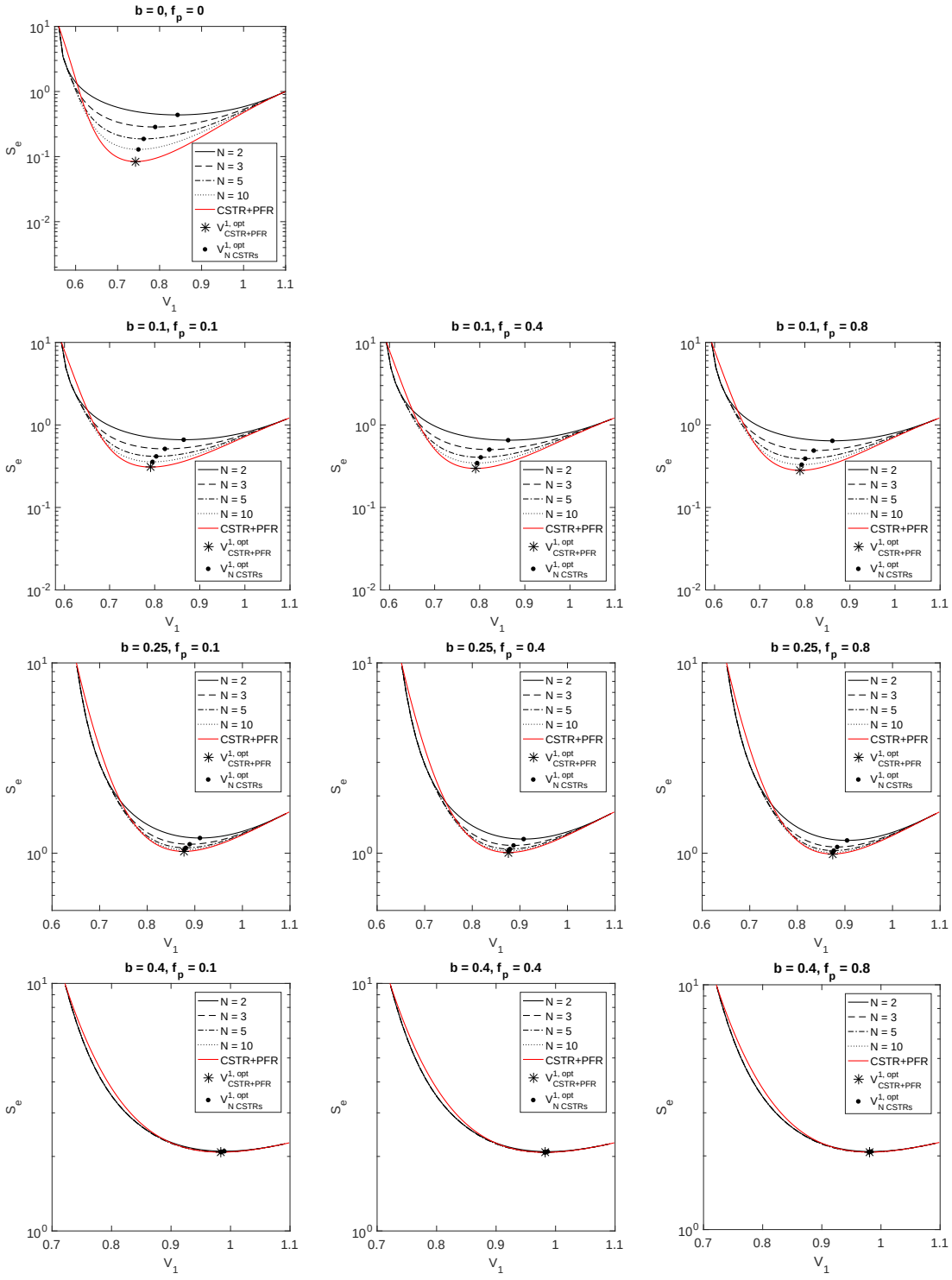

Figure 6. The optimal volume of the first CSTR, V_1^{opt}, for different configurations and different decay rates, b, and fractions between inert biomass and substrate, f_p. Note that each row have different axes.

The behavior pointed out in Figure 6 is even more visible in Figure 7. There is a certain value of b where only the first CSTR is needed ($V_1^{opt} = V_{tot}$). This value varies between 0.53-0.54 (Table 3). Although only illustrated for the CSTR+PFR in this report, the same

behavior was seen for N CSTRs in series as well. The influence from f_p is small but can be seen in Figure 7 where both V_1^{opt} and $S_e(V_1^{opt})$ increase when f_p increases.

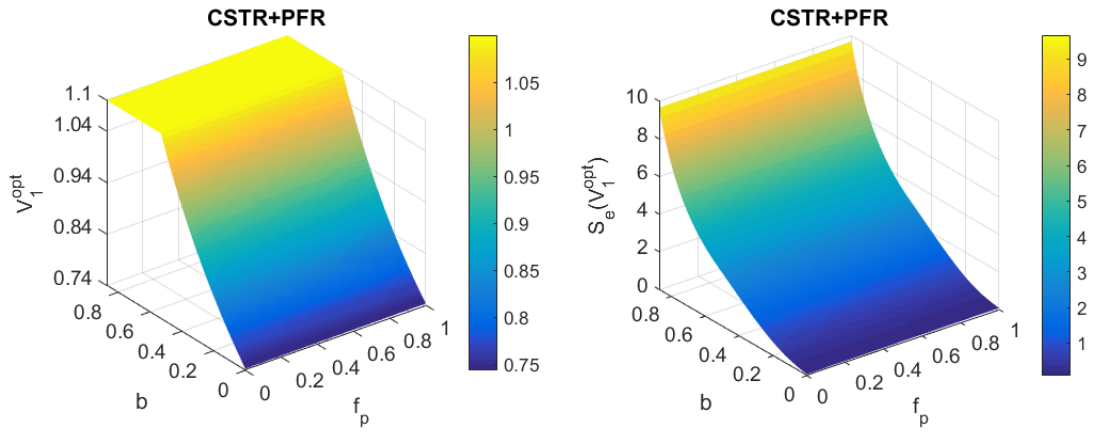

Figure 7. The optimal volume of the first CSTR, V_1^{opt}, and the effluent substrate level, $S_e(V_1^{opt})$, for different decay rates, b, and fractions between inert biomass and substrate, f_p, for a CSTR+PFR.

Table 3. Break values of b

Configuration	b_{break}
2 CSTRs	0.54
5 CSTRs	0.54
CSTR+PFR	0.53

The ratio between the optimal volume of the first reactor for CSTRs in series and the CSTR+PFR, and the ratio between the corresponding effluent substrate levels were calculated. Both ratios are greater than one for almost all values of b and f_p for both 2 and 5 CSTRs in series (Fig. 8 and 9). The CSTR+PFR is more effective than the CSTRs in series when b is low since it requires a smaller first volume and still generates a lower substrate level in the effluent. For $b > b_{break}$, there is no difference between the configurations (ratio = 1).

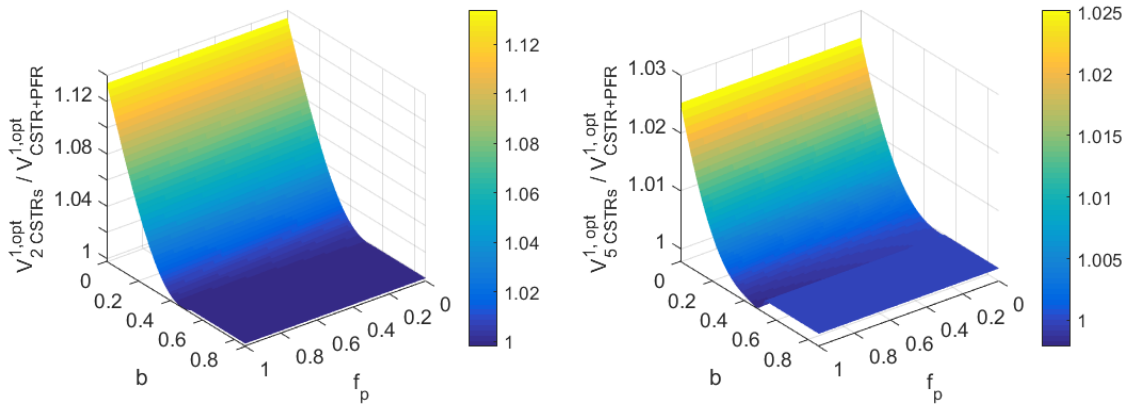

Figure 8. The ratio between the optimal volume of the first reactor, V_1^{opt}, for N = 2 (left) or 5 (right) CSTRs in series and CSTR+PFR for different decay rates, b, and fractions between inert biomass and substrate, f_p.

