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SENSITIVITY OF BIFURCATION TRAITS TO MODEL PARAMETERS IN POLY-ß-HYDROXYBUTRYRATE PRODUCTION

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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-ß-hydroxybutyrate (PHB) is an important biopolymer whose commercial application is still limited due to the high costs associated with its production. This paper examines a continuous bioreactor proposed for the production of PHB. The influence of minor variations in the kinetic parameters of a simple cybernetic model formulated is examined. It is shown that minor variations in these parameters can have significant impacts on the bifurcation analyses obtained.

Keywords: Biopolymers, Continuous Process, Bifurcation Analysis, Cybernetic Modelling

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. Biopolymers are among the important biodegradable materials being produced today. They are polymers produced from biological sources such as plants and micro-organisms. An important class of biopolymers is that of the natural polyesters, polyhydroxyalkanoates (PHAs). Since they were first discovered, PHAs have become one of the largest groups of thermoplastic polymers known with over 100 different types currently produced from a variety of monomers (Williams *et al.*, 1999). They have a wide range of applications from biodegradable plastics to biomedical engineering. 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 (Lee *et al.*, 1999).

The metabolic processes by which PHAs are accumulated by bacterial cells is reasonably well understood. 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 (Anderson and Dawes, 1990). Although in theory, PHB production can be triggered by engineering a deficiency in one of several nutrients, it is normal practice to employ ammonia as the limiting nutrient under excess glucose (Anderson and Dawes, 1990). Under these conditions, the cells tend to accumulate a large amount of the polymer.

In spite of the significant amount of research that has been undertaken, the industrial production 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 expense being that of the carbon source (Lee *et al.*, 1999). Therefore, recent efforts have focussed on genetically modifying microorganisms such as *Escherichia coli* to produce PHB. The advantages of using such microorganisms are that they grow fast and can be lysed easily, thereby reducing production time and costs (Madison and Huisman, 1999).

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While the genetic modifications described above 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 and nonlinear model predictive control 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 is a very useful tool in this regard 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. An implicit assumption is that the model employed 'is' the process, *i.e.* the model is an ideal and exact representation of the process. A mathematical model of any process relies on certain parameters to qualitatively and quantitatively simulate experimental observations. These parameters are usually estimated from a given set of experimental data. However, unmeasurable process disturbances often reduce the accuracy of the data and minor discrepancies are often observed between the model predictions and the experimental data. A second consideration is that, unlike parameters in empirical models that are estimated using a wealth of data, parameters in mechanistic models are often obtained from a limited data set.

Bifurcation analysis has been employed to study a range of biological processes. In these cases, the results obtained from the bifurcation analysis corresponded at least qualitatively with experimentally observed phenomena. Based on the successes of these studies, one might be tempted to accept the findings of a bifurcation analysis of a particular process without performing sufficient experiments. The objective of this paper is to demonstrate the potential dangers of making such a decision by examining the effect of variations in parameter values on predictions of the bifurcation analysis.

2. 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 (Kompala *et al.*, 1986) 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 microorgranisms are available in the literature (Yoo and Kim, 1994; Gadkar *et al.*, 2003). The first model (Yoo and Kim, 1994) 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 (Gadkar *et al.*, 2003) 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.

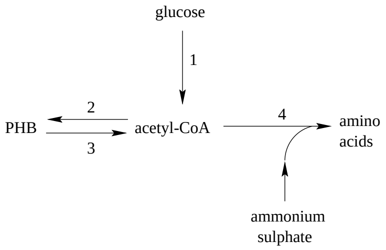

Fig. 1. New cybernetic model of PHB synthesis in *Alcaligenes eutrophus.*

The model formulated here (see Figure 1) considers four reactions, each representing one of the pathways in the detailed model described above (Gadkar *et al.*, 2003). In this scheme, 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. Cells are assumed to be comprised of PHB and residual biomass. Residual biomass is defined as all metabolites excluding PHB present in the cell. Acetyl-CoA and amino acids are assumed to be the precursors for cell growth. The rates of these reactions and for the reaction producing residual biomass are assumed to follow variations of Monod's kinetics.

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 second set of cybernetic variables seeks to maximise the production of PHB and residual biomass from reactions 2 and 4. However, in defining the corresponding variables, the reaction rates 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 rates of production of PHB and amino acids from acetyl-CoA but on the availability of glucose and ammonium sulphate.

