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Functional States Recognition System for Fed-batch Cultivation of *Saccharomyces cerevisiae*

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Summary: Free software for entering and documenting data EpiData is here used for design of a system for functional states recognition during a fermentation process. The identification of the current process state is based on the predetermined rules, rendering specific metabolic mechanisms. Developed system is further applied for a fed-batch cultivation of *Saccharomyces cerevisiae*.

Key words: Functional states, Fed-batch process, Yeast metabolism, Information based system, Database system.

1. INTRODUCTION

To develop an accurate and complete model of fermentation processes (FP) is not an easy task due to a lot of reasons [3, 5, 6]. FP are rather complex systems which still can be described only in a roughly simplified way. These processes combine some interactions and reactions, different in their nature: purely physical, chemical and physical-chemical, purely biological and biochemical. Those different by nature reactions are in a mutual commitment. Besides the intracellular processes, characterized with a complicated structure of the metabolism, which mechanisms are still not fully understood, the variation of the local conditions in the bioreactor, caused by the fluid dynamics of the multiphase system, have to be also considered, even in a very simplified way. This complexity of FP leads to description with a big number of factors that have influence on the process.

Based on the presented peculiarities it could be summarized that in terms of mathematical modelling FP are characterized by a complicated structure of organization and interdependent characteristics, which determine their non-linearity and non-stationary properties. These processes are known to be very complex and their modelling may be a rather time consuming and thus a costly task. However, it is neither necessary nor desirable to construct comprehensive process models that can describe the system in all possible situations with a high degree of accuracy. In many cases, the globally valid conventional numeric models, which describe the overall process behaviour, 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. At the same time, the metabolic way of the culture growth is a very important peculiarity of FP. There are processes, where the product of metabolism is directly connected with the growth of the population – primary metabolites. There are others as well, at which this connection is missed or it is slightly expressed – secondary metabolites. In general, the metabolic way of growth strongly depends on the fermentation conditions.

As an alternative to the global model approach, the multiple model approach could be considered, which allows taking into account the metabolic changes and helping in that way in modelling and control of complex processes such as FP. The main idea of the multiple model, and in particular – of the functional state approach is to use a two-level hierarchy. At the first level the process is divided into macrostates, called *functional states* (FS), according to behavioural equivalence. In each FS the process is described by a conventional type of model, called a *local model*, which is valid in this FS only. In each FS certain metabolic pathways are active enough to dominate the overall behaviour of the process. 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.

Definitely, the step of the recognition of current functional state is an important task for development of an adequate model and further high-quality control. This is the problem, to which the present work is devoted to. There is a lot of software for data entering, documentation and processing. In this work EpiData is chosen, because it is free and easy to work with. EpiData is a Windows 95/98/NT/2000 based program (32 bit) [4]. Its development is initiated by Jens M. Lauritsen and the program is designed by Jens

M. Lauritsen and Michael Bruus [1]. One of the main advantages of EpyData is the possibility simple text lines to be converted to a dataentry form for which further control could be added or conditional jumping to other fields or calculations could be done [1]. EpiData is released as a freeware by *The EpiData Association*, *Odense Denmark*, which main idea is to make the program free and accessible to more scientists, specialists and students.

The aim of this paper is a system for functional state recognition during a fermentation process to be designed using EpiData. Developed system is further applied for a fed-batch cultivation of *Saccharomyces cerevisiae*.

2. FUNCTIONAL STATES DURING FED-BATCH CULTIVATION OF *SACCHAROMYCES CEREVISIAE*

Based on the metabolic mechanisms and a lot of investigations, Zhang et al. [5, 6] have supposed that the whole yeast growth process can be divided into at least five FS in batch and fed-batch cultures (Table 1).

Table 1

Functional state I - First ethanol production state
The process is defined to be in this state when the sugar concentration is above the critical level and there is sufficient dissolved oxygen. In this state ethanol is produced. *Rule for FS I recognition*: $S > S_{crit}$ and $O_2 > O_{2crit}$
Functional state II - Mixed oxidative state
The process enters this state when the sugar concentration decreases to be equal to or below the critical level and there is sufficient dissolved oxygen in the broth. Both sugar and produced ethanol are co-metabolized through the oxidative pathways in the state. *Rule for FS II recognition:* $S \leq S_{crit}$, $O_2 \geq O_{2crit}$ and $E > 0$
Functional state III - Complete sugar oxidative state
The process is defined to be in this state when there is no ethanol available, the sugar concentration is not higher than the critical level and the dissolved oxygen is above its critical level. In this state, sugar is completely oxidized to water and carbon dioxide. *Rule for FS III recognition:* $S \leq S_{crit}$, $O_2 \geq O_{2crit}$ and $E = 0$
Functional state IV - Ethanol consumption state
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. *Rule for FS IV recognition:* $S = 0$ and $O_2 \geq O_{2crit}$
Functional state V - Second ethanol production state
The conditions for this state are that both concentrations, for sugar and for dissolved oxygen, are below the corresponding critical levels. When the dissolved oxygen becomes the limiting factor for yeast growth, ethanol is produced. *Rule for FS V recognition:* $S \leq S_{crit}$, $O_2 < O_{2crit}$ and $E > 0$

