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A complete computer monitoring and control system using commercially available, configurable software for laboratory and pilot plant *Escherichia coli* fermentations

RS Blackmore, JS Blome and JO Neway

Somatogen Inc, 2545 Central Ave, Boulder, CO 80301, USA

The advent of inexpensive computers and associated control and data acquisition software makes possible the development of sophisticated, configurable, integrated monitoring and control systems for small-scale laboratory and pilot-scale fermentors at low cost. We describe here the implementation of such a system, the interfacing of off-line instruments to enhance real time data analysis, low level process control and several substrate feeding protocols.

Keywords: fermentation; fermentation monitoring; fermentation control; fermentation software; *Escherichia coli*

Introduction

Accurate monitoring and control of the fermentation process is critical for understanding and controlling the reproducibility of fermentation processes [6,29]. This goal is most easily met by coupling an appropriate computer system to the fermentors [8]. Much has been written concerning the interfacing of computers to monitor and control fermentors [2], however this can involve expensive computer systems and considerable software development. This has typically restricted such systems to large fermentation facilities [2,9,25], but the availability of inexpensive personal computers during the 1980s has opened up a new avenue, as applications were developed on these computers to implement fermentation monitoring and control [28]. However, the cost of developing such software for more than one or two fermentors can be considerable, making such an approach impractical for large fermentation development labs or pilot plants containing many fermentors. However, as the personal computer has become more powerful and much cheaper, the available software has become increasingly sophisticated.

The last few years have seen considerable progress in the development of commercial configurable monitoring and control software packages for personal computers. As these packages are intended for generic industrial monitoring and control applications which occur over the same time frame as the fermentation process, they can be usefully applied to the fermentation process. The advantage of such a system is that much of the difficult and specialized programming, for example implementation of pre-emptive multi-tasking, necessary for simultaneous process monitoring and control of multiple fermentors, has already been done. Development efforts can instead be focused on the unique requirements of the particular fermentation process and the instruments, measurements and control systems, it uniquely may require. Many of the adaptive growth models and other control strategies previously described in the literature [1,11,16,17,19,23] can then be incorporated into the control strategy without the necessity of having to write, or re-write, source code.

This combination of sophisticated, configurable monitoring and control software and increasingly powerful personal computers, has made possible the construction of an integrated fermentation computer monitoring and control system, previously possible only with costly distributed control systems which are not practicable for smaller scale fermentation operations in either research or production environments. Although many fermentor manufacturers provide personal computer software to monitor and control fermentors, this software often falls short in that it does not allow the integration of fermentors from other manufacturers into a single system. More importantly, it usually does not allow the addition of other analytical instruments to provide an integrated, real time picture of the fermentation process or provide sufficient useful feedback to control the process.

In the work presented here, we describe the construction of an integrated monitoring and control system based on inexpensive personal computers and commercially available configurable software. The system allows simultaneous process monitoring and control of air, oxygen and glucose feed for six 15-L fermentors, one 20-L, one 100-L and one 600-L fermentor, all performed in fed-batch mode for the production of recombinant proteins in *E. coli*. We describe the performance of the system, integration of some of the instruments and devices commonly used in fermentation experiments and discuss some of the possibilities for this system.

Materials and methods

The configurable software used in this work was the 'Genesis' system, provided by Iconics Inc of Foxborough, MA, USA. The Genesis system software communicates to digital devices (ie analog to digital converters or on-line analytical

Correspondence: JO Neway, Somatogen Inc, 2545 Central Ave, Boulder, CO 80301, USA
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instruments) via software drivers. An individual driver is required for each digital hardware type. The Genesis software was operated on three IBM PC compatible computers with an 80486 central processing unit (CPU) running at 33 or 66 MHz with 8 Mbytes RAM each. Communication between these units was achieved using the Iconics 'GenNet' computer networking option.