The kinetic parameters were first selected so that the model was in qualitative agreement with the biology of the process. A continuous bioreactor was then considered and bifurcation diagrams were constructed with the dilution rate as the chosen bifurcation parameter. The aim of this exercise was to evaluate the ability of the chosen model structure to capture intricate bifurcation behaviour. In constructing these diagrams, a steady state was first identified by simulation. The steady state locus around this point was traced using the bifurcation analysis software AUTO (Doedel, 2001). The bifurcation diagrams thus obtained for different glucose feed concentrations are shown in Figures 2. The ammonium sulphate feed concentration was kept constant at 2.54 g/l. From Figure 2a, it can be seen that at low glucose feed concentrations, the reactor rapidly undergoes washout while at higher glucose feed concentrations the residual biomass concentration remains relatively constant. Further, multiple steady states are observed separated by a region of instability. With respect to the process productivity, from Figure 2b it can be seen that the productivity increases sharply with an increase in the glucose feed concentration. This result is in qualitative agreement with the biology of the system as when the bacteria are exposed with an excess of glucose, the excess substrate is channeled into PHB. Based on the results of these analyses, it can be concluded that the model, though relatively simple, is capable of explaining complex bifurcation phenomena such as steady-state multiplicity.

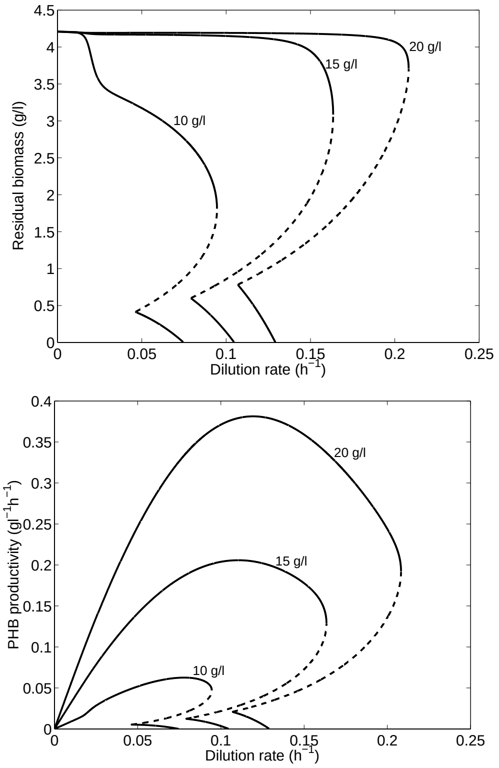

Fig. 2. Bifurcation diagrams for the model formulated for different feed glucose concentrations. Randomly selected values for the kinetic parameters are used.

3. BIFURCATION ANALYSIS OF A CONTINUOUS REACTOR PRODUCING PHB

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 (Yoo and Kim, 1994) (56 experimental data points) were used as a reference in finding the values of the 11 kinetic parameters in the model. Values were initially obtained by trial-and-error and were refined using least squares parameter estimation in gPROMS^{TM} (Process Systems Enterprise) (see Figures 3).

In this process, the two main control variables are the dilution rate and the ratio of the concentrations of glucose and ammonium sulphate in the feed stream. A bifurcation analysis was first undertaken with the glucose feed concentration as the bifurcation parameter. The bifurcation diagrams thus obtained are shown in Figures 4. The dilution rate and the feed concentration of ammonium sulphate were 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 very low, there exists no stable steady state for the model. This is consistent with the biology of the system as if insufficient glucose is provided to the cells, then the growth rate of cells will be less

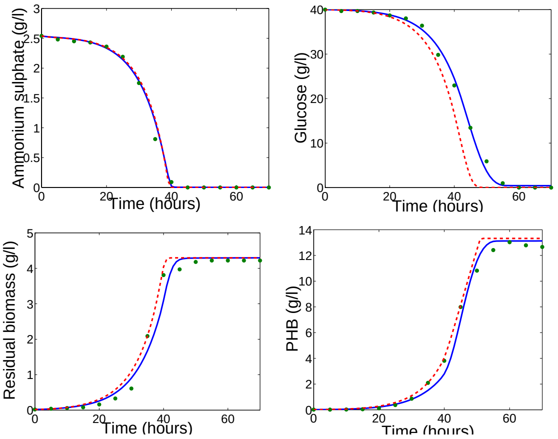

Fig. 3. Comparison of the simulation results of the formulated model with published experimental data. Legend: —— estimated parameters, - - - modified parameters.

