Cytotechnology **26:** 227–236, 1998.

Development of a versatile computer integrated control system for bioprocess controls

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Received 17 July 1997; accepted 15 December 1997

Key words: bioreactor, computer control, data acquisition, glucose control

Abstract

A general approach is described for the implementation of a networked multi-unit computer integrated control system. The use of data acquisition hardware and graphical programming tools alleviates tedious programming and maintains potency and flexibility. One application of the control system, the control of a mammalian cell perfusion culture based on a key nutrient glucose concentration, was demonstrated. The control system offers customized user interface for all process control parameters and allows the flexibility for continued improvement and implementation of new tailored functions. The temperature, pH, dissolved oxygen and glucose level were accurately controlled.

Introduction

As new continuous processes such as perfusion culture are gaining more popularity, the role of computer integrated control systems are no longer confined to data logging and integrated management over bioreactors. Special attention has been placed on meeting nutrient requirements of high cell density arose from continuous processes. As opposed to a conventional fixed setpoint control of physical parameters, such as temperature, pH, dissolved oxygen and agitation, the control of continuous processes involves the manipulation of one or more feed streams for a controlled delivery of nutrients and is more sophisticated and complex. Due to the complexity, a computer integrated control system becomes indispensable in such applications.

The availability of new sensors and the understanding of cellular metabolism make advanced control strategies possible. This in turn helps address the complexity and uncertainty of a cellular system for better process controls. For optimal media usage and product yield, a number of approaches have been proposed. Maintaining nutrients within desired ranges reduces by-product generation and increases nutrient utilization efficiency. Control strategies vary from regulating the level of a single key nutrient such as glucose (Konstantinov *et al.*, 1996) and a single feed stream of balanced nutrients to multiple feeding of glucose and glutamine. Dynamic feeding can also be implemented through indirect methods such as oxygen uptake rate (OUR) measurement (Kyung *et al.*, 1994) and computer-controlled optimal growth (Knorre *et al.*, 1991). Consequently, better product yield and culture physio-state were attained. In addition to feeding control, new control strategies can accurately control dissolved oxygen concentration through an adaptive predictive control (Diaz *et al.*, 1995). The application of estimation methods can assist the control, monitoring and understanding of bioprocesses. Two pronounced methods are various extended Kalman filters and artificial neural network. Both are capable of realistic estimation of a bioprocess system from incomplete and fault-prone raw process data (Karim and Rivera, 1992).

Given the complexity and variety of control strategies, an ideal computer integrated control system should be flexible enough to accommodate various controls. In addition, it should offer customized and optimized functions toward a particular control strategy and application. These desired features are not all present in today's commercial computer integrated

