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Bioprocess Monitoring, Control and Data Management Software SHIVA

Programski paket SHIVA za praćenje i regulaciju bioprocesa te obradbu podataka

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Summary

Monitoring, control and data management are very important parts of every process. For this purpose, especially when a lot of data has to be collected and processed, the computer is a necessity. For all important biotechnological processes the development of an user-friendly software appears to be necessary due to lack of such specialised programs on the market. Because of the availability, personal computer platform was chosen. Program package is divided in two parts named SHIVA I and SHIVA II. SHIVA I can be used for control of bioprocess and offers measurement and regulation of biotechnological variables (T, pH, dissolved oxygen,...), alarms, graphic presentation, bioprocess documentation, data interchange, etc.

SHIVA II is designed for management and analysis of data acquired in bioprocess experiments: curve fitting and interpolation, parameter optimisation, calculations on data series, derivation and integration, simulation and fitting of mathematical models. It runs under DOS, Windows and OS/2 environment with latter two enabling multitasking. SHIVA package therefore enables user assistance with his experiments on two levels: firstly by monitoring and control of bioprocess and secondly by analysing acquired data and planning future experiments.

Sažetak

Praćenje i kontrola važne su aktivnosti u upravljanju procesima. Zbog toga je, pogotovo kad ima puno podataka, nužna primjena računala. Mogućnosti koje nam ona nude na tom području postaju dostupne širem krugu korisnika (user-friendly). Programski paket može se podjeliti u dva dijela nazvana SHIVA I. i SHIVA II. Program SHIVA I. primjenjuje se za kontrolu bioprocesa i omogućava mjerenje i reguliranje bioloških varijabli (T, pH, otopljeni kisik,...), alarme, grafički prikaz, dokumentaciju bioprocesa, izmjenu podataka s drugim programima i slično.

Program SHIVA II. služi za obradbu i analizu podataka izmjerenih tijekom bioprocesa: uklanjanje slučajnih pogrešaka i interpolacija, optimiranje parametara, proračuni iz vremenskih nizova podataka, deriviranje i integriranje, simulacija i izbor najpogodnijih matematičkih modela. SHIVA I. i II. rade s programima DOS, Windows i OS/2. Posljednja dva omogućavaju istodobnu uporabu više programa (multitasking). Programski paket služi pri izvođenju pokusa na dvije razine: za kontrolu i regulaciju bioprocesa te planiranje budućih pokusa.

Introduction

Traditional as well as modern bioprocesses exploiting biological potential of microorganisms, plant and animal cells currently supply a wide variety of products to the pharmaceutical, food, agrochemical and chemical industries. Maximum economic benefit from existing and new bioprocesses can only be obtained if the fermentation/culturing process is well understood, well controlled and to some extent optimised *(1)*. For achiev-

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ing these demands a computer supported by a good software can be a valuable tool. The software should govern the operation of the computer, its interfaces and peripheral devices and may dictate the overall performance of the process. There is a lack of generalised fermentation monitoring and control programs on the market *(2)*. In addition many programs available for data analysis are rarely compatible with monitoring and control programs. For that purpose two programs SHIVA I and SHIVA II are introduced. Both are 100 % compatible, mouse-aware, fully menu driven programs. A full on-line help system is provided, describing all of the main program features along with a description of how to navigate the program.

Materials and Methods

The program package SHIVA was written in computer language Borland Pascal version 7.0 (Borland International, 1994) on IBM compatible 80486 PC.

Results and Discussion

a) Program description

SHIVA I was especially developed for monitoring and control of bioprocesses. The program can be applied in various types of bioprocesses (submerged, solid-state) in different operation modes (batch, fed-batch, continuous, recycle) enabling accurate process control and optimum providing conditions. Its main options are presented in Fig. 1.

Every bioprocess is defined by its name, acquisition time and variables (on-line, off-line, indirect and regulated). In description window all particularities of the process are written. According to the process name new directory on the hard disk is created in which all data from current process are being saved in order to ensure data safety. Every variable has its own file which enables high flexibility in data managing and analysis.

