Modelling and Bifurcation Studies of a Two-Stage Continuous Bioreactor for the Production of Poly-β-hydroxybutyrate (PHB)

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Abstract

There is growing interest in the chemical engineering community in the development of environmentally friendly products such as biopolymers. Poly-β-hydroxybutyrate (PHB) is an important biopolymer whose commercial application is still limited due to the high costs associated with its production. This paper examines the various configurations of continuous bioreactors proposed for the production of PHB in an effort to identify the optimal configuration with respect to the productivity of the system. It is found that a single reactor with cell recycle offers the greatest productivity as compared to two reactors in series or a simple single reactor configuration.

Keywords : Biopolymers, Continuous process, Bifurcation analysis, Model parameters, Model structure

1 Introduction

Over the past few decades, society's dependence on man-made materials, and polymers in particular, has increased dramatically. However, the non-biodegradable nature of these products has resulted in a situation where we are now generating millions of tons of municipal waste every year. A promising solution to this problem is to use biodegradable materials which do not need recycling and could be disposed off in landfills without fear of soil contamination. The fact that our supply of fossil fuels (from which a large proportion of plastics, polymers, paints and fibres are produced) is dwindling rapidly provides an added incentive to use biochemicals *in lieu* of conventional products. Further, though the cost of production of a particular product via a biochemical route may be more expensive than from petrochemical resources, the reduction in the need for clean-up chemicals helps negate these additional costs [1].

Biopolymers are among the important biodegradable materials being produced today. They are polymers produced from biological sources such as plants and micro-organisms. At present, they are being used in a variety of applications from the construction industry [2] to biomedical engineering [3]. An important class of biopolymers is that of the natural polyesters, polyhydroxyalkanoates (PHAs), which are polymers of hydroxyalkanoates. The

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first PHA to have been discovered was poly-3-hydroxybutyrate or PHB (also called poly-β-hydroxybutyrate) from the bacterium *Bacillus megaterium* in 1926 [4]. Since then, PHAs have become one of the largest groups of thermoplastic polymers known with over 100 different types currently produced from a variety of monomer types [5]. They have a wide range of applications from biodegradable plastics [6] to implantable medical devices [7] and tissue engineering [5]. As a result, they are the subject of much attention within the chemical engineering community and a lot of research has been undertaken towards improving the production of these biopolymers [8].

1.1 Intracellular accumulation of PHAs

The metabolic processes by which PHAs are accumulated by bacterial cells is now relatively well understood [9]. Bacteria synthesise PHAs as a carbon and energy reserve material when their growth is limited due to the unavailability of a nutrient such as nitrogen, sulphur or phosphorous [10]. By polymerising soluble intermediates into insoluble molecules, the cells do not undergo alterations of their osmotic state and leakage of these valuable compounds out of them is prevented [11]. Thus, PHAs are functionally similar to starch in plants and glycogen in animals. More than 300 different micro-organisms are known to synthesise and intracellularly accumulate PHAs [8]. Of these, the bacterium *Alcaligenes eutrophus* (also called *Ralstonia eutropha*) has been the most widely used organism because it is easy to grow and because its physiology and biochemistry leading to the synthesis of PHAs are being extensively studied [12].

Historically, the PHA poly-β-hydroxybutyrate (PHB) has been studied the most and has also been responsible for commercial interest in this class of polymers [11]. In most organisms, PHB is synthesised from acetyl coenzyme A (acetyl CoA) by a sequence of three reactions catalysed by β-ketothiolase, acetoacetyl-CoA-reductase and poly-β-hydroxybutyrate synthase [10, 13, 9]. While it is well known that PHB synthesis is regulated at the enzyme level [10], not much is known about the regulation of PHB accumulation and the interactions of these regulatory effects with cell metabolism [9]. In contrast to PHB synthesis, the mechanism as well as the regulation of PHB degradation are poorly understood [14]. However, it has recently been suggested that PHB synthesis and degradation are cyclic and PHB is degraded to acetyl CoA by the enzymes i-poly-β-hydroxybutyrate depolymerase, 3-hydroxybutyrate dehydrogenase, acetoacetyl:succinyl-CoA transferase and ketothiolase [14].

1.2 Commercial production of PHB

Though, in theory, any nutrient can be supplied in limitation, PHB production is normally carried out by limiting cells on ammonia in the presence of excess glucose [15]. Under these conditions, the cells tend to accumulate a large amount of the polymer. Optimal feeding strategies first involve subjecting the cells to normal nutrient conditions to aid cell growth. This is then followed by a period of nitrogen limitation. Under these conditions, the PHB content increases significantly and PHB productivity of up to 4.94 g $l^{-1}h^{-1}$ can be obtained [8]. This reasoning also formed the basis for a patent by ICI in which two reactors in series are used to produce PHB [16].

In spite of the significant amount of research that has been undertaken, the bacterial production on a large-scale of PHB has been limited. One of the problems preventing the wide-spread commercial application of PHB is its high production cost, the most significant contribution being from the carbon source [8]. Other sources such as methanol have been evaluated but it was found that the bacteria employed had a low PHB content and that the PHB produced was difficult to extract [17]. Therefore, recent efforts have focussed on genetically modifying microorganisms such as *Escherichia coli* to produce PHB. The advantages of using such microorganisms is that they grow fast and can be lysed easily, thereby reducing production time and costs [11]. Other more radical approaches include the genetic modification of plants to produce PHAs on a large scale [18].