In an industrial aerobic yeast growth process where oxygen is often limited, more FS might exist. For instance, a state with conditions of $O_2 < O_{2crit}$ and $S > S_{crit}$, and a state with $S = 0$, $E > 0$ and $O_2 < O_{2crit}$ might be possible. Since all of experimental data came from laboratory scale cultivations, these FS do not occur frequently.

In principle FS I can appear in all batch, fed-batch and continuous yeast growth processes. FS IV normally appears only in batch culture that is why it will be omitted in this work. The functional states FS II, FS III and FS V are normally found in fed-batch and continuous cultures [6]. It should be noted that the FP could be only in one FS at any time. However, a certain FS can appear in the process more than once during one run.

3. DESIGN OF FUNCTIONAL STATES RECOGNITION SYSTEM

The design of a system for recognition of functional states during *S. cerevisiae* fed-batch cultivation is based on a data set of cultivation, performed in the *Institute of Technical Chemistry, University of Hanover, Germany* [3]. The data consists of on-line measurements of substrate (glucose, S, [g/l]) and dissolved oxygen (O_2, [%]), as well as the off-line data for biomass (yeast, X, [g/l]) and product (ethanol, E, [g/l]). These process variables are considered as the most informative for the current process status. The functional states recognition system is built on the rules for recognition, described in Table 1.

After the definition of main process variables, namely [S, O_2, X, E], a structure of the system has to be designed. This process includes e few steps, described in details in [2]. Each variable is defined in a data file (in this case *New_states.ges*) by adding information for three main features of the data, namely *the name of the input field* (e.g. *S*), *text described the variable* (e.g. substrate) and *a field definition* (e.g. #.#### for the numeric format of the substrate concentration). Besides the process variables, the date and the hour of the current cultivation are also included for the system journal requirements. The defined structure for the considered here fed-batch cultivation of *S. cerevisiae* is shown on Fig. 1.

At this stage of the system development the process data is manually introduced by the operator. Each variable is limited in range of its possible value. A possibility for the elimination of type-errors is provided (Fig. 2).

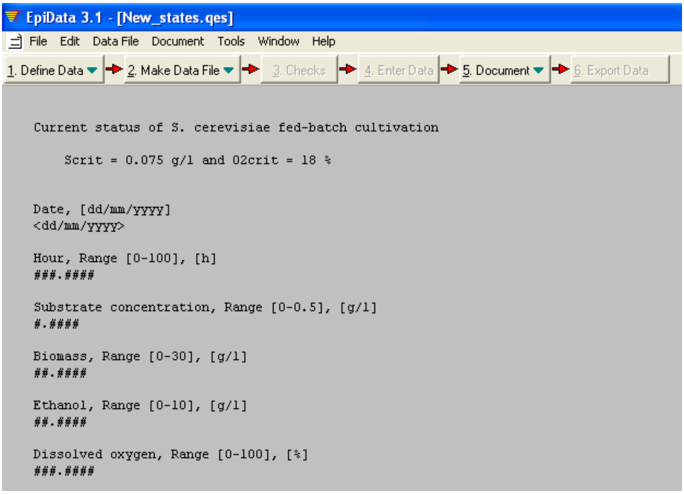

Fig. 1 Structure of the data entry

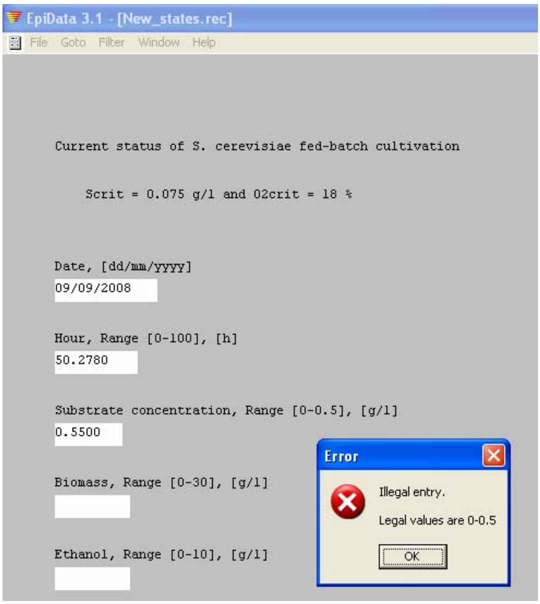

Fig. 2 Possibility for the elimination of wrong entry

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The recognition rules presented in Table 1 are implemented in the designed system (Fig. 3) taking into account the critical levels of substrate concentration and dissolved oxygen. Based on Zhang et al. [5, 6] and the experience with the *S. cerevisiae* strain and cultivations, the following values are assumed for fed-batch mode:

