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A Systematic Petri Net Approach for Multiple-Scale Modeling and Simulation of Biochemical Processes

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Abstract A method to exploit hybrid Petri nets for modeling and simulating biochemical processes in a systematic way was introduced. Both molecular biology and biochemical engineering aspects are manipulated. With discrete and continuous elements, the hybrid Petri nets can easily handle biochemical factors such as metabolites concentration and kinetic behaviors. It is possible to translate both molecular biological behavior and biochemical processes workflow into hybrid Petri nets in a natural manner. As an example, penicillin production bioprocess is modeled to illustrate the concepts of the methodology. Results of the dynamic of production parameters in the bioprocess were simulated and observed diagrammatically. Current problems and post-genomic perspectives were also discussed.

Keywords Biochemical engineering · Modeling and simulation · Multiple-scale · Petri nets · Fed-batch bioprocess · Penicillin fermentation

Notation

F_{in}	Combined inlet flow rate of all additions ($dm^3\ h^{-1}$)
F_{out}	Outlet flow rate of evaporated water ($dm^3\ h^{-1}$)
G	Glucose concentration ($g\ dm^{-3}$)
G_{in}	Glucose concentration in the feed ($g\ dm^{-3}$)
K_d	Autolysis rate constant (h^{-1})
K_h	Penicillin hydrolysis rate constant (h^{-1})
K_p	Product saturation constant ($g\ dm^{-3}$)
K_s	Substrate saturation constant ($g\ dm^{-3}$)
K_x	Growth saturation constant ($g\ g\text{-}DW^{-1}$)
N	Soluble organic nitrogen concentration ($g\ dm^{-3}$)
P	Penicillin concentration (as potassium salt) ($g\text{-}PenGK\ dm^{-3}$)

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S	Substrate concentration (g dm^{-3})
t	Time (h)
V	Culture broth volume (dm^3)
X	Biomass concentration (g-DW dm^{-3})
$Y_{x/s}$	Substrate to biomass yield (g-DW g^{-1})
$Y_{p/s}$	Substrate to penicillin yield (g-PenGK g^{-1})
ξ	Specific substrate consumption rate for maintenance (g g-DW^{-1} h^{-1})
ξ_{max}	Maximum specific substrate consumption rate for maintenance (g g-DW^{-1} h^{-1})
μ	Specific biomass growth rate (h^{-1})
μ_{max}	Maximum specific biomass growth rate (h^{-1})
π	Specific penicillin production rate (g g-DW^{-1} h^{-1})
π_{max}	Maximum specific penicillin production rate (g g-DW^{-1} h^{-1})
σ	Specific substrate consumption rate (g g-DW^{-1} h^{-1})

Introduction

During the last few decades, the science of industrial biotechnology has undergone rapid development. The scope of the field includes biotechnological production processes or fermentation processes, in which a considerable diversity of products are produced (e.g., food products, solvents, enzymes and pharmaceutical products, etc.), as well as biological wastewater treatment processes. In order to enhance the production of target products in fermentation process, genetic improvements of metabolic pathways and effective process design and control are highly desired.

Meanwhile, the post-genomics era provides us with extensive numerical molecular data that greatly improves our understanding of industrial strains at both genomic and proteomics level. So far, a total of 1,349 microbial genomes (1,257 bacterial, 92 archaeal) have been fully sequenced. From the standpoint of metabolic engineering, these developments have opened a new realm of exciting possibilities. Metabolic analysis and engineering strategies can now be built on a sound genomic basis. Computational approaches such as metabolic control analysis [1], flux balance analysis [2], and elementary mode analysis [3] have been employed with success for years.

The ability to predict performance and refine bioprocesses by modeling and simulation is now increasingly important due to the high capital and operating costs of large scale production bioprocesses. While mathematical modeling for bioprocess control is a very tricky and time consuming work, knowledge-based bioprocess operation has been preferably employed, using experiences of operators. In the last decade, rapid progress has been made in developing tools for controllability analysis based on linearized process models. Computing intelligent techniques, such as fuzzy logic [4] and neural network [5] and their combination [6], are implemented for process control and operation.

Petri net (PN) is one of the several mathematical modeling languages for the description of distributed systems. It was first introduced and formally defined by Prof. Dr. Carl Adam Petri in the 1960s [7]. Since then, the Petri net formalism has been extended and developed, and both theory and application aspects of this model have been flourishing. Petri nets provide the primitives of the description of the synchronization of concurrent processes, while programming languages provide the primitives for the definition of data type and the manipulation of data values [8]. An approach for analyzing recipe-controlled chemical production processes by means of CP-nets was developed and has been mainly to be used to examine processes from the chemical industry [9]. We are motivated to model and simulate gene-controlled bioprocesses with Petri nets in this work.

