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Modelling of a Sequencing Batch Reactor for Producing Polyhydroxybutyrate with Mixed Microbial Culture Cultivation Process Using Neural Networks and Operation Regime Classification

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A neural network based methodology for the modelling of a sequencing batch reactor (SBR) for producing Polyhydroxybutyrate (PHB) with Mixed Microbial Cultures (MMC) is proposed. The advantages of applying MMC for more effective production of PHB have already been documented and mechanistic models were developed, however, the lack of good understanding and the ability to describe phenomena involved in the complex nature of the bioprocess led to unsuccessful release of reliable and accurate mechanistic models. In order to perform successful process control and optimisation, empirical models developed from process operational data should be capitalised. Bootstrap aggregated neural networks are used in this study to enhance model accuracy and reliability. In the case of PHB production through SBR using MMC, the two feeding substrates of acetate and ammonia were found to play dominant roles in PHB production trajectory and different process operation regimes exist depending on the concentrations of these substrates. This paper proposes a method for the classification of such operation regimes and building neural network models corresponding to these regimes using bootstrap aggregated neural networks.

1. Introduction

PHB is one of the constituents of Polyhydroxyalkanoates (PHAs) synthesized by bacteria that are nourished by acetate. PHAs share similar mechanical properties to those of polypropylene, with a fundamental difference of being completely biocompatible, biodegradable and being produced from renewable biological resources. In addition to the conventional applications known for petrochemical plastics, PHAs have already found their place in surgical applications for implants, sutures and surgical pins. Therapeutic applications of PHAs were reported for controlled release of active pharmaceuticals and carriers of nutrients. Applications of PHAs in artificial organs, artificial blood vessels and materials for wound treatment based on biocompatibility of the PHAs were published by Chen (2010).
Since the 1980s many companies have tried to commercialise PHA on a pilot or the industrial scale. Chen (2009) tabulated more than twenty companies engaged in production of PHA in UK, Austria, Germany, USA, Japan, Brazil, Italy and China. Although some of the PHA related projects were terminated due to relatively stable petroleum prices, some other companies such as ADM Metabolix (USA) and Bio-On (Italy) produced PHA in a scale of fifty and ten thousand tonnes per year respectively (Chen, 2009). The main barrier in widespread production of PHAs has been the cost difference in comparison with the petro-products. In 1998, Biopol, a commercially available bioplastic product, was marketed at around 17 times the price of synthetic plastic (Ramadas et al., 2010). This price difference was reduced to 9 euro/kg for PHAs versus 1 euro/kg for synthetic plastics in 2002 (Serafim et al., 2004). Despite all efforts, production cost of PHA is still considerably higher than the equivalent petrochemical plastics.
In order to minimize the overall cost of PHA, production steps should be identified and optimized accordingly. Large scale production of PHA occurs via microbial fermentation. Development and selection

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of the best feeding strain, optimization of the shake flask step, pilot plant and industrial scale up studies along with the PHA extraction and purification methods are the main development stages (Chen, 2009).
PHAs are intercellular products of various microorganisms. More than 300 different microorganisms have been documented capable of synthesizing PHA. Although industrial production processes are based on the use of pure cultures, development of MMC by cloning microorganisms with the PHA synthase genes has recently gained attention. Application of MMC enables process operation with no need for sterilization resulting in cost effective production. Additionally, application of a mixture of organisms with different properties maximise production in varying operation conditions (Dias et al., 2006).
Optimisation of PHA production from MMC in SBR using a mechanistic model is reported by Dias et al. (2005), however, the development of detailed mechanistic models is very challenging. This paper presents a data driven modelling method through operating regime decomposition and bootstrap aggregated neural networks. Neural network models offer a simple procedure to build relatively accurate models from process operation data. Additionally, these empirical models open new windows in the application of optimization techniques addressed for this type of processes.