The digital computer interface chosen for communication with the analog fermentor instrumentation was the GE Genius distributed I/O system (GE Fanuc Automation Inc, Charlottesville, VA, USA). This system is a Local Area Network (LAN) with the individual LAN nodes performing Analog to Digital (A to D), Digital to Analog (D to A) and relay switching functions. Communication to the computer is entirely digital. This gives high noise immunity as the LAN blocks can be located very close to the instrument of interest. The GE Genius blocks were connected together in the LAN and to the Genesis computer system using shielded twisted pair cable according to the manufacturer's instructions. The LAN blocks were configured according to the manufacturer's instructions. LAN communication was set up at the manufacturer's standard rate of 153.6 Kbaud. Communication between all digital equipment and the computer software was accomplished using individual software drivers for each type of device. These drivers were either obtained from Iconics or written in-house. For the 15-L and 20-L fermentors, additions of glucose and base were measured using Mettler PJ6 and BB3 balances respectively (Mettler PM30 balances were used for the 100-L fermentor), with RS-232 standard connections to the computer system via two Digichannel COM/Xi Intelligent Asynchronous Communications Boards (Digiboard, Eden Prairie, MN, USA). Additions of glucose and base to the 600-L fermentor were measured using Toledo Scale mode 8510 indicator and 9325 analog output to A to D blocks on the GE Genius Lan. These additions to the 600-L fermentor were controlled by opening or closing pneumatic valves. Glucose and base additions to all other fermentors were made using either Harvard Apparatus Model 1203 or 66 peristaltic pumps.

Unless otherwise stated, the dissolved oxygen (DO) level was controlled by a local fermentor controller increasing the agitation speed of the fermentor. Oxygen and air mass flow were controlled using Sierra Instruments (Sierra Instruments, Monterey, CA, USA) series 840 flow controllers under the direct control of the computer system. Total mass flow of air and oxygen was held constant at 1 vessel volume per minute (vvm). Unless otherwise stated, pH was controlled by a local fermentor controller opening a valve or switching on a pump to add 30% (v/v) ammonium hydroxide which provided both pH control and a nitrogen supply.

Fermentations were operated at 15 L, 20 L, 100 L (LSL Biolafitte Inc, Princeton, NJ, USA) or 600 L (Abec Inc, Allentown, PA, USA) scale using Somatogen standard operating procedures with defined media and strains of *E. coli* K12 developed by Somatogen for hemoglobin expression. Mass spectrometer measurements of fermentor exit gas streams were made using a Perkin Elmer model MGA1200 mass spectrometer as follows: A 3.2 mm outside diameter (o.d), 1 mm inside diameter (i.d.) stainless steel tube was connected to the fermentor exit gas stream after the exit filter but before the back pressure regulator. The gas sample was transferred as a consequence of the pressure difference between the fermentor and atmosphere through this tube to a valve manifold near to the mass spectrometer. Each gas stream was connected to a 3-way valve which was normally open to the atmosphere to allow continuous purging of the gas transfer line at a flow rate of approximately 0.5 L per min. The computer system controlled the selection of each valve (and therefore fermentor), to switch each gas stream to the sample inlet of the mass spectrometer. The computer allowed an 8-s purge time followed by a 2-s measurement time. With eight fermentor gas streams to be sampled per mass spectrometer unit, the mass spectrometer measurements for any given fermentor could be updated every 80 s. Glucose measurements were made by taking a sample of the fermentation broth, centrifuging a 1-ml sample to remove the bacterial cells and testing the supernatant broth using a YSI Model 2700 Select Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). Cell mass was monitored using the OD measured at 600 nm by first diluting a fresh sample of bacterial cells taken from the fermentor with an 8 g L^{-1} NaCl solution to a final OD less than 0.6 OD at 600 nm. The OD was measured using a Novaspec II spectrophotometer (Pharmacia LKB Biochrom Ltd, Cambridge, UK).