1.3 Objectives

While the approaches described above do hold significant promise, efforts at maximising the efficiency of existing processes for producing PHB are also invaluable as insights into these processes may help in the formulation of future PHB production strategies. Progress in the fields of flow cytometry [19] and nonlinear model predictive control [20] is enabling improved process monitoring and control. However, for these developments to significantly improve the efficiency of a process, a systematic analysis needs to be undertaken to determine the operating conditions under which maximum productivity is obtained.

Bifurcation theory [21] is a very useful tool in this regard [22] as it provides insight into features such as steady states and limit cycles which can in turn help determine the optimal operating conditions for a given process. A bifurcation analysis employs a mathematical model of the process being studied to predict stable and unstable stationary and periodic steady states. Bifurcation analysis has been employed to study a range of biological processes such as cell interactions [23], microbial growth, hybridoma cultures and yeast cells in continuous bioreactors [24, 25, 26], the eukaryotic cell cycle [27] and cell population balance models [28, 29]. In this paper, bifurcation theory is used to study three configurations for the continuous production of PHB - a single reactor, a single reactor with recycle and two reactors in series - in order to determine the optimal system in terms of PHB productivity. A cybernetic model of PHB accumulation in the bacterium *Alcaligenes eutrophus* is formulated and used in conducting these studies.

2 Analysis of PHB production in continuous cultures of *Alcaligenes eutrophus*

2.1 Cybernetic modelling of PHB accumulation

While several approaches are available to model PHB production, one modelling approach that has been very successful in describing biological systems is the cybernetic modelling approach [30] which was originally formulated to describe 'the Diauxie effect' first observed by Monod. In this approach, cells are construed to be optimal strategists that seek to maximise a particular goal (usually cell growth) given the existing environmental conditions. Two cybernetic models of PHB synthesis in microorganisms are available in the literature [31, 32]. The first model [31] assumes that cells are composed of two components, namely residual biomass and PHB. Though this model was successful in predicting PHB production in the bacterium *Alcaligenes eutrophus*, it failed to take into consideration the underlying metabolic processes. In order to address this deficiency, Gadkar and co-workers [32] formulated a detailed cybernetic model that takes into consideration the metabolic pathways by which the carbon and nitrogen sources are utilised for cell growth and PHB synthesis. In this paper, a model of intermediate complexity was formulated accounting for the underlying metabolic processes.

As with the earlier models, cells are assumed to be comprised of PHB and residual biomass. Residual biomass is defined as all metabolites excluding PHB present in the cell. The model formulated here (see Figure 1) considers four reactions, each representing one of the pathways in the detailed model described above. The first reaction represents the glycolytic pathway and accounts for glucose assimilation and conversion to acetyl-CoA. The second reaction represents the PHB synthesis pathway while the third reaction represents the reverse phenomenon. The last reaction accounts for nitrogen assimilation and conversion, together with acetyl-CoA, to amino acids. The stoichiometry of these reactions is given in Table 1. Acetyl-CoA and amino acids are assumed to be the precursors for cell growth. The requirements of these precursors are assumed to be 22.5 mmol of acetyl-CoA and 9.0 mmol of amino acids per gram of residual biomass.

The rates of these reactions and for the reaction producing residual biomass are assumed to follow

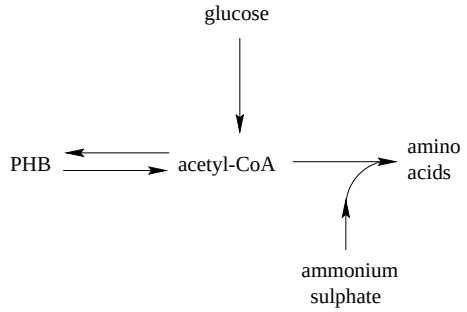


Figure 1: A cybernetic model of PHB synthesis in *Alcaligenes eutrophus*.

variations of Monod's kinetics [33] and are given by

$$r_j = \mu_j^{max} \left(\frac{e_j}{e_j^{max}} \right) \prod_{q=1}^{n} \frac{C_{a_q}}{K_{j,q} + C_{a_q}} \tag{1}$$

Here, μ_j^{max} is the maximum specific rate of the jth reaction; e_j^{max} is the maximum specific concentration of the enzyme e_j catalysing the jth reaction; C_{a_q} is the concentration of the substrate, a_q; and $K_{j,q}$ represents the half-saturation constant of the substrate, a_q. The reaction producing residual biomass is assumed not to be enzyme-catalysed.

The specific rate of enzyme synthesis is given by

$$r_{e_j} = \alpha_e^* + \alpha_e u_j \tag{2}$$

where α_e^* is the specific rate of constitutive enzyme synthesis and α_e denotes the maximum specific rate of enzyme synthesis. The latter is assumed to be regulated by the cybernetic variable u_j.