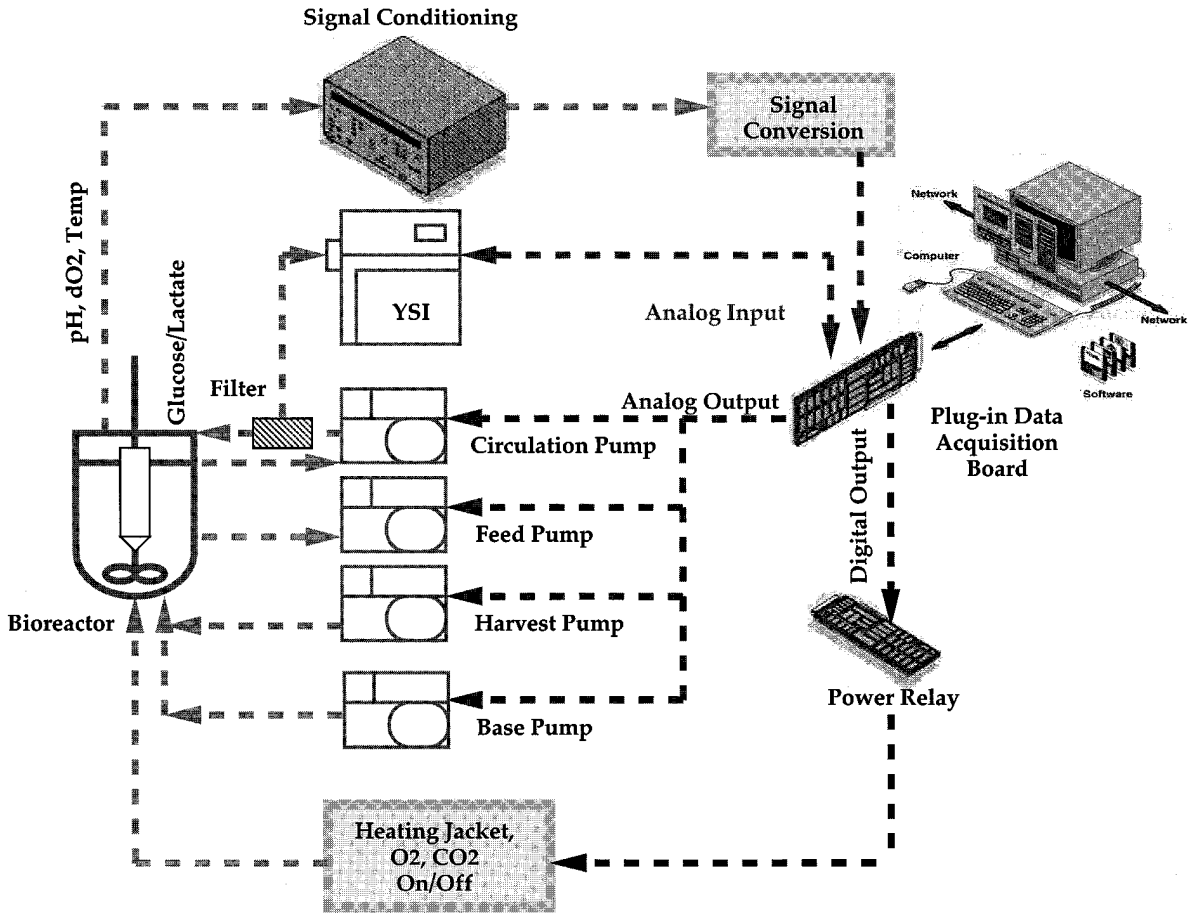

Figure 1. Perfusion bioreactor and computer control: Diagram of peripheral devices and signal flows.

control systems. An important factor to consider is value which act as a leverage between desired features and overall cost on control system acquisition and maintenance. A self-designed computer integrated control system can offer all the flexibility, customization, optimization and cost efficiency. Partially self-designed control systems have been proven to meet the requirements of particular applications and control strategies (Kyung *et al.*, 1994; Diaz *et al.*, 1995; Konstantinov, 1996; Konstantinov *et al.*, 1996). Full potentials can be realized through a completely self-designed control system (Buntemeyer *et al.*, 1994). The wide availability of data acquisition hardware and graphical programming tools makes the design of a control system easy. For a computational intensive algorithm, a real time linkage can be established between a control system and an external algorithm.

We present the development of a computer integrated control system and one application of the system to mammalian cell perfusion culture control based on glucose level. The control system employs data acquisition hardware and a graphical programming tool and was developed within a month period of time. Special attentions have been placed on optimized and customized functions and user interface, flexibility and cost efficiency. The potentials of a self-designed control system will be illustrated in details. CHO cell culture data will also be presented in conjunction with the performance of the control system.

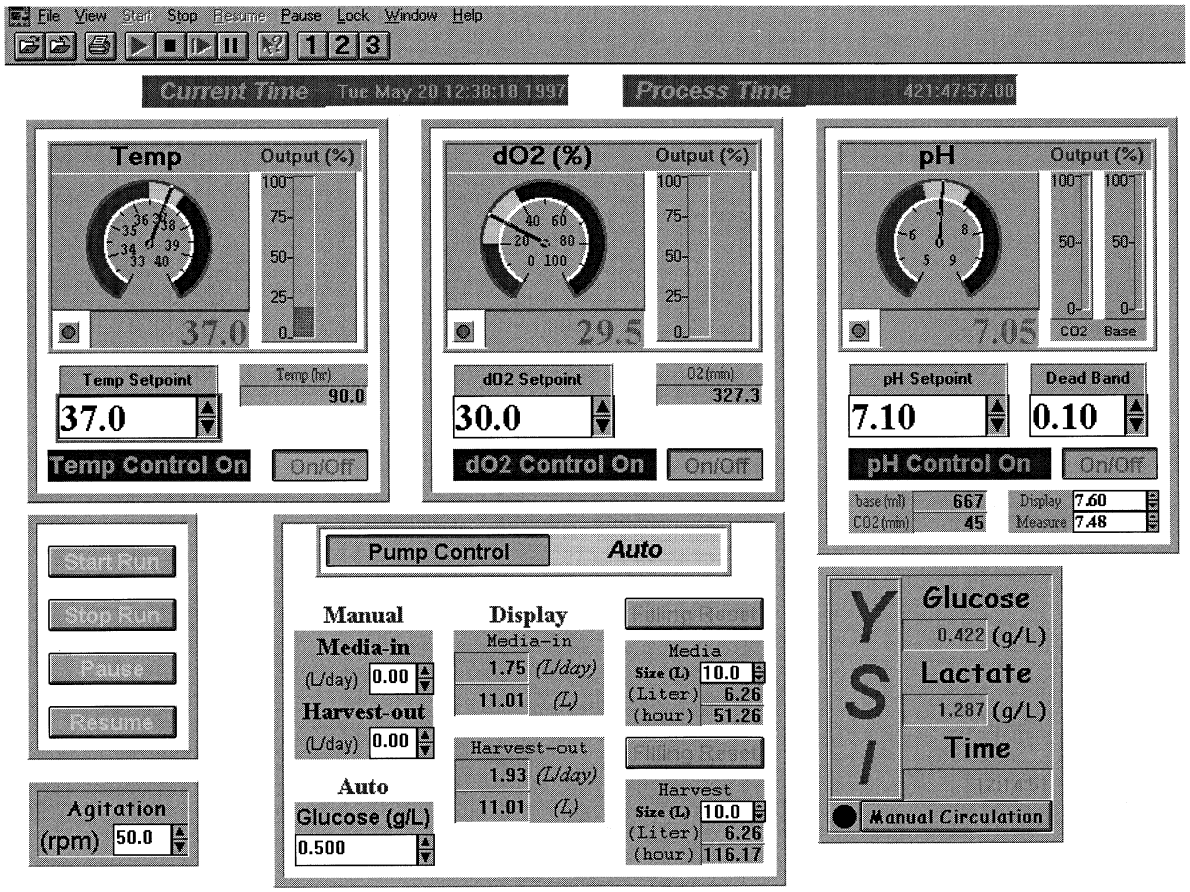

Figure 2. Screen I: Process control display and input. See detail description in Results and Discussion section.

