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A System for Functional States Recognition of *Escherichia coli* Fed-batch Cultivation

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Summary: EpiData (free software for entering and documenting data) is used for design a system for functional states identification based on specific metabolic mechanisms.

Keywords: Functional States, Information Based System, Dataentry, Database System, Metabolism, Mathematical Description, Fed-batch Processes, *Escherichia coli*.

1. INTRODUCTION

Bioprocesses, and particularly cultivation processes, are characterized by a complicated structure of organization and independent characteristics, which determines their nonlinearity and non-stationary. In many cases, the globally valid conventional numeric models, which describe the overall process behavior, cannot be used in on-line monitoring and control, either because they do not describe the process well enough or contain too many poorly known parameters. Simple unstructured models, which account for key process variables (biomass, substrate and product concentrations) do not reflect metabolic changes and are unsuitable for many tasks [1, 8, 9, 11, 12]. The multiple model approach is an alternative concept, which helps in modelling and control of complex processes such as bioprocesses.

Halme [11, 12] introduces the functional state concept to describe and analyze the current biological state of bioprocesses, and applies the approach in expert system-based fault diagnosis and in control of bioprocesses. The main idea is to use a two-level hierarchy where at the first level the process is divided into macrostates, called functional states, according to behavioural equivalence. In each functional state the process is described by a conventional type of model, called a local model, which is valid in this functional state only. In each functional state certain metabolic pathways are active enough to dominate the overall behaviour of the process. The biological behaviour is quite similar during each functional state. At the second hierarchical level some numeric detection algorithms and/or rules based on expert knowledge can be used for the recognition of the functional states and state transitions.

There is a lot of software for data entering, documentation and processing of data. We chose an EpiData, because it is free and easy for work. In EpyData can write simple text lines and the program converts this to a dataentry form for which we can add further control of entry, conditional jumping to other fields or calculations [3, 10].

The aim of this paper is to design and work out a system for recognition of functional state of E*scherichia coli* fed-batch cultivation.

2. FUNCTIONAL STATES OF *ESCHERICHIA COLI* FED-BATCH CULTIVATION

The whole *E. coli* growth process can be divided into at least five functional states, according to the physiological behaviour of the microorganisms in the process [5-7]:

First acetate production state (FS I)

The rules for recognition of *FS I* during the *E. coli* cultivation process are:

$$S > S_{crit}\,, pO_2 > pO_{2crit} \text{ and } A > 0\,, \tag{1}$$

where S is concentration of substrate (glucose), [g/l]; pO_2 – concentration of dissolved oxygen, [%]; A – concentration of acetate, [g/l]; S_{crit} and pO_{2crit} – critical values of substrate and dissolved oxygen concentrations.

In this state the main carbon source is the glucose and the acetate excretion is the result of a metabolic “overflow” mechanism.

Overflow metabolism has been attributed to an enzymatic limitation in the TCA cycle.

Mixed oxidative state (FS II)

The process enters this state when the following conditions are available:

$$S \leq S_{crit} \text{ , } pO_2 \geq pO_{2crit} \text{ and } A > 0 \text{ .} \tag{2}$$

Here both sugar and produced acetate are co-metabolized through the oxidative pathways in the state. The bacteria *E. coli* are able to re-oxidize the reduced metabolic intermediates that were accumulated during the first functional state. In this functional state accumulated acetate is also metabolized.

Complete sugar oxidative state (FS III)

The rules for recognition of *FS III* have to meet the requirements:

$$S \leq S_{crit} \text{ , } pO_2 \geq pO_{2crit} \text{ and } A = 0 \text{ .} \tag{3}$$

In this state, sugar is completely oxidized to water and carbon dioxide. There is sufficient quantity of dissolved oxygen in the media which helps with the reoxidizing of the metabolic intermediates in the cell (NADH and FADH).

Ethanol consumption state (FS IV)

The process enters this state when the following conditions are available:

$$S = 0, pO_2 \geq pO_{2crit} \text{ .} \tag{4}$$

The process is defined to be in this state when ethanol is available but no sugar is in the broth, and the dissolved oxygen concentration is above the critical level. Ethanol is the only carbon source for yeast growth.