To procure aseptic conditions the whole system should be sterilised. Although common *sterilisation time* is 20 min at 121 °C, software enables these parameters to be different according to the process requirements.

Fig. 1. A schematic presentation of SHIVA I options

Slika 1. Shematski prikaz mogućnosti programskog paketa SHIVA I.

To monitor the bioprocess, key variables should be followed. The best solution are sensors connected on-line to the process. To obtain correct data, changes of sensors characteristics should be compensated through *sensor calibration*. Three different calibration options are available. When calibration in one point is chosen, only sensor offset is adjusted, while in two points offset as well the slope of the sensor is calculated. Finally, the sensor parameters can be entered also as numerical values via keyboard.

According to the way the data are obtained, they can be divided in three groups: *on-line, off-line and indirect data*. On-line data are obtained from sensors directly connected to the data acquisition unit and to the computer. Acquisition time can be user-defined depending on the process dynamics. Off-line data, obtained from instruments not connected to the computer or by other analytical methods, can be entered during or after the process via keyboard and immediately graphically compared with the on-line data. Indirect data, that can not be measured directly, are calculated by computer according to mathematical equation defined by user.

To lead the process in its optimal way, simple regulation loops combined with more sophisticated algorithms *(3,4)* must be applied. SHIVA I enables different *regulation modes*. If fixed set point is selected, the computer keeps constant value of regulated variable during the whole process. Optional time profile allows set-point changing with time according to the predefined profile. The most advanced regulation mode is event control. A short procedure written by user defines under which conditions the action is taken. For that purpose a special designed regulation language was developed. It permits use of measured or indirect variables as well as logical (AND, OR, NOT) and comparison (= , => , =< , < , >) operators.

In *graphical representation* up to 6 parameters can be monitored on the same graph. This can be data of the current experiment (on-line graphics), data from previous experiments or combination of both. Last option is very useful when comparison between current and previous experiments is of interest.

Computerised monitoring of the bioprocess brings another feature. Since we know certain limitations or presume responses to some actions, we can classify their breach as a sign of unplanned behaviour and alarm process operator. There are 5 different *types of alarms* included:

- Sensor alarms occur when measured value is outside the logical range and so it is an indication of sensor malfunction.
- Regulation alarms indicate that regulation action was not successful, e.g. pH is decreasing although a base is added.
- Process alarms take place when measured value is outside user-defined interval.
- Interrupt alarms occur when the program is interrupted, e.g. because of power supply blackout (system returns to normal operation, when power supply is established again; time and duration of power supply blackout are written in the LOG file).
- Hardware alarms show that there are problems with hardware (e.g. read/write error, disk full, etc.).

When the alarm occurs, the message is written in the status row. Information about time and type of alarm is saved in LOG file and can later serve for documentation.

All data are written in *files* in a special format that enables fast data management. Files can be transformed from this format to ASCII format and vice versa to simplify communication with other popular software packages (WinWord, QPro, Excel, etc.).

SHIVA II is a further logical step from SHIVA I. After the data is obtained it has to be carefully analysed. Because of SHIVA I and SHIVA II compatibility all obtained bioprocess data can be immediately analysed even within running experiment. Its main features are presented in Fig. 2.

Common first step in data analysis is the removal of undesirable noise. This can be done with *smoothing*. Three options are available: Fourier series, Splines and orthogonal polynomials. By latest option a polynomial equation from first to ninth order, depending on user's selection, is calculated. It is particularly useful, when a calibration curve is to be established and the equation form is not predefined. However, to fit experimental data by a defined equation in which values of parameters have to be calculated, optimisation methods should be applied. SHIVA II offers two optimisation methods (one gradient and one nongradient) to perform fast and accurate parameter calculations *(5)*. As an optimisation criterion a statistical correlation index *(6)* is used. Results in all options are presented in graphical form. In procedures where equation or parameter values are calculated, results are displayed and written into a text file.

To calculate kinetics of the studied process, the slope of the straight line, supposed to be proportional to kinetic rate, is commonly calculated. However, this method sometimes leads to incorrect results due to variable kinetic rate.