$$S_{crit} = 0.075 \text{ [g/l] and } O_{2crit} = 18 \text{ \%}.$$

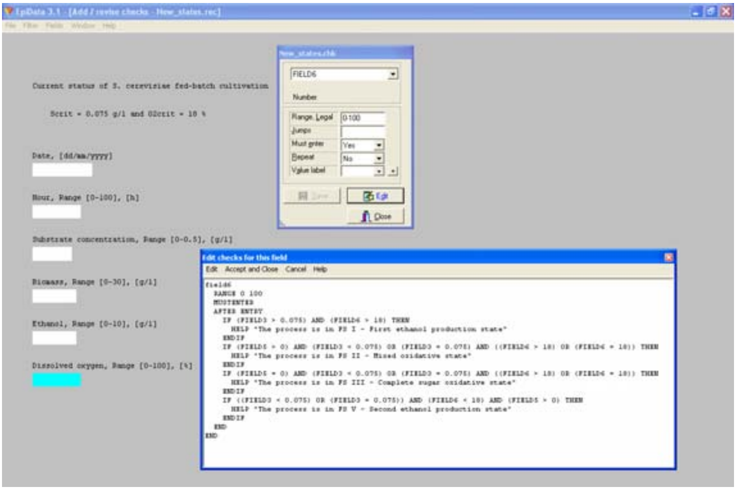

Fig. 3 Rules for functional states recognition

Based on the implemented rules and adding data from the cultivation process, the designed system will recognizes the current status of the process at each moment. Entering scores of experimental data records, the designed system leads to recognition of four functional states during this cultivation, namely:

- *complete sugar oxidative state* (*FS III*)
- *first ethanol production state* (*FS I*)
- *first ethanol production state (FS I)*, *after changing of* S_{crit}
- *mixed oxidative state (FS II)*.

The system for the functional state recognition responds to each data record. The following figures show the results after the recognition, respectively Fig. 4 for *complete sugar oxidative state* (FS III) and Fig. 5 - for *first ethanol production state* (FS I). Figures for the rest two recognized functional states are not here presented because of the similar view.