Second acetate production state (FS V)

The conditions for this state are:

$$S \leq S_{crit} \text{ , } pO_2 < pO_{2crit} \text{ and } A > 0 \text{ .} \tag{5}$$

When the dissolved oxygen becomes the limiting factor for *E. coli* growth, acetate is produced. Due to the low level of dissolved oxygen metabolic intermediates can not be reoxidized.

The described functional conditionals are used for designing and creating of the system for functional states recognition. The models in each current state are used for output user information.

The current data in the system are real experimental data from *E. coli* fed-batch cultivation process [2].

3. EPIDATA

EpiData is a Windows 95/98/NT/2000 based program (32 bit) for DataEntry. EpiData development is initiated by Jens M. Lauritsen, MD. PhD, Denmark [3, 10]. Program released as freeware by: The EpiData Association, Odense Denmark. Program design: Jens M.Lauritsen & Michael Bruus.

The aim of this free software is that in EpiDate must write simple text lines and the program converts this to a dataentry form for which we can add further control of entry, conditional jumping to other fields or calculations.

The idea of the EpiData Association is this program to be free and more scientists, specialists and students to use it [3].

The EpiData software is very suitable for descriptions of biotechnological processes [4].

4. SYSTEM STRUCTURE

After defining the functional states and process variables must be define a system structure. The process included some steps:

4.1. Define data

Data is defined by writing three types of information for each variable in file States.qes:

A. Name of input field (variable, e.g. v1 or exposure);

B. Text describing the variable. (e.g. mode of something or "day of beginning");
C. A field definition, e.g. ## for two digit numerical.

Other options: e.g. colour of background and fields, line height etc.

In our case the variables are three – substrate concentration [g/l], dissolved oxygen concentration [%] and acetate concentration [g/l].

For journal of system we include also date and hour of current cultivation. The defined data in file States.qes are shown on Fig. 1.

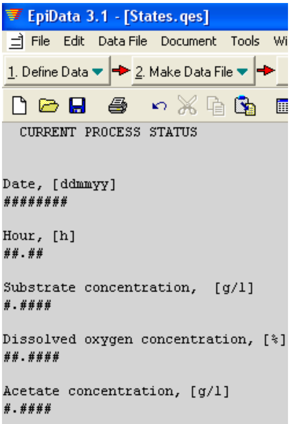

Fig. 1

After defining a file States.qes before making data file States.rec we can see a data entry form with option “preview data form” and if it is correct this step is ready.

4.2. Data file

When data entry form is suitable for user must be make a data file (Fig. 2). This file States.rec is main file, in which is collected all information about different cultivation. The information of bioreactor is writing in it. The different cultivation can be recognizing according the date of cultivation.

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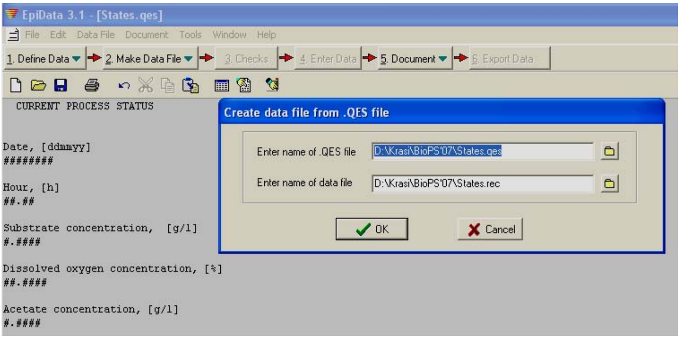

Fig. 2

This file has output to some program for data processing such as dBaseIII, Excel, Stata, SPSS and SAS.

4.3. Data entry

In such creation file is collected data (Fig. 3).

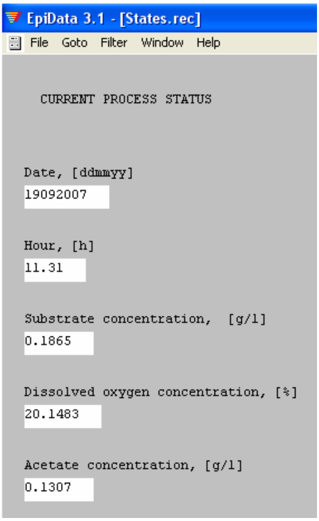

Fig. 3

4.4. Add/Revise Checks - at Entry of Data

In EpiData is one more useful function. This is step “*Add/Revise Checks”*. Here is written all conditionals that must be executing. For example the different variables have different range of values; also here can be write the “*if - else*” conditions.

