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An Advisory System for On-line Control of 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 an advisory system for on-line control of a fermentation process. Based on the preliminary developed system for functional state recognition, presented here system will advise the user which new functional state can be reached and what kind of control actions have to be taken. New-designed system appears as an expert system and comprises knowledge of well-trained operators of cultivation processes. Developed advisory system is further applied for a fed-batch cultivation of *Saccharomyces cerevisiae*.

Key words: Advisory system, Information based system, Database system, On-line control, Functional states, Fed-batch process.

1. INTRODUCTION

In the contemporary biotechnology there is an obvious need of improved process control and optimization. An important step for process optimization and high-quality control is the development of adequate models. Fermentation processes (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 task. Many mathematical models of FP have been proposed [6, 8, 9] but just a few have been used to optimize industrial plants. The common modelling approach is the development of an overall nonlinear model of FP that performs satisfactorily through the entire operating range. Unfortunately, this approach has a lot of disadvantages. As much more detailed description of the process is aimed as more complex model will be obtained. Complex global models are characterized with a big number of parameters, which complicate the model identification and simulation. Moreover, a global model is not able to describe the metabolic changes during the entire operating range and the parameter non-stationary. In order to avoid mentioned above disadvantages, some alternative approaches have to be searched. There is a strong intuitive appeal in building systems which operate robustly over a wide range of operating conditions by decomposing them into a number of simpler modelling or control problems. This appeal has been a factor in the development of increasingly popular multiple-model approach, and in particular – functional state modelling approach, to cope with strongly nonlinear and time-varying systems [6, 8, 9]. Using this approach complicated problems are decomposed into subproblems that can be solved independently. Then the individual solutions of the decomposed problems lead to the global solution of the complex problem.

The multiple-model based approach is an appropriate tool for monitoring and control of complex processes such as FP [6, 8, 9]. When modelling and control FP, as the most successful methodology could be considered the decomposition into operating regimes. The process is decomposed into different stages, corresponding to the stages, through which the process carries out. These approaches often give a simplified and transparent nonlinear model or control representation. In addition, the local approaches have computational advantages, lend itself to adaptation and learning algorithms and allow direct incorporation of high-level and qualitative plant knowledge into the model.

When using functional state modelling approach, the step of the recognition of current functional state is an important task for development of an adequate model. Next, based on the current recognized functional state, advices for the further process control could be disposed to the users. This is the problem to which the present work is devoted to - using EpiData an advisory system to be designed, based on the preliminary developed system for functional state recognition [5]. EpiData, a Windows 95/98/NT/2000 based program (32 bit), is chosen because it is free and easy to work with [4, 7]. The program is designed by Jens M. Lauritsen and Michael Bruus [3] and it is released as a freeware by *The EpiData Association*, *Odense Denmark*. Developed here advisory system is further demonstrated for a fed-batch cultivation of *Saccharomyces cerevisiae*.

2. FUNCTIONAL STATES MODELLING APPROACH TO *SACCHAROMYCES CEREVISIAE* FED-BATCH CULTIVATION

The main idea of the functional state modelling approach is that the process is divided into macrostates, called *functional states* (FS), according to behavioural equivalence. In each FS the yeast metabolism is dominated by certain metabolic pathways. Based on a lot of investigations, Zhang et al. [8, 9] have supposed that the whole yeast growth process can be divided into at least five functional states in batch and fed-batch cultures (Table 1).

Table 1

Functional state	Rules for recognition
first ethanol production state (FS I)	$S > S_{crit}$ and $O_2 > O_{2crit}$
mixed oxidative state (FS II)	$S \leq S_{crit}$, $O_2 \geq O_{2crit}$ and $E > 0$
complete sugar oxidative state (FS III)	$S \leq S_{crit}$, $O_2 \geq O_{2crit}$ and $E = 0$
ethanol consumption state (FS IV)	$S = 0$ and $O_2 \geq O_{2crit}$
second ethanol production state (FS V)	$S \leq S_{crit}$, $O_2 < O_{2crit}$ and $E > 0$

A yeast growth process switches from one functional state to another when the metabolic conditions are changed. The functional state diagram of the process can be illustrated as it is shown in Fig. 1 [8, 9].

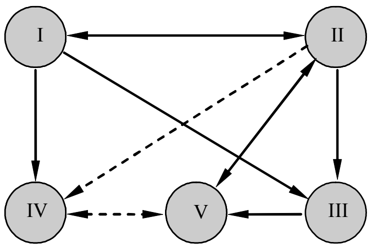

Fig. 1 Functional state diagram

Zhang et al. [8, 9] has reported that 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. The functional states FS II, FS III and

FS V are normally found in fed-batch and continuous cultures [8, 9]. The solid arrows in Fig. 1 indicate the necessary or normal transition between various functional states of the process. The dotted arrows indicate that the transitions take place when the mode of culture changes between batch and fed-batch cultures. It should be noted that FP could be only in one functional state at any time. However, a certain functional state can appear in the process more than once during one run.

