Competition between two microbial populations in a sequencing fed-batch reactor and its implications for waste treatment applications

Sitaram Dikshitulu
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Competition between two microbial populations in a sequencing fed-batch reactor and its implications for waste treatment applications

Dikshitulu, Sitaram, Ph.D.
New Jersey Institute of Technology, 1993
COMPETITION BETWEEN TWO MICROBIAL POPULATIONS
IN A SEQUENCING FED-BATCH REACTOR AND ITS IMPLICATIONS
FOR WASTE TREATMENT APPLICATIONS

by
Sitaram Dikshitulu

A Dissertation
Submitted to the Faculty of
New Jersey Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

Department of Chemical Engineering,
Chemistry, and Environmental Science

May 1993
ABSTRACT

Competition Between Two Microbial Populations in a Sequencing Fed-Batch Reactor and its Implications for Waste Treatment Applications

by
Sitaram Dikshitulu

Pure and simple competition between two microbial populations in a sequencing fed-batch reactor (SFBR) was studied both at the theoretical and experimental level. Competition occurred for a single chemical pollutant which could serve as the sole carbon and energy source for both competitors. A mathematical model describing the process under inhibitory kinetics (as is usually the case with hazardous and toxic substances) was derived and theoretically analyzed. The model predicts that the dynamics of a SFBR, and the kinetics of biodegradation, result in a complex set of operating regimes in which neither species, only one species, or both species survive in a steady cycle. The model also predicts the existence of multiple outcomes, achievable from different start-up conditions, in some domains of the operating parameter space.

The experimental system involved phenol as the model pollutant, and two species capable of utilizing phenol as their sole carbon and energy source. These species were *Pseudomonas putida* (ATCC 17514) and *Pseudomonas resinovorans* (ATCC 14235). A methodology was developed to accurately determine the kinetics of phenol biodegradation by each individual species in pure culture batch experiments. It was found that both species biodegrade phenol following Andrews' kinetics. The experimentally determined kinetic parameters were then used with the model equations to predict the behavior of a SFBR employing both species together.

The model predictions were experimentally tested by inoculating a SFBR with both species and operating under conditions falling in different regimes of the operating
parameter space. In all cases there was excellent agreement between the predicted and measured concentrations of phenol, total biomass, and the biomass of each individual species. All different types of behavior of the system predicted by the analysis of the model were experimentally confirmed including the existence of multiple outcomes under the same operating, but different start-up conditions.

This study shows how serious discrepancies can arise in scale-up of biological treatment systems if population dynamics are not taken into account. This study also confirms experimentally the theory of microbial competition in periodically forced bioreactors.
Competition Between Two Microbial Populations in a Sequencing Fed-Batch Reactor and its Implications for Waste Treatment Applications

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"The Effect of Population and Substrate Interactions on SBR Design Optimization"- EPA Annual Symposium on Bioremediation of Hazardous Wastes: Research, Development and Field Evaluations, Dallas, TX (May 4-6, 1993)


"Biodegradation of Single and Multiple Hazardous Substances in a Sequencing Batch Reactor: Theory and Experimental Results."- AIChe Annual Meeting, Chicago, IL (November 11-16, 1990)
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Hazardous compounds can be eliminated from aqueous environments by using biological methods. A significant advantage of biological treatment is that it can be very cost effective in comparison to other waste treatment processes such as incineration, adsorption, or catalytic destruction. Most commonly used systems for biological treatment of wastewaters involve fixed film systems and activated sludge systems. In fixed film systems organisms grow attached to a surface while in activated sludge systems organisms grow in suspension. In both cases mixed, rather than pure, cultures of microorganisms convert the contaminants to carbon dioxide, water, new cell mass and other end products.

Wastewaters are usually treated in continuous flow systems (CSTRs) using activated sludge and a series of tanks; the contaminated water flows from one tank into the next on a continuous basis and virtually all tanks have a predetermined liquid volume.

An innovative, alternate wastewater technology is that of Sequencing Batch Reactors (SBRs). SBRs are time oriented systems, with flow, energy input and tank volume varying according to some predetermined, periodic, operating strategy. SBRs result in an unsteady state operation. These systems have a number of advantages such as a strong control over organism selection (43), cycling between anoxic and aerobic periods of operation, greater flexibility in meeting changes in feed conditions, better control in the settling characteristics of the sludge, effective control of the process and the quality of discharge, and the fact that they do not need a separate clarifier.

Even though SBRs are currently used quite successfully with activated sludge systems (35, 80), and have been studied by a number of researchers (20, 35, 41-44, 65), they have largely been approached empirically with regard to mixed microbial populations.
The current approach to bioremediation of contaminated wastewaters is to use a so called "activated sludge". This is a highly heterogeneous microbial culture consisting of species indigenous to a particular site. Although, it is reasonable to expect that indigenous microbial populations will be adapted to the pollutants, there is no reason to believe that optimal pollutant degrading strains will always be present. Finn (24) has argued that biological treatment could be significantly improved by using well defined and not necessarily complex cultures. Microbial cultures can be viewed as catalysts in the classical chemical engineering sense. Unless the microbial system is well characterized, it is almost impossible to duplicate results and derive rational design criteria. One of the main problems with non-characterized heterogeneous cultures is that the effects of species interactions are neglected. Such interactions are always present in mixed cultures and lead to changes in biomass composition depending on the conditions of operation. These composition changes imply a change in the catalyst, in the chemical engineering sense, and thus, in the rate of the process. One of the most common interactions among microbial populations inhabiting a common environment, is competition for nutrients and other resources. Knowledge of the dynamics of interactions arising among microbial populations inhabiting a bioreactor is important when a biomass of a specific species composition is required.

In the present study the effect of microbial competition on the dynamics of a sequencing batch reactor has been investigated. A special case of SBR was considered; namely, one in which reaction occurs during the fill phase and settling of solids is neglected. In such a case, a SBR can be called a Sequencing Fed-Batch Reactor (SFBR). Pure and simple competition by two species for a single substrate was considered. A theoretical model describing the process was developed, analyzed, and then experimentally validated. The model equations were numerically solved for different values of some key parameters of SFBR operation. The various possible outcomes of competition were examined for their stability and attainability during actual operation. Two species,
*Pseudomonas putida* (ATCC 17514) and *Pseudomonas resinovorans* (ATCC 14235) both capable of growing on phenol (which was selected as the model toxic compound for the study) as the sole carbon and energy source, were selected for the experimental validation of the model. The kinetics of phenol biodegradation by the aforementioned species were revealed from pure culture batch experiments. These kinetic parameters were used in the numerical studies with the model. Numerical simulations revealed the regions of various outcomes in the operating parameter space and guided the design of experiments for model validation. The experimental results from SFBR operation agreed very well with the model predictions.
CHAPTER 2
LITERATURE REVIEW

2.1 Kinetics of Phenol Biodegradation

Phenol which has been selected as the model compound for the present study, is a toxic substance and its microbial degradation can best be described by a mechanism of substrate inhibition. The Haldane (29) or Andrews' (3) equation has been predominantly used to describe the degradation of phenol by pure as well as mixed cultures (3, 16, 23, 62, 70). Allsop et al. (1) have concluded that the inhibition of phenol permeases or hydroxylases by high concentrations of phenol is the probable cause for the inhibitory degradation kinetics. Janke et al. (45) reported that the conversion of phenol to catechol by a strain of Pseudomonas putida having only the meta cleavage pathway was inhibited by phenol levels exceeding 23.5 g m⁻³, although the data did not allow for a distinction between permease and hydroxylase association inhibition. Wedding et al. (78) reported the in vitro complexing of phenol with cytochrome b₅ reductase (NADH) and (NAD⁺) in porcine cell homogenates. Since these co-enzymes are necessary for phenol hydroxylase activity, the formation of complexes suggests a permease or hydroxylase based reaction interference by phenol (1). Studies on phenol uptake rate by Sokol (68, 69) and Sokol and Howell (70) also support a permease-or hydroxylase- based interference. Since the mechanism used in deriving the Haldane equation assumes a direct linkage between phenol concentration and phenol uptake, such an interference may be termed Haldane-like. Kinetics described by this behavior have also been reported as Andrews (3) kinetics.

The kinetics of phenol biodegradation have been investigated in continuous (13,15, 32, 47, 79), as well as batch experiments (27, 32, 50, 56, 62, 75-77). Kinetic parameters
Table 2.1  Andrews model parameters for microbial populations degrading phenol.

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<tr>
<th>Reference</th>
<th>Culture</th>
<th>T</th>
<th>pH</th>
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<td></td>
<td></td>
<td>°C</td>
<td></td>
<td>h$^{-1}$</td>
<td>g m$^{-3}$</td>
<td>g m$^{-3}$</td>
<td>g g$^{-1}$</td>
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<td>Chi and Howell (13)</td>
<td><em>Pseudomonas sp.</em></td>
<td>30</td>
<td>-</td>
<td>0.369</td>
<td>5.94</td>
<td></td>
<td>227</td>
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<td>Colvin and Rozich (15)</td>
<td>UMC</td>
<td>22</td>
<td>7.2</td>
<td>0.19</td>
<td>7.9</td>
<td>139</td>
<td>0.9</td>
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<td>Colvin and Rozich (15)</td>
<td>UMC</td>
<td>22</td>
<td>7.2</td>
<td>1.07</td>
<td>79</td>
<td>172</td>
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<td>D'Adamo, Rozich, and Gaudy (16)</td>
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<td>25</td>
<td>6.8-7.0</td>
<td>0.204-0.363</td>
<td>5-266</td>
<td>142-1199</td>
<td>-</td>
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<td>Guptapal (27)</td>
<td><em>P. resinovorans</em> (ATCC 14235)</td>
<td>28</td>
<td>7.2</td>
<td>0.947</td>
<td>13.06</td>
<td>151.88</td>
<td>0.596</td>
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<td>&lt;1</td>
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<td>0.388</td>
<td>1.06</td>
<td>903</td>
<td>0.70</td>
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<td>Jones, Jansen, and McKay (47)</td>
<td><em>P. putida</em> (NCIB 8250)</td>
<td>20</td>
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<td>0.44</td>
<td>&lt;1</td>
<td>110</td>
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<td>Kotturi, Robinson, and Inniss (50)</td>
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<td>7.0</td>
<td>0.119</td>
<td>5.27</td>
<td>377</td>
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<td>Molin and Nilsson (59)</td>
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<td>6.7</td>
<td>0.4</td>
<td>&lt;3</td>
<td>500</td>
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<td>0.16-5.2</td>
<td>7.5-19.4</td>
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<td>Tang and Fan (76)</td>
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<td>Wang (77)</td>
<td><em>P. putida</em> (ATCC 17514)</td>
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<td>7.2</td>
<td>1.55</td>
<td>27.9</td>
<td>12.4</td>
<td>0.537</td>
</tr>
<tr>
<td>Yang and Humphrey (79)</td>
<td><em>P. putida</em> (ATCC 17514)</td>
<td>30</td>
<td>0.6</td>
<td>0.567</td>
<td>2.39</td>
<td>106.0</td>
<td>0.85</td>
</tr>
</tbody>
</table>

UMC: Unidentified mixed culture
obtained from these studies are shown in Table 2.1. The initial rate method is used in batch experiments whereas steady state measurements are required for continuous experiments. The large medium requirement is the major disadvantage of continuous culture experiments. Batch experiments require an accurate estimation of the initial rate of microbial growth; for this to be achieved, a large number of measurements is needed, and the experimenter must have the ability to distinguish between the various stages of growth. The batch method is based on the use of data from the exponential phase of growth. Unless the inoculum size is small enough, especially at low initial substrate concentrations and for fast growing organisms, one cannot get enough data for an accurate determination of the rate (51).

Determination of biodegradation kinetics by mixed cultures is problematic when the mixed culture is treated as a single functional population. This can be seen from the following studies with phenol. D'Adamo et al. (16) studied phenol biodegradation by using a heterogeneous seed population obtained from a municipal treatment plant. The degradation of a synthetic medium containing phenol (50-1000 g m⁻³) was studied in batch experiments and the specific growth rates were fitted to the Andrews' (3) model. These authors reported values of μ* from 0.131 to 0.363 h⁻¹, Kₛ from 5 to 266 g m⁻³, and Kᵣ from 142 to 1199 g m⁻³, from experiments performed over a period of six months. The wide ranges of the biokinetic constants clearly suggest that the species composition of the mixed culture had been altered over the period the experiments were performed.

Pawlowsky and Howell (62) used a mixed culture derived from soil and activated sludge organisms to degrade a synthetic medium containing phenol. The mixed culture consisted of a diverse population ranging from spherical and rod shaped bacteria to filamentous forms, and of distinctive predators such as protozoa and a few rotifers. These mixed cultures were acclimated in a chemostat with a feed medium containing 100 g m⁻³ phenol. With a 6 hour residence time, spherical bacteria dominated with several predators present, whereas with a 4 hour residence time filamentous bacteria were dominant with
very few predators present. Two sets of batch experiments were performed using seeds from the two chemostat runs. The fitted biokinetic parameters - \( \mu^*, K_s, K_I \) - for the two sets were \( 0.26 \text{ h}^{-1}, 25.4 \text{ g m}^{-3}, 173.0 \text{ g m}^{-3} \) and \( 0.223 \text{ h}^{-1}, 5.86 \text{ g m}^{-3}, 934.5 \text{ g m}^{-3} \), respectively. From these data it is clear that kinetic constants are meaningless when they do not refer to a specified species composition.

Szetela and Winnicki (75) obtained cultures from an aeration tank operated by a municipal sewage treatment plant. The microorganisms were adapted to biodegrade phenol using it as their sole carbon and energy source. The adaptation process was carried out with the organisms immobilized in an anion exchange resin bed. The cultures were subsequently isolated from the carrier by mixing at a high intensity. A set of batch experiments- with phenol in the range of 20-800 g m\(^{-3}\) were performed. There was no scatter in the specific growth rate data, something that was observed in the work of Pawlowsky and Howell (62). The reason for this could be that the adaptation method used, caused the isolation of microorganisms with similar characteristics.