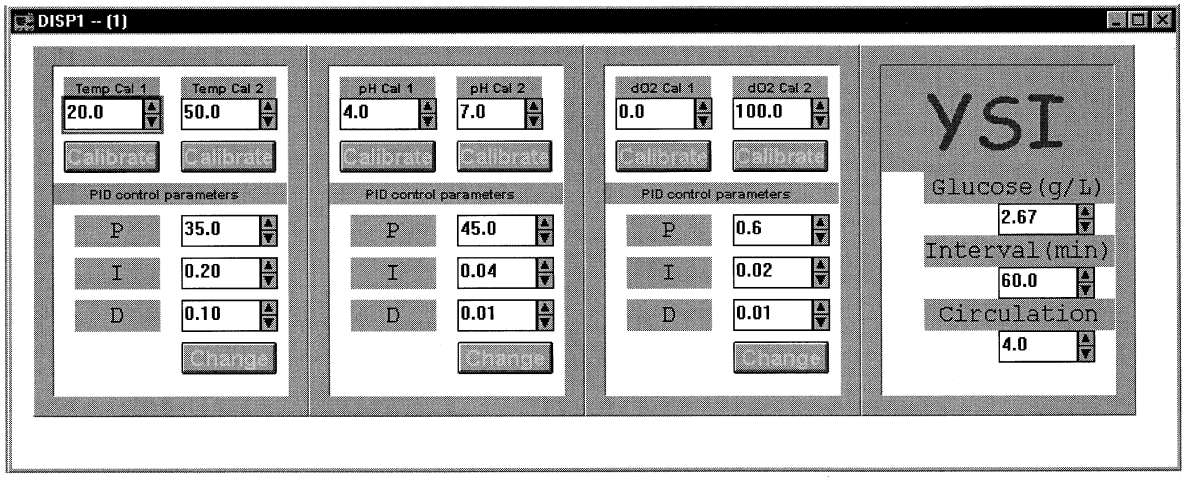

Figure 3. Screen II: Calibration and setting. See Results and Discussion section.

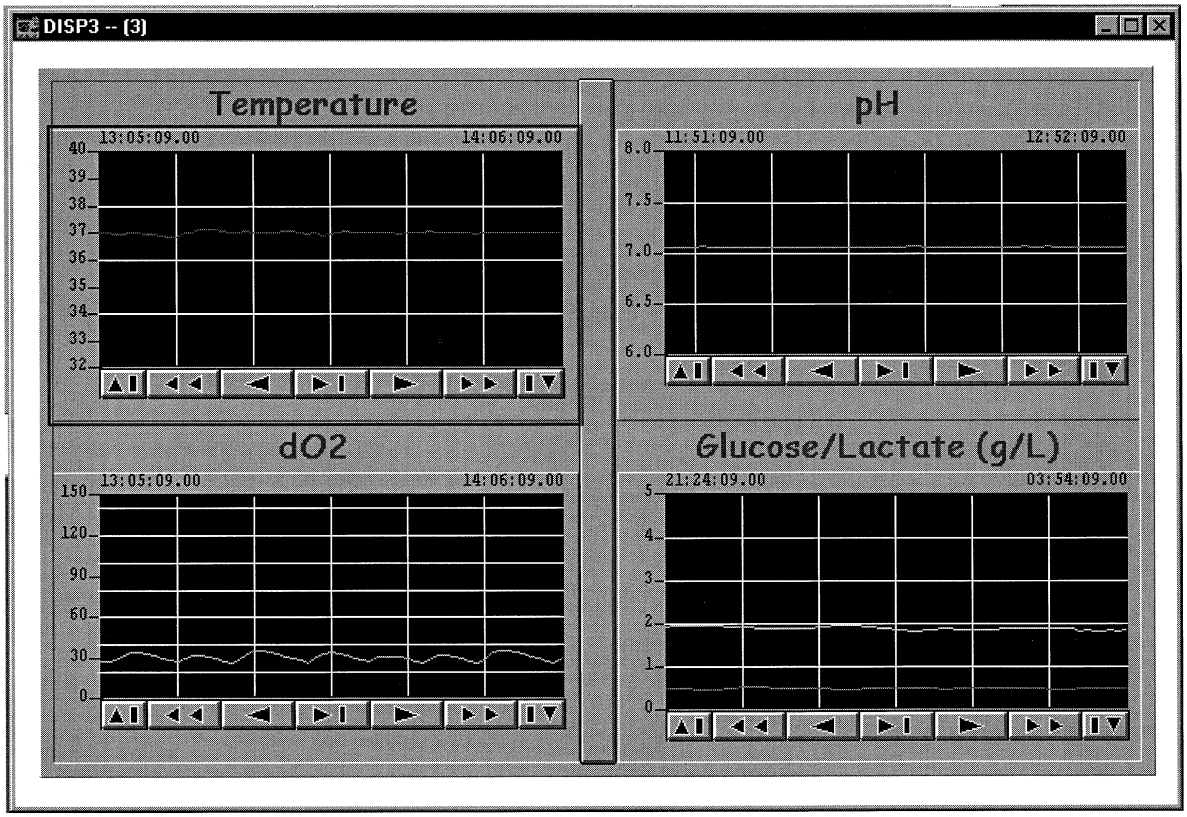

Figure 4. Screen III: Historical data displays for temperature, pH, DO and glucose/lactate.