Biochemical Processes

The behavior of bioprocessing systems is extremely complex, with the occurrence of subtle or even paradoxical phenomena. They are either discrete or continuous systems, and also hybrid systems, where these two extreme views of dynamic systems are applied to different subsystems. When the product is composed of discrete parts, it can be quantified in a discrete manner, by an integer number. For example, manufacturing systems and assembly lines are typical systems where various discrete parts of some device are assembled. In contrast, if the product is a fluid as it is mainly the case in process systems, like chemical or food industry, the characterization becomes more difficult. Typically, three categories of such processes which are distinguished: the batch process, fed-batch process, and the continuous one (Fig. 1).

Batch fermentation refers to a partially closed system in which most of the materials required are loaded onto the fermentor, decontaminated before the process starts and then removed at the end. The only material added and removed during the course of a batch fermentation is gas exchange and pH control solutions. In this mode of operation, conditions are continuously changing with time. Therefore the fermentor is an unsteady-state system; although in a well-mixed reactor, conditions are supposed to be uniform throughout the reactor at any instant time. The principal disadvantage of batch processing is the high proportion of unproductive time (down time) between batches, comprising the charge and discharge of the fermentor vessel, the cleaning, sterilization, and re-start process [10].

Fed-batch processes are commonly used in industrial fermentations, for example, in the production of baker's yeast, some enzymes, antibiotics, growth hormones, microbial cells, vitamins, amino acids, and other organic acids. The reason for this diversity of use is related to the theoretical analysis of many fermentation processes, which revealed the extended or exponentially fed-batch cultures to be optimal, particularly in terms of productivity. Fed-batch fermentations have also gained popularity because of the improved control possibilities, such as computer-coupled fermentation systems. The fed-batch reactor displays many operating characteristics typical of continuous systems. However, due to the inherent mode of operation of this process, the system can never really reach a true steady state, which makes the control of fed-batch reactors a very challenging task [11].

Continuous culture can outperform batch culture by eliminating the inherent down time for cleaning and sterilization, and the long lags before the organisms enter a brief period of high productivity. It is sometimes possible to maintain very high rates of product formation for a long period time with continuous cultivation. Continuous culture is superior to batch culture in several ways for research. Interpretation of results is difficult for batch culture because of changing concentrations of products and reactants, varying pH, and redox potential and a complicated mixture of growing, dying, and dead cells. On the other hand, there is less complexity in the data generated from continuous cultures dynamic equilibria or small excursions from steady state. In other word cause and effect relationships tend to

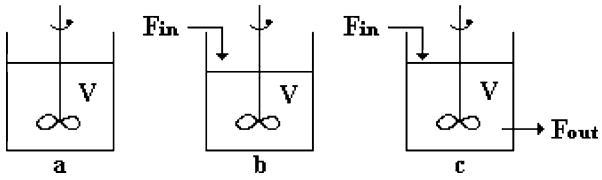

Fig. 1 Three types of bioprocesses: **a** batch process, **b** fed-batch process, and **c** continuous process

be more direct and obvious. Nevertheless, although bioreactor productivity is higher in continuous cultures, there is no such great improvement over batch cultures of the relatively higher number of tanks and resources needed for operation and sterilization to support the continuously operated vessel [12].

Petri Nets

Petri net is a graphic oriented language of modeling, specification, simulation, verification, and control of systems. It offers a formal way to represent the structure of a discrete event system, simulate its behavior, and draw certain types of general conclusions on the properties of the system. It is in particular well suited for systems in which communication, synchronization, and resource sharing are important [13]. A large amount of investigations on Petri nets have been compiled in the literature, and various applications have chosen Petri nets as their control models due to its intuitively understandable graphical notation (http://www.informatik.uni-hamburg.de/TGI/PetriNets/).

A Petri net is a directed bipartite graph in which the nodes represent transitions and places. The directed arcs describe which places are pre- and/or post-conditions for which transitions. The formal definition is the followings.