Fig. 2. A schematic presentation of SHIVA II options
Slika 2. Shematski prikaz mogućnosti programskog paketa SHIVA II.

In such situation *derivation*, a simple but powerful operation, is the method of choice. It calculates kinetics for very small intervals. All changes in kinetics are easily detected and intervals with constant kinetics are determined.

A final step of a system description is a construction of the *mathematical model*. A typical mathematical model consists of a set of differential and algebraic equations coupled with process constraints. It is obvious that a model can be constructed only by a human. On the other hand, evaluation of model parameters from experimental data can be long and boring work. This is the part where computer shows its performance. SHIVA II allows calculation of up to 20 parameters from up to 20 series of experimental data. Since procedures of numerical integration

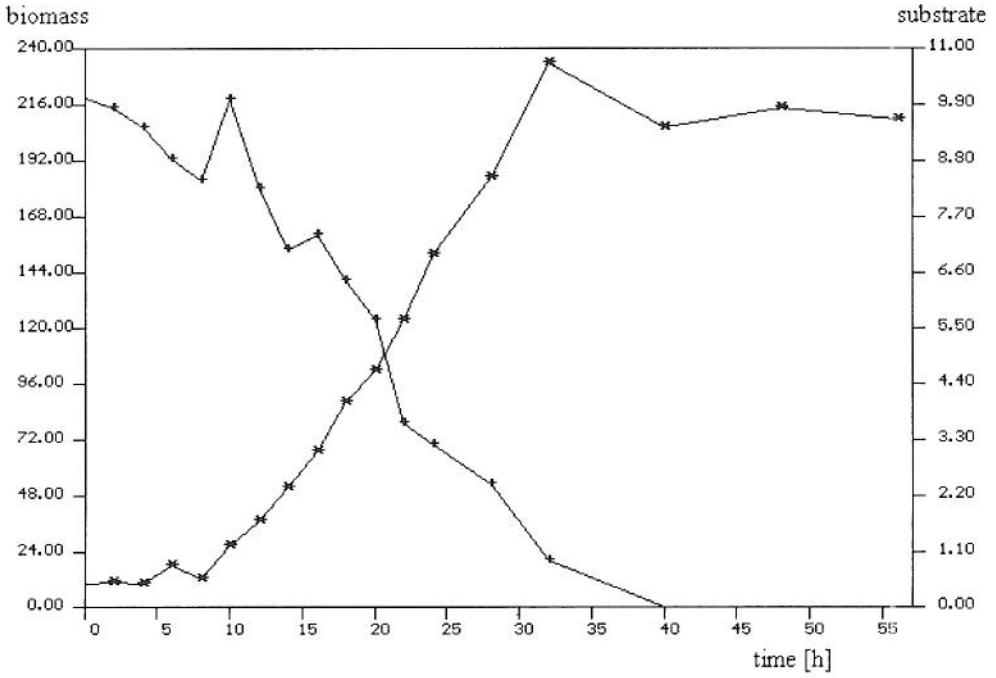

Fig. 3. Experimental data of yeast bioprocess: biomass (*) and substrate (+)
Slika 3. Eksperimentalni podaci u proizvodnji kvasca: biomasa (*) i supstrat (+)