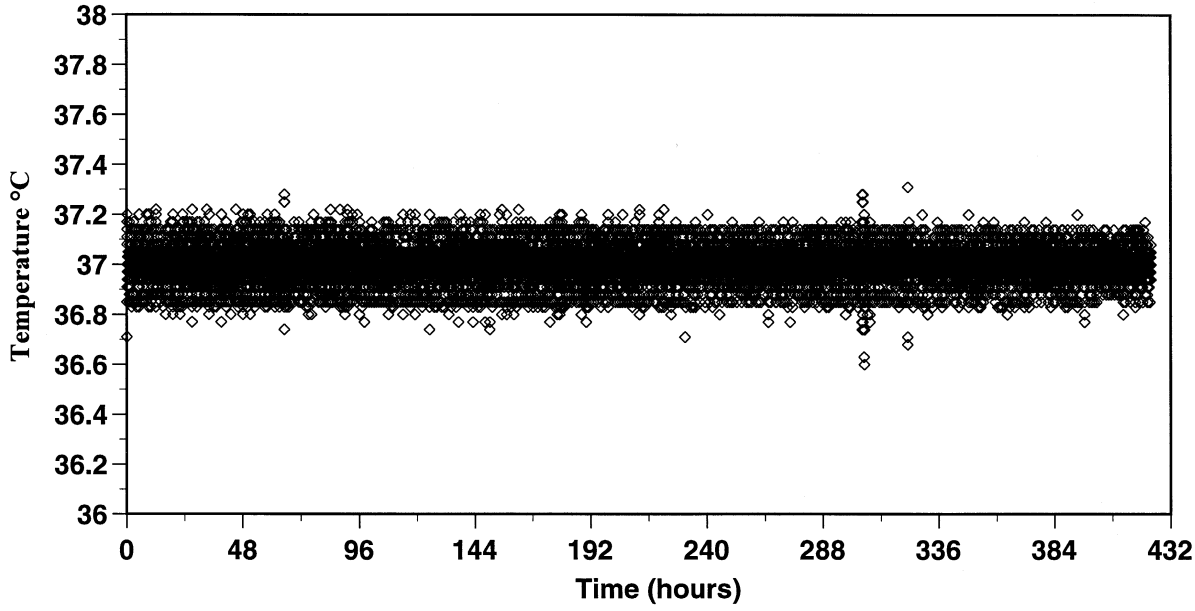

Figure 5. Temperature control profile in an initial test run with a setpoint of 37 °C.

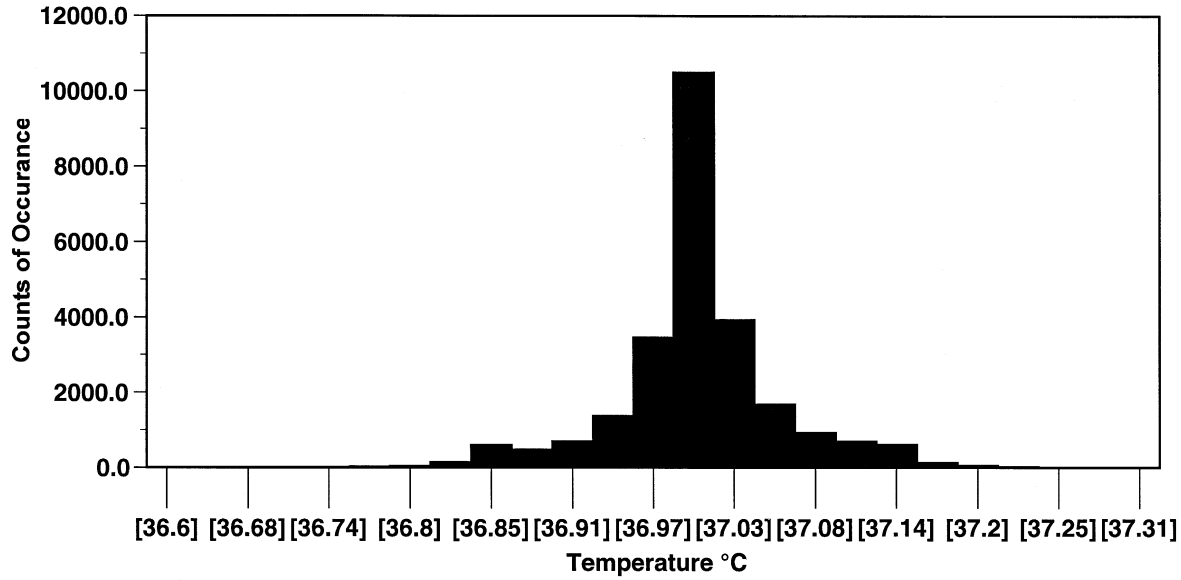

Figure 6. Temperature distribution and histogram from the initial test run.