Two sets of cybernetic variables are employed in the model. The first set seeks to maximise the production of acetyl-CoA from reactions 1 and 3. The corresponding cybernetic variables are given by

$$u_1 = \frac{r_1}{r_1 + r_3} \qquad u_3 = \frac{r_3}{r_1 + r_3} \tag{3}$$

$$v_1 = \frac{r_1}{\max(r_1, r_3)} \qquad v_3 = \frac{r_3}{\max(r_1, r_3)} \tag{4}$$

The second set of cybernetic variables seeks to maximise the utilisation of acetyl-CoA to produce PHB and amino acids. However, in defining the corresponding variables, the rates of the reactions corresponding to glucose and ammonium sulphate assimilation are employed. This strategy was used with the reasoning that, from a biological perspective, the choice of which reaction to maximise is dependent not on the concentrations of PHB

Table 1: Reaction stoichiometry for the formulated cybernetic model

no.	**Reaction**
1	glucose → 2 acetyl-CoA
2	2 acetyl-CoA → PHB
3	PHB → 2 acetyl-CoA
4	2 acetyl-CoA + ammonium sulphate → amino acids

and amino acids but on the availability of glucose and ammonium sulphate.

$$u_2 = \frac{r_1}{r_1 + r_4} \qquad u_4 = \frac{r_4}{r_1 + r_4} \tag{5}$$

$$v_2 = \frac{r_1}{\max(r_1, r_4)} \qquad v_4 = \frac{r_4}{\max(r_1, r_4)} \tag{6}$$

The mass balances for the ten species that comprise this model are given below.

$$\frac{d\mathbf{X}_{ext}}{dt} = \mathbf{S}_{ext}\mathbf{r}c + D(\mathbf{X}_{ext}^{feed} - \mathbf{X}_{ext}) \tag{7}$$

$$\frac{d\mathbf{X}_{int}}{dt} = \mathbf{S}_{int}\mathbf{r} - r_g\mathbf{X}_{int} \tag{8}$$

$$\frac{d\mathbf{e}}{dt} = \mathbf{r}_e - (r_g + \beta_e)\mathbf{e} \tag{9}$$

$$\frac{dc}{dt} = r_g c - Dc \tag{10}$$

Here, $\mathbf{S}_{ext}$ and $\mathbf{S}_{int}$ are the stoichiometric matrices corresponding to the vectors of extracellular and intracellular metabolites, $\mathbf{X}_{ext}$ (mmol/l) and $\mathbf{X}_{int}$ (mmol/g-residual biomass) , respectively; D is the dilution rate (h^{-1}); r_g is the rate of synthesis of residual biomass (g $l^{-1}h^{-1}$); $\mathbf{e}$ is the vector of enzymes (μmol/g-residual biomass); $\mathbf{r}_e$ is the vector of enzyme synthesis rates (μmol g-residual biomass$^{-1}h^{-1}$); β_e is the constant rate of enzyme degradation (h^{-1}); and $\mathbf{r} = [r_1 \quad r_2 \quad r_3 \quad r_4 \quad r_g]$.

In order for the model to be quantitatively accurate, reasonable values of the model parameters must be used. The experimental data of Yoo and Kim [31] was used as a reference in finding the values of the parameters μ_j^{max} and $K_{j,q}$. Values were initially obtained by trial-and-error and were refined using least squares parameter estimation in gPROMS^{TM} (Process Systems Enterprise, UK). The values thus obtained are listed below in Table 2. Figures 2 compare the simulation results with the experimental data used for parameter estimation.

Table 2: Model parameters for metabolic reactions

reaction j	μ_j^{max} trial and error	μ_j^{max} least squares	$K_{j,q}$ trial and error	$K_{j,q}$ least squares
1	4.75	4.72888	35.0	35.1306
2	5.0	5.31675	0.00154	0.001723
3	0.5	0.892636	1.72	1.71235
4	5.75	5.8635	0.0001	0.181775 (AMS)
			0.0001	9.9555x10^{-5} (acetyl-CoA)
g	4000.0	3992.11	10.0	10.3149

The values of the constants used in the rates of enzyme synthesis were chosen arbitrarily and are given in Table 3.

Table 3: Model parameters for enzyme synthesis reactions

α_e^*		0.05 μg g-residual biomass^{-1} h^{-1}
α_e		0.95 μg g-residual biomass^{-1} h^{-1}
β_e		1.0 h^{-1}
e_j^{max}	$j = 1, \ldots, 4$	1.00 μg g-residual biomass^{-1}