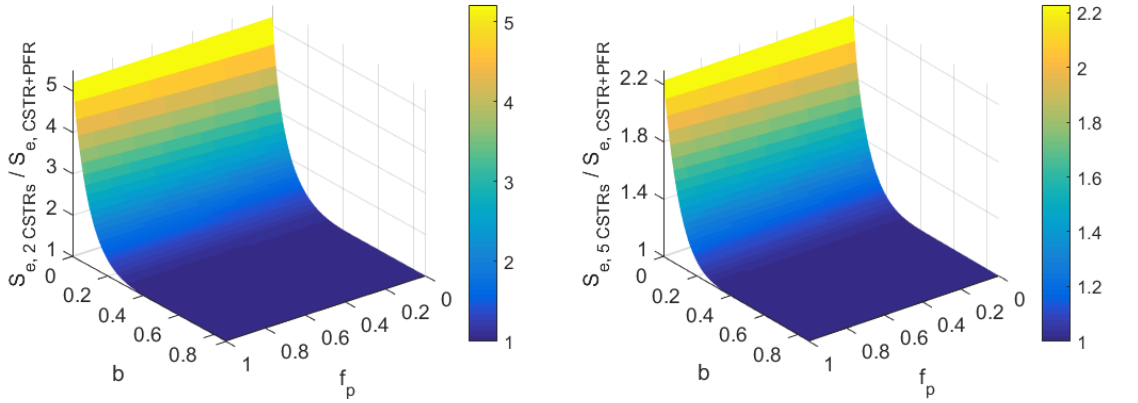

Figure 9. The ratio between the effluent substrate level, S_e, for N = 2 (left) or 5 (right) CSTRs in series and CSTR+PFR for different decay rates, b, and fractions between inert biomass and substrate, f_p.

4.3 OPTIMAL AND SUBOPTIMAL DESIGN FOR *N* CSTRs

The different configurations used for this example give similar results. When N is increasing, configuration (a) converges to (b) (Fig. 10). As b increases, both the difference between the configurations (Fig. 11) and the variation along N decrease. The latter can be seen in Figure 10, where the relative difference from low to high N is decreasing when b increases. The same behavior has previously been shown in Figure 6.

From Figure 10 one can also see that the overall substrate level is increasing when b increases. Increasing f_p, on the other hand, cause a decrease of S_e (Fig. 10). An interesting effect that f_p has on the substrate levels is that for low N, the difference between configuration (a) and (b) decreases with increasing f_p. However, for high N, the difference between the configurations increases with increasing f_p (Fig. 12).

This analysis have shown that the number of CSTRs will have less influence on the results when the decay rate increases, and that only optimizing the first volume of the bioreactors is a good approximation of the optimal design, especially when b is high.

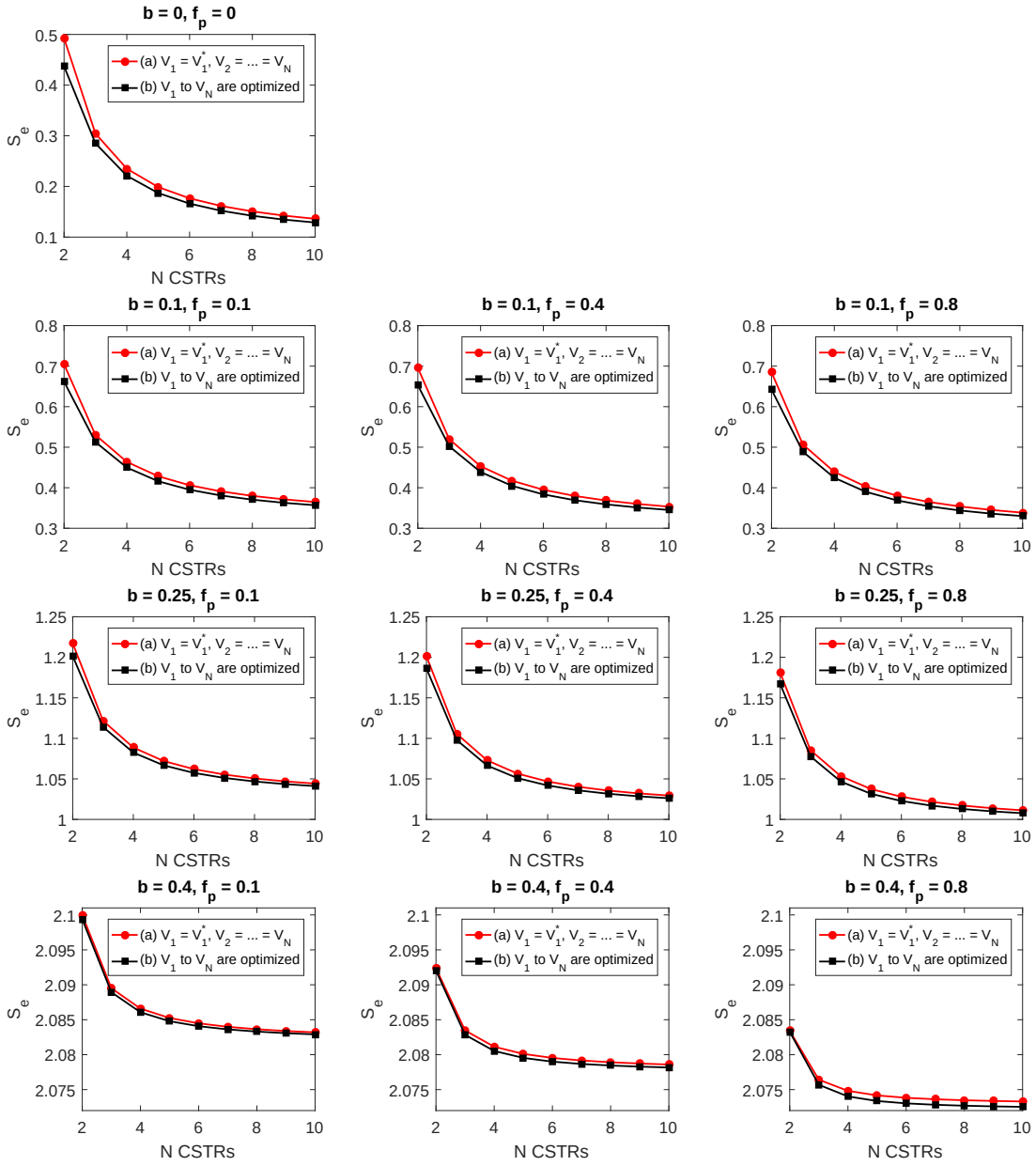

Figure 10. Optimal and suboptimal design for N CSTRs in series for two different configurations: (a) $V_1 = V_1^*$, $V_2 = ... = V_N$, and (b) V_1 to V_N are optimized, evaluated for different decay rates, b, and fractions between inert biomass and substrate, f_p

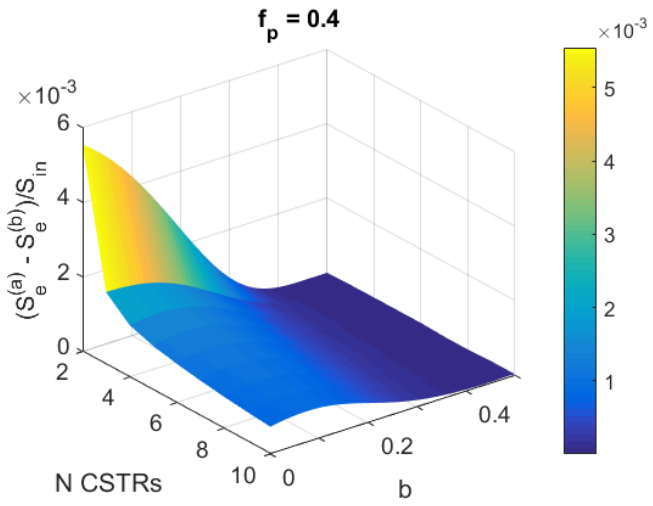

Figure 11. Difference between the substrate level from configuration (a) and (b), divided by S_{in}. b is varying between 0.0-0.50 with a stepsize of 0.01 while f_p was kept constant at 0.40.

