

**GENERALIZED NET FOR CARBON DIOXIDE MONITORING OF  
FERMENTATION PROCESSES**

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**Abstract:** Monitoring of exhaust gases is of significant importance for optimal carrying out of fermentation processes. Since fermentation activity is reflected in O<sub>2</sub> consumption and CO<sub>2</sub> production, levels of exhaust gases get information for any deviation from the optimal metabolism of the microorganism. Already developed generalized net (GN) model for control of O<sub>2</sub> is here extended with a loop for monitoring of CO<sub>2</sub>, rendering also respiratory quotient variation. Using presented here GN, better monitoring and control of exhaust gases is ensured and higher process efficiency is achieved.

### **1. Introduction**

Fermentation processes are used in the manufacturing of countless products from simple yeasts to complex enzymes and pharmaceutical products. All fermentation processes require the active culturing of live biological components in various types of growth media. Optimal control of cultures growth provides the desired end products. Proper control of both liquid nutrients and atmospheric gases levels are important [11]. Considering atmospheric gases, it is of interest to monitor the dissolved oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) levels.

In aerobic fermentation air is forced into the fermenter to provide the cultures with needed O<sub>2</sub>. The cultures converts O<sub>2</sub> into CO<sub>2</sub> during the process and CO<sub>2</sub> is released. O<sub>2</sub> is the most important gaseous substrate in aerobic fermentation, while CO<sub>2</sub> is the most important gaseous by-product of the process. In anaerobic fermentation the air flow and corresponding O<sub>2</sub> level is controlled more closely. This is particularly important because the yield of the reactor is directly proportional to the ability to maintain desired O<sub>2</sub> and CO<sub>2</sub> concentrations during the fermentation cycle [3, 11, 12, 13].

Exhaust gas monitoring is a powerful tool for process control because the ratio between CO<sub>2</sub> production and O<sub>2</sub> consumption reflects any deviation from the optimal metabolism of the microorganism. The relation of the volume of released CO<sub>2</sub> to the volume of consumed O<sub>2</sub> is called a respiratory quotient (RQ). When fermentation is carried out in a batch mode, RQ is reliable indicator of the efficiency of the production process and the status of the fermenter. The production of CO<sub>2</sub> by yeast in a batch culture can indicate the stage of growth for that culture [4]. During the exponential phase, yeast cells grow by fermenting the available sugar and producing ethanol and CO<sub>2</sub> as byproducts. The amount of CO<sub>2</sub> generated during the exponential growth phase can be directly correlated to optical density values which are generally used to determine the progression of the growth phase.

The monitoring of CO<sub>2</sub> concentration is of significant importance also in the enzyme production process [14]. The values of CO<sub>2</sub> concentration are used to get a picture of the state of the process. The level of CO<sub>2</sub> is an indication of the metabolic activity of the mould or bacteria, and the information is used to control the feed of new nutrient pulses to the process.

While traditional instrumentation on fermenters provides data for control loops, thereby ensuring constants conditions it is not well suited for the analysis of fermentation metabolism. Production stuff soon realized that a better indication of the emissions of CO<sub>2</sub> and O<sub>2</sub> could help them determine the respiration data in the fermenter and thereby increase the efficiency of production. This fact presumes to be developed an effective loops for observation of CO<sub>2</sub> levels during the fermentation processes. As an appropriate tool this task to be realized, generalized nets (GNs) theory could be applied. Until now GNs have been used for modelling of parallel processes in several areas [1, 2], among that biotechnological processes [10]. The framework of generalized nets has already several implementations to fermentation processes, in particular: modelling of different operational modes [10], optimal process control [5] and control of specific physics-chemical parameters, namely pH [8], temperature [7, 9] and O<sub>2</sub> [6]. In order to get detailed picture of exhaust gases dynamics, a generalized net model for CO<sub>2</sub> monitoring is here developed.

## 2. Generalized net model for carbon dioxide monitoring

The generalized net model described oxygen control system [6] is here extended to monitor CO<sub>2</sub> and to calculate the respiratory quotient. Extended GN is presented in Fig. 1. GN model is developed for fed-batch operational mode of fermentation processes. As it has been demonstrated in [10], the presented GN model can be easily transformed for batch or continuous mode.