On the base of models (1 ÷ 5) we are defining this conditionals. On Fig. 4 is shown the procedure of defining of Field5 (acetate concentration).

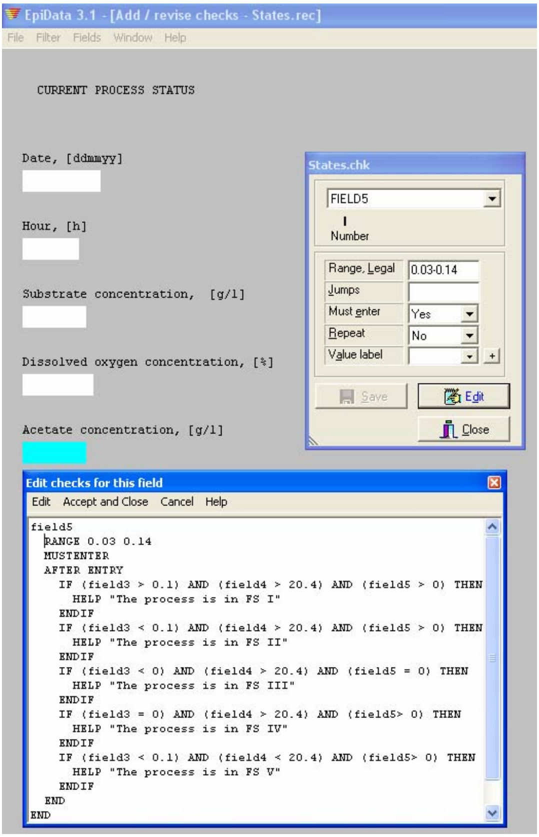

Fig. 4

The procedure is easy to realize, because the command are given in separated Help.

4.5. Output forms

The output form with process data can be design with dBaseIII, Excel, Stata, SPSS and SAS. On Fig. 5 is shown a current state of cultivation and the local model of the state.

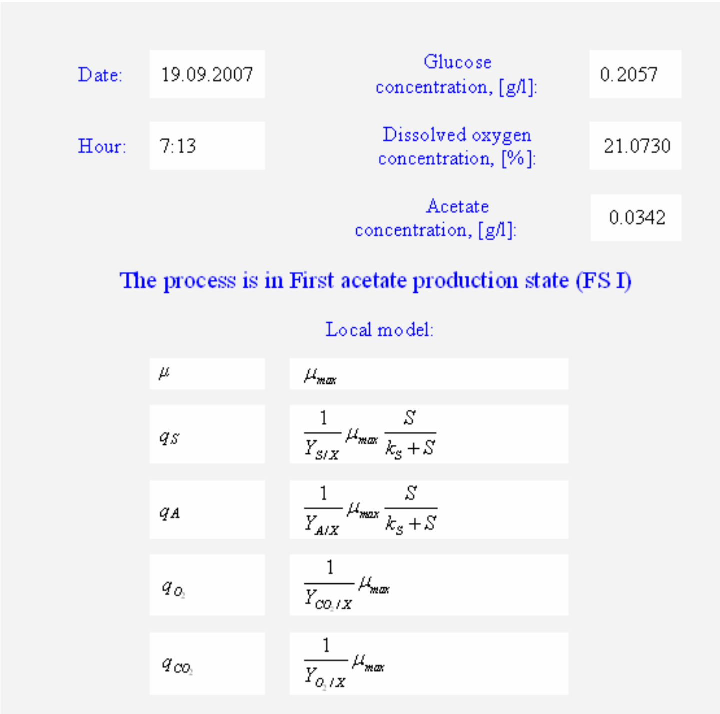

Fig. 5

6. CONCLUSION

The first version of a system for functional states recognition of *Escherichia coli* fed-batch cultivation is created. The system informs the users about current state of process and shows the model of this functional state.

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