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MODELLING OF FERMENTATION PROCESSES ON THE BASIS OF GENERALIZED NETS

T. Pencheva, O. Georgieva

Centre of Biomedical Engineering- Bulgarian Academy of Sciences
Acad. G. Bonchev Str., bl. 105, tel. 00359 2 979 3648
Sofia 1113, BULGARIA
e-mail: tania.pencheva@clbme.bas.bg

Abstract: Fermentation processes are characterized by a complicated structure of organization and interdependent characteristics, which determines their nonlinearity and non-stationary. Their specific peculiarities predetermine the elaboration of some new methods and approaches for the modelling and control of such processes. A new approach for modelling of considered processes, based on the apparatus of Generalized Nets (GN), is presented in this paper. Generalized Nets provide the opportunity to describe the logic of the fermentation process modelling. Generalized Net models are obtained for batch, fed-batch and continuous modes of fermentation processes. The GN-model for fed-batch operational mode is described in details. The GN-models of batch and continuous modes are obtained by a less number of changes in basic GN-model for fed-batch mode. The facility of models obtaining demonstrates the flexibility and the efficiency of Generalized Nets as modelling tool.

Keywords: Fermentation Processes, Batch, Fed-batch and Continuous Operational Mode, Modelling, Generalized Nets.

1. INTRODUCTION

Fermentation processes attract an increasing interest, being at the heart of both classical industrial activities and some new applications, such those related with environment, pharmacy, etc [5]. These processes are characterized by a complicated structure of organization and interdependent characteristics, which determines their nonlinearity and non-stationary. Depending on the media flow to or from the reactor, or the supply of oxygen, the operational mode of fermentation processes can be classified into three groups: batch, fed-batch and continuous.

In a batch cultivation there is no exchange of liquid medium. All the substrates are contained in the medium from the beginning, therefore, their initial concentration is quite high. After inoculation, the cells are grown and controlled until unessential medium component is exhausted or the accumulation of inhibiting products ceases the growth. In practise, pure batch cultivation is seldom found.

In fed-batch cultivation, medium components are continuously fed to the reactor, but no medium is taken out. This means that the liquid volume is increasing during the process. The fed-batch operational mode is used when the substrate concentration must be kept low for optimum growth or product formation. The advantage of improved possibilities to control the biological reaction by the substrate flow is opposed to greater effort for equipment. Both, batch and fed-batch operational mode are preferred for most industrial processes, to avoid problems with strain stability that may arise during prolonged cultivations.

In a continuous cultivation there is a permanent inflow of substrate to and outflow of medium including cells from the reactor, usually both with the same rate so that a steady state is reached. Similar to the fed-batch operational mode, continuous mode provides good possibilities to control the biological reaction by setting a proper resistance time via the flow rate. The advantage of a continuous process is that it usually can reach the highest productivity.

Due to the complexity of fermentation processes, their modelling may be a rather time consuming and thus a costly task. Hence, an elaboration of some new methods and approaches for the modelling and control of fermentation processes is needed. The application of Generalized Nets (GN) will be considered. Up to now GN are used as a tool for modelling of parallel processes in several areas [1, 2] - economics, transport, medicine, computer technologies etc. Generalized Nets provide the opportunity to describe the logic of the fermentation process modelling. Three Generalized Net models for the mentioned above fermentation operational modes are presented in this paper.

2. GENERALIZED NET MODELS

Fed-batch operational mode keeps the technical convenience of batch mode and makes the most of the advantages of continuous mode. The use of fed-batch by the fermentation industry takes advantage of the fact that residual substrate concentration may be maintained at a very low level in such mode. A low residual level of substrate may be advantageous in:

- Removing repressive effects of rapidly utilised carbon sources and maintaining conditions in the culture within the aeration capacity of the fermenter;
- Avoiding the toxic effects of a medium component.

Based on the specific peculiarities of fermentation processes and on the previous authors' papers [3, 4], the Generalized Net models of batch and continuous fermentations can be obtained from a GN-model of fed-batch fermentation. It could be achieved by a less number of changes in the GN-model of fed-batch operational mode. The GN-model of fed-batch fermentation will be considered in details. The other two models will be presented as a derivative of the first one.