Results

Fermentor and computer layout

The interconnection of the computers and the fermentors is shown in Figure 1. Each fermentor had a pump or valve to add glucose and a pump or valve for addition of ammonium hydroxide. The glucose and ammonium hydroxide feed bottles were mounted on load cells so that the total quantity of feed addition could be recorded. In the case of the 15-L and 100-L fermentors, these were connected to the computer system by individual RS-232 communication lines. In the case of the 600-L fermentor, analog signals from glucose, ammonia and the vessel weight load cells were converted to digital values and passed to the computer system by the GE Genius LAN. In addition, each fermentor had oxygen and air mass flow controllers with the mass flow setpoints sent by the computer system via the GE Genius LAN. Analog signals from the fermentor amplifiers (ie dissolved oxygen, pH, temperature and agitation) and the mass flow controllers were digitized using GE Genius analog I/O blocks. Data from the GE blocks were typically updated to the computer system once every second. Data were read from the RS-232 devices (Mettler Balances) every 30 s as the signals from these do not change rapidly.

DO, pH and temperature control were usually carried out using the local fermentor controllers, with the computer system in a supervisory mode. The alarm function in the software was configured to alert the fermentation operator if the pH, DO or temperature varied outside of specified ranges, indicating a failure in the control system (eg a faulty or jammed peristaltic pump, loss of oxygen pressure, etc).

The Genesis software can be used to control pH and DO directly using configurable proportional, integral and derivative (PID) control loops and examples of the control

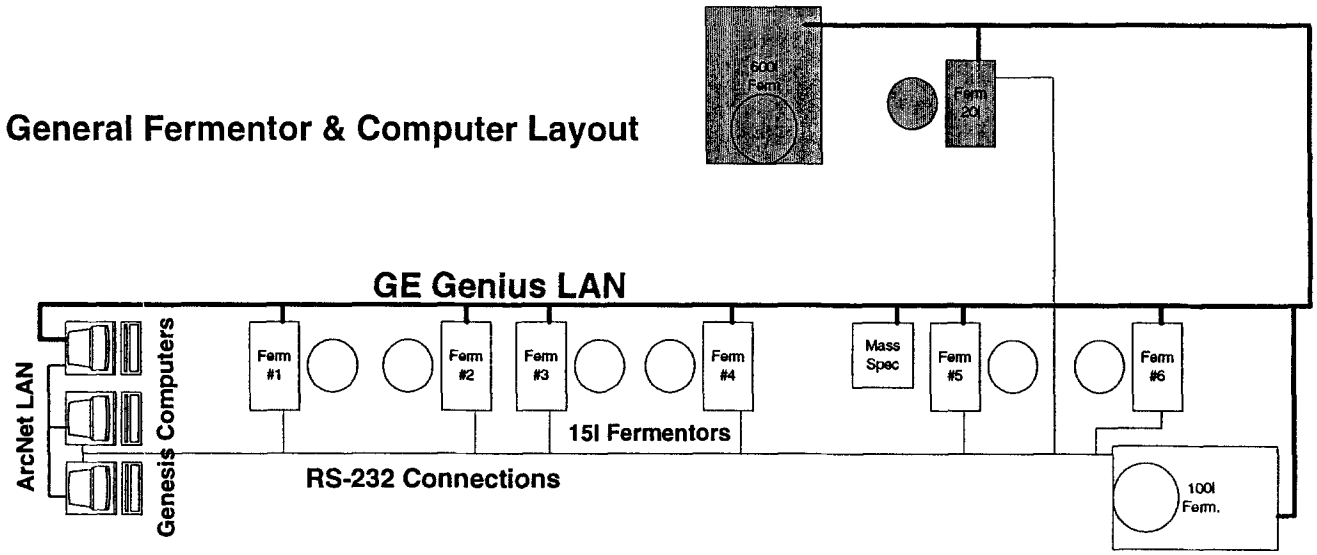

Figure 1 General fermentor and computer layout. The three networked Genesis computers are shown linked to the six 15-L; one 20-L; one 100-L and one 600-L fermentor. The GE Genius LAN (thick line) connects the A to D, D to A and relay blocks mounted next to the fermentor to the computer. The RS-232 connections (thin line) take digital measurements from the balances to the computers and return instructions to the glucose pumps. Each RS-232 device has a separate computer line.