Materials and methods

Bioreactor and control

The diagram of a perfusion bioreactor and its control setup is illustrated in Fig. 1. An Applikon 3L bioreactor (Applikon Inc., Foster City, CA) with a working volume of 2 L was equipped with a 75 μm pore size spin filter. Signals from temperature, pH and dissolved oxygen (DO) sensors were conditioned by separate modified temperature, pH and DO controllers (B&C Electronics 7615, distributed by Applikon Inc., Foster City, CA). Conditioned process signals were converted and acquired by a PCL-818L multi-purpose data acquisition card (American Advantech Corp., Sunnyvale, CA) installed in a Dell 486 computer. A strategy written in version 2.12 Genie data acquisition and control software (American Advantech Corp., Sunnyvale, CA) was used for data acquisition, display and control. Discrete proportional, integral and derivative (PID) control algorithms use feedback process signals and output digital and analog signals to various devices. The digital signals were sent to a PCLD-885 power relay output board (American Advantech Corp., Sunnyvale, CA) to control open and close of a heating jacket for temperature control and solenoid valves for oxygen and carbon dioxide in a 30 second cycle time. A PCL-727 digital to analog output card (American Advantech Corp., Sunnyvale, CA) outputs analog signals to control the speed of base, circulation, media and harvest pumps (101 U/R, Watson Marlow, Falmouth, England). Cell culture was controlled at setpoints of 37 °C, pH 7.10 +/- 0.05, 30% dissolved oxygen, and 50 rpm.

Glucose and lactate concentrations in the 3L bioreactor were monitored on-line hourly by YSI 2700 Select Biochemistry Analyzer equipped with a monitoring and control accessory (YSI Inc., Yellow Springs, Ohio). A circulation pump to circulate spent medium within the spinfilter was activated 4 minutes prior to YSI sampling time and continued for 2 minutes after sampling. Samples drawn through an A-SEP crossfiltration unit (Applikon Inc., Foster City, CA) were analyzed by YSI 2700. Glucose and lactate concentrations were sent to the data acquisition card to control the glucose level inside the bioreactor. Since a standard PID control strategy does not apply in this case where samples are taken hourly, a consumption-based algorithm was employed for the glucose control. The algorithm calculates the flow rate needed to bring glucose to a setpoint on basis of feed stream glucose level and to compensate the rates of consumption, carry-out, and changes in consumption during the previous three sample intervals. It outputs analog signals through the PCL-727 digital to analog output card to control the speed of media and harvest pumps (101 U/R, Watson Marlow, Falmouth, England). All pumps were calibrated against 0-5 V process signals and calibration data were stored in the control strategy.