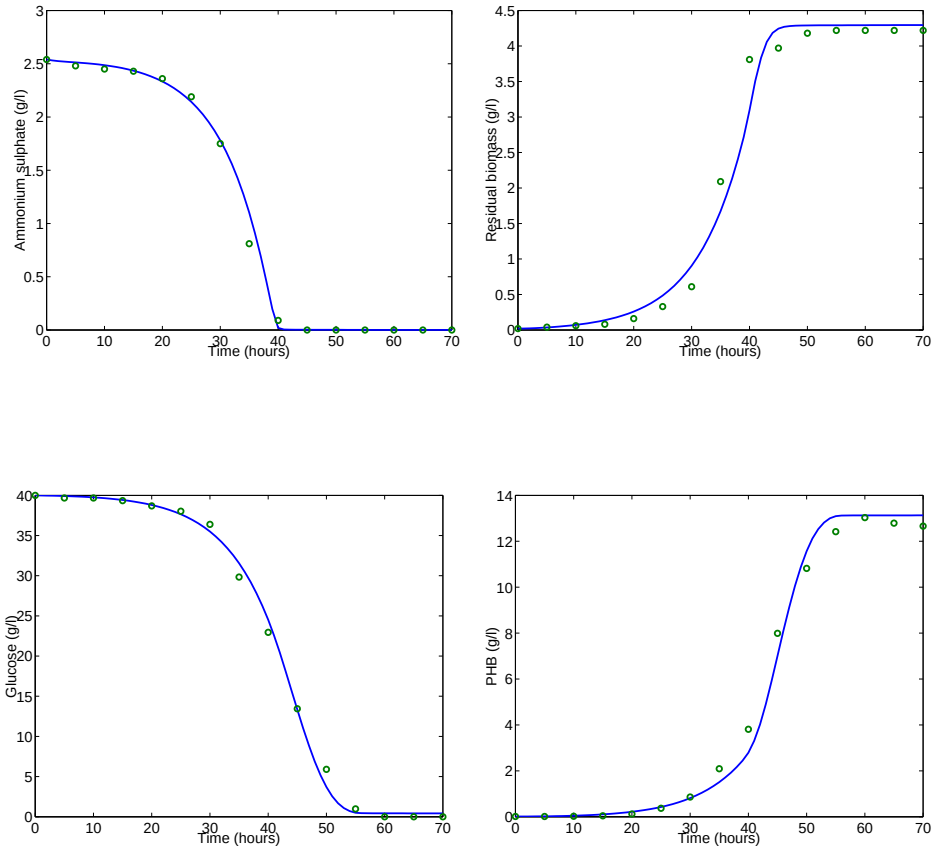


Figure 2: Comparison of the simulation results of the formulated model with published experimental data [31].

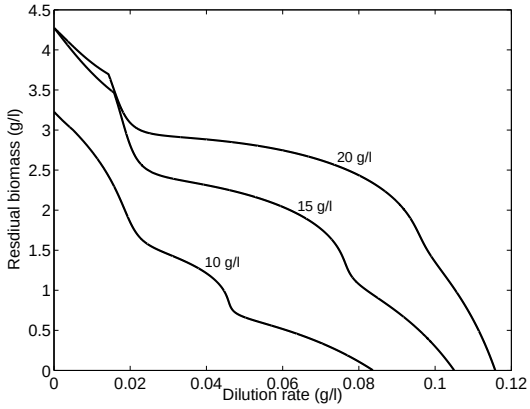
2.2 Bifurcation analysis of a single-stage continuous reactor producing PHB

In the model formulated above, the three main parameters with respect to process design and control are the dilution rate and the concentrations of glucose and ammonium sulphate in the feed stream. Of these, the latter two are inter-related and therefore only one of them needs to be analysed. In the next two sections, the effects of the dilution rate and the concentration of glucose in the feed stream on the productivity of PHB in a single stage continuous reactor are studied.

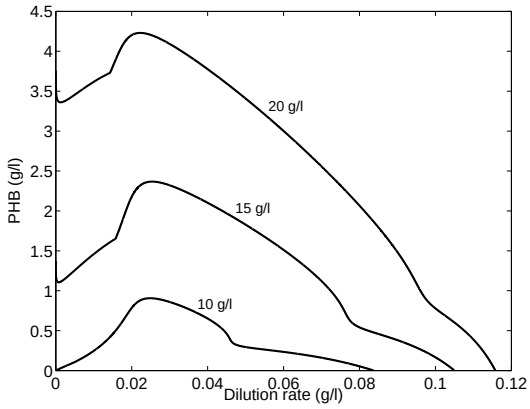
2.2.1 Effect of dilution rate

A bifurcation analysis of the model formulated above was undertaken with the dilution rate to the reactor as the bifurcation parameter. A steady state was first identified by simulation. The steady state locus around this point was traced using the bifurcation analysis software AUTO [34]. The bifurcation diagrams obtained for different feed concentrations of glucose are depicted in Figures 3. The feed concentration of ammonium sulphate was kept constant at 2.54 g/l.

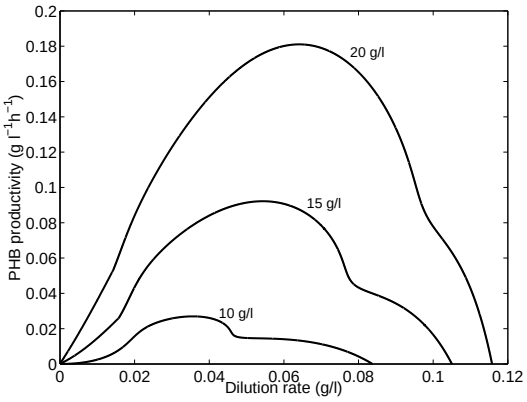
Figure 3a shows the dependence of the concentration of residual biomass on the dilution rate. It can be seen that, for a given feed concentration of glucose, the concentration of residual biomass decreases with an increase in the dilution rate until the reactor undergoes washout. In bifurcation analyses of other cybernetic models of bioreactors [26, 25], the biomass concentration in the reactor is relatively constant with an increase



(a) Residual biomass concentration



(b) PHB concentration



(c) PHB productivity

Figure 3: Bifurcation diagrams for a single reactor producing PHB for different feed concentrations of glucose with respect to the dilution rate.

in dilution rate until a point is reached when the reactor rapidly undergoes washout. Further, in this model, the rate at which the residual biomass decreases is not regular. The reason for this unusual behaviour has not been ascertained. Another important observation is that, unlike other cases [26, 25], multiple steady states are not encountered in any region. This observation was validated by undertaking exhaustive simulations, each starting at a different initial condition. Also, decreasing the glucose feed concentration resulted in a decrease in the residual biomass concentration for a given dilution rate. This is perfectly reasonable as decreasing the amount of glucose fed to the reactor has a proportional and direct effect on the growth rate of cells.