2. Process operation regimes

2.1 Classification of process operation regimes

Good process understanding is the key for accurate process modelling. With this aim, the mechanistic model developed by Dias et al. (2005) was used to investigate classification of different operation regimes. A series of factors were defined to scrutinize PHB production curves over production time as well as the curves of acetate and ammonia concentrations. Based on these factors, a number of qualitative operation regimes were identified to specify the state of the batch at any time during batch progress. Figure 1 illustrates the nine most dominant and important Regime Types (RT). Each plot consists of three subplots demonstrating acetate concentration on the top, ammonia concentration in the middle and PHB concentration at the bottom.
In the first regime (RT1), the two feeding substrates of acetate and ammonia are present in the medium and hence a gradual accumulation of PHB is observed. In the second regime (RT2), complete depletion of acetate occurs while ammonia is in excess. Based on experimental results, it was concluded that whenever ammonia was present in the medium, cell growth and PHB formation occurred simultaneously (Dias et al., 2005). During the course of this regime, PHB concentration augmented until acetate complete exhaustion occurred. After this point, biomass concentration continues to increase in reflect of further ammonia consumption and depletion of PHB as a source of energy for metabolic activities of the cells. In Figure 1, the point when acetate concentration value becomes relatively steady is denoted by (∇) on the acetate and PHB subplots.
In the third regime (RT3), ammonia consumption rate decrease to a small value followed by complete exhaustion of acetate. In this case, cells have already started to consume PHB as an energy source for cell growth and metabolic activities in the absence of an external carbon source. In Figure 1, the ammonia consumption halt point is depicted by ($\triangle$) on both ammonia and PHB subplots. In the fourth regime (RT4), unlike RT2, ammonia fades away prior to acetate depletion. In this case, biomass concentration undergoes an initial increase followed by a stagnation stage in the absence of the nitrogen source. In this regime PHB constantly increases by continuous consumption of the carbon source.
In the fifth regime (RT5), unlike the third regime, complete depletion of ammonia occurs prior to the complete consumption of the acetate. In this case, cell growth and PHB formation continue until there is no ammonia in the medium. After this point up to the point when acetate is completely consumed, no further growth occurs in the biomass; however, PHB is formed by consumption of the carbon source. When both carbon and nitrogen sources are wiped out, PHB is consumed as an energy source.
In the sixth regime (RT6), acetate concentration relatively stabilises near a constant value followed by complete exhaustion of ammonia. In this regime, biomass concentration reaches a maximum value in reflect of total consumption of ammonia. Afterwards, PHB accumulation continues until most of the cell cultures reach their maximum capacity of preserving PHB as their intercellular product. Acetate consumption rate reduces to a small value based on the carbon needs of cells for their metabolic activities and further augmentation of PHB by the unsaturated cells. In this regime, PHB formation was not fully accomplished by the end of the batch.
The seventh regime (RT7) appears following the sixth regime when sufficient time is allocated to the process until a steady positive value for PHB is obtained. This point in time is demonstrated by (+) signs on the PHB subplots in Figure 1. The eighth regime (RT8) appears by further continuation of the seventh regime when the total PHB concentration remains at its maximum value for over 10 % of the total process analysis time interval. This point in time is illustrated by vertical (O) signs on the PHB subplots in Figure 1.

In the ninth regime (RT9), PHB concentration reaches a maximum constant value, maintains that concentration for more than 10 % of the total analysis time and then PHB depletion occurs due to complete acetate depletion used for metabolic activities of cells.
It is important to note that only the most dominant and important operation regimes are introduced in this paper and it does not reflect all the regime types obtained from simulation results.