2.1. Development of the Generalized Net model for fed-batch fermentation

GN-model, described a fed-batch operational mode, is presented in *Fig. 1*.
The token α enters GN 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:

$$Z_1 = \langle \{l_1, l_6\}, \{l_5, l_6\}, r_1 = \begin{array}{c|cc} & l_5 & l_6 \\ \hline l_1 & \text{false} & \text{true} \\ l_6 & W_{6,5} & \text{true} \end{array}, \square_1 = \vee\,(l_1, l_6) \rangle$$

where $W_{6,5}$ = "need of new concentration of substrate, on the dependence of value in position l_{19}". Due to the specific peculiarities of fed-batch fermentation, the accumulation of substrate has to be avoided because of possibility of appearance of inhibition effect. This fact determines the maintenance of substrate concentration at some low level. It is realized by the changes of concentration of feeding rate in dependence of substrate concentration in the fermenter.

The token α obtains the characteristics "concentration of the substrate added to the fermenter" in place l_5, "amount of medium feed in storage" in place l_6 and "substrate concentration in the fermenter" in position l_{19}.

The token β enters GN in place l_2 with an initial characteristic "concentration of dissolved oxygen". The form of the second transition condition of the GN-model is:

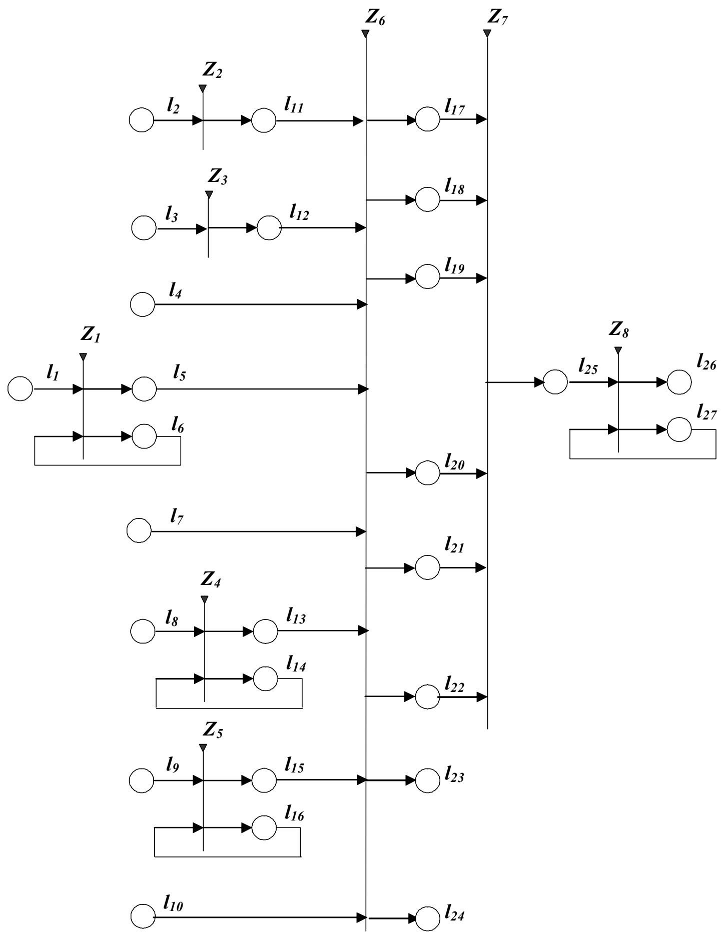

Fig. 1. Generalized Net model of fed-batch fermentation

$$Z_2 = <\{l_2\},\{l_{11}\},\ r_2 = \begin{array}{c|c} & l_{11} \\ \hline l_2 & W_{2,11} \end{array},\ \square_2 = \wedge\,(\,l_2,\, l_{17},\, l_{23})>$$

where $W_{2,11}$= "existence of a token in place l_{23}". The new concentration of dissolved oxygen depends on the value in position l_{17}.

The token β obtains the following characteristics:

- in place l_{11} - "concentration of dissolved oxygen added to the fermenter";
- in place l_{17} - "concentration of the dissolved oxygen in the fermenter";

The token ξ, which comes from place l_{10}, acquires a new characteristic in place l_{23} - "request for control of concentration of dissolved oxygen by oxygen flow rate".

The token γ enters GN with an initial characteristic "rotation speed of stirrer" in place l_3. The form of the third transition condition of the GN-model is:

$$Z_3 = <\{l_3\},\{l_{12}\},\ r_3 = \begin{array}{c|c} & l_{12} \\ \hline l_3 & W_{3,12} \end{array},\ \square_3 = \wedge\,(\,l_3,\, l_{17},\, l_{24}\,)>$$

where $W_{3,12}$ = "existence of a token in place l_{24}". The new rotation speed of stirrer depends on the value in position l_{17}.