Colvin and Rozich (15) studied phenol degradation kinetics in a two stage continuous culture system. Mixed cultures from a wastewater treatment plant were used. The reactors -which were connected in series- were inoculated with phenol acclimated biomass. The dilution rate of the two reactors was different. The steady state biomass composition was different in the two reactors. Batch experiments were then performed, using biomass from each of the reactors, with phenol in the range of 50 - 800 g m\(^{-3}\). A wide range of specific growth rates, for each of the phenol concentrations studied, was observed. This clearly indicates again that the kinetic constants have to be associated with a specific biomass composition. These results also suggest that the mode of operation (e.g., two reactors in series) can alter the biomass composition. Instead of the foregoing conclusions, the authors claim that the two-stage continuous flow method could not be used as a technique for determining the biokinetic constants for heterogeneous populations.
Most of the existing studies on phenol biodegradation (or biodegradation of any other compound) involve unidentified mixed cultures. This is due to the fact that the current practice in the waste treatment industry is to use activated sludge. This practice evolved from technologies that had been developed earlier for treatment of municipal wastes, and relies on rules-of-thumb and highly empirical approaches to reactor design. However, such a "black box" approach often fails when addressing problems in hazardous waste treatment as the activated sludge may not have the desired and/or constant biomass composition. Furthermore, a highly heterogeneous culture may lead to reduced biodegradation rates. For example, Jones et al. (47) studied the biodegradation of phenol in a two stage chemostat. An activated sludge containing numerous bacterial populations was used, but only two species of the *Acineobacter-Moraxella* group were found responsible for the biodegradation. The survival of the other species in the system could be attributed to predation on the phenol degrading species, thereby reducing the efficiency of the reaction system. These authors concluded that industrial wastewaters can be treated by bacterial ecosystems much simpler than those involved in municipal sewage treatment units.

### 2.2 Species Interactions

The wide range of kinetic parameters observed when mixed cultures are used, shows that bacterial interactions need to be considered while studying the dynamics of the system. Knowledge of the system dynamics is required in order to design effective and optimal treatment units. Furthermore, a control strategy may be necessary for the attainment and maintenance of steady state conditions as industrial processes are never free from fluctuations in input variables. To study the dynamic responses in a rational way, mathematical models must be constructed and analyzed. These models must be taking into
account the species interactions while for the kinetics they should involve those of the individual species present in the mixed culture.

The interactions among microbial populations in mixed cultures have been reviewed by Fredrickson (26) and Slater (66). Competition, alone or in combination with another interaction appears to be the most common microbial interaction. Microbial competition, is defined as the situation in which two populations use a resource and that resource has a dynamical effect on at least one of them. The pattern of microbial competition studied the most, is pure and simple competition. Microbial populations are engaged in pure and simple competition when they interact in no other way except competition for a single nutrient whose availability affects the growth rate of all populations involved in the interaction (26).

Pure and simple competition in a chemostat which operates under time invariant inputs has been the subject of several experimental (30, 31, 46, 58) and theoretical (4, 8, 9, 12, 36, 38, 63) studies. The conclusion from these studies is that if the chemostat is ideal and the resource competed for is not biologically renewable within the reactor, there is no steady state which may involve more than two populations. Even the coexistence of two species is only mathematically predicted and cannot be practically realized. It requires operation at discrete values of the chemostat dilution rate, which is not practically feasible owing to the random fluctuations in the flowrate (72). As pointed out by Powell (64), the system of ordinary differential equations at the discrete dilution rate values where coexistence is obtained, is structurally unstable. Baltzis and Fredrickson (8) observed that if the bioreactor is non-ideal in the sense that cells attach to the vessel walls, steady state coexistence of two species is possible over a wide range of operating parameter values provided that the population which grows slower under the given operating conditions is the one which exhibits wall attachment. Coexistence, not at a steady state but rather in a state of sustained oscillations (limit cycles), is possible in an ideal chemostat whose inputs are constant, provided that pure and simple competition occurs for a resource which is
biologically produced within a reactor by a third bacterial population which is not involved in the competitive pattern (9).

If the pattern of competition is not pure and simple, steady state coexistence of microbial competitors has been found to occur. For example, coexistence of two bacterial populations under substrate competition and product inhibition has been observed experimentally (18). Coexistence has been also observed in cases where competition occurs for multiple substrates. In such cases coexistence is even easier to attain when each population grows faster on a different substrate. Coexistence of two organisms competing for mixed nutrients has been observed by Lewis (55). Lester (53) has reported coexistence of six species. Competition for mixed substrates by microbial populations in a chemostat has been also studied by Yoon et al. (81). These researchers found that coexistence of several species at steady state was possible and concluded that diversity of nutrient sources in municipal wastes may be a key factor in supporting a heterogeneous population. Industrial wastes on the other hand may not have such a wide diversity of nutrients, thereby a simpler ecosystem should be expected in industrial waste bioremediation units.

Hutchinson (40) was the first to suggest that seasonal variations may be an explanation for the observed diversity of competing species in physical habitats. Using this idea for the case of pure and simple competition between two populations in a chemostat, a number of theoretical studies have shown that periodic variation of the chemostat dilution rate (12, 57, 71, 73), or of the limiting nutrient concentration in the feed (28, 37, 67), allows the two microbial populations to coexist in a stable state of sustained oscillations. These studies have assumed that the species growth rate adapts instantaneously to the changes occurring in the reactor. Under this assumption, a necessary condition for coexistence is that the specific growth rate curves of the two competitors cross each other and thus, under periodic operation of the chemostat, the competitive advantage alternates between the two populations so that they are able to
coexist. Pavlou et al. (63) have shown that a time delay in the adaptation of the growth rate may allow for coexistence of two species even in cases where the specific growth rate curves do not cross. The only experimental work reported in the literature that shows that pure and simple competitors persist in a reactor where conditions vary periodically is the one by Davison and Stephanopoulos (17). These investigators used pH as the varying parameter and operated the chemostat for part of the cycle under one pH and for the rest of the cycle under a different pH value.

The present study is the first to show that periodic variation of the inlet flowrate (as in a SFBR) can lead to coexistence of two pure and simple competitors.

2.3 Stability Analysis

The analysis of the dynamics of any chemically or biochemically reacting system from the stability viewpoint is of paramount importance in assessing the performance of a reactor. Stability analysis of bioreactors where mixed cultures are employed is even more important and complex as the dynamics get complicated by the presence of species interactions. Biochemical reactors with mixed cultures often exhibit multiple types of behavior involving steady and/or oscillatory states. Most chemical and biochemical systems are described by nonlinear differential equations. Stability of steady states can be determined by studying the character of the eigenvalues of the Jacobian matrix (2). These results are local in character due to the required linearization. They provide useful information which can then be used in generalizing the results for the global behavior of the system usually by the use of numerical methods. The stability of the oscillatory states is more difficult to determine and is based on the Poincaré theory and the so-called Floquet multipliers of the system. Review of these mathematical methods is out of the scope of this dissertation. It needs only to be stated that for the SFBR system considered here there are only oscillatory states, due to the external forcing of the flowrate, and that the stability of
these cycles was numerically determined using proper software originally developed by Doedel (21).

2.4 Periodic Operation of Reactors

Periodic operation of chemical reactors has experienced a revival in chemical engineering research in recent years. Periodic processes may have advantages over steady state processes in terms of productivity or selectivity (6, 7, 22, 34).

In biological treatment of wastes, an example of periodic reactor operation is that of Sequencing Batch Reactors (SBRs). In recent years SBRs have become more popular in waste treatment as a result of automated control, improved decanting mechanism, and aeration equipment that is resistant to plugging during start/stop operation. SBRs are currently used successfully with activated sludge systems (44, 75) as their operation is flexible and can be altered to accommodate a wide variety of waste waters. SBRs have been proposed as a means of negating filamentous bulking in activated sludge systems, as the ratio of fill to react periods affect the settleability of the sludge (20, 33). Earlier research (14, 20, 35, 41-44, 65) has shown that SBRs present a number of advantages over conventional activated sludge systems, including: cycling between anoxic and aerobic periods of operation, greater flexibility in meeting changes in feed conditions, better control in settling characteristics of the sludge, effective control of the process and the quality of discharge, and the fact that they do not require a separate clarifier. In classical SBRs, reaction occurs only during the "react" period. More recent work has demonstrated that if the reaction starts during the fill period, the presence of intermediates which are toxic and/or inhibitory for the biomass (such as nitrite in the denitrification process), can be substantially reduced during SBR operation (11, 54). Baltzis et al.(10), have also shown that in general, the volumetric efficiency of the SBRs is greater than that of the CSTRs; SBRs achieve the same level of treatment, at an equivalent throughput, in a
volume much smaller than the CSTRs, something which may result in significant savings in mixing and aeration costs.

A lack of widely accepted design standards is the major obstacle for bringing the SBR technology to a broader practical application (5). Most of the studies on SBRs are empirical and have not resulted in final conclusions. SBR technology can be substantially improved if a fundamental approach is used for analyzing the process involved. For waste treatment which involves mixed cultures, such a fundamental approach needs to consider species interactions. This dissertation studies microbial competition in a periodically operated reactor used for treating a hazardous waste and contributes to the basic understanding of SBR technology and to the further development of the theory of microbial competition.
CHAPTER 3

OBJECTIVE

The objective of this dissertation was to study the dynamics of two species which are involved in pure and simple competition for phenol in a Sequencing Fed-Batch Reactor (SFBR). The approach adopted was as follows:

1. Develop an experimental methodology in order to obtain accurate kinetic parameters for the pure cultures.
2. Mathematically model the SFBR system, and solve the equations numerically, using the previously determined kinetic parameters.
3. Explore the different types of behavior in the operating parameter space.
4. Experimentally verify all possible outcomes of competition predicted by the mathematical model.
A sequencing batch reactor operates in a cyclic mode. Each cycle is comprised of five distinct periods: fill, react, settle, draw, and idle. In most cases SBRs use activated sludge. The settle phase, in these cases, is required to separate the solids from the liquid. In the special case considered here there are no settle and idle phases and the biomass remains suspended in the reactor throughout the cycle. It is also assumed that reaction occurs throughout the cycle. This special case of SBR can be called Sequencing Fed-Batch Reactor (SFBR).

The flowrate of inlet (untreated waste) during the fill phase is constant, and the same is true for the flowrate of the outlet (treated waste) during the draw-down phase. The values of the two flowrates are not necessarily equal. Constant flowrates imply linear changes in the volume of the reactor contents. The minimum volume of reactor contents (beginning of fill-phase, and end of draw-phase) cannot be zero in order for at least some biomass to remain
in the vessel. Biomass is not externally fed to the reactor. Figure 4.1 shows a schematic of the variations of the volume of the reactor contents during the cyclic operation.

If the beginning of a cycle is set as time zero, then $t_1$ indicates the end of the fill phase, $t_2$ denotes the end of the period during which the reactor operates without any input or output, and $t_3$ marks the end of the cycle which immediately starts repeating itself. The reactor is originally inoculated with two phenol degraders which will be called species 1 and 2. The specific growth rate of both species follows Andrews' inhibitory expression, i.e.,

$$\mu_j = \mu_j^* S / (K_j + S + S^2/K_{jj}), \quad j = 1, 2.$$ 

Assuming that the bacterial cells do not exhibit any tendency for attachment to the reactor walls, that biomass maintenance requirements are negligible, and that the density of reactor contents is constant and equal to that of the untreated waste (inlet), the process can be described in general, by the following equations:

**Overall mass balance:**

$$\frac{dV}{dt} = Q_f - Q$$

where,

$V$ = Volume of the reactor contents, m$^3$

$t$ = time, hours

$Q_f$ = flow rate at which the untreated waste is fed into the reactor, m$^3$ h$^{-1}$

$Q$ = flow rate at which the treated waste is removed from the reactor, m$^3$ h$^{-1}$

**Mass balance on substrate:**

$$\frac{d(VS)}{dt} = Q_f S_f - QS - \frac{\mu_1 b_1 V}{Y_1} - \frac{\mu_2 b_2 V}{Y_2}$$

where,

$S$ = concentration of the phenol in the reactor, g m$^{-3}$

$S_f$ = concentration of the phenol in the untreated feed stream, g m$^{-3}$

$\mu_j$ = specific growth rate of species $j$ on phenol, h$^{-1}$

$b_j$ = biomass concentration of species $j$, g m$^{-3}$
Y_j = yield coefficient of species j on phenol, (grams- biomass / grams- substrate)

Substituting (1) in (2) we get,

\[
\frac{dS}{dt} = \frac{Qf}{V}(S_f - S) - \frac{b_1}{Y_1}\mu_1 - \frac{b_2}{Y_2}\mu_2
\]  \hspace{1cm} (3)

Mass balance on individual species:

\[
\frac{d(Vb_j)}{dt} = -Qb_j + \mu_j b_j V
\]  \hspace{1cm} (4)

Substituting (1) in (4) we get,

\[
\frac{db_j}{dt} = (\mu_j - \frac{Qf}{V})b_j, \hspace{0.5cm} j = 1, 2
\]  \hspace{1cm} (5)

Equations (1), (3), and (5) in dimensionless form are given as,

\[
\frac{dV'}{d\theta} = Q' - Q'
\]  \hspace{1cm} (6)

\[
\frac{du}{d\theta} = \frac{Qf}{V'}(u_f - u) - \beta[x_1f_1(u) + \eta x_2f_2(u)]
\]  \hspace{1cm} (7)

\[
\frac{dx_j}{d\theta} = [\beta f_j(u) - \frac{Qf}{V'}]x_j, \hspace{0.5cm} j = 1, 2
\]  \hspace{1cm} (8)

where,

\[
V' = \frac{V}{V_m}, \hspace{0.5cm} \theta = \frac{tQ_f*\sigma_1}{V_m}, \hspace{0.5cm} Q'_f = \frac{Q_f}{Q_f*\sigma_1}, \hspace{0.5cm} Q' = \frac{Q}{Q_f*\sigma_1}, \hspace{0.5cm} u = \frac{S}{K_1},
\]

\[
u_f = \frac{S_f}{K_1}, \hspace{0.5cm} \beta = \frac{\mu_1 V_m}{Q_f*\sigma_1}, \hspace{0.5cm} \eta = \frac{Y_1}{Y_2}, \hspace{0.5cm} x_j = \frac{b_j}{Y_1K_1}, \hspace{0.5cm} j = 1, 2,
\]

\[
\sigma_1 = \frac{t_1}{t_3}, \hspace{0.5cm} \sigma_2 = \frac{t_2 - t_1}{t_3}, \hspace{0.5cm} \sigma_3 = \frac{t_3 - t_2}{t_3}, \hspace{0.5cm} \omega = \frac{K_2}{K_1}, \hspace{0.5cm} \phi = \frac{\mu_2^*}{\mu_1^*},
\]

\[
\delta = \frac{V_0}{V_m}, \hspace{0.5cm} \gamma_j = \frac{K_1}{K_{lj}}, \hspace{0.5cm} j = 1, 2.
\]
where $Q_0^*$ is a reference flowrate taken as the flowrate during the fill-phase, $V_0$ and $V_m$ are the minimum and the maximum volume, respectively, of the reactor contents during the cycle.