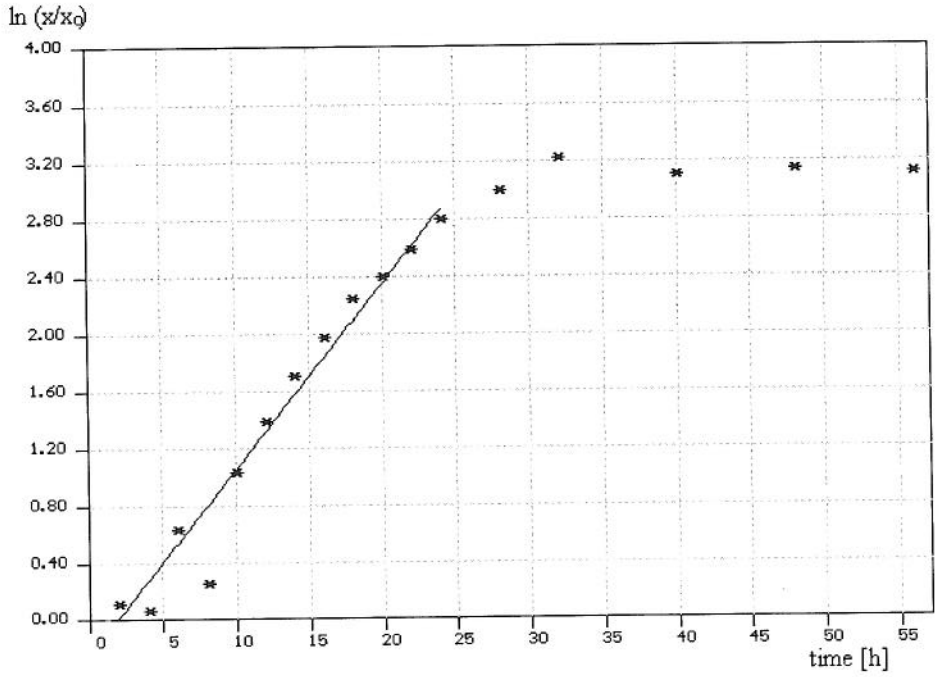

Fig. 4. Transformed biomass. Initial part is fitted by the straight line
Slika 4. Transformirani podaci za biomasu kvasca. Početni je dio prilagođen pravcu

and optimisation methods are included, computer can manage algebraic as well as differential equations. All results are presented graphically. Parameter values are displayed on the screen and written in a text file.

For model behaviour studies, *simulation* is an indispensable tool. It enables examination of possible process schemes and sensitivity of model parameters. The simulation option uses the same user-written models as for modelling. Only the values for all model parameters should be defined.

In data analysis many new *files with transformed data* are created. This can easily lead to the confusion with file names so special attention was given to this problem. The computer assigns unique extension to newly created files. If for example the file BIOMASS.SHI is smoothed, to the new file with smooth biomass data the name BIOMASS.T01 is assigned. Character T means that a transformation was made while 01 indicates it is the first file with biomass transformed data. Every extension is coupled with description window, where type of transformation is written.

b) Application example

Let us examine a simple case of yeast bioprocess. There are two series of data: biomass concentration and substrate concentration. Both data were obtained from off-line measurements (Fig. 3). The maximal specific

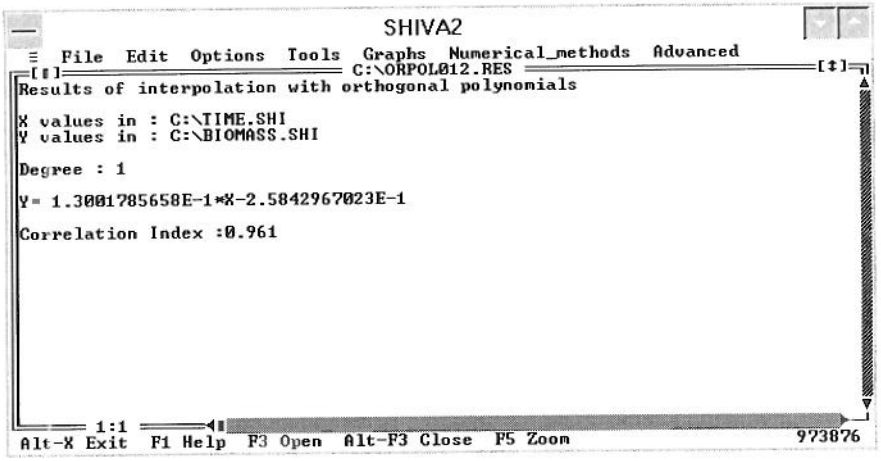

Fig. 5. Results of fitting. Information about type of fitting, used data files, mathematical equation and correlation index are given
Slika 5. Rezultati uklanjanja slučajnih pogrešaka. Dane su informacije o upotrijebljenim podacima, matematičkim jednadžbama i indeksu korelacije