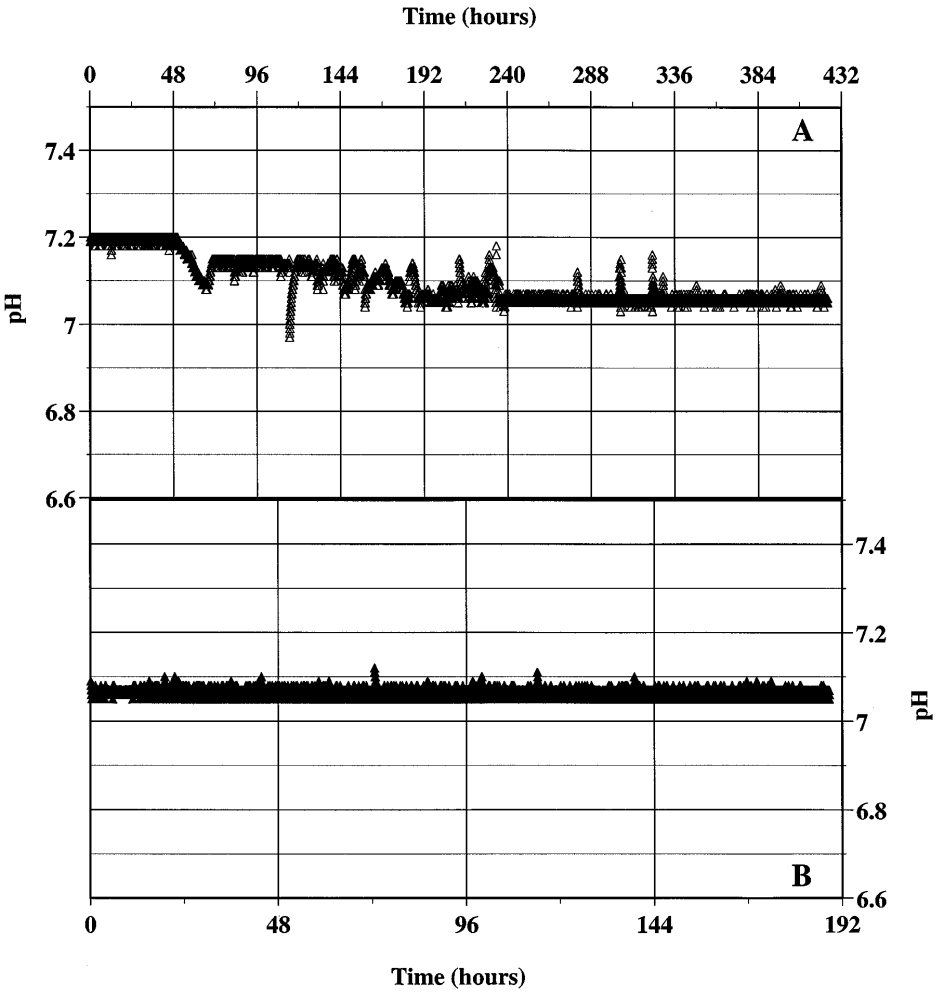

Figure 7. pH control profile with a setpoint of 7.10±0.05. A: Initial test run; B: Second run after PID parameter adjustment.

The control program acquires data and controls the process every second. Process data were logged to a file every minute. Daily bioreactor samples were centrifuged and stored in a –20 °C freezer for later product concentration analysis.

Cell line, media and cell cultures

An anchorage-dependent CHO clone, CHO.M-CIF.46, expressing monocyte-colony inhibition factor (M-CIF) was used in this computer-controlled perfusion culture. The CHO.M-CIF.46 clone was amplified to 100 μM methotrexate (MTX). Routine T-flask passage was carried out every 3–5 days at 1:2 – 1:4 split ratio in MEM alpha$^-$ medium supplemented with 5% dialyzed fetal bovine serum (DiFBS) and 100 μM MTX. Seed cells were maintained in spinners under MTX selection (50 μM) using a completely modified MEM (CM-MEM) medium containing 5% DiFBS and with a microcarrier load of 2.5 g/L (Cytodex I, Pharmacia, Uppsala, Sweden). Bioreactor seed culture was prepared in a modified HGS-CHO-3 medium without any MTX, and the same medium was used in the bioreactor.

Results and discussion

Customized multi-screen displays

The control program we developed interacts with users through three main screens. It was designed to perform flexible standard process controls such as temperature, pH and dissolved oxygen as well as more advanced

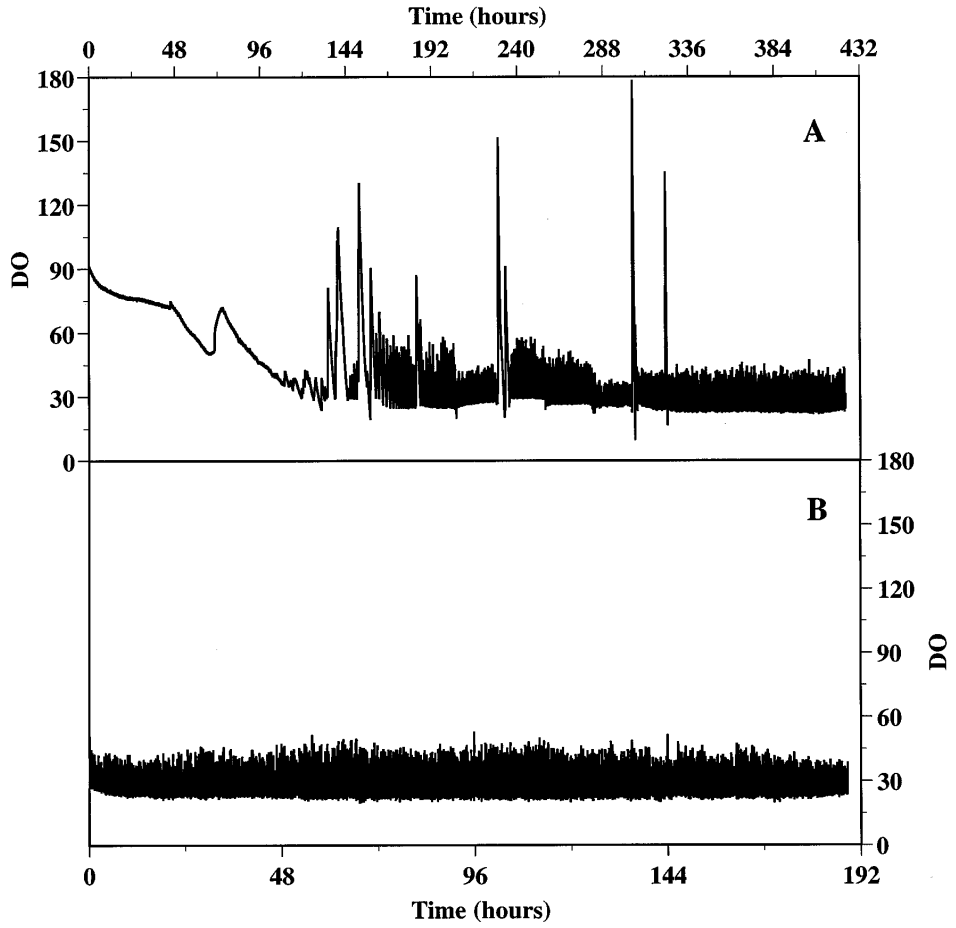

Figure 8. DO control profile with a setpoint of 30% . A: Initial test run; B: Second run after PID parameter adjustment.