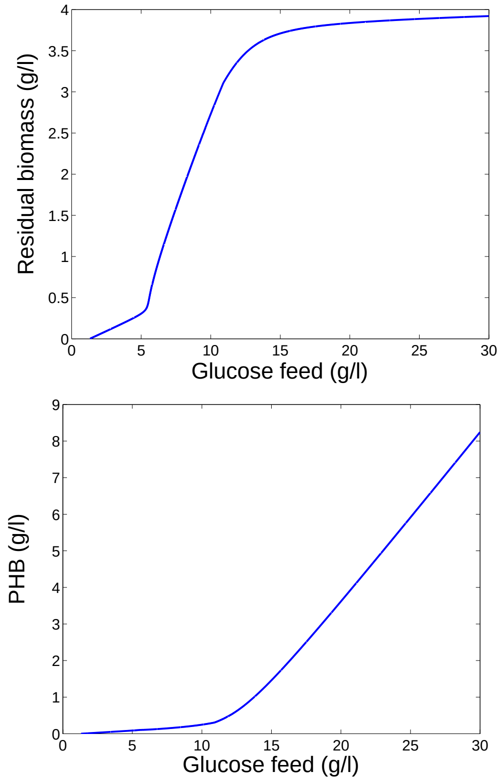

Fig. 4. Bifurcation diagrams for a single reactor producing PHB with respect to the feed concentration of glucose.

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 while from Figure 4b it can be seen that very little, if any, PHB accumulation takes place. This is again reasonable as when the carbon source is not in excess, in general, no PHB accumulation takes place. Further, under these conditions, an increase in the amount 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 conditions, 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 (Kim *et al.*, 1994). 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.

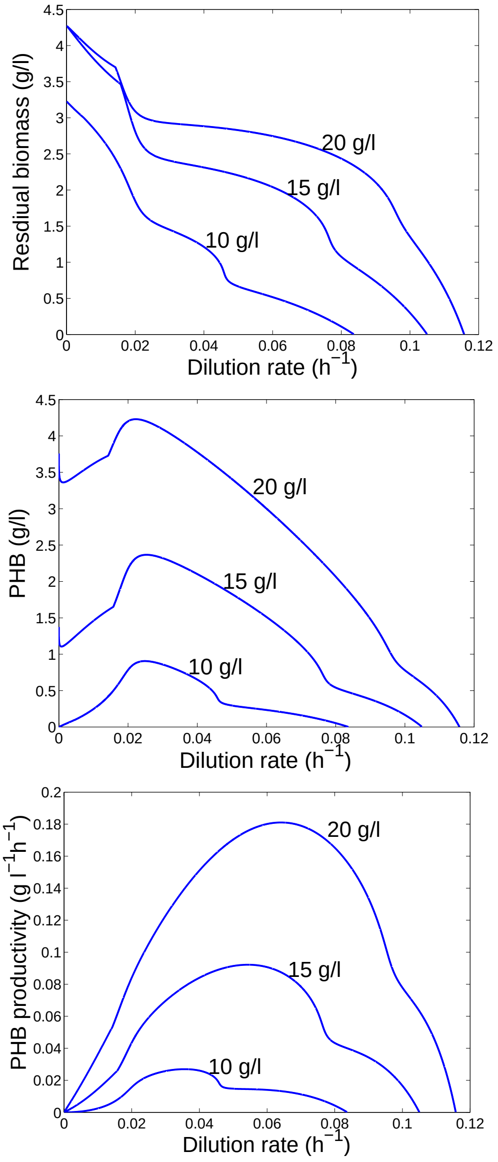

Fig. 5. Bifurcation diagrams for a single reactor producing PHB for different feed concentrations of glucose with respect to the dilution rate.

A bifurcation analysis of the model formulated was then undertaken with the dilution rate to the reactor as the bifurcation parameter. The bifurcation diagrams obtained for different feed concentrations of glucose are depicted in Figures 5. The feed concentration of ammonium sulphate was again kept constant at 2.54 g/l. Figure 5a 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. 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 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 5b. 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 reactions occurring in continuous reactors. Also, as expected, decreasing the glucose feed concentration has a significant 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 5c, 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 5b and 5c, 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 5c. 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.

4. INFLUENCE OF MODEL PARAMETERS ON BIFURCATION ANALYSES

Two important considerations in fitting a model to experimental data are the amount and quality of the available data and the number of model parameters to be estimated. The quality of available data is dependent on the accuracy of the measurement techniques and reproducibility of the experiments. While the latter is usually rigorously accounted for, unmeasurable disturbances in the operating conditions can result in minor variations in the measured data. These variations introduce uncertainty into the estimated values of the parameters and, consequently, possible inaccuracies in the bifurcation analyses. Secondly, a sufficient amount of data is necessary in order to obtain confidence in the estimated values of parameters. If insufficient data is available, then multiple sets of parameter values may be obtainable giving the same accuracy with respect to the ability of the model to reproduce experimental observations.