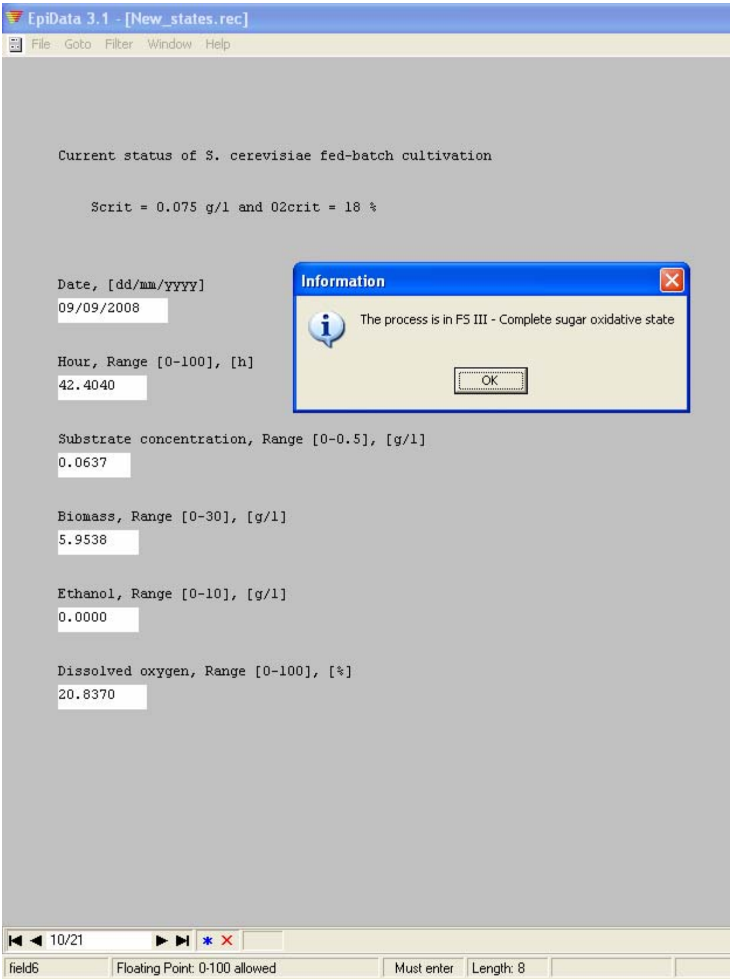

Fig. 4 Information about the recognized functional state – FS III

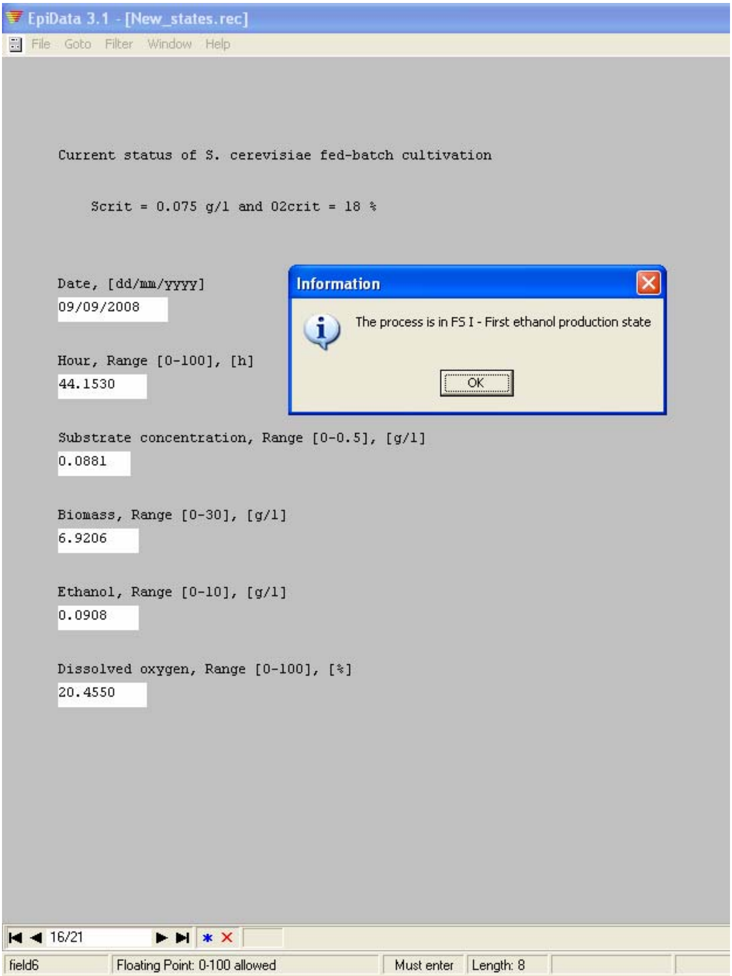

Fig. 5 Information about the recognized functional state – FS I

The EpiData allows the data collected to be exported in different formats, e.g. text format, dBase III, Excel, etc. This program feature makes possible the exported data to be presented in more informative way. Fig. 6 presents the whole fed-batch cultivation of *S. cerevisiae*, divided in all together four functional states after the application of developed here functional states recognition system.

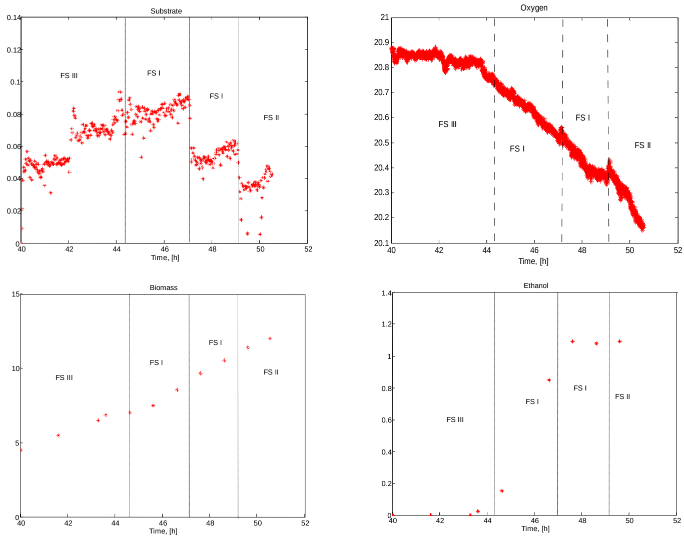

Fig. 6 Recognized functional states during fed-batch cultivation of *S. cerevisiae*, using designed system

4. CONCLUSION

A system for functional states recognition during a fermentation process has been here developed based on the free software EpiData. The identification of the current process state is based on the predetermined rules, rendering specific metabolic mechanisms. In this way the system could be very helpful as a first very important step of the development of the process model, comprised of more transparent and clear local models. Developed recognition system is successfully applied for a fed-batch cultivation of *S. cerevisiae*, leading to recognition of four functional states.

As a further possibility for improving of developed recognition system is a similar system to be developed and tuned in such way to receive the process data directly from the different sensors in bioreactor. That will allow such a system to be used even for on-line control and optimal carrying out of the cultivation process.

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