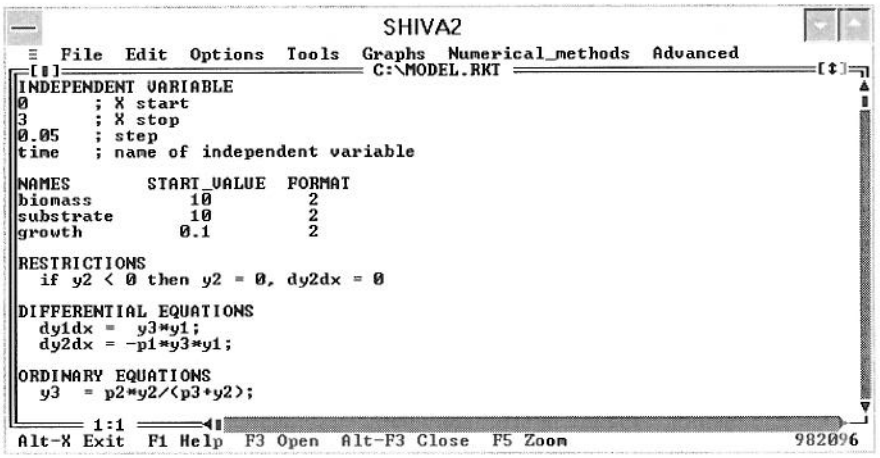

Fig. 6. A simple mathematical model using Monod kinetics for growth rate. Model parameters to be evaluated are p1, p2 and p3
Slika 6. Jednostavan matematički model u kojem se koristi Monodova kinetika za brzinu rasta. Parametri modela su p1, p2 i p3

growth rate is to be determined as well as the yield coefficient $Y_{X/S}$.

According to the simple mass balance for biomass, maximal specific growth rate can be calculated from equation:

$$\ln (x/x_0) = \mu t$$

By using calculation option a new data series containing $\ln(x/x_0)$ values is generated. Initial part of this series is fitted by a straight line (Fig. 4). Maximal specific growth rate is equal to slope of the straight line and can be read from results file (Fig. 5).

For yield determination data is fitted by a very simple model (Fig. 6). The unknown parameters are $Y_{X/S}$, K_S and μ_{max}. The graphical comparison between experimental data and calculated data is shown in Fig. 7 while the results of modelling are presented in Fig. 8.

In the process of modelling a little lower ($\mu_{max} = 0.11\ h^{-1}$ from modelling and $\mu_{max} = 0.13\ h^{-1}$ from fitting) result for maximal specific growth rate was achieved. From the model parameters also K_S and $Y_{X/S}$ were estimated.

All operations shown were performed in a couple of minutes, thus many different models and transformations can be verified in a short time.