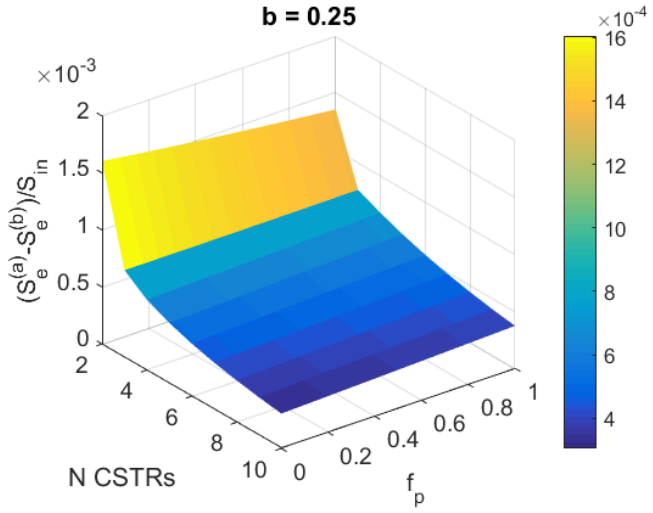

Figure 12. Difference between the substrate level from configuration (a) and (b), divided by S_{in}. f_p is varying between 0.0-1.0 with a stepsize of 0.01 while b was kept constant at 0.25.

4.4 OPTIMAL DESIGN FOR A GIVEN EFFLUENT SUBSTRATE CONCENTRATION

The optimal volumes achieved when solving problems 2PFR and 2N are denoted V_{opt} and $V(N)$ respectively. Results are presented as the ratio between the solutions. The ratio $V_{opt}/V(N)$ increases as N increases, which means that the solution to problem 2N converges to the solution to problem 2PFR when N increases (Fig. 13). When the requirements are strict (i.e. the ratio between S_e and S_{in} is low) there is quite a big differ-

ence between the solutions. With less strict requirements, the difference is less prominent.

The variables b and f_p affect the results in opposite ways. As b increases, $V_{opt}/V(N)$ decreases, especially for low values on N, while increasing f_p leads to an increase of $V_{opt}/V(N)$. Furthermore, the variation between N = 2 and N = 5 increases as b increases (lines further apart), while it decreases as f_p is increased. Once again, the effect from this, is less prominent with less strict requirements on the substrate effluent level.

Note that in Figure 13, S_{min} is marked with a red, dashed line when $S_e < S_{min}$. The values of S_{min} can be found in Table 3.

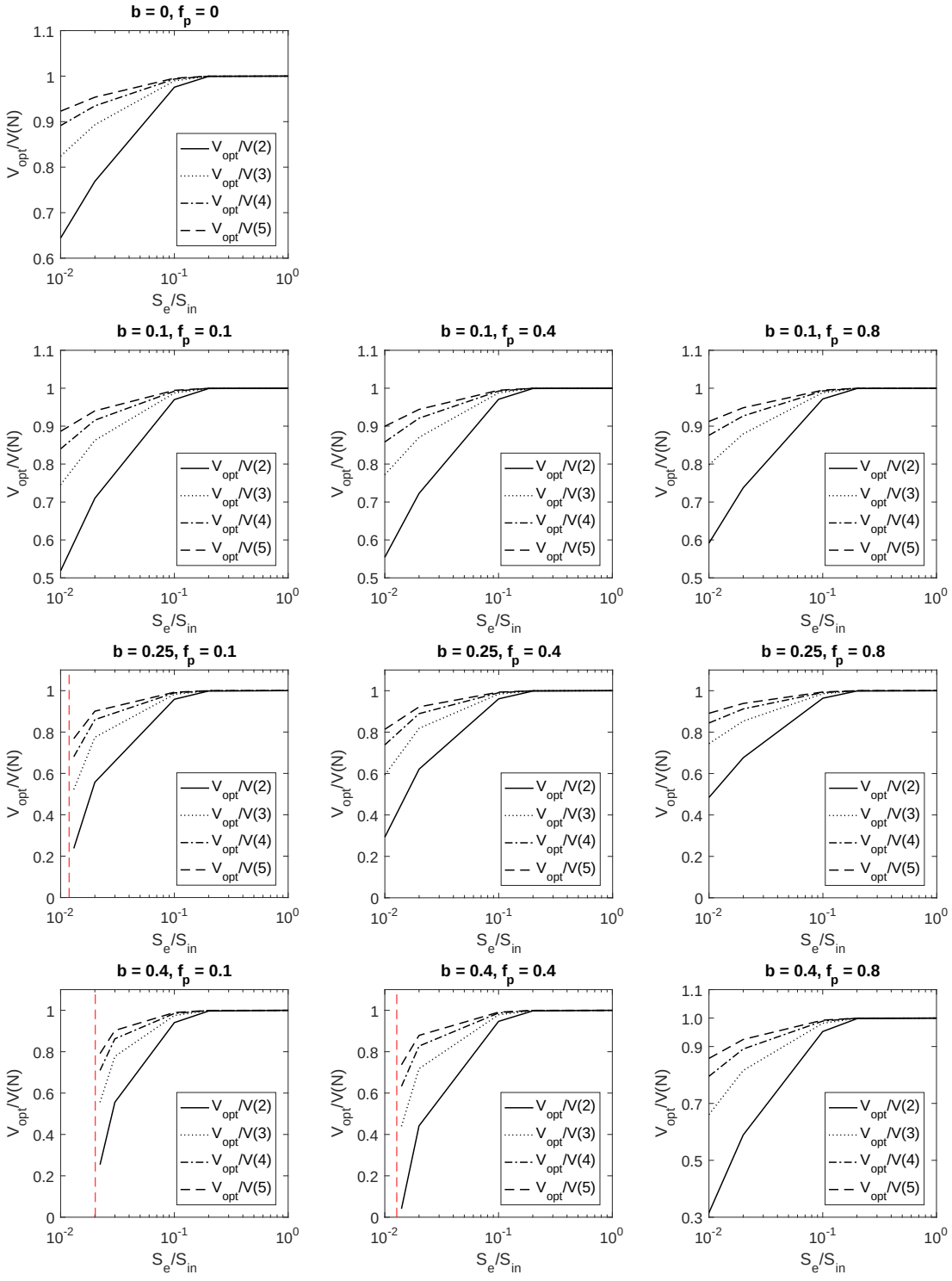

Figure 13. Minimum total volume required for a given substrate effluent level, S_e. The red, dashed lines shows the minimum possible substrate level S_{min} for the cases when the required $S_e < S_{min}$.

5 DISCUSSION

In this study, a system of differential equations was used to describe the dynamics of the substrate, biomass and inert biomass concentrations in bioreactors. The objective of the study was to extend the work by Zambrano et al. (2015) by adding the biomass decay rate and one more ODE to describe the inert biomass. A numerical analysis of several CSTRs in series and of one CSTR connected to a PFR has been carried out. It was done by solving two optimization problems: (1) minimize the effluent substrate concentration by optimize the volumes, given a total volume, and (2) minimize the total volume needed to obtain a certain substrate conversion. The optimization problems were solved and illustrated with four examples. The configuration of N CSTRs in series was compared to a CSTR followed by a PFR.

The study showed that the solution of N CSTRs in series converges to the solution of the CSTR+PFR in the first optimization problem. With no decay of biomass, the results provided here, are the same as was found by Zambrano et al. (2015). The decay rate affects the results by lessen the difference between the configurations, i.e. the solutions are almost the same when the decay rate is high. Not surprisingly, the decay rate will cause a higher effluent substrate level and a larger optimal minimum volume. Introducing a decay rate causes biomass to die, and either becomes substrate or inert biomass. With a higher amount becoming substrate (i.e. lower f_p), the effluent substrate level increases. From all minimizing problems, it was found that b has a bigger impact on the results than f_p has.

5.1 RESPONSE FOR A GIVEN V_1

The steady-state solution for N CSTRs converges towards the solution for the CSTR+PFR for a certain V_1. It was also found that the substrate level decreases along the reactors while the biomass concentrations increased (Fig. 5). This analysis was mostly done to illustrate the process in the reactors and to determine if the system was operating in the desired way.

5.2 OPTIMAL DESIGN FOR V_1

Evaluating the response for different V_1 showed that the optimal volume and the effluent substrate level decreases as N decreases, once again showing that the solution for N CSTRs converges to the one for the CSTR+PFR. The configuration used had less importance when b increased (Fig. 6). For a certain value on b, there was no longer any need for more than one CSTR, no matter what configuration was used (Table 3). This can be related to the wash-out volume, which is depending on the decay rate (Eq. 24). With a high decay rate, a large first volume is needed to prevent wash-out, thus the remaining volume is quite small and will not contribute to the conversion of substrate in any large extent. For the case with the CSTR+PFR, this means that only a CSTR is needed when b is large enough and the benefits of the PFR will therefore be slightly lost. If V_1 is large for the case of N CSTRs in series, the remaining volume will be very small. Slicing it up in $N-1$ small volumes (as in Fig. 4) will cause it to mimic a PFR, especially if N is large as

well. For example, when $b = 0$, more than 10 CSTRs in series will be needed to mimic the CSTR+PFR, but for $b = 0.54$ there is practically no difference between the configurations even for $N = 2$. In conclusion, when b is large, a large first volume is needed and the difference between N CSTRs and the CSTR+PFR is very small (Fig. 6 and 7).