Functions $f_1(u)$ and $f_2(u)$ represent -in dimensionless form- the specific growth rate of species 1 and 2, respectively, and are given as

$$f_1(u) = \frac{u}{1 + u + \gamma_1 u^2} \quad \text{and} \quad f_2(u) = \frac{\phi u}{\omega + u + \gamma_2 u^2}$$

During the first phase of the cycle (fill-period), we have $Q' = 0$ and $Q_f = 1/\sigma_1$. Subject to the initial condition $V' = \delta$ at $\theta = 0$, equation (6) when integrated, yields

$$V' = \delta + \frac{1}{\sigma_1} \theta$$  \hspace{1cm} (9)

The first phase of the cycle ends at time $\theta_1$ at which $V' = 1$. Thus, equation (9) implies that

$$\theta_1 = \sigma_1 (1 - \delta)$$  \hspace{1cm} (10)

During the second phase of the cycle, $Q_f' = Q' = 0$ while during the third and final phase, $Q_f' = 0$ and $Q' \neq 0$. If a steady cyclic pattern of operation is to be reached, the volume of the material fed into the reactor during the first period of the cycle must be equal to the volume of the reactor contents emptied during the third phase of the cycle. Hence,

$$Q_f t_1 = Q(t_3 - t_2), \quad \text{or} \quad Q' = \frac{1}{\sigma_3}$$  \hspace{1cm} (11)

Considering equation (11) and that $Q_f' = 0$, the volume change during the third phase of the cycle is given by

$$V' = 1 - \frac{1}{\sigma_3} (\theta - \theta_2)$$  \hspace{1cm} (12)

which is obtained by integrating equation (6) subject to the condition $V' = 1$ at $\theta = \theta_2$.

At the end of the cycle ($\theta = \theta_3$), we have $V' = \delta$ and thus, equation (12) implies that
\[ \theta_3 - \theta_2 = \sigma_3 (1 - \delta) \quad (13) \]

From the definition of \( \sigma_3 \) one can easily see that \( \theta_3 - \theta_2 = \sigma_3 \theta_3 \), which combined with equation (13) results in
\[ \theta_3 = 1 - \delta \quad (14) \]

From the foregoing analysis it can be concluded that the system is described by the following sets of equations:

I. First phase of cycle, i.e., \( 0 \leq \theta \leq \sigma_1 (1 - \delta) \)
\[
\frac{du}{d\theta} = \frac{uf - u}{\delta \sigma_1 + \theta} - \beta [x_1 f_1 (u) + \eta x_2 f_2 (u)] \quad (15)
\]
\[
\frac{dx_j}{d\theta} = [\beta f_j (u) - \frac{1}{\delta \sigma_1 + \theta}] x_j , j = 1, 2 \quad (16)
\]

II. Second and third phase of the cycle, i.e., \( \sigma_1 (1 - \delta) \leq \theta \leq 1 - \delta \)
\[
\frac{du}{d\theta} = -\beta [x_1 f_1 (u) + \eta x_2 f_2 (u)] \quad (17)
\]
\[
\frac{dx_j}{d\theta} = \beta f_j (u) x_j , j = 1, 2 \quad (18)
\]

The analysis of the model equations [(15)-(18)] is discussed in the next section. It should be mentioned that if one sets \( x_2 = 0 \) the equations given above reduce to a model describing biodegradation of a single pollutant in a SFBR by a pure culture.
4.2 Analysis of Model Equations

The system of model equations (15)-(18) possesses four classes of periodic solutions: (1) extinction of both species (washout state): \( x_1(t) = 0, x_2(t) = 0, u(t) = u_f \); (2) extinction of species 2 only (1-state): \( x_1(t) > 0, x_2(t) = 0, 0 < u(t) < u_f \); (3) extinction of species 1 only (2-state): \( x_1(t) = 0, x_2(t) > 0, 0 < u(t) < u_f \); (4) coexistence of the two species (coexistence state): \( x_1(t) > 0, x_2(t) > 0, 0 < u(t) < u_f \). It is important to determine the stability characteristics of these solutions, since only stable solutions are actually attainable by the system. The stability of the periodic solutions is determined by their characteristic (or Floquet) multipliers. From equations (15)-(18) one can easily see that after transients decay and the system reaches an actual periodic orbit, the following stoichiometric relation holds: \( u(t) - x_1(t) - \eta x_2(t) = u_f \). Computer simulations show that this relation is valid well before the final orbit is reached. Because of the stoichiometric relation, the system of three differential equations is asymptotically dynamically equivalent to a system of two differential equations. Thus, the stability of the periodic states of the system can be studied by considering two (rather than three) Floquet multipliers.

Depending on the nature and magnitude of the two Floquet multipliers, \( \lambda_1 \) and \( \lambda_2 \), a periodic solution falls into one of the following categories: (1) stable node, when \( \lambda_1 \) and \( \lambda_2 \) are real, and \( |\lambda_1|, |\lambda_2| < 1 \); (2) stable focus, when \( \lambda_1 \) and \( \lambda_2 \) are complex conjugate, and \( |\lambda_1|, |\lambda_2| < 1 \); (3) saddle, when \( \lambda_1 \) and \( \lambda_2 \) are real and \( 0 < |\lambda_1| < 1 < |\lambda_2| \); (4) unstable node, when \( \lambda_1 \) and \( \lambda_2 \) are real and \( 1 < |\lambda_1|, |\lambda_2| \); (5) unstable focus, when \( \lambda_1 \) and \( \lambda_2 \) are complex conjugate and \( 1 < |\lambda_1|, |\lambda_2| \). For a periodic solution to be stable, the magnitude of both its characteristic multipliers must be less than one, i.e., both of them must lie inside the unit circle in the complex plane.

The system considered here involves four operating parameters, namely \( \beta, u_f, \delta \), and \( \sigma_1 \). As these operating parameters are varied, the periodic states, as well as their characteristic multipliers, change. Thus, at certain values of the operating parameters, at
least one characteristic multiplier of a periodic solution crosses the unit circle in the complex plane, and the stability characteristics of the periodic state change. This is where a bifurcation of the periodic solution occurs. Two types of bifurcation are of relevance for the system considered in the present study. They both occur when one real multiplier ($\lambda_1$) crosses the unit circle at +1. The first is a transcritical bifurcation, when two periodic solutions exchange their stability characteristics. Depending on the location of the second multiplier, there may be an exchange between a saddle and a stable node when $|\lambda_2| < 1$, or a saddle and an unstable node when $|\lambda_2| > 1$. The second is a saddle-node bifurcation, when a saddle and a node collide and disappear. This is also called turning point or limit point bifurcation. Depending on the location of the second multiplier, this may be a saddle-stable node (saddle-sink) bifurcation when $|\lambda_2| < 1$, or a saddle-unstable node (saddle-source) bifurcation when $|\lambda_2| > 1$. Several other types of bifurcation are possible and have been observed in similar systems (53), but do not occur in the system studied in this dissertation.

Among the operating parameters, $\beta$ and $u_F$ seem to be the most important ones since they may be undergoing variation during operation due to changes in inlet flowrate and strength of the waste (phenol concentration), respectively. Hence, one would like to know the dependence of the dynamic behavior of the system on the values of $\beta$ and $u_F$, i.e., the range of values of these parameters over which each one of the periodic states of the system is stable. This is accomplished by constructing the operating diagram of the system, i.e., a diagram in the $\beta - u_F$ plane where the regions of qualitatively different dynamic behavior of the system are marked. In order to construct the operating diagram, the boundaries of the various regions need to be determined, i.e., the curves in the $\beta - u_F$ plane on which a qualitative change in the dynamic behavior of the system (a bifurcation) occurs. To do this, the periodic solutions (stable and unstable) of the system are computed, their characteristic multipliers calculated, and a pair of $\beta$ and $u_F$ values determined for which one of the characteristic multipliers becomes unity. A continuation algorithm (21) is used for
tracing the curve in the 2-parameter space ($\beta - u_0$) on which the characteristic multiplier remains at unity. A more detailed description of the numerical methods used in this study is given in the literature (48, 49, 60, 61). This analysis of the model equations is general and applies to any system of two species competing for a single substrate.

A characteristic operating diagram for the system considered here, is given on page 47 after the determination of the kinetic parameters from independent batch experiments.
CHAPTER 5

EXPERIMENTAL SYSTEM

5.1 Materials and Apparatus

5.1.1 Chemicals and Microorganisms

Phenol (crystals-CH-9470, Fluka Chemie AG., Switzerland) was used as the toxic substance whose biodegradation was studied in pure and mixed culture experiments. The bacterial strains used were *Pseudomonas putida* - ATCC 17514 and *Pseudomonas resinovorans* - ATCC 14235 (American Type Culture Collection, Rockville, MD). The chemicals used for the synthetic media preparation were: dihydrogen potassium phosphate, $\text{K}_2\text{HPO}_4$ (P288-3 Fisher Scientific, Fairlawn, NJ); potassium phosphate, monobasic $\text{KH}_2\text{PO}_4$ (P284-500 Fisher Scientific); ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ (A702-3, Fisher Scientific); magnesium sulfate, $\text{MgSO}_4$ (M63-500, Fisher Scientific); manganese sulfate $\text{MnSO}_4$ (2550-1, J. T Baker Chemical Co., Phillipsburg, NJ); and ferric chloride, $\text{FeCl}_3$ (Matheson Coleman and Bell, Norwood, Ohio).

5.1.2 Growth medium

The synthetic waste medium was prepared by adding phenol, as required, from a stock solution of 6 kg m$^{-3}$ to a 50 mM phosphate buffer ($\text{K}_2\text{HPO}_4$ and $\text{KH}_2\text{PO}_4$) solution of pH 7.2. Other chemicals which were added (per liter of buffer) are 0.5g $(\text{NH}_4)_2\text{SO}_4$, 0.1g $\text{MgSO}_4$, 0.01 g $\text{MnSO}_4$, 0.0005g $\text{FeCl}_3$, and 100 ml of tap water. The synthetic growth medium was sterilized in an autoclave ($121^\circ\text{C}$, 20 min). A nutrient broth (BBL-11479, Becton Dickinson and Co, Cockeysville, MD) solution was used for reviving the cultures from freeze-dried samples as well as for maintaining the cultures. Nutrient agar (001-01-8, Difco labs, Detroit, MI) was used for storing the cultures.
5.1.3 Experimental Set-up

A 4-liter jacketed lucite reactor with an internal diameter of 0.14 m was used. The reactor was fitted with two air dispersion tubes (Pyrex brand-coarse, 211-138B), a dissolved oxygen probe (New Brunswick Scientific Co., series 900), a thermometer, feed and discharge lines and a sampling port. A magnetic stirrer was used for mixing the reactor contents. A schematic diagram of the experimental setup is shown in Figure 5.1. The cyclic operation of the reactor was automated by using a programmable sequence controller (Omron, model SCY-PO). Variable flow pumps (Sage Instruments-375A / Manville Corp.-C17125 LP) were used for feeding and discharging the reactor. Water was circulated in the reactor jacket using a circulating hot water bath (Endocal RTE-8), in order to maintain the reactor contents at 28.5°C. The reactor contents were aerated at a rate of 800 ml min⁻¹ by using an air pump. When required, the air stream was enriched with oxygen (Industrial grade, Liquid Carbonic Ltd.) The same reactor was used in batch experiments for determination of the kinetic parameters. During batch experiments the controller was not activated.

5.2 Analytical Procedures

5.2.1 Biomass Assay

The total biomass concentration in the reactor was determined by measuring the optical density of the samples. This method has been widely practiced as reported in the literature (79). The optical density was measured with a spectrophotometer (Varian-DMS 200) at a wavelength of 540 nm, with deionised water as the reference sample. A calibration plot was prepared between optical density and biomass concentration¹ as follows: *P. putida*, was grown in the synthetic growth medium, with phenol as the sole carbon and energy

¹The units of concentration used in this study are g m⁻³. In the literature on hazardous wastes it is more common to see the unit of parts per million (ppm). However, there is an ambiguity whether it is on a weight/weight, weight/volume, or volume/volume basis.
Figure 5.1: Schematic of the experimental unit: (1) constant temperature water bath for feed tank; (2) feed tank, i.e., closed tank containing the untreated waste; (3) programmable sequence controller which is used for automating the SFBR cyclic mode of operation; (4) peristaltic feed pump; (5) solenoid valve; (6) jacketed reactor; (7) circulating water bath; (8) thermometer; (9) dissolved oxygen (D.O.) probe; (10) air diffusion tubes; (11) magnetic stirrer; (12) air pump; (13) oxygen regulating valve; (14) peristaltic discharge pump; (15) discharge tank.
source. The biomass was collected by filtration using a Nylaflon Membrane filter (No 66600, Gelman Sciences Inc., Ann Arbor, MI), and was resuspended in deionised water. This ensured that the salts present in the growth medium would not be present when the weight of the biomass was determined. The optical density of this biomass resuspension was measured and subsequently a 100 ml sample was dried, on aluminum pans, at 60°C in order to determine the weight of the biomass. The original biomass resuspension was diluted with different amounts of deionised water and the optical density of the resulting solutions was measured. The calibration data are given in Table 5.1 while the calibration curve is given in Figure 5.2. There is a linear relationship till an optical density of 0.6 with a slope of 273.3827 g m⁻³ per unit of optical density.

For the SFBR mode of operation, where both bacterial species were used, determination of the concentration of each individual species was required. Unfortunately, both species give rise to colonies of the same shape and size on nutrient agar. However, tests with oxi-ferm tubes (Roche Diagnostic Systems, Nutley, NJ) showed that *P. resinovorans* was citrate positive (in 24 hours) while *P. putida* was not.

On an agar medium with sodium citrate as the sole carbon source it was found that *P. resinovorans* colonies appeared after a 24h incubation at 30°C, while the appearance of *P. putida* colonies required an incubation period of at least 36h. This property led to the use of the following methodology. A 20 µl sample from the reactor was diluted with 5 ml of deionised water. A 20 µl sample of this solution was diluted again with 5ml of deionised water. If the biomass concentration was too high, 10 µl (rather than 20 µl) samples were used in the above procedure. Samples (10 µl) taken from the final solution, were spread plated on nutrient agar and sodium citrate containing agar, using L-shaped glass rods. The plates (petri dishes) were incubated for 24 h at 30°C and then, the colonies on the plates were counted using a colony counter (Gallenkamp Inc.). The ratio of the colony count (average from two plates) from sodium citrate agar plates to the colony
Table 5.1 Data of biomass concentration and corresponding optical density readings for calibration.

