MANUAL

for working with the InSEMCoBio system.

I. Theoretical part

I.1 Graphical User Interface (GUI)

GUI, or simply a graphical user interface (in English: graphical user interface, GUI), is a type of user interface in which the elements provided to the user for control are implemented in the form of graphical images (menus, buttons, lists, etc.).

In GPI, the user has access of his choice to all visible objects of the monitor window (they are called interface elements) using a peripheral device (keyboard, mouse, etc.), and performs their immediate manipulation.

Most often, the elements of the graphical interface are implemented as icons that suggest their purpose and properties, which helps users to perform the desired functions.

The graphical interface is very reminiscent of a web page - everything is controlled with a mouse and some of the keyboard buttons.

I.2. Identification algorithms built into the InSEMCoBio system

Three types of identification algorithms are built into the system: Evolutionary, Genetic and Hybrid.

Evolutionary algorithms (EA) include evolutionary programming, evolutionary strategies, genetic algorithms, and genetic programming. They often generate approximate solutions for all kinds of problems related to optimization tasks. A detailed description of the functions of the programs implementing the Evolutionary Algorithms embedded in the system are given in the master's course textbook [1].

Genetic Algorithms (GA) are direct methods that use the basic elements of biological evolution such as crossovers and random changes between the elements (chromosomes) of the population for the optimization of technical systems. A more detailed explanation of the main features of this evolutionary strategy for a high-order biochemical model is presented in [2].

Hybrid algorithms (EA-GA Hybrid) are a combination of the two algorithms described above. When describing the operation of the system, it will be shown in practice how these algorithms work and the results of their application under the same conditions will be presented.

I.3. Models describing the processes used for proof system performance

At this stage of system development, two biotechnological processes have been introduced. One is related to protein production in culture of E. coli MC 4110. E. coli can grow in a variety of culture media both aerobically and anaerobically. Glucose is most often used as a carbon source. Under aerobic conditions, part of the glucose is oxidized to carbon dioxide, with the final electron acceptor being oxygen. E. coli is the most thoroughly studied microorganism of all living organisms. It is the microorganism of choice for genetic engineering because cultures of it can produce unlimited amounts of product as a result of an introduced gene. At this stage, several important drugs (insulin, for example) are obtained on the basis of *E. coli* MC 4110.

MANUAL

for working with the InSEMCoBio system.

$$dX/dt = mu * X - F/V * X \tag{1}$$

$$dS/dt = -1/Y_{XS} * mu * X + (S_0 - S) * F/V$$
(2)

$$d02/dt = 1/Y_{0X} * mu * X + Kla * (02^* - 02) - F/V * 02$$
(3)

$$\frac{dV}{dt} = F \tag{4}$$

The second process is related to the production of gluconic acid, which is widely used in the food, pharmaceutical and other industrial fields. The process under investigation is production of gluconic acid by fermentation of *Aspergillus niger*. The main substrate is glucose. The conversion of glucose to gluconic acid is simply the oxidation of the aldehyde group of the sugar to a carboxyl group. The basic process is well known and has been the subject of a number of studies, but information on the kinetic aspects of culture and its formal modeling is of special interest and especially for control. Equations (5)-(8) are a batch process model for the production of gluconic acid from *Aspergillus niger* synthesis.

$$dX/dt = R_X \tag{5}$$

$$dGA/dt = R_{GA} \tag{6}$$

$$dS/dt = -1/Y_{XS} * R_X - 1/Y_{SGA} * R_{GA}$$
(7)

$$dO2/dt = 1/Y_{OX} * R_{GA} + Kla * (O2^* - O2)$$
(8)

Kinetic Models built into the system:

$$\mu(S) = \frac{\mu_{max} * S(t)}{K_S + S(t)} \quad \text{Mono}$$
(9)

$$\mu(S,X) = \frac{\mu_{max} * S(t)}{K_C X(t) + S(t)} \quad \text{Contois}$$
(10)

$$\mu = \mu_{max} \frac{S/X}{K_S + S/X} \quad \text{Fujimoto} \tag{11}$$

II. Activation of the system

To work with the system, MATLAB.17 or MATLAB.19 must be installed beforehand. The system is started by the marked file in the leftmost field of Fig.1. The Identification Panel appears on the screen.

II.1 Identification panel operation for the fed-batch fermentation process of E. coli MS 4110

The identification process includes optimization procedures aimed at finding the values of the coefficients of the model so that it describes the dynamics of the biotechnological process as accurately as possible with respect to the experimental data.

MANUAL

for working with the InSEMCoBio system.

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Since the training system is developed for users not familiar with programming, much of the functions of the programs given below in the figures remain hidden from the user. Only some of them are listed for activation by performing the following steps. The result after activating them is shown in parentheses:

- process selection Choose Fermentation Process (E. coli MC4110 Fed-batch);
- process model selection (Set model)
- selection of kinetics Kinetic Models (Mono);
- choosing an identification algorithm Choose Algorithm (Evolutionary Algorithm);

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Fig.2.

MANUAL

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Equations (1, 2 and 4) included in the process model are marked and are in gray. The omitted equation (3) is left in black. Mono (9) was chosen as the kinetic model.

The identification procedure is started by pre-entering the experimental data by pressing the Load data button and then with the RUN command at the lower right end of the middle field of the panel. Figures No. 3 and 4 show the completed identification procedures of the Best Solution model when different values of the identification algorithm (Set Algorithm Parameters) and values of the parameters of the process model (Set Problem Parameters) are selected.

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Fig.3.

for working with the InSEMCoBio system.