3. DESIGN OF AN ADVISORY SYSTEM FOR ON-LINE CONTROL OF *SACCHAROMYCES CEREVISIAE* FED-BATCH CULTIVATION

The design of an advisory system for on-line control of fed-batch cultivation of *Saccharomyces cerevisiae* is based on the preliminary developed system for recognition of functional states during a fermentation process. This recognition system is described in details in [5]. The same fed-batch cultivation of *S. cerevisiae* as considered in [5] and performed in the *Institute of Technical Chemistry, University of Hanover, Germany* [6] is here used as a test set. The functional states recognition system is built on the rules for recognition, described in Table 1.The system for recognition of functional state is learned in such way to recognize all five FS, presented in Table 1 and Fig. 1, although not all of them appears during the test experimental data set. Further, these five recognized FS, with some explanations and possible control actions, are consequently presented.

If the recognition system identifies that the process is in *first ethanol production state* (FS I), the advisory system responds as shown in Fig. 2. If the user likes to switch the process to another functional state, dissolved oxygen has to be kept to be sufficient and substrate concentration has to be decreased to be equal to or below the critical level. Then:

- If there is ethanol in the broth, the process can be switched to *mixed oxidative state* (FS II).
- If there is no ethanol in the broth, the process can be switched to *complete sugar oxidative state* (FS III).
- If there is no substrate in the broth, the process can be switched to *ethanol consumption state* (FS IV).

If the recognition system identifies that the process is in *mixed oxidative state* (FS II), the advisory system responds as shown in

Fig. 3. If the user likes to switch the process to another functional state, it is necessary:

- Substrate concentration to be kept to be equal to or below the critical level, dissolved oxygen to be sufficient and no ethanol to be available in the broth – then the process can be switched to *complete sugar oxidative state* (FS III).
- If the user likes to switch the process to *first ethanol production state* (FS I), sufficient dissolved oxygen has to be kept and the culture has to be fed so substrate concentration to increase above the critical level.
- If the user likes to switch the process to *second ethanol production state* (FS V), substrate concentration has to be kept to be equal to or below the critical level, but the dissolved oxygen has to be decreased below the critical level.
- If the process accidentally switches from fed-batch to batch mode - to *ethanol consumption state* (FS IV, shown with dotted arrows in Fig. 1), sufficient dissolved oxygen has to kept, but no substrate has to be available in the broth.

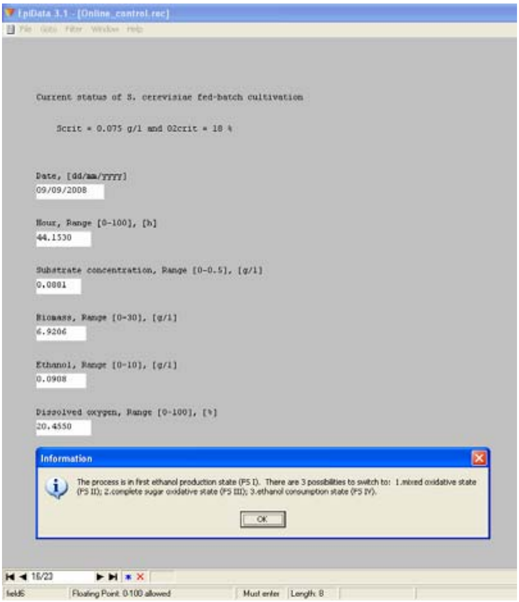

Fig. 2 Advisory system at *first ethanol production state* (FS I)