Ideally, the optimal volume of the first reactor should be smaller for the configuration CSTR+PFR than for N CSTRs in series, no matter what b and f_p was used. Or in other words, the ratio between the optimal volumes for the N CSTRs and the CSTR+PFR should have been greater than or equal to one for all values of b and f_p. However, probably due to limitations in the numerical representation in Matlab, one can see that this is not the case (Fig. 8). Even though the ratio is less than one for some values of b and f_p, it is very close to one, meaning that they are practically the same. As has been proven before (e.g. Bischoff (1966)), the configuration of one CSTR followed by a PFR is in a sense the boundary of N CSTRs in series, and it should thereby not be possible to have a ratio less than one.

5.3 OPTIMAL AND SUBOPTIMAL DESIGN FOR N CSTRs

The optimal and suboptimal design of N CSTRs in series was studied. It was found was that optimizing all volumes of the CSTRs will give a lower effluent substrate level, especially when N is small. As N was increased, the difference between only optimizing the first volume and optimizing all volumes decreased. These results indicate that a good approximation to the optimal design is to only optimize the first volume and keep the remaining volumes equally sized. The results are in agreement with Hill and Robinson (1989) and de Gooijer et al. (1996). The parameters b and f_p have opposite effect on the results. While the effluent substrate level increases as b increases, it decreases when f_p increases (Fig. 10). The impact from b is bigger when N is small, which subsequently indicates that the difference between optimizing all volumes and only optimizing the first volume is less when b increases (Fig. 11). Thus, for a high decay rate, it is an even more accurate approximation for the optimal design of CSTRs in series to only optimize the first volume and divide the remaining volume equally between the rest of the reactors. This can be related to what was found when finding the optimal volume V_1^{opt} for N CSTRs and the CSTR+PFR. V_1^{opt} for N CSTRs are equal to V_1^{opt} for the CSTR+PFR when b is large. This means that the first volume of the two configurations used in Example 2.4.5 will be approximately the same when b is large. Optimally designing the remaining volumes or just distributing them equally will then have less impact on the effluent substrate level. The overall substrate level in the reactors decreases when increasing f_p, which was expected. Increasing f_p causes a bigger difference between the optimization procedures if N is large, while if N is small, the difference decreases (Fig. 12). This can be explained by once again considering the size of V_1. When V_1 for configuration (b) goes towards V_1^* (i.e. when b is high), the volume distribution for configuration (a) converges towards (b) when $N = 2$. With V_1 for configuration (b) close to V_1^*, the optimization of the remaining volumes will have bigger effect on the effluent substrate level when N is large. The effect is visible when using more than two CSTRs, but is more prominent when N increases ($N \rightarrow 10$ in this study; Fig. 10).

5.4 OPTIMAL DESIGN FOR A GIVEN EFFLUENT SUBSTRATE CONCENTRATION

The second optimization problem was evaluated by comparing the solutions to problem 2N with the one from problem 2PFR. The total volume needed for different requirements on the effluent substrate level was found for the two configurations. Results show that the ratio between the configurations is closer to one for large N, meaning that the total volumes of both configuration is similar. Increasing b leads to a lower ratio and increasing f_p leads to a higher ratio (Fig. 13). These results might seem somewhat surprising since the previous examples have shown that increasing b causes the two configurations to converge towards each other (see e.g. Fig. 6). The analysis did show that a larger total volume is needed when b increases, but the volume for the CSTRs in series increases more than for the CSTR+PFR. For previous examples, there has been an upper limit of the total volume, which causes both configurations to reach the boundary when b increases. In this example, we have both stricter (lower) S_e and no upper boundary on the total volume, which makes it possible to reach even higher volumes. In conclusion, for this analysis, a CSTR+PFR will always be superior to N CSTRs in series for strict effluent requirements. For the less stringent requirements, the configurations do indeed become more alike. Furthermore, when the effluent substrate level is 100% of S_{in}, there is no substrate reduction in the reactors, and the total volume is the same for both configurations.

5.5 ASSUMPTIONS AND PARAMETER VALUES

Results from this study show that given a total reactor volume, the effluent substrate level can be reduced by dividing the volume in several smaller volumes. It was also shown that a fairly accurate approximation to the optimal design of bioreactors in series is to optimally design the first volume and equally distribute the following volumes. However, this study was conducted under three assumptions: (1) one main biomass consumes one main substrate, (2) the biological parameters do not change with the liquid temperature, and (3) the oxygen demand was fulfilled. Including heterogeneous biomass populations and substrates or a model to account for the oxygen and temperature dependence could be a way to make the model more realistic.

With the parameter setup used in this study, using CSTRs in series is not always superior to a single CSTR, which have been shown by de Gooijer et al. (1996) as well. Since the parameter values will affect the optimal design and the substrate conversion, accurate and plant specific parameters should be used if this method were to be implemented in practice. As was shown, the decay rate will strongly affect the results and there is a critical limit for b above which only one CSTR was needed no matter which configuration was used (Table 3). It would have been interesting to see how the other parameters, especially the growth rate, affect b_{break}. The relation between the growth rate and the decay rate is what determines the biomass, and thus the substrate levels, in the reactors and it will probably affect b_{break}. The total volume will affect b_{break} in such a way that a larger volume gives a higher b_{break}. Changing the parameter values will also affect the results from the second optimization problem (2N and 2PFR). The minimum substrate level that can be obtained in the reactors are related to the parameters in the Monod function, where an

increase of the half saturation constant would give a higher S_{min} and an increase of the maximum specific growth rate would decrease S_{min}. Given that the general shape of the curves in Figure 13 is not strongly affected by b and f_p, and that S_{min} was lower than the required S_e for most of the cases, altering K_S and μ_{max} will probably only change the results for the three cases where the required $S_e < S_{min}$. The actual values retrieved from the study will not be the same for another parameter setup, but one would probably see the same behavior as for this study.

6 CONCLUSIONS

The study has shown that the differential equation systems presented can be used to optimally distribute the bioreactor volumes for a configuration of N CSTRs in series and of a CSTR+PFR so to (1) minimize the effluent substrate level given a total volume, or (2) minimize the total volume needed to fulfill a certain requirement on the effluent substrate level. The model can be used to optimally divide a total volume into smaller ones and thereby increasing the substrate conversion, something that could be of interest in e.g. existing wastewater treatment plants with restricted space. To get a fairly accurate approximation of the optimal design, it is possible to use the approach for the CSTR+PFR to find the optimal design of the first volume, and then distribute the remaining volume equally. This approach would be more computational efficient and less time consuming than optimizing all volumes, but still provide fairly accurate results.

One of the most interesting result from the study is that there are situations where only a single CSTR is needed. An extension of this study could be to evaluate how b_{break} is affected by changing parameter values, insight that could help when deciding on if, and when, multiple CSTRs are needed. Other possible extensions would be to further develop the model to include temperature dependence, other kinds of biomass and substrates, a model for the oxygen demand, or implement it for the ASP.

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APPENDIX A - MATLAB FUNCTIONS AND SCRIPTS

FUNCTIONS

@diff_eqn - Differential equations for the CSTR

```
function F = diff_eqn(x)

global mumax Ks Y fp b Q v Sin Xin Zin

    % x = [S X Z];

    F(1) = -(((mumax*x(1)/(Ks+x(1)))/Y)-((1-fp)*b))*x(2)+(Q/v)*(Sin-x
        (1));
    F(2) = (((mumax*x(1)/(Ks+x(1)))-b)*x(2))+((Q/v)*(Xin-x(2)));
    F(3) = (fp*b*x(2))+((Q/v)*(Zin-x(3)));

end
```

@PFR - ODEs for the PFR

```
function [G] = PFR(~,x)

global mumax Ks Y fp b Q Ar

    % x = [S X Z];
    % G = [dS/dh dX/dh dZ/dh];

    G(1,1) = -(((mumax*x(1)/(Ks+x(1)))/Y)-((1-fp)*b))*Ar*x(2)/Q;
    G(2,1) = (Ar*((mumax*x(1)/(Ks+x(1)))-b)*x(2))/Q;
    G(3,1) = (fp*b*Ar*x(2))/Q;

end
```

@CSTR_V_given - Used for problem 1N

```
function [Se] = CSTR_V_given(VV)

global Q mumax Y b fp Ks Sin Xin Zin v

  Sin = 10;
  Xin = 0;
  Zin = 0;
  n = length(VV);

  % Calculating S in the first CSTR
      S1 = (((Q/VV(1))+b)*Ks)/(mumax-(Q/VV(1))-b);
      X1 = Q*(Sin1-S1)*Y/(Q+(VV(1)*b*(1-((1-fp)*Y))));
      Z1 = ((VV(1)/Q)*fp*b*X1)+Zin;
      Sin = S1;
      Xin = X1;
      Zin = Z1;