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In Fig. 5 shows the comparison of the results of the above two identifications. This is initialized by selecting (Selected) the simulations (MC) that we want to compare in the far

for working with the InSEMCoBio system.

lower right field of the panel. EA1 stands for Evolutionary Algorithm 1 and EA2 is Evolutionary Algorithm 2. In Fig. 5 the experimental data and the results of the different identification procedures are shown with different colors

In Fig.6. the identification process of the same process is shown, but the oxygen dynamics equation (8) is also added. The parameters associated with this equation are also shown under Set Problem Parameters. The identification process is demonstrated with the simulations of small Figures 1 and 3. After completion of the procedure, the final Best Solution results are given as follows: for biomass and substrate in small Figure 2, for oxygen in Figure 4.

In Fig.7, the same process is selected with parameters and the kinetic model given in Fig. 4, but another algorithm is enabled - Genetic Algorithm with its specific parameters listed in Set Algorithm Parameters.

Figures No. 8 and 9 show the identification procedures of the model with different values of the identification algorithm (Set Algorithm Parameters) selected - small Figure 1 and Best Solution small Figure 2 after the completion of the identification procedures.

In Fig. 10 the comparison of the results of the above two identifications is given. This is initialized by selecting (Selected) the simulations (MC) that we want to compare in the far lower right field of the panel. GA1 represents Genetic Algorithm 1, and GA2 is Genetic Algorithm 2. In Fig. 10 with different colors the experimental data and the results of the different identification procedures are shown.



Fig.6.

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Fig.12

for working with the InSEMCoBio system.

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			B
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Fig.13

In Fig.11, the same process is selected with parameters and the kinetic model given in Fig. 4, but another algorithm is enabled – EA-GA Hybrid with its specific parameters listed in Set Algorithm Parameters.

Figures No. 12 and 13 show the identification procedures of the model when different values of the identification algorithm (Set Algorithm Parameters) are selected - small Figure 1 and Best Solution small Figure 2 after the completion of the identification procedures.

In Fig. 14 the comparison of the results of the above two identifications is given. This is initialized by selecting (Selected) the simulations (MC) that we want to compare in the far lower right field of the panel. EA-GA1 is Hybrid Algorithm 1 and EA-GA2 is Hybrid Algorithm 2. In Fig. 14 with different colors the experimental data and the results of the different identification procedures are shown.

Fig. 15 shows a comparison of the three identification algorithms EA1, GA2 and EA-GA3 of the process from Fig. 4, but with a changed kinetic model – instead of Mono, Contois is used.

Fig. 16 shows a comparison of the three identification algorithms EA1, GA2 and EA-GA3 of the process from Fig. 4, but with a changed kinetic model – Fujimoto was used instead of Mono.

Figure 17 shows a comparison of two of the identification algorithms GA2 and EA-GA3 of the process of Figure 6 with oxygen dynamics included.

MANUAL

urrent Step	Choose Fermetation Process		Logs	
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	<pre>d02/dt = 1/Yox*mu*X + Kla*(02* - 02) - F/V*02</pre>	Fujimoto		
Gluconic Acid Process Control	I dV/dt = F			
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Fig.15.

for working with the InSEMCoBio system.







Fig.17.

for working with the InSEMCoBio system.

II.2 Identification panel work for gluconic acid production in batch fermentation of Aspergillus niger

When selecting the other process, the batch fermentation model appears on the panel - equations (5-7). After entering the data and after selecting the algorithm and kinetic model, the identification procedure is started.



Fig.18.

In fig. 18 shows the model identification procedure (5-8) with the Mono kinetic model (9) - (small Figure 1 and 2) and the final results (small Figure 3 and 4).

In fig. 19, the final results of three identification procedures are compared when choosing the euplution algorithm, the Mono kinetic model (9) and at different combinations of the values of the coefficients of the algorithm and of the process model. Different colors were used for each procedure in the comparison. The combination of algorithm and process model coefficient values can be traced in the table at the lower right end of the middle field of the panel.

In fig. 20 compared the final results when choosing another identification algorithm - genetic.

After a certain amount of identification procedures, Fig. 21 shows a comparison of the results of three evolutionary and three genetic algorithms for the same process under the same kinetic model.

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for working with the InSEMCoBio system.



Fig. 19.



Fig. 20.



for working with the InSEMCoBio system.

Fig. 21.

II.3. Demonstration of process control laws for gluconic acid production in Aspergillus niger fermentation

There are four options for managing this process. Two with intermittent feeding and two with continuous feeding. Two approaches are considered. In the first approach, the goal is to maintain a pre-set low value of the substrate (glucose) in the nutrient medium (0 or 3 g/l) - Fig. 22 and Fig. 23





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In the second pass, a constant concentration of the target product (gluconic acid) is maintained, which corresponds to the maximum productivity of the process. Fig. 24 and Fig. 25



Fig. 24.

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At Fig. 22-25 with symbols the experimental data in batch fermentation are shown, and with dashed lines the values of the main variables biomass and hl (subfigures c) and gluconic acid (subfigures a) are shown dotted lines.

Comparing the results obtained up to the eightieth hour of fermentation in subfigures a, it is found that in the intermittent management with feeding (Fig. 23 and Fig. 25) a higher concentration of the target product gluconic acid is achieved compared to the continuous management (Fig. 22 and Fig.24).

It should be noted, however, that the fed-batch process must be stopped when the volume reaches the maximum working volume (80 l), while in a continuous cultivation mode the process can last longer. This does not provide grounds for a definite conclusion as to which cultivation regime will accumulate a greater amount of the target product.

By activating the Gluconic Acid Process Control button in the left field of the panel, the results shown above can be demonstrated. A screen appears on the right that allows you to select one of the control options described above and start the procedure with the Start button.

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Fig.26.

Fig. 26 shows such a control law demonstration of Fig. 22.

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