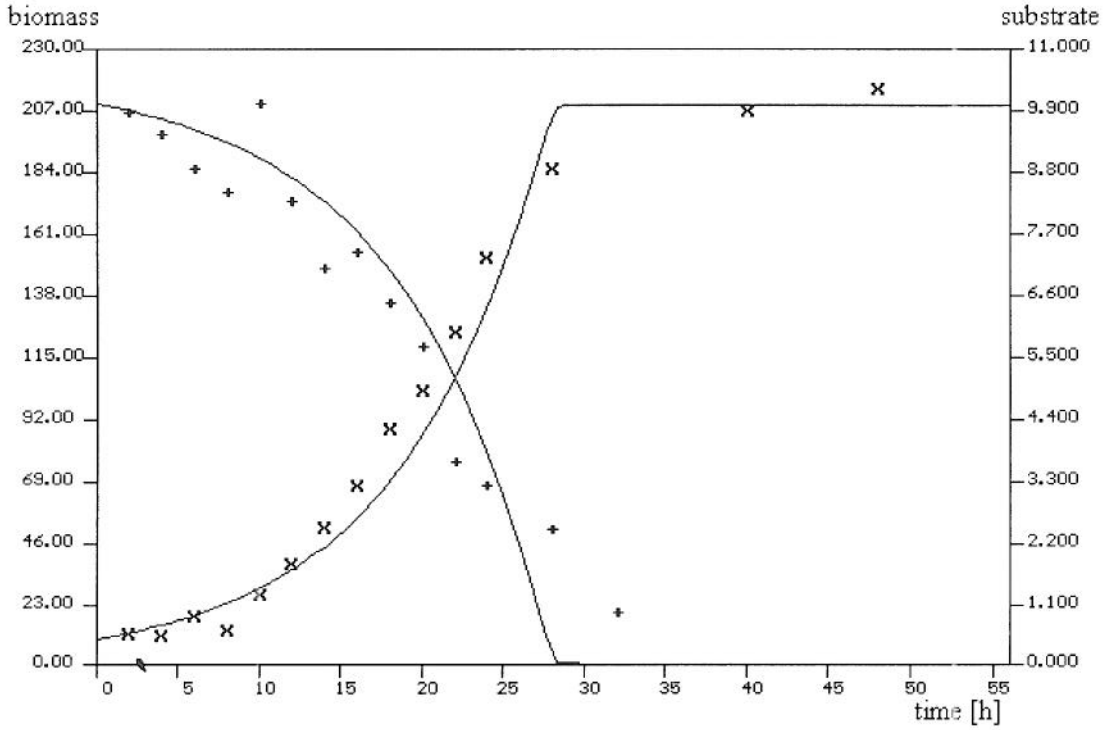

Fig. 7. Graphical comparison between experimental (markers) and calculated (solid curve) data
Slika 7. Usporedba eksperimentalnih (točke) i izračunanih (crta) podataka

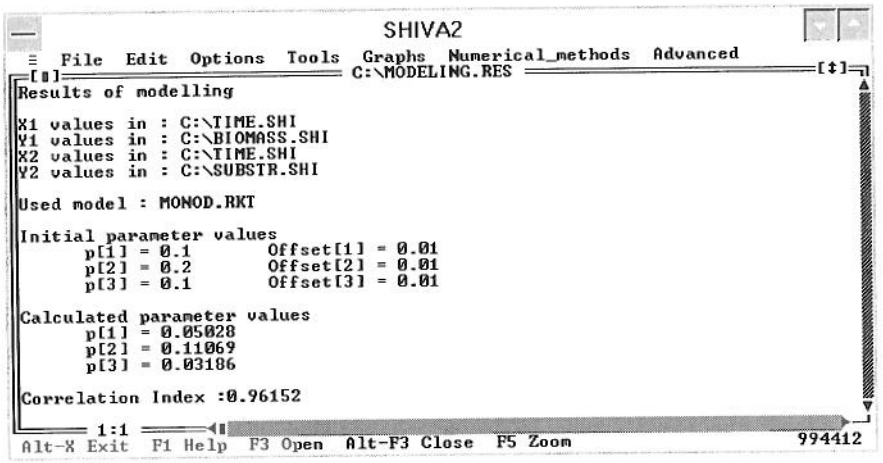

Fig. 8. Result of parameter evaluation. Type of operation, data files, used model, initial parameter values, calculated parameter values and correlation index are given

Slika 8. Rezultati evaluacije parametara. Dane su informacije o tipu proračuna, podacima, primijenjenom modelu, početnim vrijednostima parametara, izračunanim vrijednostima parametara i korelacijski indeks

Conclusions

Program package SHIVA is a user-friendly, modular structured program that can be tailored up to user's wishes. Different levels of its usage are possible according to user's needs. Easier level does not require any special knowledge about the process and can be handled also by persons not expert in the field of biotechnology. It includes data acquisition of standard predefined bioprocess variables (pH, pO_2, T, etc.), simple regulation modes (fixed set point, time profile) as well as noise removal with smoothing procedures. By more expert users new indirect data can be defined to be used as additional information about the bioprocess and for the control. A great help in bioprocess understanding and advanced control strategy are simulation and modelling options. Models can be very simple consisting of mathematical equations fitted to experimental data with no physical meaning, developed to be applied in event control. On the other hand also complex models, revealing internal structure of bioprocess and interaction between different compartments can be built. In this, process simulation is an indispensable tool for parameter sensitivity analysis. Software package SHIVA can be a helpful tool for simple as well as for advanced requirements in bioprocess monitoring and optimisation.

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