The token γ obtains the characteristic "rotation speed of stirrer" in place l_{12} and l_{18}.

The token ξ acquires a new characteristic in place l_{24} - "request for control of dissolved oxygen concentration by the rotation speed' variation".

The control of pH in the fermenter is realized by adding base or acid in dependence of current value of pH. The correction of pH is shown in *Fig. 1* in transitions Z_4 and Z_5.

The token ε enters GN with an initial characteristic “amount of acid” in place l_8 and the token δ - with an initial characteristic “amount of base” in place l_9.

The forms of the fourth and fifth transitions of the GN-model are:

$$Z_4 = <\{l_8, l_{14}\},\{l_{13}, l_{14}\}, r_4 = \begin{array}{c|cc} & l_{13} & l_{14} \\ \hline l_8 & false & true \\ l_{14} & W_{14,13} & true \end{array}, \square_4 = \vee (l_8, l_{14}) >$$

$$Z_5 = <\{l_9, l_{16}\},\{l_{15}, l_{16}\}, r_5 = \begin{array}{c|cc} & l_{15} & l_{16} \\ \hline l_9 & false & true \\ l_{16} & W_{16,15} & true \end{array}, \square_5 = \vee (l_9, l_{16}) >$$

where

- $W_{14,13}$ = “add acid if the value in position l_{22} has been increased”;
- $W_{16,15}$ = “add base if the value in position l_{22} has been decreased”.

The token ε obtains the characteristics “concentration of acid added to the fermenter” in place l_{13}, “amount of acid in storage” in place l_{14}. The token δ obtains the characteristics “concentration of base added to the fermenter” in place l_{15} and “amount of base in storage” in place l_{16}. Tokens ε and δ are combined in place l_{22} and the new token λ acquires characteristic “concentration of pH in the fermenter”.

The tokens φ and η enter GN in places l_4 and l_7 respectively, with characteristics “initial concentration of substrate” and “initial concentration of biomass”. The token ξ enters GN in place l_{10} with an initial characteristic “choice of control variable to keep up the concentration of dissolved oxygen”. The form of the sixth transition condition of the GN-model is:

$$Z_6 = < \{l_4, l_5, l_7, l_{10}, l_{11}, l_{12}, l_{13}, l_{15}\}, \{l_{17}, l_{18}, l_{19}, l_{20}, l_{21}, l_{22}, l_{23}, l_{24}\}, r_6, \square_6 >$$

r_6=

	l_{17}	l_{18}	l_{19}	l_{20}	l_{21}	l_{22}	l_{23}	l_{24}
l_4	false	false	true	false	false	false	false	false
l_5	true	false	true	true	true	true	false	false
l_7	false	false	false	false	true	false	false	false
l_{10}	false	false	false	false	false	false	$W_{10,23}$	$W_{10,24}$
l_{11}	true	false	false	false	false	false	false	false
l_{12}	false	true	false	false	false	false	false	false
l_{13}	false	false	false	true	false	true	false	false
l_{15}	false	false	false	true	false	true	false	false

$$\square_6 = (\wedge(l_4, l_5, l_7, l_{10}), \vee(l_{11}, l_{12}), \vee(l_{13}, l_{15})).$$

The predicates $W_{10,23}$ and $W_{10,24}$ determine which control variable will be used to keep up the concentration of dissolved oxygen, respectively:

- $W_{10,23}$ = “request for control by dissolved oxygen”;
- $W_{10,24}$ = “request for control by rotation speed of stirrer”.

The tokens obtain the following characteristics:

- the token α in place l_{20} - “fermenter volume”;
- the token η in place l_{21} - “concentration of biomass in the fermenter”.

The form of the seventh transition condition of the GN-model is:

$$Z_7 = < \{l_{17}, l_{18}, l_{19}, l_{20}, l_{21}, l_{22}\},\{ l_{25} \}, r_7, \square_7 = \wedge(l_{17}, l_{18}, l_{19}, l_{20}, l_{21}, l_{22}) >$$

$$
r_7 = \begin{array}{c|c}
 & l_{25} \\ \hline
l_{17} & true \\
l_{18} & true \\
l_{19} & true \\
l_{20} & true \\
l_{21} & true \\
l_{22} & true
\end{array}
$$

All tokens, excepting the token α in position l_{20}, are combined in one token ρ in place l_{25} with characteristic "concentration of dissolved oxygen, rotation speed, concentration of substrate, volume, concentration of biomass and concentration of pH".

