Bioautomation, 2005, 2, 17-23 ISSN 1312-451X

Generalized Net Model of *Brevibacterium flavul 22LD* Fermentation Process

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Received: December 21, 2004 **Accepted: March 31, 2005**

Published: April 28, 2005

Abstract: *In order to render the specific peculiarities of the fermentation processes, as well as to avoid the complexity of mathematical description with systems of differential equations, the elaboration of some new methods and approaches for their modelling and control is predetermined. As a new, alternative approach for modelling of fermentation processes, an application of generalized nets is presented in this paper. The theory of generalized nets is applied to the fermentation process of Brevibacterium flavul 22LD for L-lysine production. A generalized net model of considered process is developed. For comparison and completeness, model with differential equations is also provided. The generalized nets model developed for the fed-batch cultivation of Brevibacterium flavul 22LD allows changing the concentration of the feeding solution and the aeration rate. In this way some inhibition effects are prevented and a possibility for optimal carrying out of the considered fermentation process is provided.*

Keywords: *Generalized Nets, Modelling, Brevibacterium flavul, L-lysine.*

Introduction

The aminoacids have a widespread application in different economic plants as fodder mixtures and food supplements, as well as in medicine, chemical and pharmaceutical industries. On a worldwide scale about 30 kinds of aminoacids have been produced. More than 60% of them have been developed by a microbial way. Among them a high percentage have glutamic acid and L-lysine.

L-lysine is one of the irreplaceable aminoacids whose content in animal protein is relatively high in the difference from plants, where the content is relatively low. So, some microbial production is necessary. The microbial produced L-lysine is added to the fodder mixtures that need to be used in stock farming. Based on a lot of experiments and process knowledge [6], it is demonstrated that the microorganisms *Brevibacterium flavul 22LD* are highly sensitive to the concentration of L-threonine in the culture broth. Thus it is very important for the concentration of L-threonine to be kept under some critical level during the fed-batch cultivation of the production of L-lysine. More details about the concrete fed-batch cultivation of *Brevibacterium flavul 22LD* for the production of L-lysine, as well as for the process conditions and process parameters, can be found in [6].

The first step of optimization of a real biotechnical production process is to be developed an adequate model. The model must describe those aspects of the process that significantly affect the process performance. In order to render the specific peculiarities of the biotechnological processes, to avoid the complexity of mathematical description with systems of differential

equations, as well as to fulfil the requirements for their hi-quality control, the elaboration of some new methods and approaches for their modelling and control is predetermined.
As a new, alternative approach for modelling of fermentation processes, an application of Generalized Nets (GN) will be presented in this paper. Up to now GNs have been used as a tool for the modelling of parallel processes in several areas [1]- economics, transport, medicine, computer technologies, and so on. The idea of using GNs for the modelling of fermentation processes is suggested by the fact that GNs provide the opportunity to describe the logic of modelling of this kind of processes. The authors are among the pioneers of applying generalized nets for the modelling of fermentation processes [5, 7, 8, 9].

In this paper the theory of generalized nets is applied to the fermentation process of *Brevibacterium flavul 22LD* for L-lysine production. The GN approach here is quite different from the commonly used differential equation models. For comparison and completeness, model with differential equations is also provided.

Fermentation process description

The rates of cell growth, substrate consumption, product formation and oxygen consumption are commonly described as follows according to the mass balance [6]:

$$\frac{dX}{dt} = \mu.X - D.X \tag{1}$$

$$\frac{dS}{dt} = D.(S_0 - S) - k_5.\mu.X - k_6 X - k_7.\eta.X \tag{2}$$

$$\frac{dL}{dt} = k_8.\eta.X - D.L \tag{3}$$

$$\frac{dThr}{dt} = -k_{13}.\mu.X - D.Thr \tag{4}$$

$$\begin{aligned}\frac{dC}{dt} &= K_L a(C^* - C) - k_{14}.\mu.X - \\ &- k_{15} X - k_{16}.\eta.X - D.C\end{aligned} \tag{5}$$

$$\frac{dV}{dt} = D.V \tag{6}$$

$$\mu = k_1 \frac{Thr.C}{(k_2 + Thr)(k_3 + S_0 - S)(k_4 + C)} \tag{7}$$

$$\eta = k_8 \frac{S.C}{(k_9 + S)(k_{10} + S)(k_{11} + C)(k_{12} + C)} \tag{8}$$

$$D = F/V \quad , \tag{9}$$

where X is the concentration of biomass, [g/l]; S - concentration of substrate, [g/l]; L - concentration of L-lysine, [g/l]; Thr - concentration of L-threonine, [g/l]; C - concentration of dissolved oxygen, [%]; F - feeding rate, [l/h]; D - dilution rate, [h^{-1}]; V - bioreactor volume, [l]; S_0 - concentration of the feeding solution, [g/l]; μ, η - specific rates, [h^{-1}]; k_1 - k_{16} - coefficients; $K_L a$ - volumetric oxygen transfer coefficient, [h^{-1}].