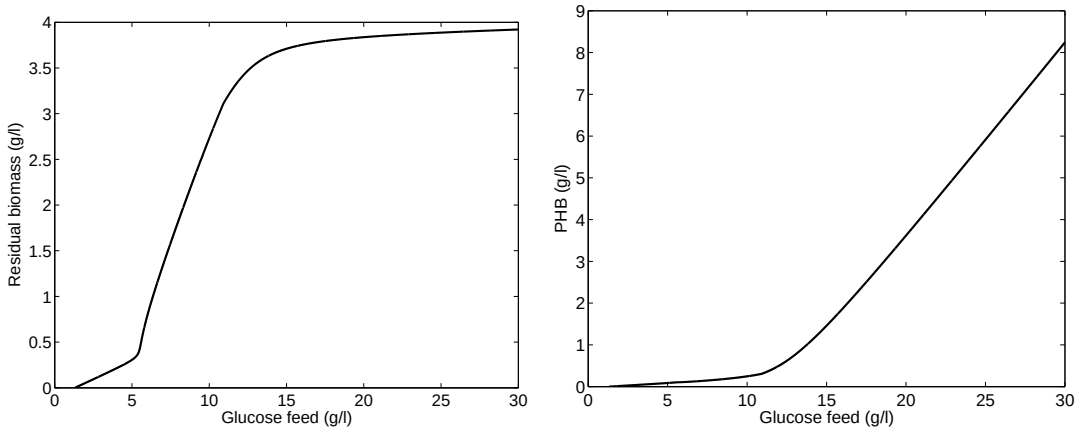
The variation of PHB concentration in the reactor with dilution rate is qualitatively different from that of the residual biomass, as seen in Figure 3b. In this case, the concentration of PHB first increases with an increase in the dilution rate before undergoing a decrease. However, the decrease is more rapid than for the case of residual biomass. Given the reaction network adopted for this system, this behaviour is reasonable as it is similar to that of intermediates and products in series-parallel reactions occurring in continuous reactors. Also, as expected, decreasing the glucose feed concentration has a profound effect on the PHB concentration in the reactor. This is reasonable as, when faced with lower glucose concentrations, cells tend to accumulate less PHB. This result shows that the model, though relatively simple, is qualitatively accurate.

From the point of view of process design and control, the most important variable is the PHB productivity, which is the rate at which PHB is produced in the reactor. From Figure 3c, it can be seen that, initially, the PHB productivity increases with an increase in the dilution rate before undergoing a rapid decrease at high dilution rates. Further, by comparing Figures 3b and 3c, it can be seen that the dilution rate at which the PHB productivity is maximum does not necessarily correspond to the dilution rate at which the PHB concentration in the reactor is maximum. These observations are reasonable as the productivity of a reactor is dependent not just on the concentration of the desired product in the reactor, but also on the dilution rate. The effects of reducing the glucose feed concentration to the reactor are best observed in Figure 3c. It can be seen that at high glucose feed concentrations, doubling the glucose feed concentration results in a corresponding increase in PHB productivity. However, at low glucose concentrations, *i.e.* when glucose is not in excess or barely so, there is almost no production of PHB.

2.2.2 Effect of glucose feed concentration

While the analyses discussed above provide some insight into the effect of the feed concentration of glucose on the productivity of the reactor, a bifurcation analysis with the glucose feed concentration as the chosen bifurcation parameter will theoretically confirm the observations made above whilst providing further insight. Figures 4 depict the bifurcation diagrams obtained when the glucose feed concentration was selected as the bifurcation parameter. In these figures, the dilution rate and the feed concentration of ammonium sulphate have been kept constant at 0.01 h^{-1} and 2.54 g/l respectively.

From Figure 4a, it can be seen that when the feed concentration of glucose is less than approximately twice the feed concentration of ammonium sulphate, there exists no stable steady state for the model. This is consistent with the model formulation as well as with the biology of the system as, under continuous conditions, if insufficient glucose is provided to the cells, then the growth rate of cells will be less than that required to prevent reactor wash out. Beyond a threshold glucose concentration, the concentration of residual biomass in the reactor increases sharply with an increase in the glucose feed concentration. From Figure 4b, it can be seen that very little, if any, PHB accumulation takes place for the same glucose feed concentrations. This is again consistent with the present understanding of the metabolism of PHB producing bacteria. When the carbon source is not in excess, in general, no PHB accumulation takes place. Further, under these conditions, an increase in the amount



(a) Residual biomass concentration

(b) PHB concentration

Figure 4: Bifurcation diagrams for a single reactor producing PHB with respect to the feed concentration of glucose.

of substrate available corresponds to a proportional increase in the growth rate of the cells.

Above a second threshold glucose concentration, the behaviour of the system undergoes a significant change as can be seen from Figures 4. At these high feed concentrations of glucose, the concentration of residual biomass remains relatively independent of the glucose feed concentration while the PHB concentration increases linearly. This is, again, a perfectly reasonable observation as these high glucose feed concentrations correspond to an excess of carbon source. Under such an excess of carbon, as is well known, the cells channel the excess substrate to produce PHB while the residual biomass content remains relatively constant. It has been observed that glucose concentrations need to be maintained in the region of 10 to 20 g/l to achieve high cell and PHB concentrations [35]. As this is evident from Figures 4, it can be said that the model formulated here is a reasonably accurate representation of the process under consideration.