```

```
  % Calculating S for the rest of the CSTRs
  for j=2:n

      v = VV(j);
      x = [Sin, Xin, Zin];

      Z = fsolve(@diff_eqn, x);

      Sin = Z(1);
      Xin = Z(2);
      Zin = Z(3);

      Sstat(j) = Sin;
      Xstat(j) = Xin;
      Zstat(j) = Zin;

  end

  Se = (Sstat(n)); % Output = substrate level in the effluent

end
```

@PFR_V_given - Used for problem 1PFR

```
function [Se] = PFR_V_given(VV)

global Q mumax Y b fp Ks Sin Xin Zin Ar

  Sin = 10;
  Xin = 0;
  Zin = 0;

  % Calculating S in the CSTR
  S1 = (((Q/VV(1))+b)*Ks)/(mumax-(Q/VV(1))-b);
  X1 = Q*(Sin-S1)*Y/(Q+(VV(1)*b*(1-((1-fp)*Y))));
  Z1 = ((VV(1)/Q)*fp*b*X1)+Zin;

  % Levels in effluent from CSTR = level in influent to PFR
  Sin = S1;
  Xin = X1;
  Zin = Z1;

  % Calculating Se from PFR
  h0 = 0;
  h = VV(2)/Ar;
  hspan = [h0 h];
  x0 = [Sin, Xin, Zin];
  [h,X] = ode45(@PFR,hspan,x0);

  % Output = substrate level in the effluent of the PFR
  Se = X(end,1);
  h = h;

end
```

@volume - Used for problems 2N and 2PFR

```
function [V] = volume(VV)

    V = sum(VV);

end
```

@nlcon1 - Non-linear constraints for problem 2PFR

```
function [C,Ceq] = nlcon1(VV)

global Q mumax Y b fp Ks Sin Xin Zin Se Ar

  Sin = 10;    % Substrate level in the influent
  Xin = 0;     % Biomass level in the influent
  Zin = 0;     % Inert biomass level in the influent

  % Calculating Se from CSTR
  S1 = (((Q/VV(1))+b)*Ks)/(mumax-(Q/VV(1))-b);
  X1 = Q*(Sin-S1)*Y/(Q+(VV(1)*b*(1-((1-fp)*Y))));
  Z1 = ((VV(1)/Q)*fp*b*X1)+Zin;

  Sin = S1;
  Xin = X1;
  Zin = Z1;

  % Calculating S(h) for PFR
  h0 = 0;
  h = VV(2)/Ar;
  hspan = [h0 h];
  x0 = [Sin, Xa_in, Xi_in];
  [~,X] = ode45(@PFR,hspan,x0);
  Se_PFR = X(end,1); % Output = substrate level in the effluent of the
      PFR
  C = [];
  Ceq = Se_PFR - Se;

end
```

@nlcon2 - Non-linear constraints for problem 2N

```
function [C,Ceq] = nlcon2(VV)

global Q mumax Y b fp Ks Sin Xin Zin v Se

  Sin = 10;    % Substrate level in the influent
  Xin = 0;     % Biomass level in the influent
  Zin = 0;     % Inert biomass level in the influent

  n = length(VV);

  % Calculating S in the first CSTR
  S1 = (((Q/VV(1))+b)*Ks)/(mumax-(Q/VV(1))-b);
  X1 = Q*(Sin-S1)*Y/(Q+(VV(1)*b*(1-((1-fp)*Y))));
  Z1 = ((VV(1)/Q)*fp*b*X1)+Zin;
  Sin = S1;
  Xin = X1;
  Zin = Z1;

  % Calculating S for the rest of the CSTRs
  for j = 2:n

      v = VV(j);
      x = [Sin, Xa_in, Xi_in];
      Z = fsolve(@diff_eqn, x);

      Sin = Z(1);
      Xin = Z(2);
      Zin = Z(3);
      Sstat(j) = Sin;
      Xstat(j) = Xin;
      Zstat(j) = Zin;

  end

  C = [];
  Ceq = Sstat(end) - Se;

end
```

SCRIPT TO GENERATE DATA FOR FIGURE 4

```
% Response for a given V1
% The program calculates the substrate level in each CSTR/along h. V1
    is fixed at 1.2*V1min for b = 0.1.

global Q b mumax Sin Ks N Vtot Vr Y fp Xin Zin v Ar

Ar = 0.428; % Area
Vtot = 1.1; % Total volume
Q = 1;      % Inflow = outflow
mumax = 2;  % Maximum specific growth rate
Y = 0.8;    % Yield factor
b = 0.1;    % Decay rate
fp = 0.4;   % Amount that becomes inert
Ks = 1.2;   % Half saturation constant
Sin = 10;   % Substrate level in the influent
Xin = 0;    % Biomass level in the influent
Zin = 0;    % Inert biomass level in the influent
Sstat = zeros(3,10);
Xstat = zeros(3,10);
Zstat = zeros(3,10);

% Finding V1
V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);   % Wash-out volume
V1 = 1.2*V1min; % If b = 0, use V1 = 0.711864407;

% Calculating the substrate level for N CSTR
i = 1;
for N = [3, 5, 10];

    j = 1;

    Vr = (Vtot-V1)/(N-1);
    V = Vr.*ones(N,1);
    V(1) = V1;

    % Calculating S for the first CSTR and storing values as input to
       the next CSTR
    Sin = 10;
    Xin = 0;
    Zin = 0;
    S1 = (((Q/V(1))+b)*Ks)/(mumax-(Q/V(1))-b);
    X1 = Q*(Sin-S1)*Y/(Q+(V(1)*b*(1-((1-fp)*Y))));
    Z1 = ((V(1)/Q)*fp*b*X1)+Zin;
    Sin = S1;
    Xin = X1;
    Zin = Z1;
    Sstat(i,j) = Sin;
    Xstat(i,j) = Xin;
    Zstat(i,j) = Zin;

    % Calculating S for the rest of the CSTRs
    n = length(V);

```

```
52      for j=2:n
53
54          v = V(j);
55          x = [Sin, Xin, Zin];
56          Z = fsolve(@diff_eqn, x);
57
58          Sin = Z(1);
59          Xin = Z(2);
60          Zin = Z(3);
61          Sstat(i,j) = Sin;
62          Xstat(i,j) = Xin;
63          Zstat(i,j) = Zin;
64
65      end
66
67      h(1:N,i) = 0:Vr/Ar:(Vtot-V1)/Ar;
68      i = i+1;
69
70  end
71
72  % Calculating the substrate level in the CSTR+PFR
73  VCSTR = 1.2*V1min; % If b = 0, VCSTR = 0.711864407;
74  VPFR = Vtot-VCSTR;
75
76      % Calculating Se from the CSTR
77      Sin = 10;
78      Xin = 0;
79      Zin = 0;
80      S1 = (((Q/VCSTR)+b)*Ks)/(mumax-(Q/VCSTR)-b);
81      X1 = Q*(Sin-S1)*Y/(Q+(V(1)*b*(1-((1-fp)*Y))));
82      Z1 = ((VCSTR/Q)*fp*b*X1)+Zin;
83
84      % Se from CSTR = input to PFR
85      Sin = S1;
86      Xin = X1;
87      Zin = Z1;
88
89      % Solve ODEs to find S(h)
90      h0 = 0;
91      hPFR = VPFR/Ar;
92      hspan = linspace(h0,hPFR,100);
93      x0 = [Sin, Xin, Zin];
94      [hPFR_vec,X] = ode45(@PFR,hspan,x0);
```

SCRIPT TO GENERATE DATA FOR FIGURE 5

```
% Optimal design for V1
% The program calculates the effluent substrate level for V1 = V1min: Vtot. The volume V1 (volume of CSTR) is fixed at given value

% Objective function:       PFR_V_given
% Linear constraints:       V1 > V1min
%                           Vi > 0
%                           sum(V) = Vtot
% Lower and upper bound:    0 < V2,...,VN < Vtot

options = optimset('TolCon',1e-14,'TolFun',1e-14,'DiffMinChange', 0, 'Algorithm', 'interior-point');

global Q b mumax Sin Ks Y fp Xa_in Xi_in Ar Vtot

Ar = 0.428; % Cross-sectional area of the PFR
Vtot = 1.1; % Total volume
Q = 1;      % Inflow = outflow
mumax = 2;  % Maximum specific growth rate
Y = 0.8;    % Yield factor
b = 0.0;    % Decay rate
fp = 0.0;   % Amount that becomes inert
Ks = 1.2;   % Half saturation constant
Sin = 10;   % Substrate level in the influent
Xin = 0;    % Biomass level in the influent
Zin = 0;    % Inert biomass level in the influent


% Evaluating response from V1 for N CSTRs, where N = [2, 3, 5, 10]
V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);   % Wash-out volume

k = 1;

for N = [2,3,5,10]

i = 1;

    for V1 = V1min:0.01:Vtot

        % Setting conditions for fmincon
        % Sum(V1,...,VN) = Vtot
        % V1 = V1 (V1 fixed at chosen value, V2,...,VN optimized)
        Aeq = zeros(N);
            Aeq(1,:) = 1;
            Aeq(2,1) = 1;
        Beq = zeros(N,1);
            Beq(1) = Vtot;
            Beq(2) = V1;

        % 0 < V2,...,VN < Vtot; V1 > V1min
        lb = zeros(1,N); lb(1) = V1min;
        ub = Vtot*ones(1,N);