<table>
<thead>
<tr>
<th>Sample.No</th>
<th>Optical Density U.O.D</th>
<th>Biomass Concentration g m⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.087</td>
<td>21.2</td>
</tr>
<tr>
<td>3</td>
<td>0.167</td>
<td>42.4</td>
</tr>
<tr>
<td>4</td>
<td>0.244</td>
<td>63.6</td>
</tr>
<tr>
<td>5</td>
<td>0.320</td>
<td>84.8</td>
</tr>
<tr>
<td>6</td>
<td>0.399</td>
<td>106.0</td>
</tr>
<tr>
<td>7</td>
<td>0.473</td>
<td>127.2</td>
</tr>
<tr>
<td>8</td>
<td>0.544</td>
<td>148.4</td>
</tr>
<tr>
<td>9</td>
<td>0.604</td>
<td>169.6</td>
</tr>
<tr>
<td>10</td>
<td>0.667</td>
<td>190.8</td>
</tr>
<tr>
<td>11</td>
<td>0.733</td>
<td>212.0</td>
</tr>
</tbody>
</table>

Figure 5.2: Calibration curve for determination of biomass concentration from optical density readings

Slope = 273.3827
Correlation = 0.999
Table 5.2 Data of phenol concentration and corresponding peak area from HPLC measurements

<table>
<thead>
<tr>
<th>Phenol g m⁻³</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.02</td>
<td>70389</td>
</tr>
<tr>
<td>15.07</td>
<td>212362</td>
</tr>
<tr>
<td>25.12</td>
<td>345631</td>
</tr>
<tr>
<td>50.24</td>
<td>686040</td>
</tr>
<tr>
<td>75.36</td>
<td>1016599</td>
</tr>
<tr>
<td>100.48</td>
<td>1357182</td>
</tr>
<tr>
<td>150.72</td>
<td>1999270</td>
</tr>
</tbody>
</table>

Figure 5.3 Calibration curve for determination of phenol concentration from HPLC peak area

slope = 13238.71

correlation = 0.999
count from the nutrient agar plates represented the fraction of the total biomass consisting of *P. resinovorans*, and was translated to biomass concentration by multiplying the total biomass (from optical density reading) with this fraction. The difference between the total biomass concentration and that of *P. resinovorans*, was the biomass concentration of *P. putida*. This approach minimized the error coming from the serial dilutions of the sample and did not require any knowledge of, or assumption regarding the size of the cells.

5.2.2 Phenol Assay

Phenol concentration was measured as follows. A sample (about 5 ml) was taken from the reactor, and a drop of 6N HCl solution was added to it to kill the microorganisms and to ensure that all phenol was in the molecular form. The sample was then filtered through a 0.2 μm filter paper (Gelman Sciences Inc., Ann Arbor, MI) and the filtrate was immediately processed on a HPLC (SP8800- Spectra Physics Analytical, Piscataway, NJ). The mobile phase was a 55:45 (per volume) ratio of A:B, where A is methanol with 1% acetic acid, and B is deionized water with 1% acetic acid. The flow rate was 1 ml/min. The column used was a Lichrospher 50-RP-Select B (EM Separations, Gibbstown, NJ). The UV detector, a spectra 200 (Spectra Physics Analytical), was set at a wavelength of 280 nm. The data were processed on an IBM-compatible computer using a PE Nelson model 2600 chromatography software Rev 5.10. The data/computer interface was a PE Nelson 900 series interface (PE Nelson Systems Inc, Cupertino, CA). A calibration curve was originally prepared and then frequently tested against standard phenol solutions to ensure the validity of the readings on actual samples. The calibration data are shown in Table 5.2

5.3 Experimental Procedure

5.3.1 Kinetics of Phenol Biodegradation

The kinetics of phenol biodegradation were studied in pure culture batch experiments with each one of the species employed. A number of batch experiments were performed, with
each one of the species, with initial phenol concentrations in the range of 20 to 180 g m\(^{-3}\). In all experiments the initial biomass concentration was kept low (about 4 g m\(^{-3}\)). Small inocula lead to an extended exponential growth phase, thus allowing for enough data collection for determination of the kinetics. The pure cultures, grown in nutrient broth solution, were initially acclimatized to the synthetic medium containing phenol as the sole carbon and energy source at a concentration of 100 g m\(^{-3}\) as follows. A 2 ml sample from the culture which was cultivated in the nutrient broth, was transferred in 100 ml of the synthetic medium and placed in an incubator shaker (250 rpm) at 28°C until all phenol had been depleted. At that point, a 2 ml sample was transferred in 100 ml of fresh synthetic medium, to form the secondary culture, and the procedure was repeated once more to get the tertiary culture. When phenol had been depleted again, the culture was declared a stock culture and inocula were taken from it to run experiments at various phenol concentrations. The stock cultures were maintained by spiking them periodically with phenol and by occasionally transferring a loop to fresh medium, when it was felt that chemicals other than phenol needed replenishment.

The batch experiments were performed in the jacketed lucite reactor shown in Figure 5.1 with a working volume of 1.5 liters. Before each experiment the reactor was cleaned with a dilute solution of hydrogen peroxide to avoid any contamination and then thoroughly washed with water. The synthetic medium with the desired initial phenol concentration was loaded to the reactor at 28.5°C and inoculated with the acclimatized culture. The inoculum size was such that the initial biomass concentration was around 4 g m\(^{-3}\) (optical density = 0.015). The temperature of the reactor contents was maintained at 28.5°C, while the pH of the synthetic medium was 7.2. Samples of about 5ml were drawn at intervals of about 15 minutes to measure the biomass and phenol concentration. The pH, dissolved oxygen and temperature were also measured.
5.3.2 SFBR Experiments

The reactor was operated in the cyclic mode by activating the programmable timer. During the fill phase, the feed rate was constant and the volume of the reactor contents increased from the $V_0$ to $V_m$. In the second phase, the reactor operated in a batch mode. The contents of the reactor were drawn down to $V_0$ in the third phase. Immediately after the end of the third phase the cycle repeated itself. The synthetic waste with the desired phenol concentration was prepared in 20 liter feed tanks and sterilized in an autoclave. The tubing was also sterilized, and the reactor cleaned with a dilute solution of hydrogen peroxide and thoroughly washed with water, before each experiment. SFBR experiments were first performed with each of the two species (pure culture) in order to see if the model could describe these simpler systems before starting the more complex mixed culture experiments. For mixed culture SFBR experiments the feed concentration and the initial conditions in the reactor were chosen in ways that would allow verification of the different types of behavior of the system predicted by the preprepared operating diagram.

The total biomass and phenol concentration were determined as in the case of a batch reactor. Individual biomass concentrations were also measured at appropriate intervals by spread plating the samples on nutrient agar and citrate based agar plates as explained earlier. The dissolved oxygen was carefully monitored. If the dissolved oxygen fell below 85% of its saturation value in water, pure oxygen was mixed with the airstream used for aeration. The experiments were allowed to run for a prolonged period of time in order for the system to reach its eventual steady cycle.

Wall growth was not observed. Furthermore, attachment of cells to the walls of the lucite reactor (to form invisible mono- or oligo- layers) did not seem to occur. This was confirmed as follows. At the end of some preliminary experiments, the reactor was completely drained, filled with tap water and drained again, and finally filled with the synthetic waste. The vessel was allowed to run (in batch mode) over a long period of time and was aerated as during the actual experiments. The measurements indicated that no
change occurred in the phenol concentration which implied that cells of the two species did not attach to the lucite. However, attachment of biomass to the air dispersion tubes was observed during preliminary runs. In subsequent experiments, the air dispersion tubes were changed frequently in order to avoid interference from this phenomenon.
CHAPTER 6
RESULTS AND DISCUSSION

6.1 Determination of Kinetic Parameters

The kinetics of phenol biodegradation were determined from batch experiments. The initial biomass concentration was kept low (about 4 g m\(^{-3}\)) in all experiments. If the initial biomass concentration \(b_0\), is not significantly lower than the product of the yield coefficient and the initial phenol concentration \((YS_0)\), the growth will not be in the exponential phase (25). A semilogarithmic plot of the biomass concentration versus time was prepared for each of the experimental runs. The initial data fell on a line but data towards the end of the run deviated from linearity. In order to understand this behavior and to decide how kinetics can be best determined, a theoretical analysis of the growth in a batch reactor was performed and is presented in the following subsections.

6.1.1 Effect of Initial Biomass Concentration: The kinetic parameters of a species exhibiting inhibitory behavior (Andrews model) were chosen as \(\mu^* = 0.9259\ \text{h}^{-1}\), \(K_s = 11.248\ \text{g}\ \text{m}^{-3}\), \(K_I = 141.142\ \text{g}\ \text{m}^{-3}\), and \(Y = 0.6045\). The mass balance equations on the substrate and biomass (equations 3 and 5 of Chapter 4 with \(b_2 = Q_f = 0\)) were integrated using a fourth order Runge-Kutta method with double precision. Two cases were considered. The initial biomass concentration was assumed to be 10 g m\(^{-3}\) in case A and 100 g m\(^{-3}\) in case B. The initial phenol concentration in both cases was assumed to be 100 g m\(^{-3}\).

The growth of biomass was observed by preparing a semilogarithmic plot of biomass concentration versus time. These plots are shown in Figures 6.1 and 6.2. From these figures it is clear that the exponential growth phase lasts longer when the initial biomass concentration is low. This suggests that in order to get a good number of data in
the exponential phase the inoculum must be small in real experiments. It should be mentioned though that if the biomass concentration is too low, it is hard to detect its changes through optical density readings as the values fall below the minimum detectable level of the spectrophotometer. Based on these considerations it was decided to run the batch experiments with an initial biomass concentration of about 4 g m\(^{-3}\) (OD = 0.015). Furthermore, especially from Figure 6.1 it can be seen that after a certain period of time (about 2 hours for this example) there is a deviation from linearity. This deviation is an inherent feature of Andrews kinetics and suggests that not all data of the run can be considered for determining the specific growth rate.

6.1.2 Determination of the Parameters for the Specific Growth Rate Expression: For determining the parameters in the specific growth rate expression, regression of the specific growth rate values as a function of concentration to the Andrews' (or any other model) is required. Values for the specific growth rate are obtained as slopes of the linear regime of the Ln biomass versus time data. The question is whether this slope should be taken as the specific growth rate value corresponding to the substrate concentration in the
beginning of the run or to the average value of substrate concentrations in the linear regime of the Ln biomass versus time plot. To decide this, a number of numerical experiments were performed as follows. The Andrews' constants reported in the previous subsection were used and perfect biomass data were obtained and plotted as in Figure 6.1 for various initial phenol concentrations. The initial biomass concentration was assumed to be 5 g m\(^{-3}\) in all cases. The slopes were determined and attributed to either the initial phenol concentration or to the average phenol concentration of the linear regime. To further elucidate the fact that data not falling in the linear regime should not be taken into account, the procedure was repeated by using all biomass data up to the point where phenol was depleted. These generated specific growth rate values as function of phenol concentration were regressed to the Andrews model by using a non-linear regression routine based on the Gauss-Marquardt method (19). The question was which methodology would lead to values for the kinetic parameters which would be close to the originally assumed values.

Table 6.1 shows the generated data for the following cases:

Case I: Specific growth rate calculated from biomass data points in the linear regime and attributed to the initial phenol concentration.

Case II: Specific growth rate calculated from biomass data points in the linear regime and attributed to the average phenol concentration in the initial linear range.

Case III: Specific growth rate calculated from all biomass data points in the growth phase and attributed to the initial substrate concentration.

Case IV: Specific growth rate calculated using all biomass data points in the growth phase and attributed to the average substrate concentration in the growth phase.

The details of generating these data are shown in Appendix D.
Table 6.1 Substrate concentrations (initial and average) and specific growth rates

<table>
<thead>
<tr>
<th>Initial Substrate concentration g m⁻³</th>
<th>Average substrate concentration for the linear range g m⁻³</th>
<th>Specific growth rate (initial points) h⁻¹</th>
<th>Average substrate concentration for all points g m⁻³</th>
<th>Specific growth rate (all points) h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>17.287</td>
<td>0.5222</td>
<td>10.60</td>
<td>0.4056</td>
</tr>
<tr>
<td>25</td>
<td>21.925</td>
<td>0.5558</td>
<td>13.51</td>
<td>0.4606</td>
</tr>
<tr>
<td>30</td>
<td>27.04</td>
<td>0.5757</td>
<td>16.41</td>
<td>0.5036</td>
</tr>
<tr>
<td>35</td>
<td>30.40</td>
<td>0.5834</td>
<td>19.53</td>
<td>0.5359</td>
</tr>
<tr>
<td>40</td>
<td>35.35</td>
<td>0.5897</td>
<td>22.94</td>
<td>0.5589</td>
</tr>
<tr>
<td>60</td>
<td>50.86</td>
<td>0.5827</td>
<td>31.34</td>
<td>0.5684</td>
</tr>
<tr>
<td>80</td>
<td>73.12</td>
<td>0.5531</td>
<td>41.345</td>
<td>0.5628</td>
</tr>
<tr>
<td>100</td>
<td>93.73</td>
<td>0.5184</td>
<td>51.41</td>
<td>0.5436</td>
</tr>
<tr>
<td>125</td>
<td>117.28</td>
<td>0.4799</td>
<td>63.25</td>
<td>0.5123</td>
</tr>
<tr>
<td>150</td>
<td>143.15</td>
<td>0.4419</td>
<td>75.60</td>
<td>0.4804</td>
</tr>
<tr>
<td>200</td>
<td>190.87</td>
<td>0.3834</td>
<td>103.75</td>
<td>0.4205</td>
</tr>
</tbody>
</table>

The regressed kinetic parameter values for the above four cases are shown in Table 6.2

Table 6.2 Regressed Andrews parameters for cases I-IV as explained in the text

<table>
<thead>
<tr>
<th></th>
<th>( \mu^* )</th>
<th>( K_s )</th>
<th>( K_I )</th>
<th>error in regression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual</td>
<td>0.9259</td>
<td>11.248</td>
<td>141.142</td>
<td>0.0</td>
</tr>
<tr>
<td>Case I</td>
<td>0.982</td>
<td>14.60</td>
<td>133.85</td>
<td>0.18</td>
</tr>
<tr>
<td>Case II</td>
<td>0.924</td>
<td>11.043</td>
<td>143.93</td>
<td>0.17</td>
</tr>
<tr>
<td>Case III</td>
<td>1.19</td>
<td>17.99</td>
<td>61.429</td>
<td>0.57</td>
</tr>
<tr>
<td>Case IV</td>
<td>1.139</td>
<td>30.729</td>
<td>127.65</td>
<td>0.75</td>
</tr>
</tbody>
</table>
From Table 6.2 it can be concluded that the best estimates for the kinetic parameters are obtained when the specific growth rate is calculated using the points in the linear regime, and attributing it to the average phenol concentration in that regime. It should be also mentioned that the fit of data from cases I, III, and IV is very good as can be seen from the last entry of Table 6.2 which indicates the error in the regression. Nonetheless the values obtained are far away from the actual ones.