controls and displays. The user interface was designed to facilitate the reading of process parameters and control inputs. Critical process parameters and control settings can easily be visualized and supplied. Screen one (Fig. 2) displays all on-line process variables and accepts control settings. On the top of the screen, current time and process time are displayed. The temperature, pH and dissolved oxygen are shown in an easy-to-read format. Large anameters clearly tell whether a process is within controlled ranges or not by looking at which colored regions the indicator arms point to. The temperature, DO and pH setpoints can be changed anytime during the run. On a click of an 'on/off' button, an individual control starts. Display messages and indicators change to 'Control On' in a distinguishable color. The bar display next to each anameter shows the percentage of maximum output used for each process parameter control. Dosage information for the amount of time for heating, carbon dioxide, oxygen and the volume of base added are also displayed. Since pH calibration would shift after autoclave, a measurement needs to be input into the control program to correct the shift.

Pump control can be toggled between auto and manual modes. In the manual mode, pump rates are controlled by screen inputs. In the auto mode, a glucose setpoint determines the media and harvest pump rates. In either mode, the pump rates and accumulative volumes are displayed. The volumes in media and harvest containers with the time left to switch the containers are also displayed and can be reset at the time of switching the media or harvest containers.

Glucose and lactate concentrations with the time of the last new set of data are shown in a YSI block. The indicator underneath a YSI logo was designed to display the status of a circulation pump. Although the

circulation pump is automatically controlled, it still can be invoked manually.

The calibration and process control parameters can be changed at any time during a run through screen two (Fig. 3), although they usually are set at the beginning of the run. These parameters are temperature, DO and pH calibrations, and their PID control parameters. Calibration data as well as PID and control settings are all saved to a file so that the control program can be resumed in case of power or computer failure. The glucose level in a feed stream, the time interval of YSI sampling and the time to start circulation before next YSI sampling are also input from this screen.

Screen three (Fig. 4) was designed to show the historical data for temperature, pH, DO, glucose and lactate levels in four windows. The glucose and lactate window displays hourly data in a 60 hour span. Other historical data windows display minutely on-line variables for an hour. The capability of the historical data display to display key on-line process values during a complete run facilitates the process evaluation and fault detection without leaving the control program.

Additional features

These three display screens are designed to facilitate the process control. In addition to the on-line historical displays, all process data are saved every minute in a tab-delimited format ready to be opened by any spreadsheet applications for later data analysis and presentations. The control system is a multi-user system and offers 256-level access privileges for all input blocks and its applications for administrative purpose. Worth mentioning is that the dynamic controls of all process control settings such as setpoint, PID control parameters can be implemented. The control system can be remote-controlled and monitored at other locations. Imbedded dynamic data exchange (DDE) functions throughout the program can exchange data with many other applications in real time, which makes it possible to implement functions such as complicated calculations, model estimation and prediction, and HPLC linkage for on-line control. The control system also features alarm functions for any process values. The alarm functions can be so configured to alert over network and make a voice call. In addition, several control programs can run simultaneously for different bioreactors under different control strategies. The current hardware and software setup is implemented for eight bioreactor controls and a number of other bioreactors through RS-232/RS-422 serial control over programmable logic controllers (PLC). At the completion of a run, all data can be backed up to a central process database on network.

Value and cost efficiency

This control system is easy to implement and tailor-designed for a particular application. It costs a fraction of what would cost for a commercial version and offers more flexibility and customization. The control system hardware and software only costed $2000 including a computer for eight bioreactors and a number of other bioreactors, and was developed within a month period of time. Cost efficiency also represents in future maintenance because we know every detail of the control system. Additionally, as needs arise, an automated maintenance and trouble-shooting routine can be implemented in the control system for better process reliability and performance.

Performance of the control program in a CHO perfusion culture

The computer integrated control system was developed for and evaluated on a CHO cell perfusion culture using a glucose setpoint for feed control. Process control parameters were controlled within desired ranges. For a period of 424 hours, approximately 25,000 data points were collected.

The control of temperature in a bioreactor is a well established technology and a precision of $\pm$ 0.5 °C or better is routinely achieved. Typical temperature sensors include the mercury contact thermometers, electric-resistance thermometers and thermistors. Electric-resistance thermometers such as the Pt100 offer good stability, sensitivity, accuracy and linearity and are frequently used temperature sensors. The temperature control in an animal cell bioreactor is generally less complicated than in a microbial fermenter due to lower metabolic activity. Our temperature control loop employed a Pt100 temperature sensor and a standard PID control through a 30 second cycle time to control the heating jacket on and off. Although the control method was not as precise as systems with a cascade-controlled water jacket, the control system controlled temperature to 37.00 ± 0.05 (mean $\pm$ standard deviation). The temperature profile from a run is shown in Fig. 5. Most data points were within 37.1 and 36.9 °C and center-weighed at 37.0 °C. Only a few data points fell in 37.1 – 37.2 °C and 36.8 – 36.9 °C and fewer above 37.2 °C and below 36.8°C, respectively. The accuracy of the temperature control can