```

```
52          % Creating vector V with initial guesses
53          Vr = (Vtot-V1)/(N-1);
54          V = Vr.*ones(N,1);
55          V(1) = V1;
56
57          % Minimizing function CSTR_V_given with conditions as above
58          [volumes(i,1:N),fval(i)]=fmincon(@CSTR_V_given,V,[],[],Aeq,Beq,
                lb,ub,[],options);
59
60          i = i+1;
61      end
62
63      % Store the volumes and corresponding substrate levels
64      vol1plot(:,k) = (volumes(1:end,1));
65      Seplot(k,:) = fval;
66
67      k = k+1;
68
69  end
70
71  % Find V1opt for N CSTRs
72  Sin = 10;
73  Xin = 0;
74  Zin = 0;
75
76  V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);
77
78  i = 1;
79
80  for N = [2,3,5,10]
81
82      % Setting conditions for fmincon
83      % Sum(V1,...,VN) = Vtot
84      Aeq = zeros(N);
85          Aeq(1,:) = 1;
86      Beq = zeros(N,1);
87          Beq(1) = Vtot;
88
89      % 0 < V2,...,VN < Vtot; V1 > V1min
90      lb = zeros(1,N); lb(1) = V1min+(1e-03);
91      ub = Vtot*ones(1,N);
92
93      % Creating vector V with initial guesses
94      Vr = (Vtot-(V1min+(1e-03)))/(N-1);
95      V = Vr.*ones(N,1); V(1) = V1min+(1e-03);
96
97      % Minimizing function CSTR_V_given with conditions as above
98      [volumes(i,1:N),fval(i)]=fmincon(@CSTR_V_given,V,[],[],Aeq,Beq,lb,
            ub,[],options);
99
100     % Store the minimum effluent substrate level and the required V1
101     V1_opt_CSTRs(i) = volumes(i,1);
102     Se_opt_CSTRs(i) = fval(i);
103
104     i = i+1;
105
```

```
106 end
107
108 % Evaluating response from V1 for the case CSTR+PFR
109
110 l = 1;
111
112 for VCSTR = V1min:0.001:Vtot-0.001
113
114     VPFR = Vtot-VCSTR;
115
116     % Assigning initial values to parameters
117     Sin = 10;   % Substrate concentration in the influent
118     Xin = 0;  % Active biomass concentration in the influent
119     Zin = 0;  % Inert biomass concentration in the influent
120
121     % Calculating Se for CSTR
122     S1 = (((Q/VCSTR)+b)*Ks)/(mumax-(Q/VCSTR)-b);
123     X1 = Q*(Sin-S1)*Y/(Q+(VCSTR*b*(1-((1-fp)*Y))));
124     Z1 = ((VCSTR/Q)*fp*b*X1)+Zin;
125
126     % Levels in effluent from CSTR = level in influent to PFR
127     Sin = S1;
128     Xin = X1;
129     Zin = Z1;
130
131     % Calculating Se from PFR
132     h0 = 0;
133     hPFR = VPFR/Ar;
134     hspan = [h0 hPFR];
135     x0 = [Sin, Xin, Zin];
136     [hPFR_vec,X] = ode45(@PFR,hspan,x0);
137
138     % Output = substrate level in the effluent of the PFR
139     Se(l) = X(end,1);
140     hPFR_vec = hPFR_vec;
141
142     l = l+1;
143
144 end
145
146 VCSTR = V1min:0.001:Vtot-0.001;
147
148 % Find V1opt for CSTR+PFR
149 Sin = 10;
150 Xin = 0;
151 Zin = 0;
152
153 V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);
154
155 % Setting conditions for fmincon
156 % Creating vector V with initial guesses
157 V = [];
158 V(1) = V1min+(1e-03);
159 V(2) = Vtot-V(1);
160
161 % sum(V) = Vtot
```

```
Aeq = zeros(2);
    Aeq(1,:) = 1;
Beq = zeros(2,1);
    Beq(1) = Vtot;

% 0 < V < Vtot; VCSTR > V1min
lb = zeros(1,2); lb(1) = V1min+(1e-03);
ub = Vtot*ones(1,2);

% Minimizing function CSTR_V_given with conditions as above
[vol_opt_PFR,Se_opt_PFR]=fmincon(@PFR_V_given,V,[],[],Aeq,Beq,lb,ub,[])
    ;
```

SCRIPTS TO GENERATE DATA FOR FIGURE 6, 7 AND 8

The results from the two following scripts were used to create figures 7 and 8.

N CSTRs

```
% The program finds the optimum volume V^{opt}_1 for different b and fp

options = optimset('TolFun',1e-14, 'TolCon', 1e-14,'DiffMinChange', 0,
    'Algorithm', 'interior-point');

global Q b mumax Sin Ks Y fp Xin Zin

Vtot = 1.1; % Total volume
Q = 1;      % Inflow = outflow
mumax = 2;  % Maximum specific growth rate
Y = 0.8;    % Yield factor
Ks = 1.2;   % Half saturation constant
Sin = 10;   % Substrate level in the influent
Xin = 0;    % Biomass level in the influent
Zin = 0;    % Inert biomass level in the influent

% Choose N
N = 5;
fp_vec = 0:0.01:1;
b_vec = 0:0.01:0.87;

% Creating vectors to store values
volumes = zeros(length(fp_vec),N);
fval = zeros(1,length(fp_vec));
vol1_min = zeros(length(b_vec),length(fp_vec));
Se_min = zeros(length(b_vec),length(fp_vec));

l = 1;

for b = 0:0.01:0.87

    k = 1;

    for fp = 0:0.01:1

        Sin = 10;
        Xin = 0;
        Zin = 0;

        V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);

        % Setting conditions for fmincon
        % Sum(V1,...,VN) = Vtot
        Aeq = zeros(N);
            Aeq(1,:) = 1;
        Beq = zeros(N,1);
            Beq(1) = Vtot;

        % 0 < V2,...,VN < Vtot; V1min < V1
```

```
        lb = zeros(1,N); lb(1) = V1min+(1e-03);
        ub = Vtot*ones(1,N);

        % Creating vector V with initial guesses
        V(1) = V1min+(1e-03);
        Vr = (Vtot-V(1))/(N-1);
        V(2:N) = Vr.*ones((N-1),1);

        % Minimizing function CSTR_V_given with conditions as above
        [volumes(k,:),fval(k)]=fmincon(@CSTR_V_given,V,[],[],Aeq,Beq,lb
            ,ub,[],options);

        vol1_opt(l,k) = volumes(k,1); % Save V1opt
        Se_min(l,k) = fval(k); % Save Se(V1opt)

        k = k+1;

    end

    l = l+1;

end
```

CSTR+PFR

```
% The program finds the optimum volume of the CSTR, V^{opt}_1, for
    different b and fp

options = optimset('TolCon',1e-14, 'TolFun', 1e-14,'DiffMinChange', 0,
    'Algorithm', 'interior-point');

global Q b mumax Sin Ks Y fp Xin Zin Ar Vtot

Ar = 0.428; % Cross-sectional area of the PFR
Vtot = 1.1; % Total volume
Q = 1;      % Inflow = outflow
mumax = 2;  % Maximum specific growth rate
Y = 0.8;    % Yield factor
Ks = 1.2;   % Half saturation constant
Sin = 10;   % Substrate level in the influent
Xin = 0;   % Biomass level in the influent
Zin = 0;   % Inert biomass level in the influent

l = 1;

for b = 0.0:0.01:0.87

    k = 1;

    for fp = 0.0:0.01:1.0

        Sin = 10;
        Xin = 0;
        Zin = 0;