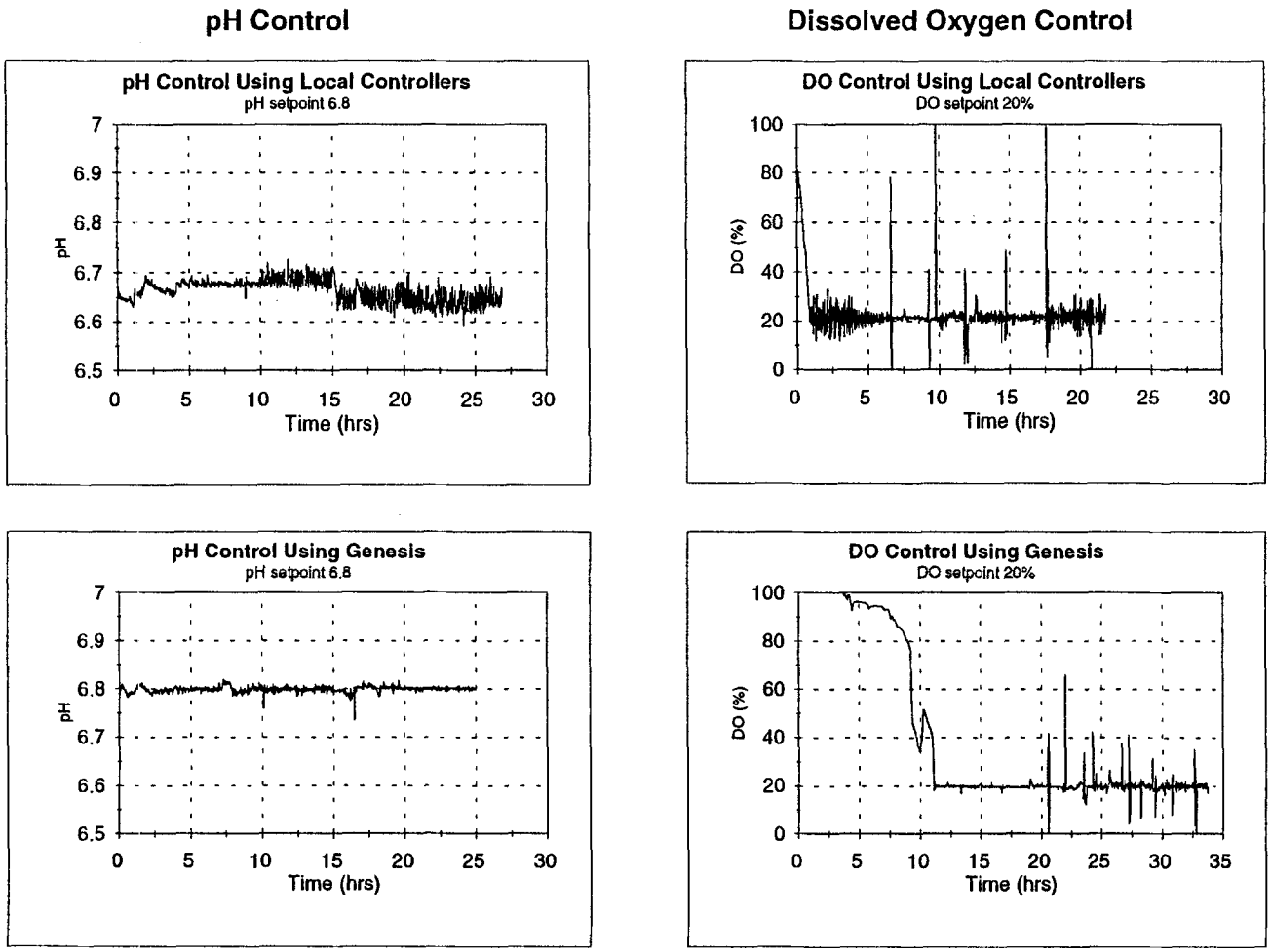

Figure 2 pH and DO control by local Biolafitte controllers and using the Genesis system. A 15-L fermentor was used for this study.