A Petri net is a 3-tuple (P,T,F), where

- $P=\{p_1, p_2, \ldots, p_n\}$ is a finite set of *places*;
- $T=\{t_1, t_2, \ldots, t_m\}$ is a finite set of *transitions*;
- $P \cap T=\Phi$, i.e., P and T are disjoint;
- F: $(P\times T)\cup(T\times P) \rightarrow N$ is a multiset of arcs, i.e., it defines arcs and assigns to each arc a non-negative integer; note that no arc may connect two places or two transitions.

A marking of a Petri net is a mapping M:$P \rightarrow N$, i.e., a multiset of its places; each place is assigned the marking (a number of tokens).

A marked Petri net is a 4-tuple $(P,T,F;M_0)$, where

- (P,T,F) is a Petri net graph;
- M_0 is the *initial marking*, a marking of the Petri net.

Ordinary Petri net models do not have such functions as quantitative aspects, so there are some extensions of Petri nets such as stochastic Petri nets [14], self-modified Petri nets [15], used to express gene regulatory networks or biochemical metabolic pathways. Hybrid Petri nets consist of discrete and continuous event systems. Therefore, it also allows quantitative analysis. In this report, we introduce hybrid Petri nets for expressing fermentation processes.

There are quite a number of Petri net tools that have been developed. Many of these, which can be found on the "Petri net world" website, are available to model and simulate bioprocesses. To name a few, these tools include Snoopy [16] and Cell Illustrator [17]. In this report, we used a hybrid Petri net tool, Visual Object Net ++ [18]. It is a small, quick, uncomplicated, and intuitive Petri net tool that supports both discrete event Petri nets and timed event/condition PN. Figure 2 shows the basis elements of VON++, and there are discrete transition, discrete place, continuous transition, and continuous place connected with normal arc, inhibitor arc, and test arc, respectively.

VON++ has a user-friendly graphical interface, which consists of object-oriented Integrated Developing Environment (IDE) and file management tool; it also represents a class hierarchy in the 'Factory' window. There are animation features enabling easy

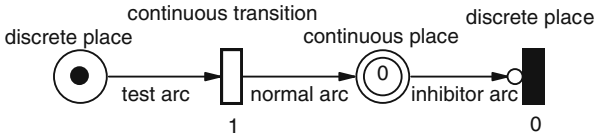

Fig. 2 Elements of the hybrid Petri net

observation of the dynamic behavior of the nets. Its object-oriented user interface allows the easy design, simulation, visualization, and documentation of hybrid PN [19].

Modeling and Simulation

Classification of mathematical models may be subdivided into analytical and discrete ones. Analytic models perform the processes of elements functioning as some functional relations (algebraic, integral-differential, finite-differential, etc.) or logical conditions. An analytical model may be studied by qualitative, analytical, or numerical methods. Dynamic models are generally based on integral and differential systems of equations. Although dynamic responses of biochemical and environmental systems are often poorly understood, models with some basic features and some empirical features have been found to correlate with actual data fairly well.

Discrete models are based on state transition diagrams. Simple models of this class are based on simple production units, which can be combined. The graphical model of Kohn and Letzkus, which allows the discussion of metabolic regulation processes, is representative for the class of graph theoretical approaches [20]. They expand the graph theory by a specific function which allows the modeling of dynamic processes. In this case, the approach of Petri nets is a new method.

Various PN formalisms (low or high level, discrete or continuous, etc.) have been used for diverse purposes within the field of production system, including modeling, analysis, simulation, control design, control implementation, monitoring, and supervision. A great number of related publications have appeared in the past decade. Scientific journals are eager to organize and publish such topics as well. For instance, a prominent special issue on Petri nets for modeling and simulation of biological network was released in 2003 by *In Silico Biology* [21] and an updated virtual issue was made in early 2010 [22]. Most recently, there is a special issue on "Petri nets for system control and automation" that collects the latest developments and achievements in the field of systems control and automation [23]. A good review paper on Petri net modeling of biological networks has highlighted the fruitful applications at the molecular level [24]. Unfortunately, limited efforts were dedicated to biochemical engineering. One of the main tasks of a bioprocess engineer handles the study, control, and maintenance of a biological process such as the production of beverages, pharmaceuticals, antibiotics, enzymes, biochemicals, enzyme-catalyzed reactions, food processing, and biological waste treatment. These processes require a well-designed optimal growth environment to obtain maximum product yield and subsequently these conditions needed to be carefully controlled at specific points or within narrow ranges which often follow specified trajectories. Environmental design involves the determination of the environmental clearance of the process, while fermentation engineering provides the means for meeting those requirements. In addition, PN models are useful to perform simulations to gain insights on the system behavior or to estimate its performance. Simulation is particularly useful in the case of batch systems and, in general, in the case of hybrid systems [25].