2.3 Bifurcation analysis of a continuous reactor with cell recycle

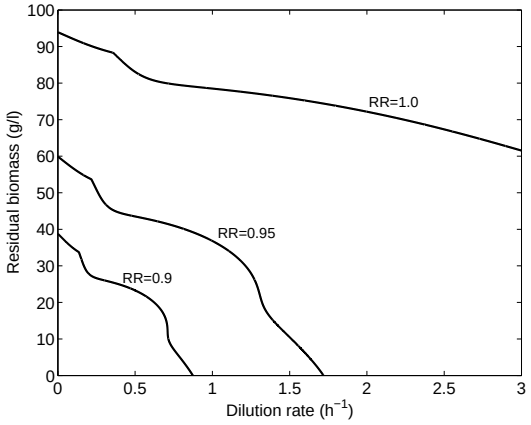
The recycling of substrate and biomass can often significantly affect the performance of a reactor. Besides increasing the utilisation of the substrate, recycling cells can often increase the concentration of biomass in the reactor and thereby increase the productivity of the system.

Consider a single reactor with a microfiltration unit downstream. A part of the exit stream is diverted to the microfiltration unit. Here, cell concentration is achieved by the removal of cell-free permeate through a microfiltration membrane. If Q_f (h^{-1}) is the flowrate to the microfiltration unit, then the flowrate of cell-free permeate, Q_p (h^{-1}), is given by Equation (11) below [36].

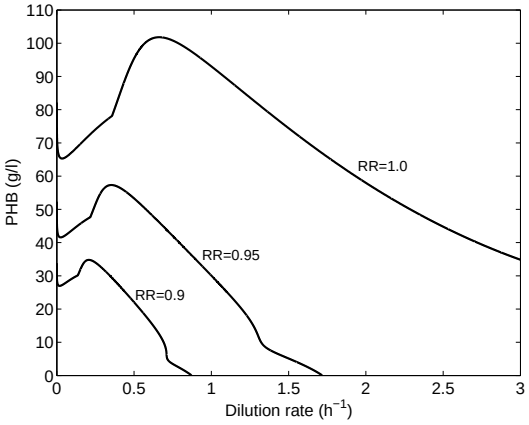
$$Q_p = Q_f \left(1 - \exp \left[-0.62 \left(\frac{a^2 L}{R^3} \right)^{2/3} \ln \left(\frac{\phi_w}{\phi} \right) \right] \right) \tag{11}$$

In this equation, ϕ is the feed cell volume fraction, ϕ_w is the cell volume fraction at the membrane surface, and a^2L/R^3 is the dimensionless channel geometry for hollow fibre membrane filtration. The value of ϕ_w has been assumed to be 0.95 and the specific volume of cells has been assumed to be 1.4 mL per g-residual biomass [37]. The value of a^2L/R^3 has been assumed to be 4 [32].

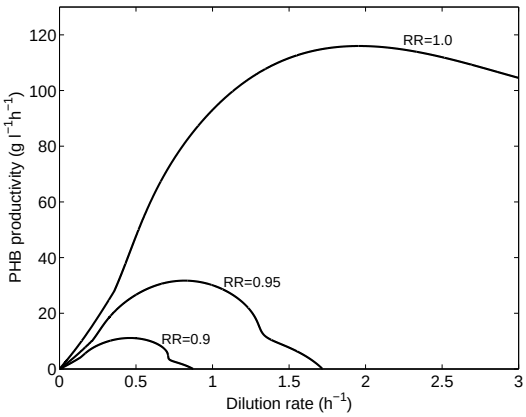
The bifurcation diagrams for this reactor with cell recycle are shown in Figure 5 for different values of the recycle ration, $RR = Q_f/D$. The feed concentrations of glucose and ammonium sulphate have been kept constant at 20 g/l and 2.54 g/l respectively. From Figure 5a, it can be seen that at recycle ratios close to 1, for a small increase in the recycle ratio, the residual biomass concentration increases sharply for a given dilution rate. Further, increasing the recycle ration also increase the maximum dilution rate at which the reactor can be operated without undergoing washout. This directly effects the steady-state concentration of PHB in the reactor, as observed from Figure 5b. In terms of PHB productivity, it can be seen from Figure 5c that increasing the recycle ratio significantly affects the maximum productivity achievable, with the increase being almost exponential. It can also be observed that there appear to be no unstable states as earlier. This was verified by undertaking several simulations to ensure that, for a given dilution rate and recycle ratio, the steady state predicted by the bifurcation analysis was the only one present. In terms of the benefits afforded by employing cell recycle, a comparison of Figures 5 with Figures 3 shows that the introduction of cell recycle significantly increases the residual biomass concentration in the reactor. This in turn increases the productivity of the system by more than two orders of magnitude. Thus, it can be concluded that the cost of introducing and maintaining a microfiltration unit will be more than compensated for by the increase in productivity obtained.



(a) Residual biomass concentration



(b) PHB concentration



(c) PHB productivity

Figure 5: Bifurcation diagrams for a single reactor with cell recycle producing PHB with respect to the dilution rate for different recycle ratios.

2.4 Bifurcation analysis of a two-stage continuous reactor producing PHB

Now that a good understanding has been obtained regarding the behaviour of a single reactor producing PHB, it is necessary to examine whether the two-stage process patented by ICI [16] offers substantial benefits over a single-stage process in terms of PHB productivity. The process consists of two reactors in series. Glucose, ammonium sulphate and other nutrients are fed to the first reactor so that no nutrient is in limitation. This results in cell growth without the excessive accumulation of PHB. Depending on the circumstances, it may also be desirable to feed the first reactor with an excess of glucose to accumulate a certain amount of PHB. In other cases, it may be desirable to operate the first reactor under carbon limitation conditions so that no PHB accumulation takes place.