6.1.3 Number of Kinetic Runs: The next question which arises regarding the methodology of obtaining the kinetic parameters deals with the number of runs required for an accurate estimation of the parameters. Furthermore, since in actual experiments there is always some error involved, the sensitivity of the kinetic parameter values to the experimental error needs to be found. To answer the foregoing questions numerical experiments were again performed using the same Andrews' constants as in the previous subsections. An error was introduced in the generated perfect data and the number of assumed batch runs was also varied. Four cases were again considered.

Case Ia Error of 5% in points before the maximum of the specific growth rate curve
(low phenol concentrations).

Case IIa Error of 5% in points after the maximum of the specific growth rate curve
(high phenol concentrations).

Case IIIa Case Ia with fewer batch runs

Case IVa Case IIa with fewer batch runs

The data for each of these cases is shown in Appendix D. The regressed values for the biokinetic parameters for the four cases above are summarized in Table 6.3. From this table,
Table 6.3 Regressed Andrews parameters for cases Ia - IVa as explained in the text

<table>
<thead>
<tr>
<th></th>
<th>$\mu^*$</th>
<th>$K_s$</th>
<th>$K_i$</th>
<th>error in regression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual</td>
<td>0.9259</td>
<td>11.248</td>
<td>141.142</td>
<td>-</td>
</tr>
<tr>
<td>Case Ia</td>
<td>0.9132</td>
<td>10.834</td>
<td>145.369</td>
<td>1.66</td>
</tr>
<tr>
<td>Case IIa</td>
<td>0.9512</td>
<td>12.396</td>
<td>140.95</td>
<td>2.35</td>
</tr>
<tr>
<td>Case IIIa</td>
<td>0.8577</td>
<td>7.430</td>
<td>161.20</td>
<td>0.48</td>
</tr>
<tr>
<td>Case IVa</td>
<td>0.9149</td>
<td>11.323</td>
<td>163.38</td>
<td>0.78</td>
</tr>
</tbody>
</table>

It is clear that even though the error from the regression used in estimating the parameters is low, there could be a significant deviation from the actual values of the parameters. This is true especially in Case IIIa where there is a deviation of 34% in the estimation of $K_s$.

From the foregoing considerations one can conclude that the kinetic parameters are more accurately estimated when a lot of data points are available at low substrate concentrations. For this reason, in the actual experiments, a number of low initial phenol concentrations were tried. However, at very low concentrations, the results were not satisfactory. This is due to the following: For very low initial substrate concentrations, in order to have an extended exponential growth phase, the initial biomass concentrations should be very low. The practical difficulty is that very low biomass concentrations cannot be accurately determined by the spectrophotometer.

6.1.4 Experimental Determination of Kinetic Constants: The kinetics of phenol biodegradation by *P. putida* (ATCC 17514) and *P. resinovorans* (ATCC 14235) were studied in batch experiments. A typical Ln (biomass concentration) versus time plot is shown in Fig 6.3. The yield coefficient, for each kinetic run, was determined as follows: biomass and phenol concentration were regressed to the equation $b = b_0 + Y(S_0 - S)$. The biomass versus phenol concentration data corresponding to Figure 6.3 are shown in Figure 6.4. The average of the yield coefficient values from all runs with a particular species was
Figure 6.3 Plot of Ln biomass concentration versus time for determination of the specific growth rate of *P. resinovorans*. Run-10.

Figure 6.4 Plot of biomass concentration versus phenol concentration for the calculation of yield coefficient of *P. resinovorans*. Run-10.
taken as the yield coefficient of that species on phenol. Data and plots for the various kinetic runs with \textit{P. resinovorans} are listed in the Appendix A (Figures A-1 to A-22). The data for \textit{P. putida} are given in Appendix B (Figures B-1 to B-34). These data are summarized in Tables 6.4 and 6.5.

\textbf{Table 6.4 Specific growth rates and yield coefficients for \textit{P. resinovorans} (ATCC 14235)}

<table>
<thead>
<tr>
<th>Expt. Run</th>
<th>Initial Phenol Concentration g m(^{-3})</th>
<th>Average Phenol Concentration g m(^{-3})</th>
<th>Specific growth rate, (\mu) h(^{-1})</th>
<th>Yield coefficient, (Y) (grams biomass/grams phenol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.43</td>
<td>16.88</td>
<td>0.512</td>
<td>0.748</td>
</tr>
<tr>
<td>2</td>
<td>26.85</td>
<td>22.71</td>
<td>0.567</td>
<td>0.589</td>
</tr>
<tr>
<td>3</td>
<td>37.88</td>
<td>34.25</td>
<td>0.624</td>
<td>0.762</td>
</tr>
<tr>
<td>4</td>
<td>41.08</td>
<td>37.81</td>
<td>0.644</td>
<td>0.804</td>
</tr>
<tr>
<td>5</td>
<td>57.05</td>
<td>52.24</td>
<td>0.607</td>
<td>0.683</td>
</tr>
<tr>
<td>6</td>
<td>62.38</td>
<td>56.56</td>
<td>0.5229</td>
<td>0.702</td>
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<td>74.04</td>
<td>70.5</td>
<td>0.5875</td>
<td>0.685</td>
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<td>8</td>
<td>92.15</td>
<td>83.32</td>
<td>0.553</td>
<td>0.626</td>
</tr>
<tr>
<td>9</td>
<td>100.24</td>
<td>82.95</td>
<td>0.4952</td>
<td>0.624</td>
</tr>
<tr>
<td>10</td>
<td>105.05</td>
<td>101.27</td>
<td>0.5056</td>
<td>0.609</td>
</tr>
<tr>
<td>11</td>
<td>150.72</td>
<td>144.41</td>
<td>0.4603</td>
<td>0.597</td>
</tr>
</tbody>
</table>

The average yield coefficient for \textit{P. resinovorans} on phenol was 0.675 with a standard deviation of 0.075.
Table 6.5 Specific growth rates and yield coefficients for *P. putida* (ATCC 17514)

<table>
<thead>
<tr>
<th>Expt. Run</th>
<th>Initial Phenol Concentration g m(^{-3})</th>
<th>Average Phenol Concentration g m(^{-3})</th>
<th>Specific growth rate, (\mu) h(^{-1})</th>
<th>Yield coefficient, (Y) (grams biomass/grams phenol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.81</td>
<td>14.742</td>
<td>0.4490</td>
<td>0.717</td>
</tr>
<tr>
<td>2</td>
<td>19.31</td>
<td>17.796</td>
<td>0.5026</td>
<td>0.802</td>
</tr>
<tr>
<td>3</td>
<td>24.85</td>
<td>22.292</td>
<td>0.5518</td>
<td>0.786</td>
</tr>
<tr>
<td>15</td>
<td>26.71</td>
<td>19.08</td>
<td>0.5140</td>
<td>0.721</td>
</tr>
<tr>
<td>4</td>
<td>31.43</td>
<td>27.835</td>
<td>0.5724</td>
<td>0.738</td>
</tr>
<tr>
<td>5</td>
<td>32.86</td>
<td>28.024</td>
<td>0.5999</td>
<td>0.748</td>
</tr>
<tr>
<td>17</td>
<td>37.28</td>
<td>26.45</td>
<td>0.5711</td>
<td>0.724</td>
</tr>
<tr>
<td>6</td>
<td>41.80</td>
<td>34.884</td>
<td>0.6044</td>
<td>0.848</td>
</tr>
<tr>
<td>7</td>
<td>51.28</td>
<td>44.95</td>
<td>0.5976</td>
<td>0.800</td>
</tr>
<tr>
<td>8</td>
<td>59.40</td>
<td>54.645</td>
<td>0.5747</td>
<td>0.752</td>
</tr>
<tr>
<td>9</td>
<td>74.03</td>
<td>70.234</td>
<td>0.5978</td>
<td>0.781</td>
</tr>
<tr>
<td>10</td>
<td>77.03</td>
<td>74.57</td>
<td>0.6050</td>
<td>0.832</td>
</tr>
<tr>
<td>16</td>
<td>78.66</td>
<td>59.56</td>
<td>0.5709</td>
<td>0.706</td>
</tr>
<tr>
<td>11</td>
<td>82.64</td>
<td>75.45</td>
<td>0.5796</td>
<td>0.726</td>
</tr>
<tr>
<td>12</td>
<td>106.14</td>
<td>98.212</td>
<td>0.5495</td>
<td>0.812</td>
</tr>
<tr>
<td>13</td>
<td>124.78</td>
<td>122.04</td>
<td>0.5330</td>
<td>0.776</td>
</tr>
<tr>
<td>14</td>
<td>154.73</td>
<td>152.17</td>
<td>0.5001</td>
<td>0.785</td>
</tr>
</tbody>
</table>

The average yield coefficient for *P. putida* on phenol was 0.768 with a standard deviation of 0.042.

The specific growth rate data for the two species were regressed to the Andrews expression by using a nonlinear regression routine based on the Gauss-Marquardt method, and the values of the kinetic parameters obtained are shown in Table 6.6.
Table 6.6 Andrews parameters for *P. putida* (ATCC 17514) and *P. resinovorans* (ATCC 14235)

<table>
<thead>
<tr>
<th>Species</th>
<th>$\mu^*$ h$^{-1}$</th>
<th>$K_s$ g m$^{-3}$</th>
<th>$K_I$ g m$^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>0.897</td>
<td>12.204</td>
<td>203.678</td>
</tr>
<tr>
<td>(ATCC 17514)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas resinovorans</em></td>
<td>1.007</td>
<td>12.985</td>
<td>117.75</td>
</tr>
<tr>
<td>(ATCC 14235)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The plots of the specific growth rates versus phenol concentrations are shown in Figures 6.5 and 6.6. The curves denote the predicted specific growth rate based on the kinetic parameters determined. Based on the kinetic parameters, a cross over of the two curves occurs at a single phenol concentration of 46.302 g m$^{-3}$. Because of the crossing of the specific growth rate curves the SFBR model predicts that if the reactor is originally inoculated with both species, coexistence of the two species is possible if the operating parameters are selected in a way such that for part of the cycle phenol concentrations favor *P. putida* and for rest of the cycle they give the competitive advantage to *P. resinovorans*.

6.2 SFBR EXPERIMENTS

6.2.1 Pure Culture Experiments: Once the kinetic parameters were determined, SFBR experiments were conducted, using only one species at a time. The conditions for the run with *P. resinovorans* (SFBR-R) were as follows:

- **fill time**: 1 hour
- **total time**: 2.5 hours
- **Minimum volume**: 0.5 liters
- **Maximum volume**: 2.00 liters
- **Phenol concentration in feed, $S_f$**: 104.0 g m$^{-3}$
- **Initial Phenol concentration, $S_0$**: 15.11 g m$^{-3}$
- **Initial biomass concentration, $b_0$**: 62.878 g m$^{-3}$
Figure 6.5 Specific growth rate curve of *P. putida* (ATCC 17514)

Figure 6.6 Specific growth rate curve of *P. resinovorans* (ATCC 14235)
This experiment was run for four cycles with readings taken in the first and fourth cycle. The phenol and the biomass concentration profiles for the first cycle are shown in Figures 6.7 and 6.8 respectively. The corresponding profiles for the fourth cycle are shown in Appendix C (Figures C-1 and C-2). The experimental data are also listed in the Appendix C. The curves in the plots represent model predictions based on the parameters obtained from the batch experiments and were generated by integrating equations (15)-(18) in chapter 4 with $b_2 = 0$. The integration was performed by using a fourth order Runge-Kutta method (The FORTRAN code is given in Appendix C). As can be seen from these curves there is excellent agreement between the predicted values and the observed values.

For SFBR experiments with a pure culture of *P. putida* (SFBR-P) the conditions were as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill time</td>
<td>0.25 hour</td>
</tr>
<tr>
<td>Total time</td>
<td>1.5 hours</td>
</tr>
<tr>
<td>Minimum volume</td>
<td>2.0 liters</td>
</tr>
<tr>
<td>Maximum volume</td>
<td>4.0 liters</td>
</tr>
<tr>
<td>Phenol concentration in feed, $S_f$</td>
<td>61.35 g m$^{-3}$</td>
</tr>
<tr>
<td>Initial Phenol concentration, $S_0$</td>
<td>0.0 g m$^{-3}$</td>
</tr>
<tr>
<td>Initial biomass concentration, $b_0$</td>
<td>38.82 g m$^{-3}$</td>
</tr>
</tbody>
</table>

The experimental data as well as the phenol and biomass concentration profiles for the first and sixth cycle are shown in the Appendix C (Table C-2; Figures C-3 to C-6). As can be seen from these figures there is excellent agreement between the predicted and the experimentally observed values. Thus, it can be concluded that the model has the capability of predicting SFBR operation with pure cultures and that the kinetic parameters determined in batch experiments are (as expected) independent of the reactor operation mode.
Figure 6.7 Biomass concentration profile for the first cycle of run SFBR-R with *P. resinovorans*.