```

```
29          V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);
30
31          % Setting conditions for fmincon
32          % Creating vector V with initial guesses
33          V(1) = V1min+(1e-03);
34          V(2) = Vtot-V(1);
35
36          % sum(V) = Vtot
37          Aeq = zeros(2);
38                  Aeq(1,:) = 1;
39          Beq = zeros(N,1);
40                  Beq(1) = Vtot;
41
42          % 0 < V < Vtot and V1 > V1min
43          lb = zeros(1,2); lb(1) = V1min+(1e-03);
44          ub = Vtot*ones(1,2);
45
46          % Minimizing function CSTR_V_given with conditions as above
47          [volumes(k,:),fval(k)]=fmincon(@PFR_V_given,V,[],[],Aeq,Beq,lb,
                ub,[],options);
48
49          vol1_opt(l,k) = volumes(k,1); % Save V1opt
50          Se_min(l,k) = fval(k);        % Save Se(V1opt)
51
52          k = k+1;
53
54      end
55
56      l = l+1;
57
58  end
```

SCRIPTS TO GENERATE DATA FOR FIGURE 9, 10 AND 11

Chosen value of b and f_p

```
% The program calculates Se when V1 to VN are optimized for different N
    . It also calculates V1* (V1opt for the case of PFR+CSTR) and Se
    for the case when V1 = V1* and V2 = ... = VN (N = 2,...,10).

options = optimset('TolFun',1e-14, 'TolCon', 1e-14,'DiffMinChange', 0,
    'Algorithm', 'interior-point');

global Q b mumax Sin Ks Y fp Xin Zin Ar Vtot v

Ar = 0.428; % Cross-sectional area of the PFR
Vtot = 1.1; % Total volume
Q = 1;      % Inflow = outflow
mumax = 2;  % Maximum specific growth rate
Y = 0.8;    % Yield factor
b = 0.0;    % Decay rate
fp = 0.0;   % Amount that becomes inert
Ks = 1.2;   % Half saturation constant
Sin = 10;   % Substrate level in the influent
Xin = 0;    % Biomass level in the influent
Zin = 0;    % Inert biomass level in the influent


% Optimize all volumes for N = 2,...,10
V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);   % Wash-out volume
i = 1;

for N = 2:1:10

    Sin = 10;
    Xin = 0;
    Zin = 0;

    % Setting conditions for fmincon
    % Sum(V1,...,VN) = Vtot
    Aeq = zeros(N);
        Aeq(1,:) = 1;
    Beq = zeros(N,1);
        Beq(1) = Vtot;

    % 0 < V2,...,VN < Vtot; V1 > V1min;
    lb = zeros(1,N); lb(1) = V1min+(1e-03);
    ub = Vtot*ones(1,N);

    % Creating vector V with initial guesses
    V(1) = V1min+(1e-03);
    Vr = (Vtot-V(1))/(N-1);
    V(2:N) = Vr.*ones((N-1),1);

    % Minimizing function CSTR_V_given with conditions as above
    [volumes(i,1:N),Se_all_opt(i)]=fmincon(@CSTR_V_given,V,[],[],Aeq,
        Beq,lb,ub,[],options);

```

```
    i = i+1;

end

% Finding V1* (V1opt for CSTR+PFR)
Sin = 10;
Xin = 0;
Zin = 0;
V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);

% Setting conditions for fmincon
% Creating vector V with initial guesses
V_PFR(1) = V1min+(1e-03);
V_PFR(2) = Vtot-V_PFR(1);

% sum(V) = Vtot
Aeq_PFR = zeros(2);
    Aeq_PFR(1,:) = 1;
Beq_PFR = zeros(2,1);
    Beq_PFR(1) = Vtot;

% 0 < V < Vtot; VCSTR > V1min
lb_PFR = zeros(1,2); lb_PFR(1) = V1min+(1e-03);
ub_PFR = Vtot*ones(1,2);

% Minimizing function PFR_V_given with conditions as above
[volumes_PFR,fval]=fmincon(@PFR_V_given,V_PFR,[],[],Aeq_PFR,Beq_PFR,
    lb_PFR,ub_PFR,[],options);

% Calculating Se for the case when V1 = V1* and V2=...=VN.
k = 1;

for N = 2:1:10;

    Vr = (Vtot-volumes_PFR(1))/(N-1);
    V = Vr.*ones(N,1);
    V(1) = volumes_PFR(1);

    % Calculating S in the first CSTR
    Sin = 10;
    Xin = 0;
    Zin = 0;
    S1 = (((Q/V(1))+b)*Ks)/(mumax-(Q/V(1))-b);
    X1 = Q*(Sin-S1)*Y/(Q+(V(1)*b*(1-((1-fp)*Y))));
    Z1 = ((V(1)/Q)*fp*b*X1)+Zin;
    Sin = S1;
    Xin = X1;
    Zin = Z1;

    % Calculating Se for the rest of the CSTRs
    n = length(V);

    for j=2:n

        v = V(j);
        x = [Sin, Xa_in, Xi_in];
```

```
        Z = fsolve(@diff_eqn, x);

        Sin = Z(1);
        Xin = Z(2);
        Zin = Z(3);
        Sstat(j) = Sin;
        Xstat(j) = Xin;
        Zstat(j) = Zin;

    end

    Se_first_opt(k) = Sstat(end);

    k = k+1;

end
```

Fixed f_p, varying b

```
% The program calculates Se when V1 to VN are optimized for different N
    . It also calculates V1* (V1opt for the case of PFR+CSTR) and Se
    for the case when V1 = V1* and V2 = ... = VN (N = 2,...,10). b is
    varying and fp is constant.

options = optimset('TolFun',1e-14, 'TolCon', 1e-14,'DiffMinChange', 0,
    'Algorithm', 'interior-point');

global Q b mumax Sin Ks Y fp Xin Zin Ar Vtot v

Ar = 0.428; % Cross-sectional area of the PFR
Vtot = 1.1; % Total volume
Q = 1;      % Inflow = outflow
mumax = 2;  % Maximum specific growth rate
Y = 0.8;    % Yield factor
Ks = 1.2;   % Half saturation constant
Sin = 10;   % Substrate level in the influent
Xin = 0;    % Biomass level in the influent
Zin = 0;    % Inert biomass level in the influent

fp = 0.4;

% Optimize all volumes for N = 2,...,10
k = 1;

for b = 0:0.01:0.5

    i = 1;

    for N = 2:1:10

        Sin = 10;
        Xin = 0;
        Zin = 0;

        V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);   % Wash-out volume
```

```
33
34          % Setting conditions for fmincon
35              % Sum(V1,...,VN) = Vtot
36              Aeq = zeros(N);
37                  Aeq(1,:) = 1;
38              Beq = zeros(N,1);
39                  Beq(1) = Vtot;
40
41              % V1min < V1 < Vtot
42              % 0 < V2,...,VN < Vtot
43              lb = zeros(1,N); lb(1) = V1min+(1e-03);
44              ub = Vtot*ones(1,N);
45
46              % Creating vector V with initial guesses
47              Vr = Vtot/N;
48              V = Vr.*ones(N,1);
49
50              % Minimizing function CSTR_V_given with conditions as above
51              [volumes,Se_all_opt(k,i)]=fmincon(@CSTR_V_given,V,[],[],Aeq
                    ,Beq,lb,ub,[],options);
52
53              i = i+1;
54
55      end
56
57      k = k+1;
58
59  end
60
61  % Finding V1*
62
63  i = 1;
64
65  for b = 0:0.01:0.5
66
67      Sin = 10;
68      Xin = 0;
69      Zin = 0;
70
71      V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);
72
73      % Setting conditions for fmincon
74      % Creating vector V with initial guesses
75      V_PFR(1) = V1min;
76      V_PFR(2) = Vtot-V_PFR(1);
77
78      % sum(V) = Vtot
79      Aeq_PFR = zeros(2);
80          Aeq_PFR(1,:) = 1;
81      Beq_PFR = zeros(2,1);
82          Beq_PFR(1) = Vtot;
83
84      % 0 < V < Vtot
85      lb_PFR = zeros(1,2); lb_PFR(1) = V1min+(1e-03);
86      ub_PFR = Vtot*ones(1,2);
87
```

```
88      % Minimizing function CSTR_V_given with conditions as above
89    [volumes_PFR(i,:),fval(i)]=fmincon(@PFR_V_given,V_PFR,[],[],Aeq_PFR,
         Beq_PFR,lb_PFR,ub_PFR,[],options);
90
91      i = i+1;
92
93  end
94
95  % Claculating Se for the case where V1 = V1* and V2=...=VN.
96  i = 1;
97
98  for b = 0:0.01:0.5
99
100 k = 1;
101
102     for N = 2:1:10;
103
104         Vr = (Vtot-volumes_PFR(i,1))/(N-1);
105         V = Vr.*ones(N,1);
106         V(1) = volumes_PFR(i,1);
107
108         % Calculating S in the first CSTR
109         Sin = 10;
110         Xin = 0;
111         Zin = 0;
112         S1 = (((Q/V(1))+b)*Ks)/(mumax-(Q/V(1))-b);
113         X1 = Q*(Sin-S1)*Y/(Q+(V(1)*b*(1-((1-fp)*Y))));
114         Z1 = ((V(1)/Q)*fp*b*X1)+Zin;
115         Sin = S1;
116         Xin = X1;
117         Zin = Z1;
118
119         % Calculating S for the rest of the CSTRs
120
121         n = length(V);
122         Sstat = zeros(1,n);
123         Xstat = zeros(1,n);
124         Zstat = zeros(1,n);
125
126         for j=2:n
127
128             v = V(j);
129
130             x = [Sin, Xin, Zin];
131
132             Z = fsolve(@diff_eqn, x);
133
134             Sin = Z(1);
135             Xin = Z(2);
136             Zin = Z(3);
137
138             Sstat(j) = Sin;
139             Xstat(j) = Xin;
140             Zstat(j) = Zin;
141
142         end
```

```
143
144         Se_first_opt(i,k) = Sstat(end);
145
146         k = k+1;
147
148     end
149
150     i = i+1;
151
152 end
```