The form of the eighth transition condition of the GN-model is:

$$
Z_8 = <\{l_{25}, l_{27}\}, \{l_{26}, l_{27}\}, r_8 = \begin{array}{c|cc}
 & l_{26} & l_{27} \\ \hline
l_{25} & false & true \\
l_{27} & W_{27,26} & W_{27,27}
\end{array}, \square_8 = \vee\,(l_{25}, l_{27})>
$$

where $W_{27,26}$ = "end of the process" and $W_{27,27} = \neg\ W_{27,26}$.

The token ρ obtains the characteristics "concentration of dissolved oxygen, rotation speed, concentration of substrate, volume, concentration of biomass, concentration of pH in the end of the process" in place l_{26} and "concentration of dissolved oxygen, rotation speed, concentration of substrate, volume, concentration of biomass, concentration of pH during the process" in place l_{27}.

Up to here, the Generalized Net model for fed-batch operational mode was described in details. In the following sections it will be presented how, by a less number of changes in the described GN-model of fed-batch operational mode, the GN-models of batch and continuous fermentations can be obtained. The two other models will be presented as a derivative of the first one.

2.2. Development of the Generalized Net model for batch fermentation

Batch culture is an example of a closed system which contains an initial limited amount of nutrient. During batch fermentation of a process, no substrate is added to the initial charge nor is the product removed until the end of the process. Similar to fed-batch fermentation, there is an accumulation of a product and nothing is removed out of the fermenter during whole fermentation process. Due to, the Generalized Net model described the batch fermentation, will be almost identical to GN-model presented in *Fig. 1* for fed-batch fermentation. The absence of feeding will cause a change in transitions Z_1 and Z_6 where predicates for activation of these transitions should be determined for this case. The form of the first transition condition of the GN-model for batch fermentation is:

$$
Z_1 = <\{l_1, l_6\}, \{l_5, l_6\}, r_1 = \begin{array}{c|cc}
 & l_5 & l_6 \\ \hline
l_1 & false & true \\
l_6 & W_{6,5} & true
\end{array}, \square_1 = \vee\,(l_1, l_6)>
$$

The token α enters GN in place l_1 with the same initial characteristic "flow rate of the medium feed" and $W_{6,5}$ = "start of the process".

The form of the sixth transition condition of the GN-model for batch fermentation is:

$$
Z_6 = <\{l_4, l_5, l_7, l_{10}, l_{11}, l_{12}, l_{13}, l_{15}\}, \{l_{17}, l_{18}, l_{19}, l_{20}, l_{21}, l_{22}, l_{23}, l_{24}\}, r_6, \square_6>
$$

$r_6=$	l_{17}	l_{18}	l_{19}	l_{20}	l_{21}	l_{22}	l_{23}	l_{24}
l_4	false	false	true	false	false	false	false	false
l_5	$W_{5,17}$	false	$W_{5,19}$	$W_{5,20}$	$W_{5,21}$	$W_{5,22}$	false	false
l_7	false	false	false	false	true	false	false	false
l_{10}	false	false	false	false	false	false	$W_{10,23}$	$W_{10,24}$
l_{11}	true	false	false	false	false	false	false	false
l_{12}	false	true	false	false	false	false	false	false
l_{13}	false	false	false	true	false	true	false	false
l_{15}	false	false	false	true	false	true	false	false

$\square_6 = (\wedge(l_4, l_5, l_7, l_{10}), \vee(l_{11}, l_{12}), \vee(l_{13}, l_{15}))$,

where $W_{5,17}$, $W_{5,19}$, $W_{5,20}$, $W_{5,21}$, $W_{5,22}$ = "start of the process", and the predicates $W_{10,23}$ and $W_{10,24}$ are the same as in fed-batch mode.

The tokens keep the same characteristics as in fed-batch mode too.

These changes are sufficient that the GN-model, presented in *Fig. 1*, to describe fermentation process in batch mode.