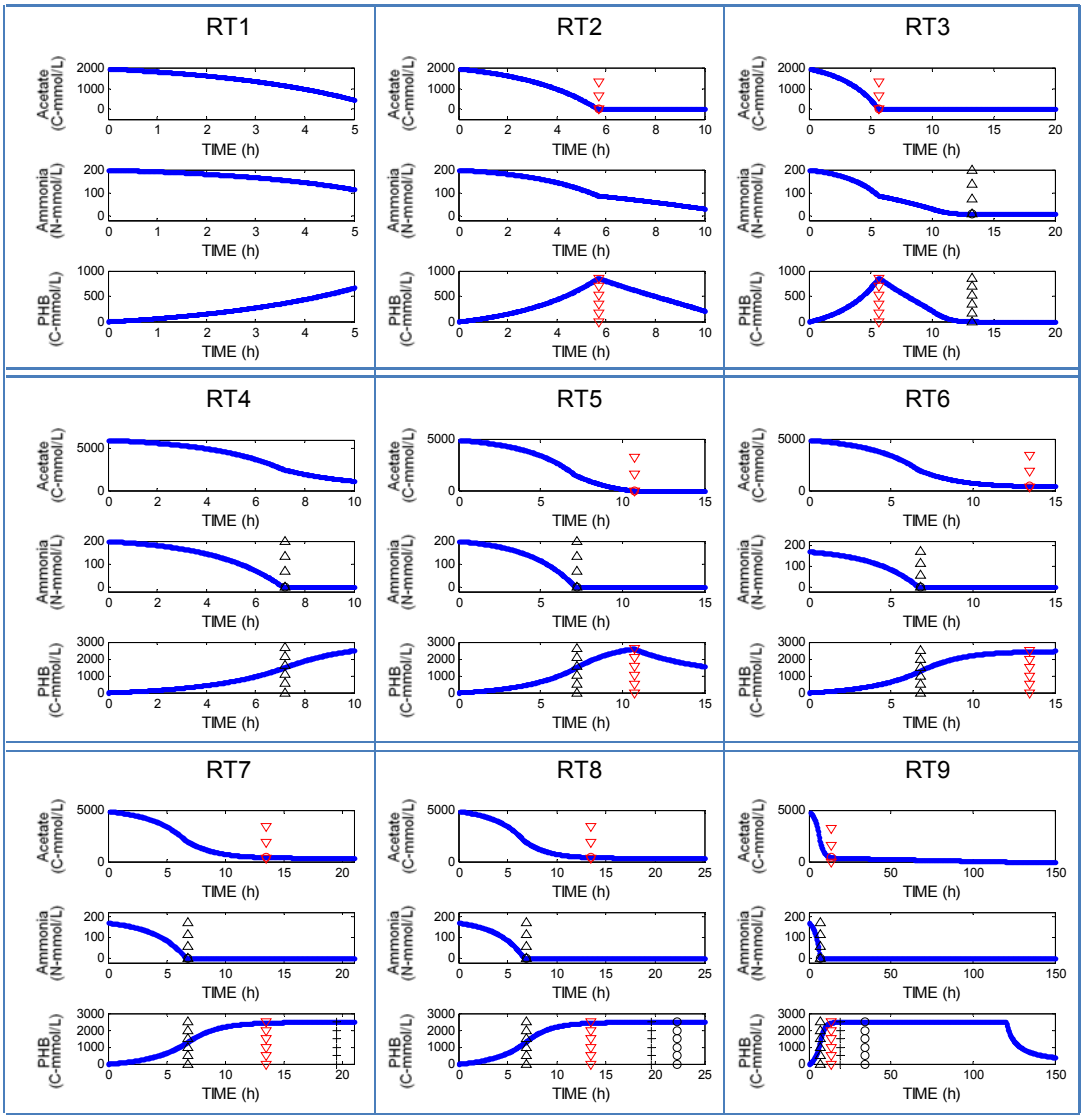

Figure 1: Qualitative demonstration of the nine operational regimes for PHB production under MMC