In this section, the sensitivity of the bifurcation behaviour to uncertainty in the model parameters is discussed. The parameter values are of the same order of magnitude as those used in the model above with the exception of the constants used in the residual biomass growth rate (data not shown). While the glucose concentration shows the largest difference, the inaccuracy is still not very significant (see Figures 3). Therefore the model can be said to be almost as accurate as the model used above.

The bifurcation diagrams obtained for the case of a single reactor using these parameter values are shown in Figures 6. Here, the dilution rate is the chosen bifurcation parameter. The feed concentrations of glucose and ammonium sulphate are kept constant at 20 g/l and 2.54 g/l respectively. The most significant difference between this analysis and that depicted in Figures 5 is with respect to the concentration of residual biomass in the reactor. In the previous case, the concentration of residual biomass only decreased with an increase in the dilution rate, irrespective of the range of the dilution rate. However, in this case, the residual biomass first increases to a maximum before decreasing. Further, the dilution rate at which washout occurs is greater than that in the earlier case.

The bifurcation diagrams with respect to both PHB concentration and PHB productivity are qualitatively similar for both cases. However, in this case, the maximum productivity achievable is almost 50% greater than that achievable in the first case. This is an extremely important difference as analyses such as these could possibly be used in the early stages of process design. Inaccurate predictions in process variables at these times could significantly affect the estimated operating costs. In the worst case, a significant overprediction could result in financial losses. This simple analysis thus shows how important it is

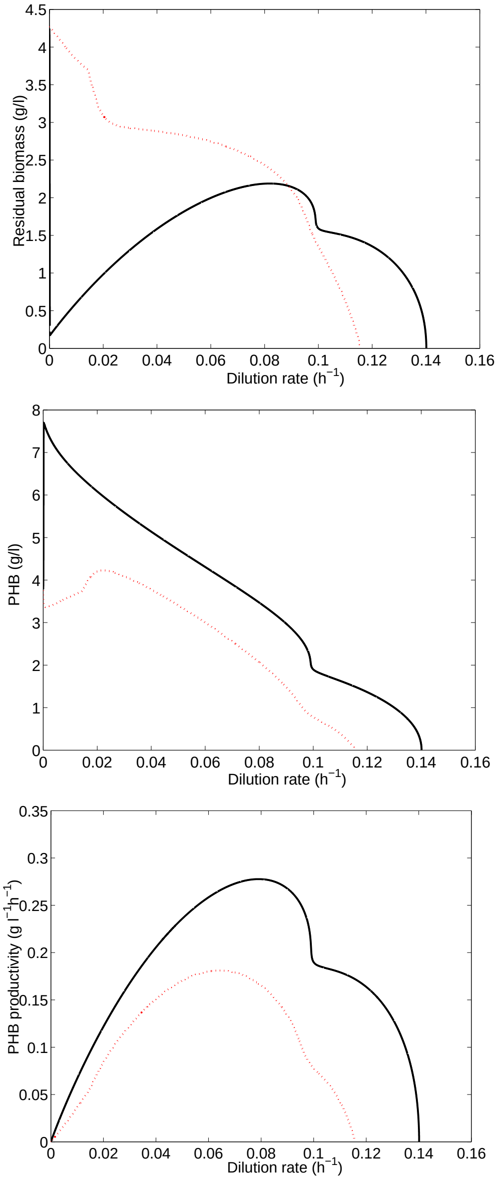

Fig. 6. Bifurcation diagrams for a single reactor producing PHB using the model with modified kinetic parameters. The dotted lines correspond to the model with estimated parameters.

to have accurate values for the model parameters and hence the importance of both detailed experimentation and robustness considerations in design and control.

5. 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 and a careful analysis of the production processes available needs to be undertaken in order to determine the optimal operating conditions. Bifurcation analysis is a useful tool for conducting such an analysis. In undertaking a bifurcation analysis, a dynamic model is usually employed to represent the process. In this study, a cybernetic modelling framework was adopted. The model structure employed has the capabilities of identifying complex and intricate bifurcation behaviour should they occur in the process (depending on model parameters and design/operation variables). Bifurcation studies were performed for the production of PHB, with the dilution rate as the bifurcation variable. Further, the influence of model parameters on bifurcation results was examined. It was observed that minor variations in the values of the kinetic parameters had a significant effect on the predictions obtained. These results evince the importance, on the one hand, of detailed experimentation for model validation and parameter estimation and, on the other hand, robustness considerations in design and control.

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