Figure 6.8 Phenol concentration profile for the first cycle of run SFBR-R with *P. resinovorans*. 
6.2.2 Mixed Culture Experiments

6.2.2.1 Operating Diagram: Before running SFBR experiments with mixed cultures, the model equations (15)-(18) (Chapter 4) were used in numerical studies in order to predict the various types of behavior of the system. The model contains 5 parameters ($\gamma_1, \gamma_2, \eta, \phi, \omega$) which depend on kinetics and are known, and four operating parameters ($\beta, \sigma_1, \delta, u_T$) which can be varied freely. After some initial simulations it was found that using values of $\delta = 0.25$ and $\sigma_1 = 0.40$ would help in the experiments in the sense that in most cases biomass concentrations would not be excessive. For these values an operating diagram was prepared. This diagram, shown in Figure 6.9, indicates what will be the behavior of the SFBR under various phenol concentrations in the untreated waste ($u_T$) and various $\beta$-values which indicate the hydraulic residence time in the reactor. The specific parameter values used for preparing the operating diagram are:

\[
\begin{align*}
\gamma_1 & = 0.059918 \\
\gamma_2 & = 0.103665 \\
\delta & = 0.25 \\
\eta & = 1.137778 \\
\sigma_1 & = 0.40 \\
\phi & = 1.122631 \\
\omega & = 1.063995
\end{align*}
\]

The numerical methods used in preparing the diagram were discussed in Chapter 2. The character of each periodic state of the system in the various regions of the diagram is shown in Table 6.7. It is observed that stable coexistence of the two species is possible in regions 13, 14, 15. Thus, if the operating conditions are such that they define a point in one of these regions, the system is predicted to operate in a state of periodic coexistence. In regions 13 and 14 the coexistence state is the only one which is stable and therefore, the system will reach this state regardless of the start-up conditions. However, in region 15 multistability, i.e., more than one stable state, is observed. Therefore, depending on the
Fig 6.9 Operating diagram for the system in the $\beta$-$u_f$ plane for $\sigma_1=0.4$ and $\delta = 0.25$
Table 6.7 Character of each periodic state in the various regions of the operating diagram (Figure 6.9) of the system*

<table>
<thead>
<tr>
<th>Region</th>
<th>Washout</th>
<th>1-state</th>
<th>2-state</th>
<th>Coexistence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>-</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>U</td>
<td>D</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>D</td>
<td>S, U</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>S</td>
<td>D, D</td>
<td>S, U</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>S</td>
<td>-</td>
<td>S, D</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>S</td>
<td>S, D</td>
<td>S, D</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>D</td>
<td>S</td>
<td>S, D</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>S</td>
<td>S, D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>S</td>
<td>S, D</td>
<td>D, U</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>D</td>
<td>S</td>
<td>D, U</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>U</td>
<td>S</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>U</td>
<td>D</td>
<td>D</td>
<td>S</td>
</tr>
<tr>
<td>14</td>
<td>D</td>
<td>D</td>
<td>D, U</td>
<td>S</td>
</tr>
<tr>
<td>15</td>
<td>S</td>
<td>D, D</td>
<td>D, U</td>
<td>S</td>
</tr>
</tbody>
</table>

* S: stable node or stable focus; U: unstable node or unstable focus; D: saddle (unstable).
1-state: survival of *P. Putida* only; 2-state: survival of *P. resinovorans* only.
initial conditions (start-up) the system will either reach a state of coexistence, or a state of extinction of both species (washout). Multistability is observed in other regions as well. For example in region 10, both the wash out state and the state of survival of *P. putida* only are stable. Multistability must be taken into account during operation, since some significant perturbation could move the system from one state to another.

The features of the operating diagram shown in figure 6.9 are exhibited by any system of two pure and simple competitors when both species follow Andrews' kinetics and their specific growth rate curves cross each other at a single nonzero value. It should be mentioned though that for other model parameter values, such systems have been found to have more complicated types of behavior - quasiperiodicity and chaos (52). If these curves cross each other at two values, the operating diagram is much more complex. The operating diagram is much simpler when the two curves do not cross each other. In such cases, neither coexistence nor a state where only the slower growing species survives is possible.

### 6.2.2.2 Description of Mixed Culture Experiments

Based on the operating diagram, regions for conducting the mixed culture experiments were selected. The basis for selecting the operating conditions for the experiments was to study all possible outcomes, i.e., survival of only one species, washout, and coexistence. The operating conditions were selected in a way which avoided very high biomass concentrations as this could lead to flocculation, oxygen limitation, mass transfer effects and possible foaming. Though these phenomena occur in real systems they were not investigated in the present study. Four experiments were performed under the conditions shown in Table 6.8 and are discussed in the following subsections.
Table 6.8 Operating conditions and initial (start-up) concentrations for mixed culture experiments

<table>
<thead>
<tr>
<th></th>
<th>SFBR -M1</th>
<th>SFBR -M2</th>
<th>SFBR -M3</th>
<th>SFBR -M4</th>
</tr>
</thead>
<tbody>
<tr>
<td>fill time</td>
<td>t₁ (h)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>&quot;react&quot; time</td>
<td>t₂ (h)</td>
<td>2.75</td>
<td>2.75</td>
<td>2.75</td>
</tr>
<tr>
<td>draw time</td>
<td>t₃ (h)</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>reference flow rate</td>
<td>Qₙ* (L h⁻¹)</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>phenol concentration in feed</td>
<td>Sₙ (g m⁻³)</td>
<td>79.15</td>
<td>202.76</td>
<td>202.76</td>
</tr>
<tr>
<td>initial phenol concentration in the reactor</td>
<td>S₀ (g m⁻³)</td>
<td>5.03</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>initial concentration of <em>P. putida</em> in the reactor</td>
<td>b₁₀ (g m⁻³)</td>
<td>10.0</td>
<td>20.0</td>
<td>10.0</td>
</tr>
<tr>
<td>initial concentration of <em>P. resinovorans</em> in the reactor</td>
<td>b₂₀ (g m⁻³)</td>
<td>59.21</td>
<td>56.20</td>
<td>10.0</td>
</tr>
<tr>
<td>minimum volume</td>
<td>V₀ (L)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>maximum volume</td>
<td>Vₘ (L)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>dimensionless feed concentration</td>
<td>uₙ</td>
<td>6.4856</td>
<td>16.6142</td>
<td>16.6142</td>
</tr>
<tr>
<td>dimensionless measure of hydraulic residence time</td>
<td>β</td>
<td>3.588</td>
<td>3.588</td>
<td>3.588</td>
</tr>
<tr>
<td>Region in Figure 6.9</td>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
Run SFBR-M1: The conditions (β and u₁) for this experiment define a point in region-3 of the operating diagram. The prediction is that the only possible steady state for the system is one in which only *P. resinovorans* (ATCC 14235) survives. The reactor was initially inoculated with both species. Figure 6.10 shows the experimental and predicted values for *P. resinovorans* concentration during the 1st, 6th, and 24th cycle of operation. The profiles for the concentration of phenol, total biomass, and biomass of *P. putida* as well as the experimental data are given in Appendix C (Table C-3; Figures C-7 to C-9). The experiment was stopped after the 24th cycle since the concentration of the total biomass was practically equal to the biomass concentration of *P. resinovorans*. As can be seen from the figures there is excellent agreement between the experimental data and theoretical predictions.

**Figure 6.10** *P. resinovorans* biomass concentration profiles for the 1st, 6th, and 24th cycle of SFBR-M1. Curves indicate model predictions. The data are correspondingly represented by □, ◇, and △.
 Runs SFBR-M2 and SFBR-M3: The conditions (β and u_f) for these experiments define a point in region 10 of the operating diagram. The difference between these two experiments is in the initial biomass concentrations. Depending on the start-up conditions the prediction is that the system would reach either a state where no population survives (washout), or a state where only *P. putida* survives.

**Figure 6.11** *P. putida* biomass concentration profiles for the 1st, 16th, 40th, 58th cycle of SFBR-M2. Curves represent model predictions. The data are correspondingly represented by □, ○, △, and ●.

Under the initial biomass concentrations for SFBR-M2 the model predicted that *P. putida* would survive. The biomass concentration of *P. resinovorans* which was predicted to washout, was initially three times higher than that of *P. putida*. This was done in order to verify the model predictions under the "worst scenario". Figure 6.11 shows the experimental and the predicted values for *P. putida* for the 1st, 16th, 40th and 58th cycle. The corresponding figures for the concentrations of phenol, total biomass, and biomass of *P. resinovorans* (Figures C-10 to C-12) as well as the experimental data (Table C-4) are
given in Appendix C. The dynamics are slow but they clearly indicate the survival of \textit{P. putida} while \textit{P. resinovorans} moves toward complete elimination from the reactor. Again, there is excellent agreement between the data and model predictions.

For SFBR-M3, the initial biomass concentration was such that the predicted outcome was the washout of both species. The initial biomass concentrations of both species were equal. Only the total biomass concentration was measured as the predicted outcome was washout. Figure 6.12 shows the phenol concentration profiles for the 1st and 24th cycle. The experimental data (Table C-5) as well as the profiles of the total biomass (Figure C-13) are given in Appendix C. Clearly, the system is proceeding towards total biomass washout. Experiments SFBR-M2 and SFBR-M3 demonstrated that multiple outcomes are possible under the same operating conditions.

\begin{center}
\textbf{Figure 6.12} Phenol concentration profiles for the 1st and 24th cycle of run SFBR-M3. Curves represent model predictions. The data are correspondingly represented by □, and ◇.
\end{center}
Run SFBR-M4: This experiment was performed under conditions which fall in region 14 of the operating diagram. The theoretical prediction is that the only possible state for the system is that of coexistence of the two species. The experiment was started with very low biomass concentrations even though the prediction was that both species would achieve high concentrations in the steady cycle. The data and the model predictions for the first cycle are shown in Figures 6.13 and 6.14. The experimental data (Table C-6) as well as the concentration profiles of phenol, total biomass, biomass of \textit{P. putida} and biomass of \textit{P. resinovorans} (Figures C-14 to C-17) are shown in the Appendix C. Figures C-14 to C-17 show the data and model predictions for the 21st and the 28th cycle. There is a clear indication that the biomass concentration of both species increases. Furthermore, while the phenol and total biomass concentration reach the steady cycle relatively fast, the individual biomass concentrations exhibit very slow dynamics. The model predicts that it would take about 1400 cycles to reach the steady cycle. For this reason, the system was perturbed by increasing the concentration of \textit{P. resinovorans}. This species was grown separately in pure culture, and a certain volume of this suspension was added to the mixed culture at the end of the 28th cycle. The concentrations and volumes were selected in a way such that the composition of the mixed culture would represent a point on the steady cycle. Figures C-14 to C-17 also show the data and the predicted values for the 4th and 11th cycle after the perturbation. Clearly the system reached a steady cycle of coexistence as predicted by the model.

During the initial operation of the reactor under conditions of SFBR-M4 the dissolved oxygen concentration levels fell to very low values. For this reason, oxygen was mixed with air before it entered the reactor, so that the dissolved oxygen levels were maintained above 6.8 g m\textsuperscript{-3}. Furthermore, the high biomass concentrations obtained in SFBR-M4 led to a higher dilution requirement for the samples before plating. This may explain why the agreement between the data and model predictions for individual biomass concentrations
Figure 6.13 Phenol concentration profile for the 1st cycle of SFBR-M4. Curve represents model prediction.

Figure 6.14 Biomass concentration profiles for the 1st cycle of SFBR-M4. Curves represent model predictions.
is not as good as in other experiments. Finally, the cycle time shown in Figures C-14 to C-17 is 4 hours even though it is reported as 6.875 hours in Table 6.8. Actually, phenol gets totally depleted in the first three hours of the cycle; for this reason after 15 cycles, the cycle time was set at 4 hours.

It can be concluded from these experiments that the model equations describe the system very well. All model predictions were experimentally validated and it should be emphasized that this is the first experimental demonstration of coexistence of two pure and simple competitors in a reactor which has periodically varying inputs.
CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

In this dissertation the dynamics of pure and simple competition between two species in a sequencing fed-batch reactor were studied both at the theoretical and the experimental level. This study offers the first experimental demonstration of coexistence of two pure and simple competitors in a spatially homogeneous reactor having a time dependent input flowrate. A detailed model has been derived, numerically solved, and experimentally validated. Predictions of complex dynamics and multiple outcomes under certain operating conditions have been experimentally shown to actually occur. The model is primarily based on the kinetics of utilization of the substrate competed for by the two populations. Kinetic expressions were revealed from independent pure culture batch experiments and for phenol, they were found to follow Andrews' inhibitory expression.

A systematic study has been performed regarding the methodology for obtaining the kinetic parameters in the Andrews expression. The results suggest that batch experiments need to be performed with low initial biomass concentrations and for a wide spectrum of initial substrate (phenol) concentrations. The values of kinetic parameters are more sensitive to rates obtained at low concentrations, thus more experiments are needed in the non-inhibitory region rather than at high (inhibitory) concentrations. For small initial biomass concentrations an exponential growth phase can be identified (linear region on a semi-log plot). The slope is the specific growth rate corresponding to the average substrate concentration during the exponential growth phase. The common practice of attributing the specific growth rate to the initial substrate concentration seems incorrect.

Regarding coexistence, it can be concluded that it requires the crossing of the specific growth rate curves of the two competitors at least at one, non-zero, value of the substrate concentration. The crossing of the specific growth rate curves of the two
populations employed in this study occurs at $S_c = 46.302 \text{ g m}^{-3}$ (or $u_c = 2.96$). In the operating diagram (Figure 6.9), the region where coexistence of two populations occurs, originates from the point $u_T = 3.793 = u_c$.

The implications of the results of this dissertation for the degradation of hazardous wastes may be illustrated by the following hypothetical example. Suppose that, as is true in many cases, a mixed unidentified culture is to be used. Initial studies are performed in batch experiments to determine the kinetics of biodegradation, and the biomass is treated as a single population. These kinetics are only apparent expressions and depend on the biomass composition. Then, a pilot SFBR study is conducted in a particular region of the operating diagram, say region 11 of Figure 6.9. The biomass composition changes with time and thus, the pilot data cannot be properly explained based on small scale experiments. If the pilot unit is operated long enough, one (or some) species will washout and performance will be stabilized. If these data are used for the final design, and as a safety factor the hydraulic residence time ($\beta$) is increased, the system will find itself in another region of the operating parameter space. Assume for example that the pilot unit is run at $u_T = 35$ and $\beta = 7.5$, and that the design of the actual unit is for $u_T = 35$ and $\beta = 8.5$. The system moves from region 11 to region 3, and if the original inoculum used in the pilot and the full-scale unit is the same, the steady-cycle biomass composition differs between the two units. The significance of the resulting scale-up error depends on the difference in the biokinetic parameters of the two species.

Another example where a mixed culture is actually needed is as follows. Consider a waste which contains recalcitrant pollutants, A and B. Assume that two strains, M and N are isolated or developed and have the ability to co-metabolize pollutants A and B, respectively. Co-metabolism of a compound implies that it cannot serve either as energy or carbon source for the bacteria; the transformation is a side effect of the growth of bacteria on another (primary) substrate. If the same primary substrate is used for species M and N, then they compete for it. Thus, treatment of pollutants A and B in the same vessel is
impossible unless a mixed culture of species M and N is maintained. Random selection of experimental conditions would lead to a lengthy and costly process development phase. This study indicates where (regions 13, 14, and 15 of Figure 3) the experiments should really be performed.

Now that a mathematical model which has been fully validated with experiments exists, a number of further studies can be performed. One can optimize SFBR design and operation by proper selection of operating parameters such as $\sigma_1$ (indicating slow or fast fill), $\delta$ (indicating the fraction of the reactor contents which should remain in the reactor at the end of a cycle), and $\beta$ (hydraulic residence time). Optimization can be performed subject to constraints, such as "maintain a mixed culture", or maintain species which can persist in $\Omega$, where $\Omega$ is a prespecified domain of the operating parameter space. The objective function for optimization can be either based on the capital cost or on maximum throughput for a given reactor volume. These optimization studies would need development of proper computer software since most existing programs are for steady state rather than for periodic operation of a reactor.