2.2 Process characteristics

The PHB production cycle by mixed microbial cultures has a “feast” phase and a “famine” phase in SBR operation. The “feast” phase is a state of operation in which nutrition required for either cell growth or formation of the microbial product is available in the operational system. On the other hand, in the “famine” phase at least one element for microbial production is absent. Although PHB decreases in “famine” phase, its occurrence is inevitable since it plays a crucial role in the process feasibility of the SBR operation. The physiological adaptation of cells during a period of nutrient shortage will result in a higher PHB formation in the subsequent “feast” phase. The time duration and the routine of executing the “famine” phase play important roles in the optimisation of the sequential batch operations (Dias et al., 2005).
In the “feast” phase of MMC, unlike the most pure cultures, cell growth and PHB accumulation occur simultaneously. Looking at Figure 1, it may be concluded that excess acetate (with consideration on operational pH range) directs the SBR operation to the “feast” phase in order to maximize PHB formation. On the other hand, ammonia feeding is the key factor in maximizing volumetric productivity of the SBR since it directly affects the cell growth rate. However, high ammonia concentration results in excess growth

and deficiency of the carbon source for PHB formation and eventually for metabolic activities. Low ammonia concentration favours PHB formation with the cost of low volumetric productivity. A trade-off solution is required for optimization purposes (Dias et al., 2005).

2.3 Characterisation plot

With the aim of characterizing operational regimes based on initial acetate, ammonia and biomass concentrations over batch progression time, a series of more than ten thousand simulated batch operations were run and scrutinized systematically. Figure 2 shows the regime types at four consecutive progression times for a given initial biomass concentration of 50 millimoles of carbon per litre (C-mmol/L). In Figure 2, the parameters used for n, m, t0, t1, t2, t3, and t4 are 350 millimoles of nitrogen per litre (N-mmol/L), 9,000 C-mmol/L, 0 hours (h), 10 h, 20 h, 30 h, and 300 h respectively. These values were selected in order to generate characterisation plots illustrating different regime types and their transformation. The characterisation plot can be used to estimate the state of a batch as defined by the regime types at any time during a batch progress. A point coordinated by the initial acetate and initial ammonia concentrations on a characterisation plot of a specific progression time (Δt), lies in an area associated with a regime type for a given initial biomass concentration. Persecution of that coordinate on different characterisation plots drawn for various progression times for a given initial biomass concentration provides an overview for the batch progression route.

If a characterisation plot was drawn in the beginning of the process, a full area of RT1 would be expected as both controlling nutrients are present at this stage. With batch progression, for any pair of acetate and ammonia concentrations, depending on the order of depletion, either RT2 (acetate depletion) or RT4 (ammonia depletion) will follow. Further progression of a batch operating in RT2 will be followed by total consumption of PHB by growing cells. The ammonia concentration decreases to zero and batch process starts to operate in RT3.

On the other side, two different operational regimes can be derived by further progression of RT4. RT5 appears when acetate concentration is exhausted after complete exhaustion of ammonia. However, in RT6 acetate consumption rate decreases to zero while acetate is still in excess. Operational regimes RT7, RT8 and RT9 consequently appear over time with further progression of RT6.