The token  $\alpha$  enters GN in place  $l_1$  with an initial characteristic “flow rate of the medium feed”. The form of the first transition of the GN model is:

$$Z_1 = \langle \{l_1, l_5\}, \{l_4, l_5\}, \begin{array}{c|cc} & l_4 & l_5 \\ l_1 & false & true \\ l_5 & W_{5,4} & true \end{array}, \vee(l_1, l_5) \rangle,$$

where  $W_{5,4}$  is “need of new concentration of substrate, depending on the value in place  $l_{11}$ ”.

The token  $\alpha$  obtains the characteristics “concentration of the substrate added to the fermenter” in place  $l_4$  and “amount of medium feed in storage” in place  $l_5$ .

The token  $\beta$  enters GN in place  $l_2$  with a characteristic “initial concentration of process variables”. As process variables could be considered substrates, biomass, products, exhaust gases (O<sub>2</sub> and CO<sub>2</sub>) etc.

The control of O<sub>2</sub> in the fermenter is realized by:

- variation of the concentration of O<sub>2</sub> added to the fermenter (transition  $Z_3$ )

or

- variation of rotation speed of stirrer (transition  $Z_4$ ) in dependence on user decision (transition  $Z_2$ ).

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

$$Z_2 = \langle \{l_3\}, \{l_6, l_7\}, \begin{array}{c|cc} & l_6 & l_7 \\ l_3 & W_{3,6} & W_{3,7} \end{array}, \wedge(l_3) \rangle,$$

where  $W_{3,6}$  is “control of  $O_2$  concentration by variation of the concentration of  $O_2$  added to the fermenter” and  $W_{3,7}$  – “control of  $O_2$  concentration by variation of rotation speed of stirrer”.

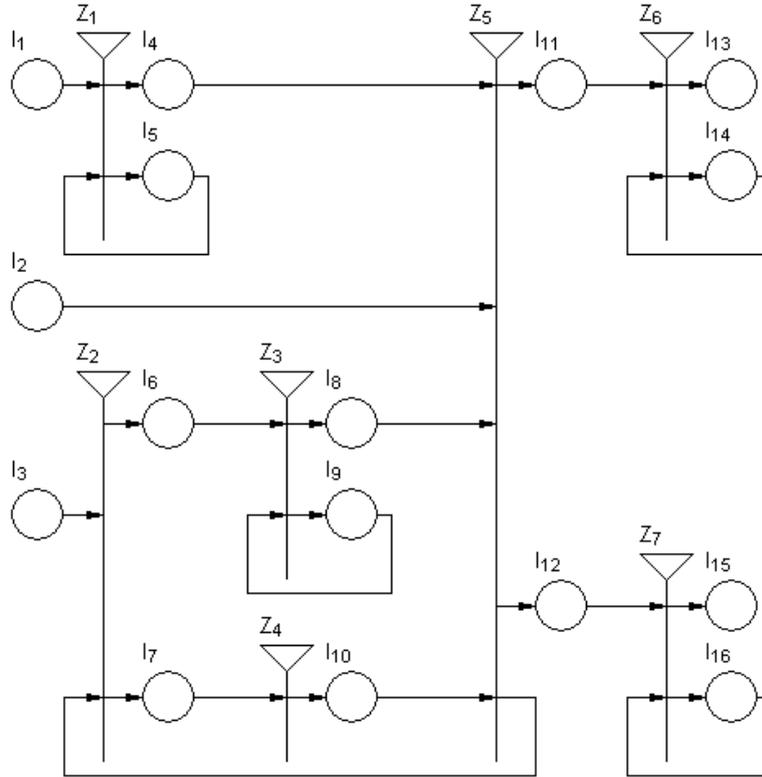


Fig. 1

The token  $\gamma$  obtains the characteristics “concentration of  $O_2$ ” in place  $l_6$  and “rotation speed of stirrer” in place  $l_7$ .