It is believed that the present study contributes towards a fundamental understanding of SBR (or SFBR) technology. At the same time it is realized that more complex systems need now to be studied. Such systems should involve multiple substrates (i.e., mixed wastes). Mixed substrates may lead to cross-inhibitory effects or to cometabolism. Such topics are of interest and it is recommended that they be studied in the future. Furthermore, questions on oxygen limitation, flocculation, and settling of biomass should also be addressed at a fundamental level. Finally, it is recommended that a closer collaboration of engineers and microbiologists be established in order to effectively address questions such as methods for fast and easy species identification, and possible formation of stable intermediates during biodegradation of complex wastes.
APPENDIX A

Experimental Data for *Pseudomonas resinovorans* (ATCC 14235)

The experimental data for determination of the specific growth rate and yield coefficient of *Pseudomonas resinovorans* (ATCC 14235) on phenol are given in this appendix. Tables A-1 to A-11 list the experimental data for the eleven batch experiments. The odd numbered figures show the plots of Ln biomass concentration versus time for the determination of the specific growth rate. The even numbered figures show the plots of biomass concentration versus phenol concentration for the determination of the yield coefficient. Yield coefficients were determined based on the data used for determining the specific growth rate (linear regime of the Ln biomass concentration versus time plot).
Table A-1  Run-1. The exponential growth phase began at $t = 1.0$ h. The average phenol concentration in the linear regime was 16.88 g m$^{-3}$

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Time (h)</th>
<th>Optical density</th>
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<th>Phenol concentration (g m$^{-3}$)</th>
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Table A-5  Run-5. The exponential growth phase began at $t = 0.75$ h. The average phenol concentration in the linear regime was $52.24$ g m$^{-3}$

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Table A-11  Run-11. The exponential growth phase began at $t = 0.75$ h. The average phenol concentration in the linear regime was 144.41 g m$^{-3}$.

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Figure A-1  Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-1.

Figure A-2  Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-1
Figure A-3  Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-2

Figure A-4  Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-2
Figure A-5  Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-3.

Figure A-6  Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-3
Figure A-7  Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-4.

Figure A-8  Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-4
Figure A-9  Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-5.

Figure A-10  Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-5
Figure A-11 Plot of \( \ln \) biomass concentration versus time for determination of the specific growth rate. Run-6.

Figure A-12 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-6
**Figure A-13** Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-7.

**Figure A-14** Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-7
**Figure A-15** Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-8.

**Figure A-16** Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-8
Figure A-17  Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-9.

Figure A-18  Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-9
Figure A-19 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-10.

Figure A-20 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-10
Figure A-21 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-11.

Figure A-22 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-11
APPENDIX B

Experimental Data for *Pseudomonas putida* (ATCC 17514)

The experimental data for determination of the specific growth rate and yield coefficient of *Pseudomonas putida* (ATCC 17514) on phenol are given in this appendix. Tables B-1 to B-17 list the experimental data for the seventeen batch experiments. The odd numbered figures show the plots of Ln biomass concentration versus time for the determination of the specific growth rate. The even numbered figures show the plots of biomass concentration versus phenol concentration for the determination of the yield coefficient. Yield coefficients were determined based on the data used for determining the specific growth rate (linear regime of the Ln biomass concentration versus time plot).
Table B-1  Run-1. The exponential growth phase began $t = 0.5$ h. The average phenol concentration in the linear regime was 14.74 g m$^{-3}$.

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<th>Time (h)</th>
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<th>Phenol concentration (g m$^{-3}$)</th>
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Table B-2: Run-2. The exponential growth phase began at $t = 0.5$ h. The average phenol concentration in the linear regime was $17.79$ g m$^{-3}$

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<th>Phenol concentration g m$^{-3}$</th>
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<th>Phenol concentration (g m$^{-3}$)</th>
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Table B-7  Run-7. The exponential growth phase began at $t = 1.0 \text{ h}$. The average phenol concentration in the linear regime was $34.88 \text{ g m}^{-3}$

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Table B-8  Run-8. The exponential growth phase began at $t = 1.25 \text{ h}$. The average phenol concentration in the linear regime was $26.45 \text{ g m}^{-3}$

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Table B-10  Run-10. The exponential growth phase began at $t = 1.0 \text{ h}$. The average phenol concentration in the linear regime was 54.64 g m$^{-3}$

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Table B-11 The exponential growth phase began at $t = 0.75$ h. The average phenol concentration in the linear regime was 70.23 g m$^{-3}$

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Table B-12  Run-12. The exponential growth phase began at \( t = 0.833 \) h. The average phenol concentration in the linear regime was 74.57 g m\(^{-3}\).

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Table B-13 Run-13. The exponential growth phase began at $t = 1.5 \text{ h}$. The average phenol concentration in the linear regime was $75.45 \text{ g m}^{-3}$

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Table B-15  Run-15. The exponential growth phase began at \( t = 1.0 \) h. The average phenol concentration in the linear regime was 98.21 g m\(^{-3}\).

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The exponential growth phase began at $t = 0.75$ h. The average phenol concentration in the linear regime was $122.04 \text{ g m}^{-3}$

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Table B-17 Run-17. The exponential growth phase began at \( t = 1.0 \) h. The average phenol concentration in the linear regime was 152.17 g m\(^{-3}\).

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Figure B-1  Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-1.

Figure B-2  Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-1
Figure B-3  Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-2.

Figure B-4  Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-2
Figure B-5 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-3.

Figure B-6 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-3
Figure B-7 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-4.

Figure B-8 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-4
Figure B-9. Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-5.

Figure B-10 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-5
Figure B-11  Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-6.

Figure B-12  Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-6
**Figure B-13.** Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-7.

**Figure B-14.** Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-7.
Figure B-15 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-8.

Figure B-16 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-8.
**Figure B-17** Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-9.

**Figure B-18** Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-9
Figure B-19 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-10.

Figure B-20 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-10.
Figure B-21 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-11.

Figure B-22 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-11
Figure B-23 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-12.

Figure B-24 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-12
Figure B-25 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-13.

Figure B-26 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-13
Figure B-27 Plot of \( \ln \) biomass concentration versus time for determination of the specific growth rate. Run-14.

Figure B-28 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-14
Figure B-29 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-15.

Figure B-30 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-15.
Figure B-31 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-16.

Figure B-32 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-16
Figure B-33 Plot of Ln biomass concentration versus time for determination of the specific growth rate. Run-17.

Figure B-34 Plot of biomass concentration versus phenol concentration for the determination of the yield coefficient. Run-17
APPENDIX C

Experimental data from SFBR experiments

The experimental data from the SFBR experiments are given in this appendix. Table C-1 and Figures C-1 and C-2 are for pure culture runs using *Pseudomonas resinovorans* (ATCC 14235). Table C-2 and Figures C-3 and C-4 are for pure culture runs using *Pseudomonas putida* (ATCC 17514). Tables C-3 to C-6 list the experimental data for the mixed culture experiments. The concentration profiles for the mixed culture experiments are shown in Figures C-5 to C-17. Some of the profiles are given in the main body of the dissertation (see Chapter 6). The FORTRAN code for the integration of the mass balance equations using fourth order Runge-Kutta method is also given.
Table C-1  Experimental data from an SFBR run (SFBR-R) with *P. resinovorans*

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Figure C-1 Biomass concentration profile for the fourth cycle of the SFBR run with \( P. \text{ resinovorans} \) - (SFBR-R). The curve indicates model predictions.

Figure C-2 Phenol concentration profile for the fourth cycle of the SFBR run with \( P. \text{ resinovorans} \) - (SFBR-R). The curve indicates the model predictions.
Table C-2 Experimental Data for SFBR run (SFBR-P) with *Pseudomonas putida*

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Figure C-3  Biomass concentration profile for the first cycle of the SFBR run with *P. putida* - (SFBR-P). The curve indicates model predictions.

Figure C-4  Phenol concentration profile for the first cycle of the SFBR run with *P. putida* - (SFBR-P). The curve indicates model predictions.
Figure C-5 Biomass concentration profile for the sixth cycle of the SFBR run with *P. putida* - (SFBR-P). The curve indicates model predictions.

Figure C-6 Phenol concentration profile for the sixth cycle of the SFBR run with *P. putida* - (SFBR-P). The curve indicates model predictions.
### Table C-3 Experimental data for SFBR run SFBR-M1

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Figure C-7 *P. putida* concentration profiles for the 1st, 6th, and 24th cycle of SFBR-M1. Curves indicate model predictions. The data are correspondingly represented by □, ○, and △.

Figure C-8 Phenol concentration profiles for the 1st, 6th, and 24th cycle of run SFBR-M1. Curves represent model predictions. The data are correspondingly represented by □, ○, and △.
Figure C-9  Total Biomass concentration profiles for the 1st, 6th, and 24th cycle of SFBR-M1. Curves indicate model predictions. The data are correspondingly represented by □, ◊, and △.
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Table C-4 (contd)

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<th>Total Biomass g m$^{-3}$</th>
<th>Phenol g m$^{-3}$</th>
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Figure C-10  Phenol concentration profiles for the 1st, 16th, 40th, and 58th cycle of SFBR-M2. Curves represent model predictions. The data are correspondingly represented by □, ◆, △, and ○.

Figure C-11  *P. resinovorans* concentration profiles for the 1st, 16th, 40th, and 58th cycle of SFBR-M2. Curves represent model predictions. The data are correspondingly represented by □, ◆, △, and ○.
Figure C-12 Total biomass concentration profile for the 1st, 16th, 40th, and 58th cycle of SFBR-M2. Curves represent model predictions. The data are correspondingly represented by □, ◆, △, and ○.
Table C-5 Experimental data for SFBR run SFBR-M3

<table>
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<th>Time (h)</th>
<th>Optical density</th>
<th>Total Biomass g m⁻³</th>
<th>Phenol g m⁻³</th>
<th>Optical density</th>
<th>Total Biomass g m⁻³</th>
<th>Phenol g m⁻³</th>
</tr>
</thead>
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Figure C-13 Total biomass concentration profiles for the 1st, and 24th cycle of SFBR-M3. Curves represent model predictions.
Table C-6 Experimental data for SFBR run SFBR-M4

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<th>Total Biomass g m⁻³</th>
<th>Phenol g m⁻³</th>
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Table C-6 (contd)

### Cycle 21

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<th>Total Biomass</th>
<th>Phenol</th>
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<th>Total Biomass</th>
<th>Phenol</th>
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Table C-6 (contd)

**Cycle 32 (Cycle 4 after perturbation as explained on p. 54)**

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<th>Total Biomass g m(^{-3})</th>
<th>Phenol g m(^{-3})</th>
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**Cycle 39 (Cycle 11 after perturbation)**

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<th>(P. \text{ resinovorans}) g m(^{-3})</th>
<th>Total Biomass g m(^{-3})</th>
<th>Phenol g m(^{-3})</th>
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Figure C-14  Phenol concentration profiles for the 21st, 28th, 32nd, and 39th cycle of SFBR-M2. Curves represent model predictions. The data are correspondingly represented by □, ◊, △, and ○.

Figure C-15  Total Biomass concentration profile for the 21st, 28th, 32nd, and 39th cycle of SFBR-M2. Curves represent model predictions. The data are correspondingly represented by □, ◊, △, and ○.
Figure C-16 *P. resinovorans* concentration profiles for the 21st, 28th, 32nd, and 39th cycle of SFBR-M4. Curves represent model predictions. The data are correspondingly represented by □, ◆, △, and ○.

Figure C-17 *P. putida* concentration profiles for the 21st, 28th, 32nd, and 39th cycle of SFBR-M4. Curves represent model predictions. The data are correspondingly represented by □, ◆, △, and ○.
**PROGRAM FOR SOLVING MODEL EQUATIONS FOR A SEQUENCING FED-BATCH REACTOR.**

CASE CONSIDERED: TWO SPECIES FOLLOWING ANDREWS KINETICS

**IMPLICIT DOUBLE PRECISION (A-H,O-Z)**

CHARACTER*10 FNAME

WRITE(*,691)

FORMAT(' enter the output file name CYCLE 1:')

READ (*,695)fname

FORMAT(A10)

OPEN (1,FILE=FNAME,STATUS='UNKNOWN')

WRITE (*,692)

FORMAT(' enter output file name all cycles:')

READ(*,695)FNAME

OPEN ( 6 ,  FILE=FNAME, STATUS='UNKNOWN')

WRITE (*,693)

FORMAT(' enter output file name CYCLE 21 : ') 

READ(*,695)FNAME

OPEN (4,FILE=FNAME, STATUS='UNKNOWN' )

WRITE (*,694)

FORMAT(' enter output file name CYCLE 28: ') 

READ ( * ,  695) FNAME

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WRITE (*,696)

FORMAT(' enter data file name:')

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READ (11,*)AMUA, AKSA, AKIA, YA, V0, VMAX, T1, T2, T3, S0, SF

WRITE (*,691)

FORMAT(' UA=',F10.5,2x,'KsA=',2x,f10.5,2X,'KiA=',2x,f10.5,2x,'YA=',f10.5)

WRITE(6,334) AMUB, AKSB, AKIB, YB

WRITE(6,335) V0, VMAX

FORMAT(2X, ' INITIAL VOL=',F5.2,2X,'Final Vol = ',F5.2)

WRITE (6,334) BA0, BB0

FORMAT(' INITIAL BIOMASS A =',F8.4,2X,'INITIAL BIOMASS B =',F8.4)

DEL=V0/VMAX

S1=T1/T3

S3=(T3-T2)*(1/T3)

GA=AKSA/AKIA

GB=AKSA/AKIB

W=AKSB/AKSA

PHI=AMIB/AMUA

ETA=YA/YB

QR=((VMAX-V0)/T1)*S1

U0=S0/AKSA

XAO=BA0/(AKSA*YA)

XBO=BB0/(AKSA*YA)

UF=SF/AKSA

BETA=AMUA*VMAX/QR

WRITE (*,*) UF, BETA, QR, S1, DEL

**DATA H,N/0.001,100/**

U=U0

XA=XAO

XB=XBO

WRITE(6,185)
FORMAT(7X,'TIME',8X,'BIO. CONC. A',2X,'BIO. CONC. B',3X,'PHENOL CONC.'),

ICOUNT=1
BA=AKSA*YA*XA
BB=AKSA*YA*XB
SPH=AKSA*U
TIME=0.0
INDEX=(ICOUNT/5)*S-icount
IF(INDEX.EQ.0) then
WRITE(6,183) time, BA, BB, SPH
WRITE(1,181) time, BA, BB, (BA+BB), SPH
ENDIF
DO 130 I=1,60
AL=1.0
T=0.001
TIME=0.0
C