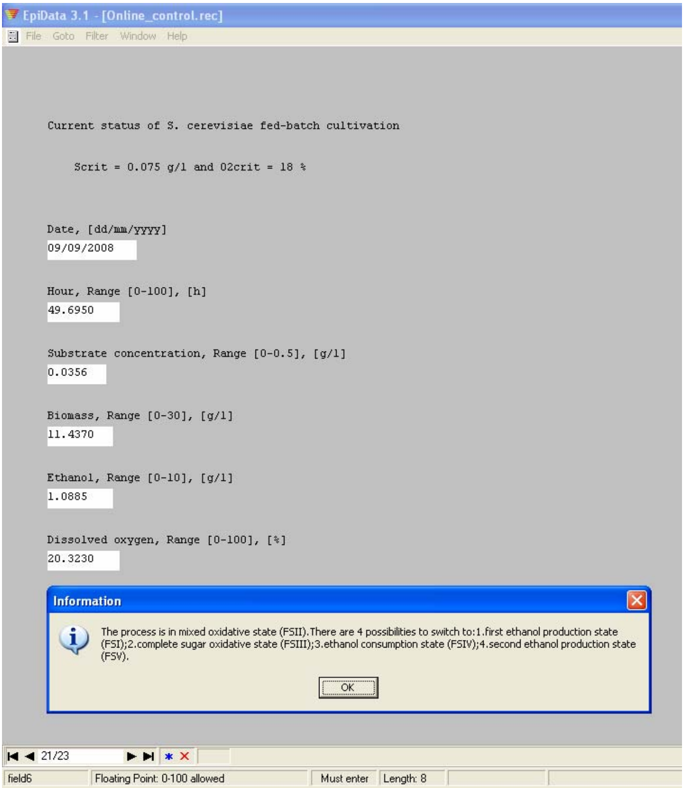

Fig. 3 Advisory system at *mixed oxidative state* (FS II)

If the recognition system identifies that the process is in *complete sugar oxidative state* (FS III), the advisory system responds as shown in Fig. 4. There is only one possibility the process to be switched to another functional state, namely to *second ethanol production state* (FS V). Aiming that the substrate concentration has to be kept to be equal to or below the critical level, but the dissolved oxygen has to be decreased below the critical level. Also, ethanol has to be available in the broth.

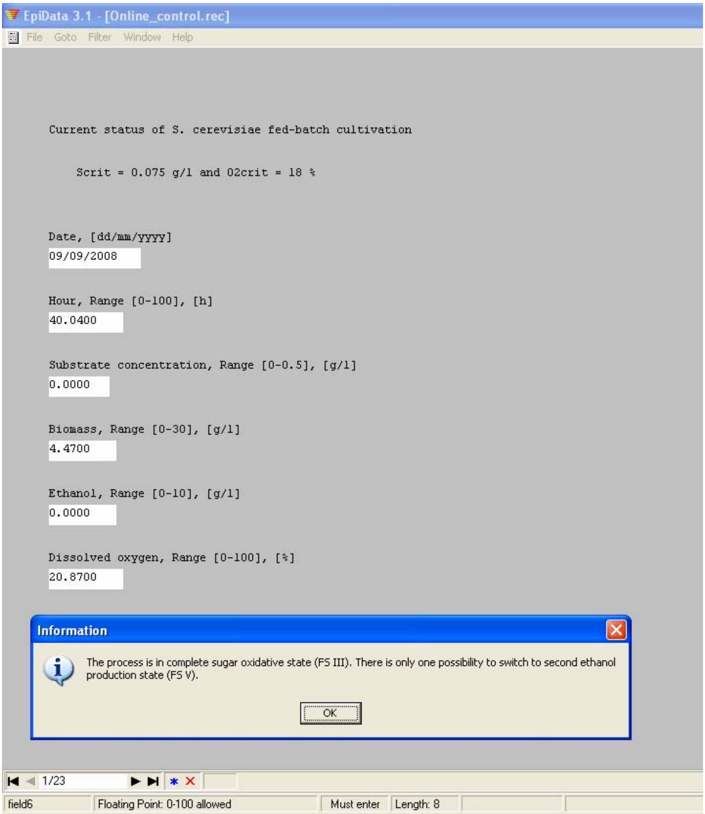

Fig. 4 Advisory system at *complete sugar oxidative state* (FS III)

If the recognition system identifies that the process is in *ethanol consumption state* (FS IV), the advisory system responds as shown in Fig. 5. FS IV normally appears only in batch culture. But if the process accidentally switches from fed-batch to batch mode (FS IV, shown with dotted arrows in Fig. 1), there is only one possibility the process to be switched to another functional state, namely to *second ethanol production state* (FS V). Aiming that substrate concentration has to be increased, but to be equal to or below the critical level, and dissolved oxygen has to be decreased below the critical level. Also, ethanol has to be available in the broth.