Definition of the concept of generalized nets

The concept of *Generalized Nets* (GN) is described in details in [1-4]. GNs are defined in a way that is principally different from the ways of defining the other types of Petri nets. The first basic difference between GNs and ordinary Petri nets is the "place - transition" relation [10]. Here, the transitions are objects of a more complex nature. A transition may contain m input places and n output places where $m, n \geq 1$.

Formally, every transition is described by a seven-tuple (Fig. 1):

$$Z = \langle L', L'', t_1, t_2, r, M, \square \rangle,$$

where

(a) L' and L'' are finite, non-empty sets of places. For the transition in Fig. 1 these are
$L' = \{l'_1, l'_2, \ldots, l'_m\}$ and
$L'' = \{l''_1, l''_2, \ldots, l''_n\}$;

(b) t_1 is the current time-moment of the transition's firing;

(c) t_2 is the current value of the duration of its active state;

(d) r is the *condition* of the transition to determine which tokens will pass (or *transfer)* from the inputs to the outputs of the transition; it has the form of an Index Matrix [4]:

$$r = \begin{array}{c|ccccc} & l''_1 & \ldots & l''_j & \ldots & l''_n \\ \hline l'_1 & & & & & \\ \vdots & & r_{i,j} & & & \\ l'_i & & (r_{i,j} \text{ - predicate}) & & & \\ \vdots & & (1 \leq i \leq m, 1 \leq j \leq n) & & & \\ l'_m & & & & & \end{array}$$

$r_{i,j}$ is the predicate that corresponds to the i-th input and j-th output place. When its truth value is *"true"*, a token from the i-th input place transfers to the j-th output place; otherwise, this is not possible;

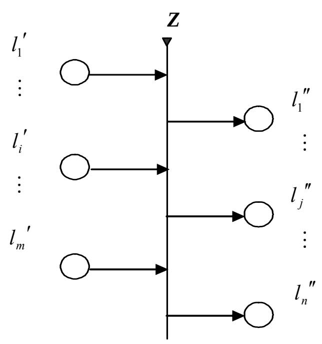

Fig. 1 A GN-transition

(e) M is an index matrix of the capacities of transition's arcs:

$$M= \begin{array}{c|ccccc} & l''_1 & \dots & l''_j & \dots & l''_n \\ \hline l'_1 & & & & & \\ \vdots & & & m_{i,j} & & \\ & & & & & \\ l'_i & \multicolumn{5}{l}{(r_{i,j} \text{ - natural number})} \\ \vdots & \multicolumn{5}{l}{(1 \le i \le m,\ 1 \le j \le n)} \\ l'_m & & & & & \end{array}$$

(f) □ is an object of a form similar to a Boolean expression. It may contain as variables the symbols that serve as labels for a transition's input places, and □ is an expression built up from variables and the Boolean connectives $\wedge$ and $\vee$ and the semantics of which is defined as follows:

$\wedge(l_{i_1}, l_{i_2}, \dots, l_{i_u})$ - every place $l_{i_1}, l_{i_2}, \dots, l_{i_u}$ must contain at least one token,

$\vee(l_{i_1}, l_{i_2}, \dots, l_{i_u})$ - there must be at least one token in all places $l_{i_1}, l_{i_2}, \dots, l_{i_u}$, where $\{l_{i_1}, l_{i_2}, \dots, l_{i_u}\} \subset L'$.

When the value of a type (calculated as a Boolean expression) is *"true"*, the transition can become active, otherwise it cannot.

Generalized net model of *Brevibacterium flavul 22LD* fermentation process

The generalized net model of the cultivation of *Brevibacterium flavul 22LD* takes into account the variation of concentration of biomass, substrate, products (L-lysine and L-threonine) and dissolved oxygen (Fig. 2).

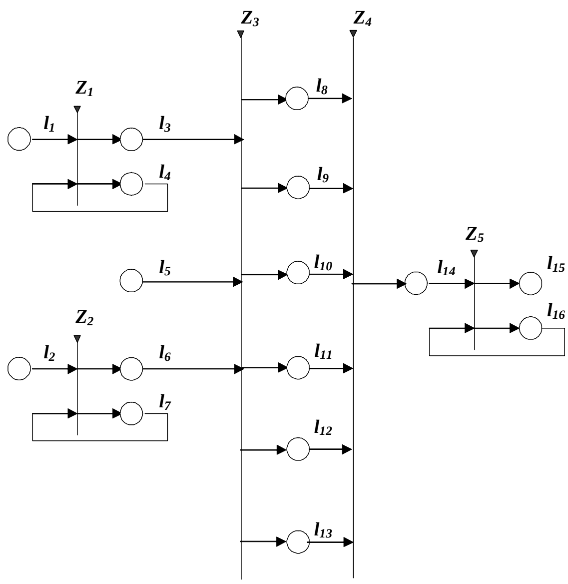

Fig. 2 Generalized net model of
Brevibacterium flavul 22LD cultivation

The token α enters the generalized net in place l_1 with an initial characteristic "flow rate of the medium feed". The form of the first transition condition of the GN model is:

$$Z_1 = <\{l_1, l_4\},\{l_3, l_4\}, r_1, \vee(l_1, l_4)>$$

$$r_1 = \begin{array}{c|cc} & l_3 & l_4 \\ \hline l_1 & false & true \\ l_4 & W_{4,3} & true \end{array}$$

where $W_{4,3}$ is “need of substrate concentration change, in the dependence on value in place l_9”.
Due to the specific peculiarities of fed-batch fermentation, the accumulation of substrate has to be avoided because of the possibility of the appearance of an inhibition effect. This fact determines the maintenance of substrate concentration at some low level to prevent increasing of concentration of L-threonine.