The cells from this reactor are fed to the second one where only glucose is fed. Any ammonium sulphate present in the second reactor can only come from the first reactor. Under these conditions, PHB is accumulated by cells to high concentrations. The cells from the second reactor are then taken to a separation stage where PHB is extracted and purified. In a single state continuous process, though it may be possible to accumulate up to 75 or 80% of cell mass as PHB, the residence times required to achieve these conditions are very high. However, by

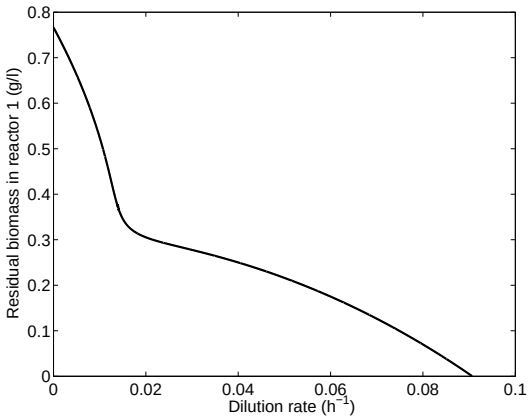
using two reactors in series, the cells can be made to achieve a PHB content of between 50 and 80% in more reasonable residence times.

In undertaking the bifurcation analysis analysis, it was decided to focus on the dilution rate to the two reactors. This choice was made based on the reasoning that while the effect of the feed concentration of glucose on the behaviour of the single reactor was understandable and expected, the effect of the dilution rate was rather more complex. Figures 6 show the bifurcation diagrams obtained with the net dilution rate to the two reactors as the chosen bifurcation parameter. The feed concentrations of glucose and ammonium sulphate to the first reactor were kept constant at 5.0 g/l and 2.54 g/l respectively. Only glucose was fed to the second reactor at a feed concentration of 15 g/l. These concentrations were chosen based on the description of the process [16] which states that the first reactor should mainly serve to promote cell growth while the second reactor should focus on PHB accumulation. Also, the total amount of glucose and ammonium sulphate fed to the two reactors as well as the dilution rate were kept equal to those values used in studying the single stage reactor in order to obtain a fair comparison.

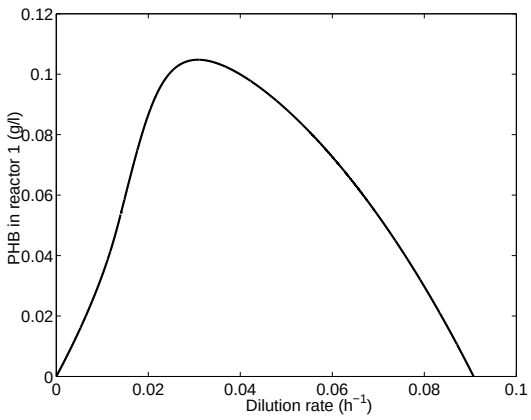
Focussing on the first of the two reactors, it can be seen from Figure 6a that the behaviour of the concentration of residual biomass in this reactor is qualitatively similar to that of the single-stage reactor. The concentration of residual biomass decreases with an increase in the dilution rate until a point is reached when the reactor undergoes washout. From Figure 6b, it can be seen that the analysis with respect to PHB in the first reactor is also qualitatively similar to that of the single-stage reactor. However, what is significant is that the concentration of PHB in the reactor is significantly lower than the concentration of residual biomass with a maximum PHB content of approximately 30% of total cell mass obtainable in this reactor. This shows that for the chosen feed concentrations of glucose and ammonium sulphate to this reactor, very little PHB accumulation occurs with cells focussing on maximising cell growth. This is in line with the recommended operating conditions for this system [16] and confirms that the chosen values of the feed concentrations to the first reactor are reasonable.

The behaviour of cells in the second reactor is very different from that in the first reactor. As can be seen from Figure 6c, the concentration of residual biomass first decreases with an increase in the dilution rate before undergoing a sharp increase. Then, after reaching a maximum, the concentration of residual biomass drops sharply and, finally, the reactor undergoes washout. A possible explanation for this unusual behaviour can be obtained by comparing Figures 6a and 6c. At low dilution rates, when the concentration of residual biomass in the first reactor is high, the concentration of ammonium sulphate in that reactor will be low (observable from Equation (7)). This is due to the fact that, as cells are focussing on cell growth in the first reactor, they maximise the uptake of ammonium sulphate. Therefore, as the concentration of residual biomass decreases with an increase in the dilution rate, the amount of ammonium sulphate taken up by the cells also decreases and the amount of ammonium sulphate present in the aqueous medium increases. However, the effects of this increase on the concentration of residual biomass in the second reactor are not immediately observable at low dilution rates. This is because, initially, as the biomass concentration in the first reactor decreases, so too does the biomass concentration in the second reactor. This is because as no ammonium sulphate is being fed separately to the second reactor, the low ammonium sulphate concentrations entering from the first stage at low dilution rates will be insufficient to counter the simultaneous reduction in the inflow of residual biomass. However, after a sufficient increase in the dilution rate, the amount of ammonium sulphate reaching the second reactor will be enough to overcome the reduction in inflow of cells. This causes a change in the behaviour of the system and the concentration of residual biomass in the second reactor increases.