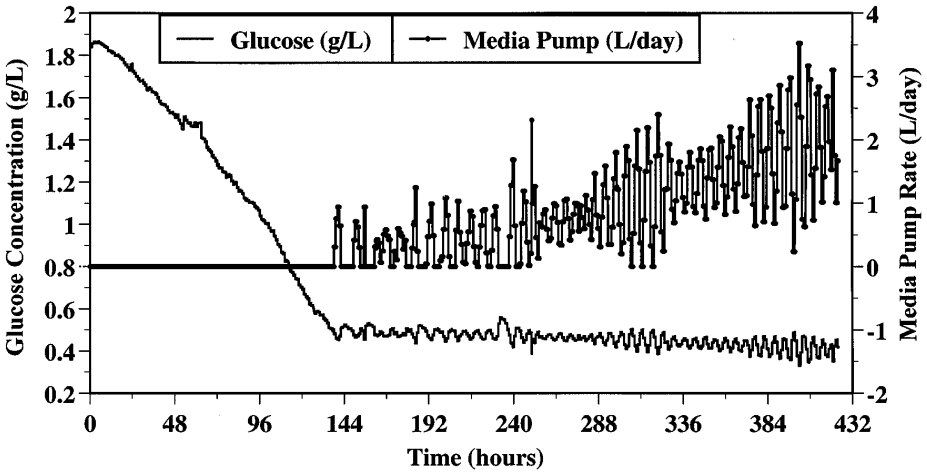

Figure 9. Glucose regulation by continuous pump rate adjustment in the initial run.

be evaluated from a distribution histogram (Fig. 6). Majority of the data points occurred at 37.0 °C. Perturbances to the control system were YSI circulation and media pump rate changes. The 'on/off' control of the heating jacket could be upgraded to a continuous power regulator for further improved control precision.

The control of pH is critical in maintaining a favorable environment for cell culture. The lactate generation by glucose metabolism will depress pH. In this study, the pH control was achieved by adding alkali solution and sparging CO_2 into the bioreactor. Initially, pH was controlled by CO_2 sparging to 7.2. (Fig. 7A). pH control and deadband were set at 7.1 and 0.2, respectively. After 60 hours, the deadband was set to 0.1. Almost all process data points were within 7.05 and 7.15. For the whole 424 hour run period, pH was at 7.10±0.05. Some dramatic pH changes during the run were caused by the PID parameter adjustments and microcarrier additions. pH changes in the CHO cell culture were gradual and slow. A simple PID control was found to be sufficient to maintain the pH in the bioreactor. pH control was fine-tuned in the second run and pH was very well controlled at 7.064±0.008 with a setpoint of 7.1 and a deadband of 0.1, as shown in Fig. 7B.

Dissolved oxygen levels are typically monitored and regulated by polarographic or galvanic sensors and PID control. The complexity of DO controls varies enormously from simple on/off valves to highly automated gas-mixing systems. Recently some bubble-free aeration systems have been described. Our bioreactor system uses an on/off solenoid valve and a microsparger. Although oxygen requirements of mammalian cells are low, a proper system design does require the careful selection of PID parameters to compensate slow oxygen transfer and slow sensor response. The DO profile in the CHO culture shown in Fig. 8A shows DO was initially high. As DO passed below the setpoint, O_2 sparging was activated. Overshooting was occasionally observed. The DO rise at 75 hours was a result of testing the control system. Since a single gas line was used for both CO_2 and O_2, many DO overshoots occurred between 120 – 250 hours were the result of CO_2 sparging in pH control, which consequently pushed the remaining O_2 in the tubing into the bioreactor. The two large overshoots at around 320 hours were caused by exhaust filter clogging. However, by fine-tuning the PID parameters, DO was controlled at 43.82±18.50 with a setpoint at 30%. By optimizing PID parameters and shortening gas delivery tubings, a far better DO control was achieved in the second run (shown in Fig. 8B). DO was controlled at 31.30±6.06% with the same setpoint. Further improvement in DO control can be achieved through a predictive control (Diaz *et al.*, 1995).

Fig. 9 shows the profile of glucose concentration. As cellular uptake of glucose occurred, glucose steadily decreased. The glucose control started at around 144 hour. Since then, glucose concentration was controlled well at 0.483±0.02 g/L with the setpoint of 0.5 g/L. The glucose readings by the YSI 2700 were very reliable. The consumption and compensation based feed control adequately controlled the glucose level. Since such a control heavily relies on the accuracy of media glucose level, bioreactor vol-

ume, and pump rates and the uniformity of mixing, the addition of an error correction term into the pump rate control could be a further improvement to the glucose control. The media pump was adjusted continuously on an hourly basis and the media pump rate data are also shown in Fig. 9. These data are valuable to be combined with off-line cell density measurements to calculate specific nutrient consumption rates throughout the run. In such a self-designed control system, the consumption rates could easily be implemented for on-screen displays.

Conclusions

A flexible computer integrated control system can be readily fabricated from data acquisition hardware and graphical programming tools. The demonstration of one application of the control system to perfusion culture control based on glucose concentration illustrated the potential of such a control system for multipurpose process controls and unlimited customization and optimization. Based on the control system, a number of control processes can be constructed and run simultaneously.

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