Properties of Fermentation Processes

In a batch process, the material is operated by finite quantities (the batches). At any time, an integer number of batches is in operation at various locations in the plant. In this configuration, the process mimics the manufacturing system introduced above, i.e., the batch of fluid in a vessel can be considered as a part. On the other hand, the complete characterization of a batch is reflected by real numbers referring to the amount of material and its operating conditions (temperature, pressure, quality, etc.). During the transport of material between two vessels, the discrete nature of the product is temporarily ignored.

Nevertheless, the distinction between discrete and continuous systems should not be considered as absolute. It is often possible to extract a discrete view in continuous processes such as chemical ones. Sometimes the continuous and discrete operations correspond to two different stages of a single production system, for example, a continuous production of fluid and the discrete packaging operations. In contrast, in other cases, very often in fine chemical processes and in food industry, the continuous and discrete operation alternates each other along the production process.

Let us suppose a simple fed-batch production system (Fig. 3). Firstly, a part of batch or raw material fluid is fed into a reactor. A reaction is executed. After that the batch is transferred into a buffer, and during this transfer, another part of batch is to be fed at a certain time point. It has to be pointed out that both the reaction and the transfer from the reactor to the buffer are continuous operations because the flow rate is constant which cannot be modified. In contrast, the batch feed and remove out of buffer or back into the reactor as batch feed are discrete operations, and the feed is proportional to the batch size. After certain cycles of fed-batch and when the operation terminates, the final batch is transferred out of the reactor. The simulation curve shows that the volume of batch waves within a stable range according to the batch feed interval and the size of feeding and removal.

Considering a continuous model of process, unit operations in transient state are sets of differential and algebraic equations of the following form. Process design of continuous fermentation is based on steady-state optimization or on heuristics. Dynamic aspects are not usually considered. The design of control systems that are able to keep the process at the optimal steady state is a task only considered at a later stage of the design process. Normally at this stage of the design procedure, it is assumed that the structure of the process is fixed, and it is costly to change the design of the process to improve its ability to maintain the setpoints. In contrast to this, it is more effective to design the process with respect to good controllability in

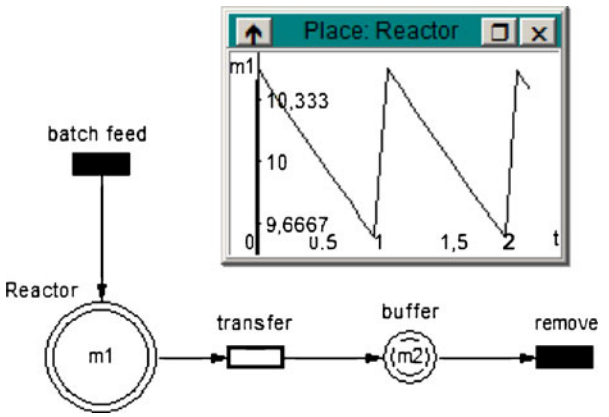

Fig. 3 Illustration of a Petri net model of Fed-batch process

order to avoid difficult control problems. In other word, dynamic aspects of the process should be considered earlier to design for improved controllability.

Petri net is useful for analytical purposes, but it is too abstract to capture essential features of this system, and the associating sensors or durations to the transitions in order to perform simulation or to derive a controller are not so straightforward. Hence, we choose to emphasize on the behavior of the bioprocess, the growth rate of microbial, product yield rate and consumption rate of substrates, etc.

The main feature of bioprocesses is that the concentration of substrates and metabolites will influence the reaction activity of biochemical processes. Therefore, the actual concentration of any metabolite is an important component of the model. As such, interpretation of VON++ as biochemical processes will be (Table 1):

The rate of bioprocesses is not defined within a Petri net, it should be specified separately. In automated control systems represented by Petri nets, execution of transitions usually depends on the presence of specific number of tokens in all staring places. However, in most chemical and biological systems, the rate of processes (transitions) is defined by the mass action law. The rate of change in the number of tokens (or concentration) is proportional to the number of tokens (or concentration) in all starting places as expressed in Fig. 4. V is the rate of firing of the transition; k is a constant (called a rate coefficient in chemical kinetics); and m3 and m4 are the concentration of place S1 and place S2 respectively. Coefficient k varies with temperature, pressure, solvent, and other factors. As a result, k will become a function of several variables.