The forms of the third and fourth transitions of the GN model are:

$$Z_3 = \langle \{l_6, l_9\}, \{l_8, l_9\}, \begin{array}{c|cc} & l_8 & l_9 \\ \hline l_6 & false & true \\ l_9 & W_{9,8} & true \end{array}, \vee(l_6, l_9) \rangle,$$

$$Z_4 = \langle \{l_7\}, \{l_{10}\}, \begin{array}{c|c} & l_{10} \\ \hline l_7 & W_{7,10} \end{array}, \wedge(l_7) \rangle,$$

where  $W_{9,8}$  is “correct the concentration of  $O_2$  if there is a token in place  $l_6$ ” and  $W_{7,10}$  – “correct the rotation speed of stirrer, on the dependence of value in position  $l_{11}$  and existence of a token in place  $l_7$ ”.

The token  $\gamma$  obtains the following characteristics:

- in place  $l_8$  – “concentration of  $O_2$  added to the fermenter”;
- in place  $l_9$  – “aeration gas in storage”;
- in place  $l_{10}$  – “stirrer speed”.

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

$$Z_5 = \langle \{l_2, l_4, l_8, l_{10}\}, \{l_{11}, l_{12}\}, \begin{array}{c|cc} & l_{11} & l_{12} \\ \hline l_2 & W_{2,11} & W_{2,12} \\ l_4 & true & true \\ l_8 & true & true \\ l_{10} & true & false \end{array}, (\wedge(l_2, l_4), \vee(l_8, l_{10})) \rangle$$

where  $W_{2,11} = W_{2,12}$  is “start of the process”. Tokens  $\alpha$  and  $\beta$  are combined in a new token  $\delta$  in place  $l_{11}$ . The token  $\delta$  obtains a characteristic “concentration of process variables (substrates, biomass, products) and stirrer speed value”. Tokens  $\beta$  and  $\gamma$  are combined in a new token  $\varepsilon$  in place  $l_{12}$ . The token  $\varepsilon$  obtains a characteristic “concentration of exhaust gases ( $O_2$  and  $CO_2$ ) and RQ value”.

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

$$Z_6 = \langle \{l_{11}, l_{14}\}, \{l_{13}, l_{14}\}, \begin{array}{c|cc} & l_{13} & l_{14} \\ \hline l_{11} & false & true \\ l_{14} & W_{14,13} & W_{14,14} \end{array}, \vee(l_{11}, l_{14}) \rangle,$$

where  $W_{14,13}$  is “end of the process” and  $W_{14,14} = \neg W_{14,13}$ .

The token  $\delta$  obtains the following characteristics:

- in place  $l_{13}$  – “concentration of process variables (substrates, biomass, products) and stirrer speed value in the end of the process”;
- in place  $l_{14}$  – “concentration of process variables (substrates, biomass, products) and stirrer speed value during the process”.

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

$$Z_7 = \langle \{l_{12}, l_{16}\}, \{l_{15}, l_{16}\}, \begin{array}{c|cc} & l_{15} & l_{16} \\ \hline l_{12} & false & true \\ l_{16} & W_{16,15} & W_{16,16} \end{array}, \vee(l_{12}, l_{16}) \rangle,$$

where  $W_{16,15}$  is “end of the process” and  $W_{16,16} = \neg W_{16,15}$ .

The token  $\varepsilon$  obtains the following characteristics:

- in place  $l_{15}$  – “concentration of exhaust gases ( $O_2$  and  $CO_2$ ) and RQ value in the end of the process”;
- in place  $l_{16}$  – “concentration of exhaust gases ( $O_2$  and  $CO_2$ ) and RQ value during the process”.

Presented GN model, based on the transitions conditions, allows controlling the  $O_2$  concentration, monitoring of  $CO_2$  concentration and calculation of RQ.

### 3. Conclusions

Exhaust gas monitoring is a powerful tool for process control and troubleshooting at all scales of fermentation, as fermentation activity is reflected in the  $O_2$  consumption and  $CO_2$  production. Any deviation from the optimal metabolism of the microorganism can be reflected in the respiratory quotient. Using the apparatus of generalized nets, a net model for monitoring of  $CO_2$  concentration during fermentation processes is here developed. Applying developed GN, optimal conditions for microbial growth process can be ensured and current process status based on the observed values of  $CO_2$  concentration and RQ can be determined. Ensuring better monitoring and control of exhaust gases is a precondition for higher efficiency of the fermentation process.

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