Fixed b, varying f_p

```
1  % The program calculates Se when V1 to VN are optimized for different N
       . It also calculates V1* (V1opt for the case of PFR+CSTR) and Se
       for the case when V1 = V1* and V2 = ... = VN (N = 2,...,10). fp is
       varying and b is constant.
2
3  options = optimset('TolFun',1e-14, 'TolCon', 1e-14,'DiffMinChange', 0,
       'Algorithm', 'interior-point');
4
5  global Q b mumax Sin Ks Y fp Xin Zin Ar Vtot v
6
7  Ar = 0.428; % Cross-sectional area of the PFR
8  Vtot = 1.1; % Total volume
9  Q = 1;      % Inflow = outflow
10 mumax = 2;  % Maximum specific growth rate
11 Y = 0.8;    % Yield factor
12 Ks = 1.2;   % Half saturation constant
13 Sin = 10;   % Substrate level in the influent
14 Xin = 0;  % Biomass level in the influent
15 Zin = 0;  % Inert biomass level in the influent
16
17 b = 0.25;
18
19 % Optimize all volumes for N = 2,...,10
20 k = 1;
21
22 for fp = 0:0.01:1
23
24     i = 1;
25
26     for N = 2:1:10
27
28         Sin = 10;
29         Xin = 0;
30         Zin = 0;
31
32         V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);   % Wash-out volume
33
34         % Setting conditions for fmincon
35         % Sum(V1,...,VN) = Vtot
36         Aeq = zeros(N);
37             Aeq(1,:) = 1;
38         Beq = zeros(N,1);
```

```
39              Beq(1) = Vtot;
40
41          % V1min < V1 < Vtot
42          % 0 < V2,...,VN < Vtot; V1 > V1min
43          lb = zeros(1,N); lb(1) = V1min+(1e-03);
44          ub = Vtot*ones(1,N);
45
46          % Creating vector V with initial guesses
47          Vr = Vtot/N;
48          V = Vr.*ones(N,1);
49
50          % Minimizing function CSTR_V_given with conditions as above
51          [volumes,Se_all_opt(k,i)]=fmincon(@CSTR_V_given,V,[],[],Aeq,Beq
                ,lb,ub,[],options);
52
53          i = i+1;
54
55      end
56
57      k = k+1;
58
59  end
60
61  % Finding V1*
62  i = 1;
63
64  for fp = 0:0.01:1
65
66      Sin = 10;
67      Xin = 0;
68      Zin = 0;
69
70      V1min = Q/(((mumax*Sin)/(Ks+Sin))-b);
71
72      % Setting conditions for fmincon
73      % Creating vector V with initial guesses
74      V_PFR(1) = V1min;
75      V_PFR(2) = Vtot-V_PFR(1);
76
77      % sum(V) = Vtot
78      Aeq_PFR = zeros(2);
79          Aeq_PFR(1,:) = 1;
80      Beq_PFR = zeros(2,1);
81          Beq_PFR(1) = Vtot;
82
83      % 0 < V_PFR < Vtot; V_CSTR > V1min
84      lb_PFR = zeros(1,2); lb_PFR(1) = V1min+(1e-03);
85      ub_PFR = Vtot*ones(1,2);
86
87      % Minimizing function CSTR_V_given with conditions as above
88      [volumes_PFR(i,:),fval(i)]=fmincon(@PFR_V_given,V_PFR,[],[],Aeq_PFR
            ,Beq_PFR,lb_PFR,ub_PFR,[],options);
89
90      i = i+1;
91
92  end
```

```
93
94  % Claculating Se for the case where V1 = V1* and V2=...=VN.
95  i = 1;
96
97  for fp = 0:0.01:1
98
99  k = 1;
100
101     for N = 2:1:10;
102
103         Vr = (Vtot-volumes_PFR(i,1))/(N-1);
104         V = Vr.*ones(N,1);
105         V(1) = volumes_PFR(i,1);
106
107         % Calculating S in the first CSTR
108         Sin = 10;
109         Xin = 0;
110         Zin = 0;
111         S1 = (((Q/V(1))+b)*Ks)/(mumax-(Q/V(1))-b);
112         X1 = Q*(Sin-S1)*Y/(Q+(V(1)*b*(1-((1-fp)*Y))));
113         Z1 = ((V(1)/Q)*fp*b*X1)+Zin;
114         Sin = S1;
115         Xin = X1;
116         Zin = Z1;
117
118         % Calculating S in the rest of the CSTRs
119         n = length(V);
120         Sstat = zeros(1,n);
121         Xstat = zeros(1,n);
122         Zstat = zeros(1,n);
123
124         for j=2:n
125
126             v = V(j);
127
128             x = [Sin, Xin, Zin];
129
130             Z = fsolve(@diff_eqn, x);
131
132             Sin = Z(1);
133             Xin = Z(2);
134             Zin = Z(3);
135             Sstat(j) = Sin;
136             Xstat(j) = Xin;
137             Zstat(j) = Zin;
138
139         end
140
141         Se_first_opt(i,k) = Sstat(end);
142
143         k = k+1;
144
145     end
```

SCRIPTS TO GENERATE DATA FOR FIGURE 12

```
% Optimal design for at given effluent substrate concentration

global Q b mumax Sin Ks Y fp Xa_in Xi_in Ar Se

Ar = 0.428; % Cross-sectional area of the PFR
Q = 1;      % Inflow = outflow
mumax = 2;  % Maximum specific growth rate
Y = 0.8;    % Yield factor
b = 0.1;    % Decay rate
fp = 0.1;   % Amount that becomes inert
Ks = 1.2;   % Half saturation constant
Sin = 10;   % Substrate level in the influent
Xa_in = 0;  % Biomass level in the influent
Xi_in = 0;  % Inert biomass level in the influent

V1min = Q/(((mumax*Sin)/(Ks+Sin))-b); % Wash-out volume

% Finding Vopt for CSTR+PFR
i = 1;

volumes1 = zeros(5,2);

Smin = ((1-fp)*b*Y*Ks)/(mumax-((1-fp)*b*Y));
Smin = 1.1*Smin;

for Se = [0.1, 0.2, 10/10, 10/5, 10];

    % Setting conditions for fmincon
    % Creating vector V with initial guesses
    V0 = V1min.*ones(1,2); V0(1)=100;

    % Linear constraint: Vi > 0
    lb = (1e-6).*ones(1,2); lb(1) = V1min+1e-6;
    ub = zeros(1,2); ub(1:end) = Inf;

    % Non-linear constraint: Se(V1,...,VN) = Se
    nonlcon1 = @nlcon1;

    % Minimizing function volume with conditions as above
    [volumes1(i,:),V_opt(i)]=fmincon(@volume,V0,[],[],[],[],lb,ub,
        nonlcon1,[]);

    i = i + 1;

end

% Finding V(N) for N CSTRs in series
l = 1;

for N = 2:1:5;

    k = 1;

```

```
53      volumes2 = zeros(5,N);
54
55      for Se = [Smin, 0.2, 10/10, 10/5, 10];
56
57          % Setting conditions for fmincon
58          % Creating vector V with initial guesses
59          V0 = V1min.*ones(N,1);
60
61          % Linear constraint: Vi > 0
62          lb = (1e-6).*ones(1,N); lb(1) = V1min+(1e-6);
63          ub = zeros(1,N); ub(1:end) = Inf;
64
65          % Non-linear constraint: SN(V1,...,VN) = Se
66          nonlcon2 = @nlcon2;
67
68          % Minimizing function volume with conditions as above
69          [volumes2(k,:),V_N(l,k)]=fmincon(@volume,V0,[],[],[],[],lb,ub,
                nonlcon2,[]);
70
71      k = k + 1;
72
73      end
74
75      l = l + 1;
76
77  end
```