Normal discrete system is easily comprehensible. We emphasize here on the continuous one which shows very useful to the dynamic system.

Figure 5 indicates how token number (or concentration) of reaction intermediate P1 changes. Mass action law assumes that particles move incessantly. However, micro-organisms are not like gas molecules. Biochemical reaction is complex in nature, with the existence of interaction delay or saturation effect. In these cases, mass action law becomes violated and should be replaced by equations which could more accurately reflect the biological interaction while the rest of the algorithm remains the same. In bioprocess, the most commonly used expression that relates the specific growth rate of the cell to the

Table 1 Interpretation of the hybrid Petri nets as bioprocesses

Petri net term	Molecular biological term	Biochemical processes term
Place (discrete and continuous)	Genes, promoters, metabolites, proteins, …	Substrate, product, reactor, vessel, metabolite, ...
Transition	Binding, bioevents, bioreaction (rate represented by functions)	Reaction or workflow
Input arc	Defines reagent of reaction	Defines input of reaction or workflow
Output arc	Defines product of reaction	Defines output of reaction or workflow
Weight function	Rate of reaction (continuous system meaningless, kinetic parameters involved)	Rate of reaction or workflow (continuous system meaningless, kinetic parameters involved)
Marking	State of reaction system (concentration of substrate and/or products)	State of reaction or workflow
Initial marking	Initial state of system	Initial state of reaction or workflow
A transition being enabled	Enough of all the reagents must be present for the reaction to complete	Enough of all the inputs must be present for the reaction or workflow to complete
A firing transition	A single reaction	A single reaction or workflow

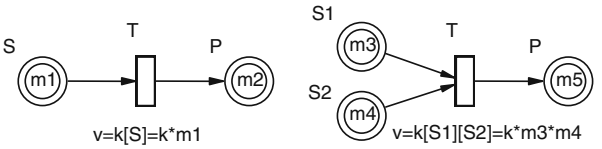

Fig. 4 Presentation of transition rate in continuous systems

substrate concentration is Monod's equation, which is given as $\mu = \frac{\mu_{\max} \cdot S}{K_s + S}$; other equations which are proposed to describe the substrate limited growth rate can also be adapted. For instance, Tessier equation, $\mu = \mu_{\max}\left(1 - e^{-KS}\right)$; Moser equation, $\mu = \frac{\mu_{\max} \cdot S^n}{K_s + S^n}$ and Contois equation, $\mu = \frac{\mu_{\max} \cdot S}{K_{sx} \cdot X + S}$, etc. [26].

Concerning bioprocess kinetics, the following well-known functions (Eqs. 1–3) are often used to describe the system in terms of biomass growth, substrate consumption, and production formation. In many cases, derivate equations are preferably presented in special bioprocesses, for example, in penicillin production, Eqs. 4–13 are to be utilized in our hybrid Petri nets model [27].

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \mu \cdot X \tag{1}$$

$$-\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{\mu \cdot X}{Y_{x/s}} + \frac{\pi \cdot X}{Y_{p/s}} + \xi \cdot X \tag{2}$$

$$\frac{\mathrm{d}P}{\mathrm{d}t} = \alpha \cdot \frac{\mathrm{d}X}{\mathrm{d}t} + \beta \cdot X \tag{3}$$

All the differential equations listed above are well-known and powerful methods to describe behaviors of bioprocesses. Using hybrid Petri nets, offer the advantage to handle them and construct bioprocesses a natural manner.

Case Study

In this section, we use a case study on penicillin production to demonstrate and evaluate the bioprocess synthesis technique.

Background

One impetus for fed-batch operation originated from developments in making penicillin production. Early investigations revealed that lactose gave better penicillin yields than other

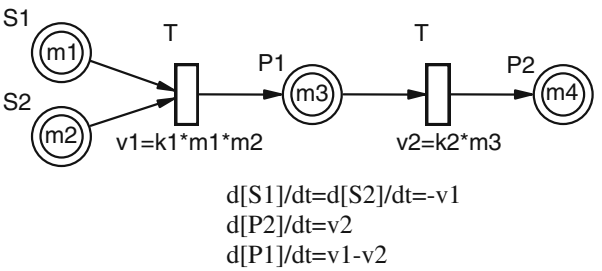

Fig. 5 Presentation of intermediate reaction rate

sugars. Lactose obtained from companies that manufacture evaporated milk was about three times the cost of glucose the usual sugar for microbial fermentations. When it was realized that the microorganisms were hydrolyzing lactose to its monomers, galactose, and glucose, expectedly by the organisms, a logical experiment would be to feed glucose instead. The result was a three to fourfold increase in penicillin yield. When feed rate was slow, product formation was restricted. On the other hand, a high feed rate was found to encourage, the organisms growth instead of penicillin production. The remarkable improvement in yield management has made it highly worthwhile to monitor glucose concentration and to establish the feed rate to be within the range for optimum yields. As a result, the market for lactose vanished as companies switched to glucose feeding.