performance are shown in Figure 2, compared to the control performance of the standard Biolafitte controllers. The latter show an offset from the setpoint due to difficulties in calibrating the Biolafitte analog outputs accurately. Oxygen and air mass flow were controlled directly by the computer system so that DO remained at 20% throughout the fermentation.

Using the mass spectrometer to semi-continuously monitor the off-gas stream from each fermentor, the following parameters were calculated in real time by the computer for each fermentor [7], oxygen uptake rate (OUR), carbon dioxide evolution rate (CER) and respiratory quotient (RQ). Examples of OUR and CER graphs are shown in Figure 3.

All of these measured and calculated data were recorded once per minute by the computer system in historian files for later analysis. Real time graphs were also available at the computer for each fermentor to allow the fermentor operators to monitor the fermentation process.

Glucose feed

Many different strategies for glucose feed during fed batch fermentation have been described [3–5,12–14,16–18,20,21,24,26,27,30,32]. Several of these were implemented on this computer system and results in terms of cell density and glucose concentration profiles are described below.

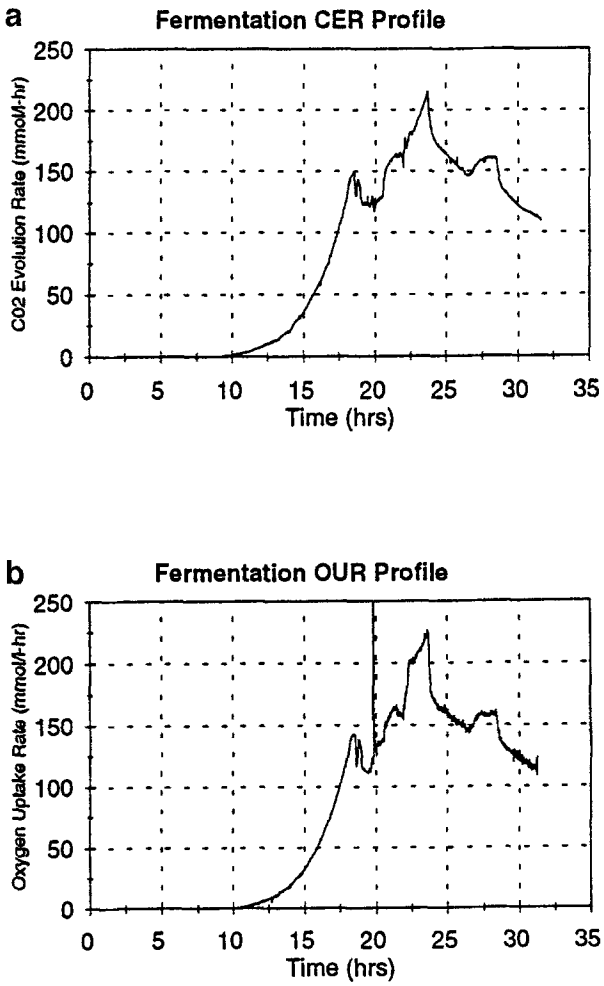

Figure 3 (a) Typical CO_2 evolution rate (CER) and (b) oxygen uptake rate (OUR) profiles for a 15-L fermentor. The sharp line at 20 h in the OUR profile is due to an increase in oxygen mass flow.