FILL PHASE

C

ICOUNT=0
WRITE(6,184)1
FORMAT(5X, 'FILL PHASE',2X,'CYCLE No.=',I2)
IF ( (I.EQ.1))then
WRITE(1,181) TIME,BA,BB,(BA+BB),SPH
ENDIF
IF(I.eq.28)THEN
WRITE(10,181) TIME,BA,BB,(BA+BB),SPH
ENDIF
IF ( I  .eq.21)THEN
WRITE(4,181) TIME,BA,BB,(BA+BB),SPH
ENDIF
ICOUNT=ICOUNT+1
CALL RUNGE (H, T, U, XA, XB, S1, DEL, BETA, UF, AL, GA, GB, W, PHI, ETA)
BA=aksa*YA*XA
BB=AKSA*YA*XB
SPH=aksa*U
T1=T-.001
T2=T1*VMAX/QR
INDEX=(ICOUNT/5)*S-icount
IF ((I.EQ.21).and.((ICOUNT.EQ.1).or.(INDEX.EQ.0))) then
WRITE(4,181) T2,bA,BB,(BA+BB),SPH
endif
IF ( (I.EQ.1).and.((ICOUNT.EQ.1).or.(INDEX.EQ.0))) then
WRITE(1,181) T2,bA,BB,(BA+BB),SPH
ENDIF
IF ((I.EQ.28).and.((ICOUNT.EQ.1).or.(INDEX.EQ.0))) then
WRITE(10,181) T2,bA,BB,(BA+BB),SPH
ENDIF
FORMAT(5(5X, F9.5))
IF(T.LE.(1.0-DEL)*S1)THEN
GO TO 50
ENDIF
WRITE(6,183) T2,bA,BB,SPH

C

REACT PHASE

C

WRITE(6,186)1
FORMAT(5X,'REACT PHASE',2X,'CYCLE No.=',I2)
AL=0
ICOUNT=ICOUNT+1
CALL RUNGE (H, T, U, XA, XB, S1, DEL, BETA, UF, AL, GA, GB, W, PHI, ETA)
BA=aksa*YA*XA
BB=AKSA*YA*XB
SPH=aksa*U
T1=T-.001
T2=T1*VMAX/QR
BA = aksA * YA *XA
BB = AKSA * YA *XB
SPH = aksA *U
T1 = T - 0.001
T2 = T1 * VMAX / QR
index = (ICOUNT / 5) * 5 - icount
if ( (i . eq. 21) . and. (index . eq. 0) ) then
WRITE(4,181) T2, bA, BB, (BA + BB), SPH
endif
if ( (i . eq. 1) . and. ((icount . eq. 1) . or. (index . eq. 0)) ) then
WRITE(1,181) T2, bA, BB, (BA + BB), SPH
endif
if ( (i . eq. 28) . and. ((icount . eq. 1) . or. (index . eq. 0)) ) then
WRITE(10,181) T2, bA, BB, (BA + BB), SPH
endif
FORMAT(4(5X,F9.5))
IF (T . LE. (1.0 - DEL) * (1.0 - S3)) THEN
GO TO 70
ENDIF
write(6,183)t2,bA,BB,SPH
C
C DRAW PHASE
C
WRITE(6,187)I
FORMAT(5X,'DRAW PHASE',2X,'CYCLE No.=',I2)
80 ICOUNT = ICOUNT + 1
CALL RUNGE (H, T, U, XA, XB, S1, DEL, BETA, UF, AL, GA, GB, W, PHI, ETA)
BA = aksA * YA *XA
BB = AKSA * YA *XB
SPH = aksA *U
T1 = T - 0.001
T2 = T1 * VMAX / QR
index = (ICOUNT / 5) * 5 - icount
if ( (i . eq. 21) . and. (index . eq. 0) ) then
WRITE(4,181) T2, bA, BB, (BA + BB), SPH
endif
if ( (i . eq. 1) . and. ((icount . eq. 1) . or. (index . eq. 0)) ) then
WRITE(1,181) T2, bA, BB, (BA + BB), SPH
endif
if ( (i . eq. 28) . and. ((icount . eq. 1) . or. (index . eq. 0)) ) then
WRITE(10,181) T2, bA, BB, (BA + BB), SPH
endif
FORMAT(4(6X,F8.4))
IF (T . LE. (1.00001 - DEL)) THEN
GO TO 80
ENDIF
write(6,183)t2,bA,BB,SPH
write (6,105)I
105 format(2x,'***************end of cycle********',i2)
130 CONTINUE
C111 continue
STOP
END
C
C INTEGRATION TO SOLVE THE MASS BALANCE ON SUBSTRATE &
EIMASS OF TWO SPECIES
FOURTH ORDER RUNGE KUTTA METHOD
SUBROUTINE RUNGE (H, T, U, XA, XB, S1, DEL, BETA, UF, AL, GA, GB, W, PHI, ETA)
IMPLICIT REAL*8 (A-H,0-Z)
FI (T, U, XA, XB) = AL * (UF - U) / (S1 * DEL + T) -
1 (XA * U * BETA) / (1.0 + U * (GA * U**2)) -
4 (XB * U * BETA * PHI * ETA) / (W + (GB * U**2))
F2 (T, U, XA, XB) = -(AL * XA) / (S1 * DEL + T) +
4 (XA * U * BETA) / (1.0 + U * (GA * U**2))
F3 (T, U, XA, XB) = -(AL * XB) / (S1 * DEL + T) +
\[ \frac{(X \cdot U \cdot \text{Beta} \cdot \Phi)}{(W + U + (GB \cdot U^2))} \]

\[ A_{K1} = H \cdot F1(T, U, XA, XB) \]
\[ B_{K1} = H \cdot F2(T, U, XA, XB) \]
\[ C_{K1} = H \cdot F3(T, U, XA, XB) \]
\[ A_{K2} = H \cdot F1(T + H/2, U + A_{K1}/2, XA + B_{K1}/2, XB + C_{K1}/2) \]
\[ B_{K2} = H \cdot F2(T + H/2, U + A_{K1}/2, XA + B_{K1}/2, XB + C_{K1}/2) \]
\[ C_{K2} = H \cdot F3(T + H/2, U + A_{K1}/2, XA + B_{K1}/2, XB + C_{K1}/2) \]
\[ A_{K3} = H \cdot F1(T + H/2, U + A_{K2}/2, XA + B_{K2}/2, XB + C_{K2}/2) \]
\[ B_{K3} = H \cdot F2(T + H/2, U + A_{K2}/2, XA + B_{K2}/2, XB + C_{K2}/2) \]
\[ C_{K3} = H \cdot F3(T + H/2, U + A_{K2}/2, XA + B_{K2}/2, XB + C_{K2}/2) \]
\[ A_{K4} = H \cdot F1(T + H, U + A_{K3}, XA + B_{K3}, XB + C_{K3}) \]
\[ B_{K4} = H \cdot F2(T + H, U + A_{K3}, XA + B_{K3}, XB + C_{K3}) \]
\[ C_{K4} = H \cdot F3(T + H, U + A_{K3}, XA + B_{K3}, XB + C_{K3}) \]
\[ U = U + (A_{K1} + 2 \cdot A_{K2} + 2 \cdot A_{K3} + A_{K4}) / 6 \]
\[ XA = XA + (B_{K1} + 2 \cdot B_{K2} + 2 \cdot B_{K3} + B_{K4}) / 6 \]
\[ XB = XB + (C_{K1} + 2 \cdot C_{K2} + 2 \cdot C_{K3} + C_{K4}) / 6 \]
\[ T = T + H \]
\[ \text{RETURN} \]
\[ \text{END} \]
APPENDIX D

Methodology for batch biodegradation kinetics and sensitivity analysis
Determination of Specific Growth Rates

In order to develop a method for measurement of specific growth rate for a species exhibiting inhibitory kinetics, the kinetic parameters were chosen as

\[ \mu^* = 0.9259 \text{ h}^{-1} \]
\[ K_s = 11.248 \text{ g m}^{-3} \]
\[ K_i = 141.142 \text{ g m}^{-3} \]
\[ Y = 0.6045 \text{ g g}^{-1} \]

The mass balance equations on the substrate and biomass (equation 3 and 5 of chapter 4 with \( b_2 = Q_f = 0 \)) were numerically integrated. Eleven substrate concentrations were studied in the range of 20 - 200 g m\(^{-3}\) (Table 6.1). The initial biomass concentration in all cases was 5 g m\(^{-3}\). The integrations were carried till the substrate concentration became zero. The biomass and substrate concentrations were recorded at 0.1 h intervals. These data were considered as equivalent to "perfect" experimental data. A plot of logarithm of biomass concentration versus time revealed that there was a linear regime, followed by a positive deviation from linearity. The data falling in the linear regime, as well as all growth data were regressed to a linear expression. Obviously, the correlation was better for the data falling in the linear regime. The slope which would be equal to the specific growth rate could be attributed to either the initial phenol concentration or the average substrate concentration. A non linear regression routine based on the Gauss-Marquardt method was used to regress the specific growth rate versus substrate data. The program listing is given at the end of this appendix. Comparison of the parameters obtained for each of the four cases showed that the best results were obtained when the slope of the linear regime was considered and attributed to the average phenol concentration in that regime. These slopes (specific growth rates) were then considered for sensitivity analysis by introducing an error (about 5%) in the data and/or by having fewer experimental points before or after the maximum. The values are given in Table D-1.
Table D-1  Specific growth rates for sensitivity analysis
The maximum occurs at $S^* = (K_s K_i)^{0.5} = 39.84$

<table>
<thead>
<tr>
<th>Substrate concentration (g m$^{-3}$)</th>
<th>Linear regime</th>
<th>Specific growth rate-actual (h$^{-1}$)</th>
<th>Specific growth rate Case Ia (h$^{-1}$)</th>
<th>Specific growth rate Case IIa (h$^{-1}$)</th>
<th>Specific growth rate Case IIIa (h$^{-1}$)</th>
<th>Specific growth rate Case IVa (h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td></td>
<td>0.5222</td>
<td>0.5483</td>
<td>0.5222</td>
<td>0.5483</td>
<td>0.5222</td>
</tr>
<tr>
<td>25</td>
<td>0-1</td>
<td>0.5558</td>
<td>0.5280</td>
<td>0.5558</td>
<td>0.5975</td>
<td>0.5558</td>
</tr>
<tr>
<td>30</td>
<td>0-1</td>
<td>0.5757</td>
<td>0.5469</td>
<td>0.5757</td>
<td>-</td>
<td>0.5757</td>
</tr>
<tr>
<td>35</td>
<td>0-1.4</td>
<td>0.5834</td>
<td>0.6125</td>
<td>0.5834</td>
<td>-</td>
<td>0.5834</td>
</tr>
<tr>
<td>40</td>
<td>0-1.4</td>
<td>0.5897</td>
<td>0.5897</td>
<td>0.5897</td>
<td>0.5897</td>
<td>0.5897</td>
</tr>
<tr>
<td>50</td>
<td>0-2</td>
<td>0.5827</td>
<td>0.5827</td>
<td>0.5535</td>
<td>0.5827</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>0-2</td>
<td>0.5531</td>
<td>0.5531</td>
<td>0.5807</td>
<td>0.5531</td>
<td>0.5531</td>
</tr>
<tr>
<td>80</td>
<td>0-2</td>
<td>0.5184</td>
<td>0.5184</td>
<td>0.5443</td>
<td>0.5184</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>0-2.5</td>
<td>0.4799</td>
<td>0.4799</td>
<td>0.5339</td>
<td>0.4799</td>
<td>-</td>
</tr>
<tr>
<td>150</td>
<td>0-2.5</td>
<td>0.4419</td>
<td>0.4419</td>
<td>0.4639</td>
<td>0.4419</td>
<td>0.4419</td>
</tr>
<tr>
<td>200</td>
<td>0-3.5</td>
<td>0.3834</td>
<td>0.3834</td>
<td>0.3642</td>
<td>0.3834</td>
<td>-</td>
</tr>
</tbody>
</table>
C PROGRAM FOR SIMULATION OF LN B VS TIME
C FOR ANDREWS MODEL
*****************************************
DOUBLE PRECISION U, AKS, AKI, Y, S0, S, B0, B, H, T
CHARACTER*10, FNAME
WRITE(*,691)
691 FORMAT ('$ ENTER OUTPUT FILENAME:')
READ(*,695)FNAME
695 FORMAT(A10)
OPEN (6, FILE=FNAME, STATUS='UNKNOWN')
OPEN(4,FILE='SIMUL1.DAT',STATUS='OLD')
READ (4,*) U, AKS, AKI, Y, S0, B0
H=0.001
WRITE(*,*) U, AKS, AKI, Y, S0, B0
S=S0
B=B0
T=0.000
SSum=S
WRITE(6,182)T, S, B, DLOG(B), SSum
182 FORMAT(5(3x,F10.6))
INDEX=1
ICOUNT=(INDEX/100)*100-INDEX
IF (ICOUNT.EQ.0) THEN
ind=ind+1
SSum=SSum+S
Savg=SSum/ind
T1=T-H
WRITE (6, 182) T1, S, B, DLOG (B), Savg
181 FORMAT(4(5X,F10.6))
ENDIF
IF (T.LE.10) THEN
INDEX=INDEX+1
GO TO 10
ENDIF
STOP
END

C SUBROUTINE FOR INTEGRATION OF THE MASS BALANCE
EQUATIONS USING FOURTH ORDER RUNGE-KUTTA
FOR MONOD MODEL

SUBROUTINE RUNGE (H, T, S, B, U, AKS, AKI, Y)
DOUBLE PRECISION U, AKS, AKI, Y, S0, S, B0, B, H, T
F1(S, B)=-((U*S)/(AKS+S+((S**2)/AKI)))*(B/Y)
F2(S, B)=((U*S)/(AKS+S+((S**2)/AKI)))*B
AK1=H*F1(S, B)
BK1=H*F2(S, B)
AK2=H*F1(S+AK1/2, B+BK1/2)
BK2=H*F2(S+AK1/2, B+BK1/2)
AK3=H*F1(S+AK2/2, B+BK2/2)
BK3=H*F2(S+AK2/2, B+BK2/2)
AK4=H*F1(S+AK3, B+BK3)
BK4=H*F2(S+AK3, B+BK3)
S=S+((AK1+2*AK2+2*AK3+AK4)/6
S=B+((BK1+2*BK2+2*BK3+BK4)/6
T=T+H
RETURN
END
REFERENCES