Penicillin fermentation provides an excellent example of the use of feed systems in the production of a secondary metabolite. Production of penicillin usually begins with the aerobic fermentation of *Penicillium chrysogenum* in batch fermentors. A carbon source (e.g., sucrose) and various nutrients such as corn steep liquor, ammonium sulfate, and inorganic salts are required for bacterial growth and product synthesis. As the penicillin is produced, it is secreted from the cells into the surrounding medium and reaches a concentration of around 20 to 35 g/l.

Details of the parameters of synthesis and mathematical functions used to describe the fermentation kinetics are collected from the literature [28] and summarized in Tables 2 and 3 and as Eqs. 4–13, respectively. It worth noticing that the feed substrate concentration and flow can assume different values during synthesis. This allows us to determine the effect of fermentation conditions on the optimal flowsheet.

$$\frac{\mathrm{d}X_{live}}{\mathrm{d}t} = \mu \cdot X_{live} - F_{in} \cdot \frac{X_{live}}{V} - K_d \cdot X_{live} \tag{4}$$

$$\frac{\mathrm{d}X_{dead}}{\mathrm{d}t} = K_d \cdot X_{live} - F_{in} \cdot \frac{X_{dead}}{V} \tag{5}$$

$$\frac{\mathrm{d}P}{\mathrm{d}t} = \pi \cdot X_{live} - F_{in} \cdot \frac{P}{V} - K_h \cdot P \tag{6}$$

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\sigma \cdot X_{live} - F_{in} \cdot \frac{S}{V} + F_{in} \cdot \frac{G_{in}}{V} \tag{7}$$

$$\frac{\mathrm{d}V}{\mathrm{d}t} = F_{in} - F_{out} \tag{8}$$

$$\mu = \frac{\mu_{\max} \cdot S}{K_X \cdot X_{live} + S} \tag{9}$$

$$\pi = \frac{\pi_{\max} \cdot S}{K_P + S} \tag{10}$$

Table 2 Fermentation parameters

μ_{max}	K_x	K_d	π_{max}	K_p	K_h	ξ_{max}	K_s	$Y_{x/s}$	$Y_{p/s}$	X(g-DW dm^{-3})	P(g dm^{-3})	G(g dm^{-3})	N(g dm^{-3})	V(dm^3)
0.130	0.131	0.006	0.011	0.0001	0.002	0.020	0.0001	0.52	1.0	1.0	0	4.0	12.0	600

Table 3 Fermentation parameters

$\Delta t(h)$	G_{in} (g dm^{-3})	F_{in} (dm^3 h^{-1})	F_{out} (dm^3 h^{-1})	Discharges
0–10	0	0	0.75	–
10–70	~240	$4-2\exp[(10-t)/25]$	0.75	–
70–240	~240	$4-t/500$	0.75	circa 60 dm^3 daily

$$\xi = \frac{\xi_{\max} \cdot S}{K_S + S} \tag{11}$$

$$\sigma = \frac{\mu}{Y_{X/S}} + \frac{\pi}{Y_{P/X}} + \xi \tag{12}$$

$$X = X_{live} + X_{dead} \tag{13}$$

Fermentor feed characteristics were also considered during synthesis. Tables 2 and 3 show that the feed glucose concentration and flow rate were both set to minimum values in all three flowsheets. Increasing the glucose concentration may increase productivity, but that also increases separation costs because of the additional substrate remaining in the fermentor effluent stream. Thus, we notice that there is a tradeoff between increasing productivity and increasing purification costs. This result leave us an opportunity for the synthesis algorithm to consider the system as a whole, instead of unit-based, and generate an optimal system wide flowsheet.