The token β enters the GN in place l_5 with a characteristic “initial concentration of biomass, substrate, L-lysine, L-threonine, dissolved oxygen and volume”.

The token α has the following characteristics:

- “concentration of the substrate added to the bioreactor” in place l_3;
- “amount of medium feed in storage” in place l_4;
- “substrate concentration in the bioreactor” in place l_9, taking into account the initial concentration of substrate in place l_5.

The token γ enters the generalized net in place l_2 with an initial characteristic “aeration rate”.
The form of the second transition condition of the GN model is:

$$Z_2 = <\{l_2, l_7\},\{l_6, l_7\}, r_2, \vee(l_2, l_7) >>$$

$$r_2 = \begin{array}{c|cc} & l_6 & l_7 \\ \hline l_2 & false & true \\ l_7 & W_{7,6} & true \end{array}$$

where $W_{7,6}$ is “need of aeration rate change, in the dependence on value in place l_{12}” in order to control of dissolved oxygen concentration at some desired value. The token γ obtains the following characteristics:

- “dissolved oxygen added to the bioreactor” in place l_6;
- “amount of dissolved oxygen in storage” in place l_7;
- “dissolved oxygen concentration in the bioreactor” in place l_{12}, taking into account the initial concentration of dissolved oxygen in place l_5.

The form of the third transition condition of the GN model is:

$$Z_3 = < \{l_3, l_5, l_6\}, \{ l_8, l_9, l_{10}, l_{11}, l_{12}, l_{13}\}, r_3, \vee(l_3, l_5, l_6)>$$

$$r_3 = \begin{array}{c|cccccc} & l_8 & l_9 & l_{10} & l_{11} & l_{12} & l_{13} \\ \hline l_3 & true & true & true & true & true & true \\ l_5 & true & true & true & true & true & true \\ l_6 & false & false & false & false & true & false \end{array}$$

The tokens α, β and γ are combined in a new token δ, which takes on the following characteristics:

- in place l_8 - "bioreactor volume";
- in place l_{10} - "concentration of biomass in the bioreactor";
- in place l_{11} - "concentration of L-lysine in the bioreactor";
- in place l_{13} - "concentration of L-threonine in the bioreactor".

The form of the fourth transition of the GN model is:

$$Z_4 = <\{l_8, l_9, l_{10}, l_{11}, l_{12}, l_{13}\}, \{l_{14}\}, r_4, \vee(l_8, l_9, l_{10}, l_{11}, l_{12}, l_{13})>$$

$$r_4 = \begin{array}{c|c} & l_{14} \\ \hline l_8 & true \\ l_9 & true \\ l_{10} & true \\ l_{11} & true \\ l_{12} & true \\ l_{13} & true \end{array}$$

In place l_{14} the token δ obtains new characteristic "concentration of biomass, substrate, L-lysine, L-threonine, dissolved oxygen and volume".

The form of the fifth transition of the GN model is:

$$Z_5 = < \{l_{14}, l_{16}\},\{l_{15}, l_{16}\}, r_5, \vee(l_{14}, l_{16}) >$$

$$r_5 = \begin{array}{c|cc} & l_{15} & l_{16} \\ \hline l_{14} & false & true \\ l_{16} & W_{16,15} & W_{16,16} \end{array}$$

where $W_{16,15}$ is "end of the process";
$W_{16,16} = \neg\ W_{16,15}$.

The token δ obtains the characteristics:

- "concentration of biomass, substrate, L-lysine, L-threonine, dissolved oxygen and volume in the end of the process" in place l_{15};
- "concentration of biomass, substrate, L-lysine, L-threonine, dissolved oxygen and volume during the process" in place l_{16}.

Analysis and conclusions

A new approach for the modelling of fermentation processes of *Brevibacterium flavul 22LD* based on the approach of generalized nets is presented in this paper. The GN model of this fermentation process presented here allows simulating the process easily and quickly.

The generalized nets models developed for the fed-batch cultivations of *Brevibacterium flavul 22LD* provide the possibility of changing the concentration of the feeding solution to depend on the substrate concentration in the bioreactor and thus prevent inhibition effect of increasing of concentration of L-threonine.

The proposed generalized nets models allow the controlling of the concentration of dissolved oxygen at some desired value, changing the aeration rate depending on the concentration of the dissolved oxygen during the process. This fact provides a possibility for optimal carrying out of the fermentation process of *Brevibacterium flavul 22LD.*

The opportunities which the GN model provides for process optimization also confirm them as very appropriate tool for modelling fermentation processes in general.

Acknowledgements

This work is partially supported from FNSF-TS-1314/2003.

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