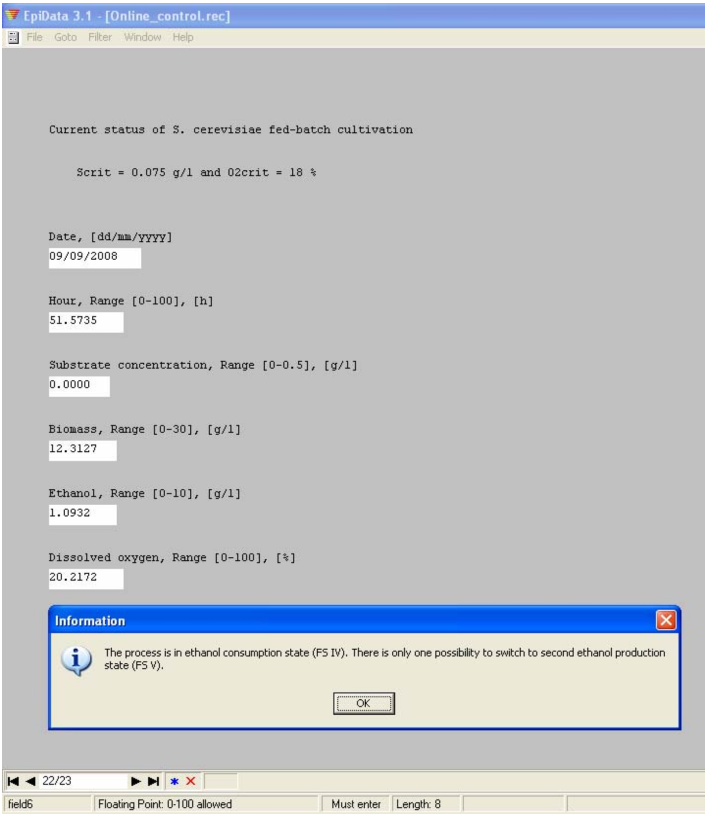

Fig. 5 Advisory system at *ethanol consumption state* (FS IV)

If the recognition system identifies that the process is in *second ethanol production state* (FS V), the advisory system responds as shown in Fig. 6.

- There is only one possibility the process to be switched to another functional state, namely to *mixed oxidative state* (FS II). Substrate concentration has to be kept to be equal to or below the critical level, but the dissolved oxygen has to be increased above the critical level.
- There is a possibility the process accidentally to switch to batch mode (FS IV, shown with dotted arrows in Fig. 1) – namely to *ethanol consumption state* (FS IV). For that purpose no substrate has to be available in the broth but sufficient dissolved oxygen has to be supplied.

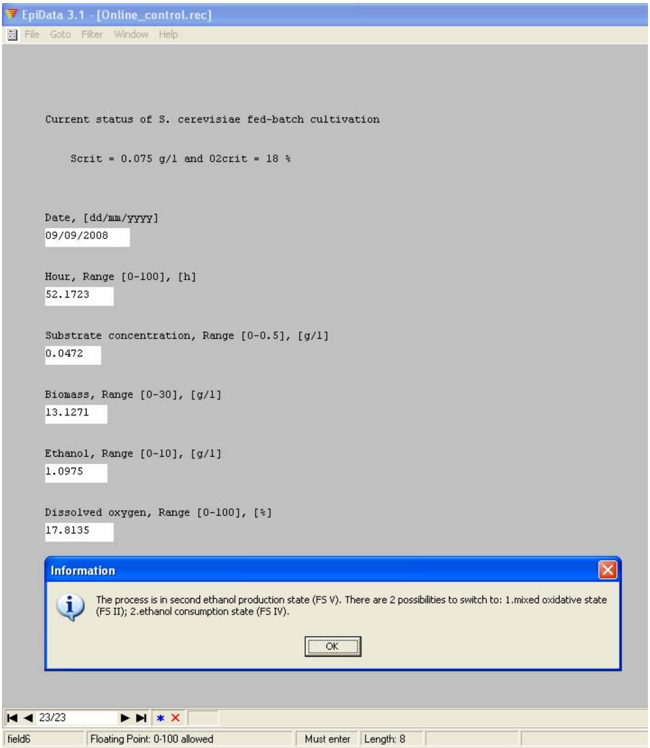

Fig. 6 Advisory system at *second ethanol production state* (FS V)

4. CONCLUSION

An advisory system for on-line control is here designed based on the preliminary developed system for the recognition of functional states during a fermentation process. New-designed advisory system could be considered as an expert system, comprising knowledge for the metabolic changes during cultivation processes. Developed system is further demonstrated for a fed-batch cultivation of *S. cerevisiae*. In all possible five functional states the user is advised for the possibilities which new functional state can be reached and what kind of control actions have to be taken. Such advisory system could be exceptionally useful if it is implemented in the automated control equipment of bioreactor. In the same time the applied controllers have to permit implementation of process knowledge [2, 8] or to be a controller with variable structure allowing taking into account the model changes during the process [1]. Thus designed advisory system could be of a high efficiency for on-line control and optimal carrying out of cultivation processes.

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