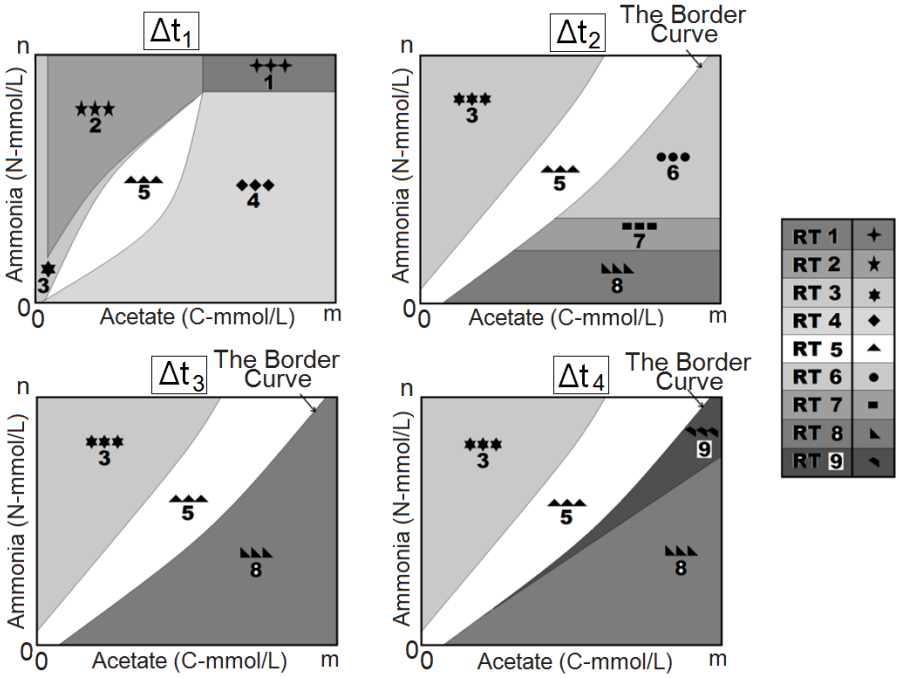

Figure 2: Characterisation plots

Looking at the first plot in Figure 2 (Δt1=t1-t0), five of the operational regimes are shown for different combinations of initial acetate and ammonia concentrations. In the second plot (Δt2), further progression of the regimes is shown following the pattern previously discussed. Additional batch progressions are shown in the third and the fourth plots. As illustrated, there is no regime change over time for a batch operating in the fifth regime RT5. Looking at these plots, it is possible to identify a border curve between the RT5 regime area and the regime areas of RT4, RT6, RT7, RT8 and RT9. This curve separates the “feast”

phase operating regimes from the “famine” phase operating regimes. Based on the carbon-nitrogen ratio of the initial nutrient concentrations, it would be possible to indicate whether a batch process would operate in a “feast” or a “famine” phase for a given initial biomass concentration.

2.4 “Feast” and “famine” phase border curve equation

Mathematical equations of the border curve separating the “feast” operation regime area from the “famine” operation regime area on the characterisation plot can play an important role in the process optimisation. Based on the generated simulation data, the following mathematical equations are proposed to characterize the border curve as a function of initial biomass concentration for a mature batch as depicted in Figure 2.

$$Y - 0.036\,(X - (4.5Z + 222.6)) = 0, \qquad Z < 10^3 \qquad (1)$$

$$Y - 0.036\,(X - (4.5\times10^{-4}Z^2 + 2.25Z + 3104.4)) = 0, \qquad 10^3 < Z < 10^4 \qquad (2)$$

In equations (1) and (2), X, Y and Z are the initial acetate, ammonia and biomass concentrations respectively in C-mmol/L, N-mmol/L and C-mmol/L. These equations estimate a border curve on the characterisation plot for a given initial biomass concentration.

3. Prediction of optimal batch time using bootstrap aggregated neural networks

Developing an optimal operation strategy requires good understanding of the process and an accurate model of the process. Neural networks have emerged as a powerful tool in developing nonlinear models for highly nonlinear industrial processes. Development of neural network models based on process input-output data is relatively straightforward and their models have been proven to be a powerful representation of complex nonlinear behaviour.