2.3. Development of the Generalized Net model for continuous fermentation

In continuous fermentation, an open system is set up. Sterile nutrient solution is added to the fermenter continuously (during whole fermentation but in determined time steps) and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system. This is the main difference from the batch and fed-batch fermentations, where during the cultivation no culture medium is taken out the fermenter. This fact causes some changes into GN-model presented in *Fig. 1*.

Some changes in the tokens' characteristics will be occurred. The most essential change is the including of a new transition which reflects the taking out of the culture medium. Therefore the GN-model for continuous fermentation acquires the following form, presented in *Fig. 2*.

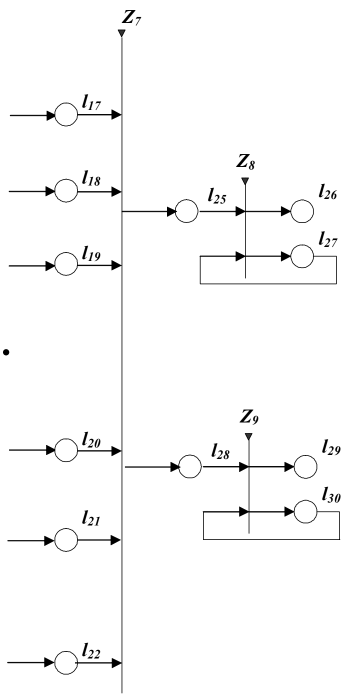

Fig. 2. Generalized Net model of continuous fermentation

The new transition Z_9 is presented as follows:

$$Z_9 = \langle \{l_{28}, l_{30}\}, \{l_{29}, l_{30}\}, r_9 = \begin{array}{c|cc} & l_{29} & l_{30} \\ \hline l_{28} & \text{false} & \text{true} \\ l_{30} & W_{30,29} & W_{30,30} \end{array}, \square_9 = \vee(l_{28}, l_{30}) \rangle$$

where $W_{30,29}$ = “end of the process” and $W_{30,30} = \neg\ W_{30,29}$.

The token α obtains the characteristics “amount of culture media, taken away from the fermenter in the end of the process” in place l_{29} and “amount of culture media, taken away from the fermenter during the process” in place l_{30}.

All tokens keep the same characteristics as in fed-batch mode. Only one exception is the token α now with an initial characteristic "dilution rate". In this case the predicate $W_{6,5}$ in transition Z_1 is "new time step".

In considered cases it is assumed that the time scale is fixed and the elementary time step is constant. It could be included a variable elementary time step, for example, the different activating points for transition Z_1, described the feeding in the case of fed-batch and continuous modes. In the other hand, a variation of time scale, according of process sensitivity to the concentration of dissolved oxygen or the value of pH, can be realized respectively for transitions Z_2 and Z_3 and for transitions Z_4 and Z_5.

Presented Generalized Net models do not pretend to be comprehensive because they do not describe a full process dynamic and all process parameters. The model can be extended with the including of accumulation of one or more fermentation products, dynamics of exhausted gases etc.

3. CONCLUSION

A new approach for modelling of fermentation processes based on the apparatus of Generalized Nets is presented in this paper. Generalized Nets provide the opportunity to describe the logic of the fermentation process modelling. So, Generalized Net models are obtained for batch, fed-batch and continuous modes of fermentation processes.

More circumstantial attention is taken out for the Generalized Net model for fed-batch operational mode. The GN-models of batch and continuous modes are obtained by a less number of changes in basic GN-model for fed-batch mode. The facility of models obtaining demonstrates the flexibility and the efficiency of Generalized Nets as modelling tool.

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REFERENCES

1. Atanassov K., Introduction in Theory of Generalized Nets, Pontika-Print, Bourgas, 1992. (in Bulgarian)
2. Atanassov K., Generalized Nets, World Scientific, Singapore, New Jersey, London, 1991.
3. Georgieva O., An Application of Generalized Nets for Modelling of Fed-batch Fermentation Process, Procceding of II-nd Int. Workshop on Generalized Nets, Sofia, 26-27 June 2001, pp. 28 - 33.
4. Georgieva O., T. Pencheva, An Application of Generalized Nets with Intuitionistic Fuzzy Sets for Modelling of Biotechnological Processes with Distributed Parameters, *Workshop on IFS and GNs, Warszawa*, 6-7 November, 2001, in press.
5. Schuegerl K., K.-H. Bellgardt, Bioreaction Engineering: Modeling and Control, Springer-Verlag Berlin Heidelberg, 2000.