Model of Penicillin Fermentation

Penicillin fermentation is a typical complex fed-batch bioprocess system. Scales involved in the system can be interpreted in two aspects, the molecular biology level and the biochemical process size. The former scale deals with metabolic network. As we know, penicillin is a secondary metabolite of fungus *Penicillium* that is produced when growth of the fungus is inhibited by stress. The synthesis pathway of penicillin is simply described as follows.

$$\alpha-\text{ketoglutarate} + \text{AcCoA} \rightarrow \text{homocitrate} \rightarrow \text{L}-\alpha-\text{aminoadipic acid}$$
$$\rightarrow \text{L} - \text{lysine} + \beta-\text{lactam}$$

The by-product L-lysine inhibits the production of homocitrate, so the presence of exogenous lysine should be avoided in penicillin production. More detailed pathway/enzyme information could be retrieved from some major biological databases, such as KEGG and BREDNA. Concerning the metabolic network modeling and simulation with Petri nets, readers are advised to refer to our previous report [29]. In this work, we focus on the latter scale.

The model is made of the main bioreactor and input and output equipments. The dynamic behavior of fermentation such as the biomass accumulation, substrate consumption and penicillin production, and broth volume changing were well described with

continuous elements; while control of the operation was outlined by discrete one. Figure 6 shows the model structure.

Simulation Results

Figure 7 represents the dynamic behavior of penicillin fermentation which is context-related and hard to be described using classical PN. The model is in well agreement with actual fermentation data, as the graphical representations of bioprocess parameters are similar to published data [28]. Glucose was used up steeply during the first 24 h, and a relatively steady phase was obtained after feeding process began. Based on Monod function, biomass increased in a typical way which showed the maximum biomass concentration (live cells) reaching about 23 g-DW dm^{-3} at 120 h. After 240 h, penicillin accumulation reached to a good harvest level, 27 g dm^{-3}. The volume of fermentation broth was controlled by an interval discharge of 60 dm^3 daily after 70 h, an outflow of 0.75 dm^3 h^{-1} and feeding after 10 h. The results are very well consistent with the bioprocesses in this example. A large complex bioprocess (a complete bioprocess of Penicillin production) can be handled with the same set of structural and behavioral properties.

Discussion and Conclusion

The case study presented in this paper shows that the hybrid Petri nets is well suited for generating bioprocess workflow and kinetics. The application of hybrid system is very useful for gaining insights into the complex behavior of bioprocesses. It is feasible to extend this methodology to any bioprocess system, as there is no special restriction of the models. By combining the Monod equation with key characteristics of penicillin fed-batch fermentation, a model was developed for describing biomass growth, substrate consumption, and penicillin formation. The proposed hybrid Petri net model possesses visualization, generality, and accuracy. The advantage compared to [28] is that hybrid Petri net approach is powerful to model the complex system with a graphical representation of the model, flexible to extend the model by incorporating new features or modules in the model without requiring changes in