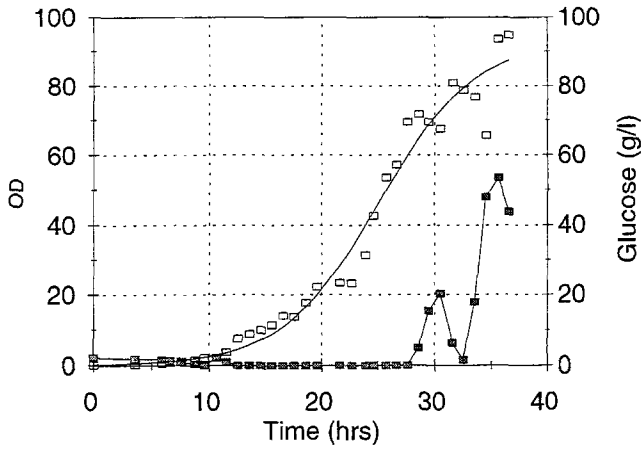

Figure 4 OD and glucose profiles observed with an exponential glucose feed strategy. Filled symbols (■) indicate glucose concentration; open symbols (□) indicate OD at 600 nm; the solid line shows the fit of a logistic growth equation to all the OD data. A 15-L fermentor was used in this study and the growth rate was 0.24 h^{-1}.

Programmed feed: In this system, the specific growth rate of the culture is controlled by the addition of a growth-limiting substance, in this case glucose, according to a predetermined profile [31]. In this work, we implemented an exponential feed profile with cell density and glucose results as shown in Figure 4. The programmed growth rate of 0.21 h^{-1} was close to that observed of 0.24 h^{-1} when a logistic equation was fitted by least squares to the OD data. Glucose remained low until 28 h when the growth rate of the culture decreased such that growth was no longer adequately described by the exponential growth equation

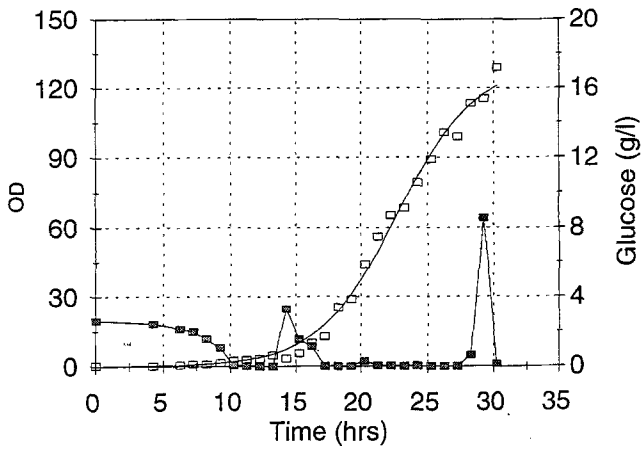

Figure 5 OD and glucose profiles observed with a DO-stat glucose feed system. Filled symbols (■) indicate glucose concentration; open symbols (□) indicate OD at 600 nm; the solid line shows the fit of a logistic growth equation to all the OD data. A 15-L fermentor was used in this study and the growth rate was 0.34 h^{-1}.