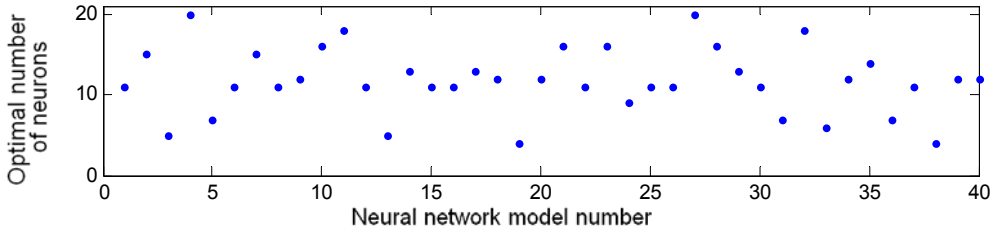

Figure 3: Optimal number of neurons for the individual neural networks

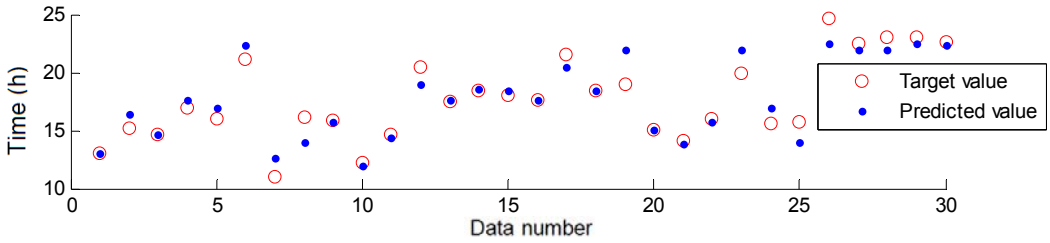

Figure 4: Model prediction performance on unseen validation data

As mentioned in this study, a successful PHB production batch undergoes the “feast” operation regime route. In order to provide a model capable of predicting batch completion time, it is essential to train the model with data obtained from batch processes operating in the “feast” operation regimes. In order to form an appropriate operational data set using a process simulation program, equations (1) and (2) were applied. The neural network model inputs are initial biomass concentration, acetate feeding concentration, and ammonia feeding concentration. The neural network model output is the time when PHB production reaches its maximum value (indication of optimal batch completion time).

Ten random values were selected in the range of 20 to 4,000 C-mmol/L for initial biomass concentration. Similarly, five values were nominated randomly in the range of 1,000 to 90,000 C-mmol/L and 60 to 2,000 N-mmol/L for each of the acetate and ammonia feeding concentrations. In the case study, a set of data was stored for 100 batches operating in the “feast” operation regimes while the other 150 batches were undesirable operations. Thirty percent of the data was reserved as the unseen validation data while the

remaining was used for model development. Data was scaled to the range of -1 and 1 prior to model development. The bootstrap aggregated neural networks (Zhang, 1999) are applied to predict optimal batch completion time (occurrence of the seventh operation regime type). Bootstrap re-sampling with replacement was used to generate 40 replications of the model development data. Each replication was randomly partitioned into a training data set and a testing data set. Each of these pairs is used to build a neural network model. For each neural network, the optimal number of hidden neurons is determined through cross validation, i.e. the network with the lowest SSE (sum squared errors) on the testing data is considered as having the optimal number of hidden neurons. The individual networks are then combined to give the aggregated neural network model. The bootstrap aggregated neural network models provide a more robust prediction capability. Moreover, it is relatively easy to deliver model prediction confidence bounds for aggregated models (Zhang, 1999).

Figure 3 shows optimal numbers of hidden neurons for each of the 40 neural network models. Predictions from these 40 models were then combined to give the final model prediction. Figure 4 shows the predicted and the target values for the optimal batch completion time on the unseen validation data set. It can be seen from Figure 4 that the model predictions are quite accurate.

4. Conclusions

Optimal production of PHB in SBR requires accurate modelling of the process. With this aim, process behaviour was scrutinised to define a number of factors enabling operation regime classification. Based on those factors, nine of the most dominant and important operation regimes were defined and characterisation plots were drawn to qualitatively model process progression routine based on its initial state. Further studies of the characterisation plot led to providing a mathematical representation for the border between the “feast” and “famine” phase operating regimes. This equation can differentiate a characterisation plot into a “feast” and a “famine” operation regime area based on the batch initial state. Knowing that the PHB production occurs in the “feast” operation regime area, the bootstrap aggregated neural network modelling technique was employed to develop models predicting the optimal batch completion time based on initial concentrations of the biomass, acetate and ammonia. It was demonstrated that the bootstrap aggregated neural network model can give accurate predictions.

For optimisation of a SBR producing PHB with mixed microbial cultures, both “feast” and “famine” operational regimes should be considered. Production occurs in the “feast” phase; however, the “famine” phase plays a crucial role in process feasibility. The optimal SBR cycles should operate near border curve of the characterisation plot differentiating the two “feast” and “famine” operation regime areas. The empirical model proved to be sufficiently accurate to estimate production batch competition operating under the “feast” phase. However, optimal duration of the “famine” phase requires further investigation. A long “famine” phase increases stability of the cell culture at the expense of losing some of the PHB products.

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