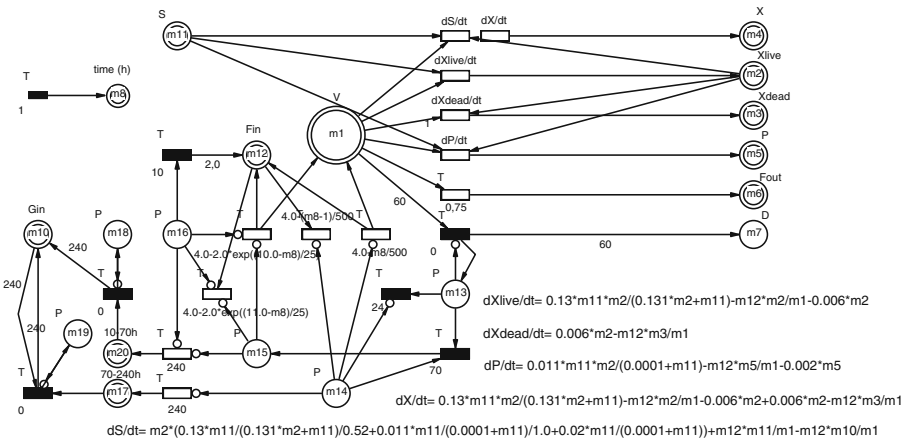

Fig. 6 Hybrid Petri nets graph of the fed-batch penicillin fermentation

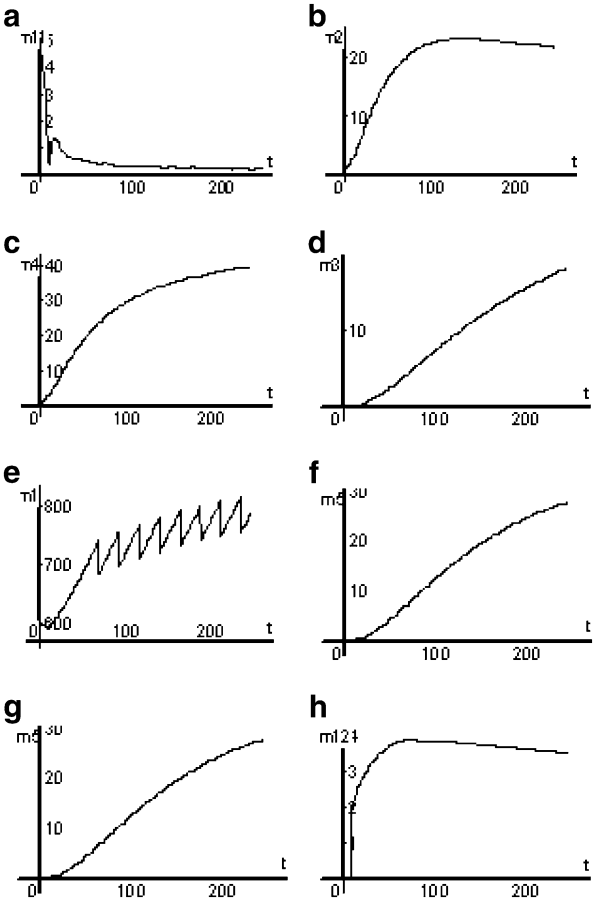

Fig. 7 Dynamic diagram of penicillin fed-batch fermentation. **a** Substrate (S); **b** living biomass (X_{live}); **c** total biomass (X); **d** dead biomass (X_{dead}); **e** volume of broth (V); **f** Penicillin (P); **g** outflow (F_{out}); **h** input (F_{in})

the overall scheme. An optimal bioprocess system can be generated because the model considers all possible connections between operation units and kinetics of the bioreactions. This elite feature allows us optimize the fermentor characteristics by changing different operation parameters appropriately.

Fermentation has been considered by some to be the most complex step in the manufacturing process since the desired product typically presents at diluted concentrations in the broth, at levels comparable to impurities [30]. However, problems often arise during the course of dealing with the whole steps of both metabolic and bioprocess scale. One serious problem is the model size. From the viewpoint of model definition, PN tends to become an unmanageable tangle when certain limits are violated; furthermore, translation in a software simulator will also become more difficult and error-prone. In this case, concepts of modularity in PNs [31], hierarchical PN [32] and object composition PN [33], could be helpful solutions. In a multi-scale hierarchical PN model, scale variables can make continuous changes in the valid scale ranges, providing theoretic bases for the implementation of a scalable map. It is possible to present two Petri net models for simulating bioprocess control flow and metabolic flow respectively from a local view and then a Petri net model for integrating bioprocess control flow and metabolic flow from a global view.

Biochemical process monitoring and control is essential for both production and regulatory reasons [34]. A main obstacle to the implementation of control strategies is the lack of reliable sensors for online measurement of the key variables [35], such as dissolved oxygen concentration (CL), carbon dioxide concentration (CO2), biomass concentration (X), and penicillin production concentration (P). Another problem is data management and online error detection, since the media is comprised of either a few complex and undefined ingredients which are difficult to characterize or several defined and simple ingredients which are straightforward to characterize individually, yet in aggregate pose a significant analytical burden [36].

Recently [37], the availability of entire genome sequences of *P. chrysogenum* and the development of functional genomics tools have allowed the elucidation of *Penicillium* syntheses via a systems biology approach. The mining and exploitation of the data obtained from genomics and the related research areas of genome-wide transcriptomics, proteomics, and metabolomics will bring us into a new era of understanding of biological systems for strain improvement. However, changes in the physiology and metabolic fluxes of microbial strains, which result from culture conditions, are often overlooked when dealing with the optimization of a fermentation in the course of transferring the improved strains to industrial practice. Generally speaking, after obtaining a high-yield production strain in the laboratory, stepwise scale-up and optimization will follow separately. Because of these reasons, the capacity of most high-yield production strains cannot be realized in industrial fermentation [38].

Fermentation is a complicated process with the multi-scale features in terms of time and space. It therefore requires a systematic model to optimize both the genetic/metabolic and fermentation scales. Hybrid Petri net approach is one of the solution. With the reliable hybrid Petri net models, coupled with significant amount of process-specific information, accurate control system design for bioprocesses is achievable.

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