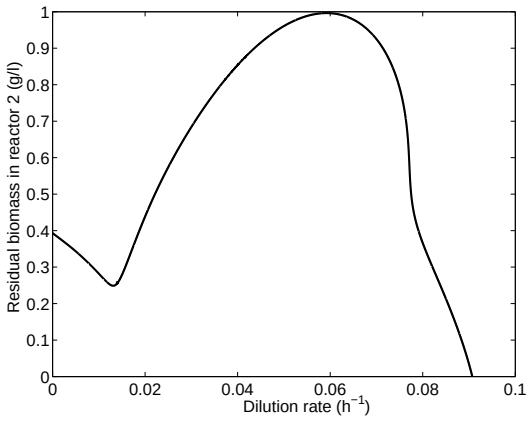
The decrease in residual biomass concentration in the first reactor also affects the PHB concentration in the second reactor. At low dilution rates, when the concentration of residual biomass in the second reactor is decreasing with an increase in the dilution rate, the constant supply of a large excess of glucose to the second reactor results in very high concentrations of PHB. The concentration of PHB decreases, however, with an increase



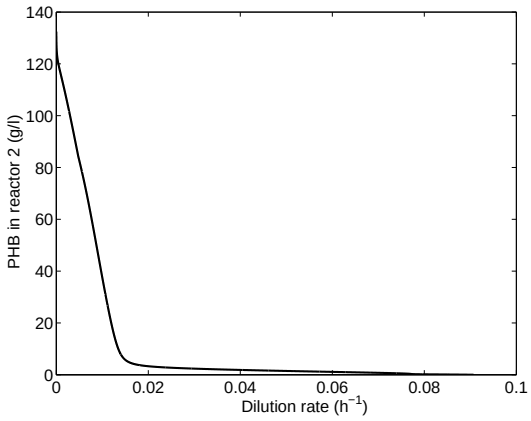
(a) Residual biomass concentration in reactor 1



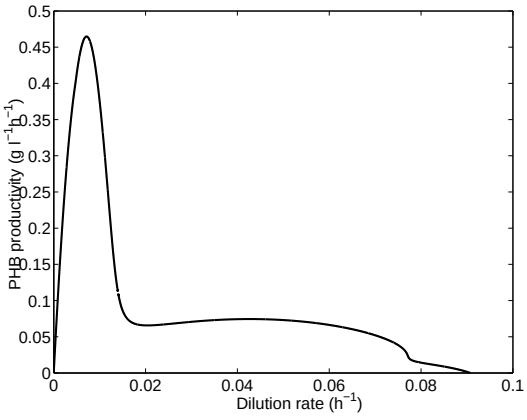
(b) PHB concentration in reactor 1



(c) Residual biomass concentration in reactor 2



(d) PHB concentration in reactor 2



(e) PHB productivity of the system

Figure 6: Bifurcation diagrams for a two-stage reactor producing PHB with respect to the dilution rate.

in the dilution rate. This is reasonable as when the concentration of cells in the reactor decreases, the ability of the system to convert glucose to PHB also decreases as the ability of the cells to assimilate extracellular glucose is reduced. By the time the dilution rate is increased to the point where a sufficient amount of ammonium sulphate is available to increase residual biomass in the second reactor, the PHB concentration in this stage is very low. These effects are more readily observable in Figure 6e, which shows the dependence of PHB productivity on the dilution rate. At low dilution rates, the productivity of the system increases rapidly with an increase in dilution rate. This is consistent with the observations made above. Then, as the cells in the second reactor begin to shift their focus on to cell growth due to the increasing availability of ammonium sulphate, the productivity of the system decreases.

In terms of the productivity of PHB, the behaviour of the two-stage reactor is significantly different from the the single-stage reactor discussed above. A comparison between Figures 6e and 3e shows that the maximum productivity achievable using the two-stage configuration is two and half times more than in a single reactor. Further, the dilution rate at which this is achieved in the two-stage system is approximately 0.01 h^{-1} as compared to approximately 0.07 h^{-1} for the single reactor. As the cost of the carbon substrate is the most important economic consideration in this process [8, 16], the dilution rate significantly affects the operating cost. In constructing the bifurcation diagrams depicted in Figures 6e and 3e, the feed concentrations of glucose and ammonium sulphate were taken to be the same (20 g/l and 2.54 g/l). Therefore, as maximum productivity is achievable in the two-stage configuration at a fraction of the dilution rate needed for the single reactor, the operating costs will be proportionately reduced. This, coupled with its greater maximum productivity, indicates that the financial rewards obtained from a two-stage process will be several orders of magnitude greater than from a single-stage process.

3 Conclusions

Biopolymers are gaining increasing importance due to the various advantages they offer over synthetic polymers. However, their commercial application is still limited due to the high costs associated with their production. In this paper, bifurcation theory has been used to determine the best reactor configuration for producing PHB. The single reactor with cell recycle and the two-stage continuous reactor examined both appear to offer significant benefits over a simple one-stage process. Further, the maximum productivity predicted by these configurations makes the continuous production of PHB a reasonable proposition.

However, the final determination of the optimal configuration will depend on other factors beyond the scope of this work for the following reasons. An important consideration with respect to the reactor with cell recycle is the maintenance of the microfiltration unit. While this configuration appears to offer the best productivity, over a long period, frequent shut down of the process to clean the membrane could significantly affect the efficiency of the process. On the other hand, the two-stage continuous process will be more complex from the point of view of control as the interactions between the reactors will have to be taken into consideration when making control actions. Further, the presence of two reactors in series will significantly increase the time constant of the system so it may not be possible to frequently change the process set point. Therefore, the final choice regarding the optimal process will be dependent on further analysis of the system.

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