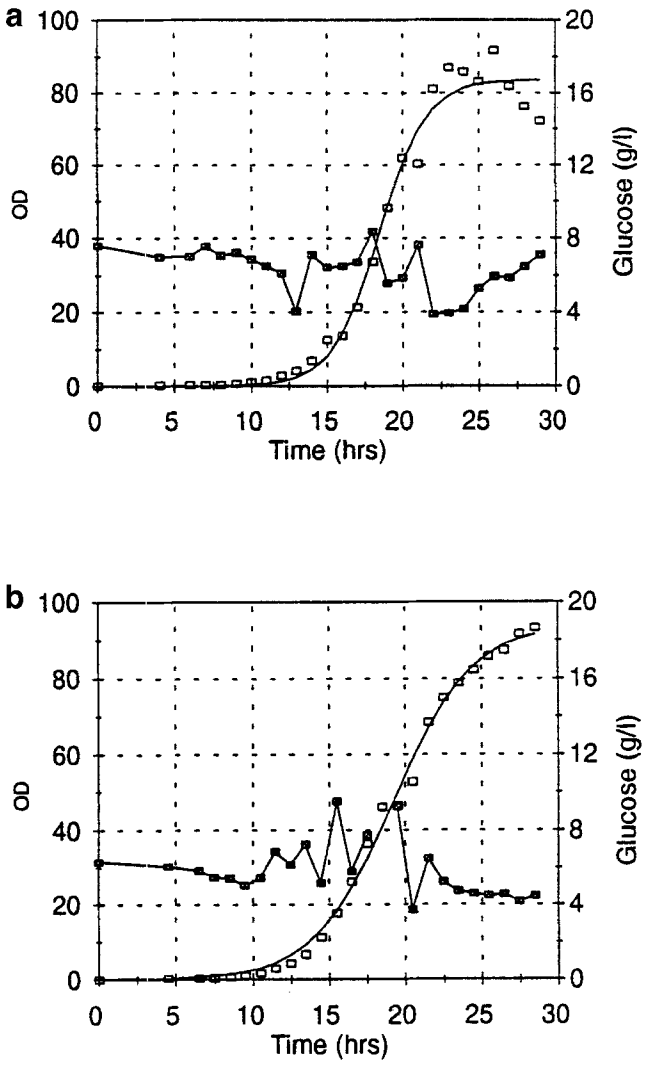

Figure 6 OD and glucose profiles observed for two fermentations with the ammonia-coupled glucose feed system. Filled symbols (■) indicate glucose concentration; open symbols (□) indicate OD at 600 nm; the solid lines show the fit of a logistic growth equation to all the OD data. The glucose setpoint was 6 g L^{-1} and a 15-L fermentor was used in this study. The growth rate was 0.64 h^{-1} for (a) and 0.39 h^{-1} for (b).

and therefore glucose began to accumulate. Once glucose accumulated to significant levels, the pump was turned off, briefly at 31 h and permanently at 36 h, resulting in a decrease in glucose concentration at these times.

DO-stat: In this feed strategy, a rise in the dissolved oxygen concentration above a target value was used as a marker of low glucose [10,22] (in this study, 40% of air saturation) and an input of 75% (w/v) glucose was added, sufficient to bring the glucose concentration to approximately 3 g L^{-1}. This method gave high cell densities and low glucose concentrations (Figure 5). Transient accumulations of glucose at 14 h and 29 h were caused by transient false DO readings above the 40% target value which led to unnecessary glucose additions.

Ammonia-coupled glucose feed: The rate of glucose uptake by *E. coli* is reflected by the rate of ammonium

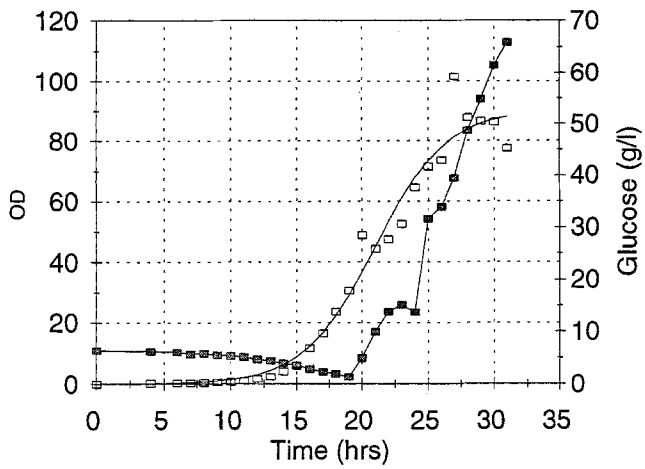

Figure 7 OD and glucose profiles observed for a fermentation using the CER-coupled glucose feed system run without correcting for the increasing CO_2/glucose yield coefficient. Filled symbols (■) indicate glucose concentration; open symbols (□) indicate OD at 600 nm; the solid line shows the fit of a logistic growth equation to all the OD data. A 15-L fermentor was used in this study and the growth rate was 0.37 h^{-1}.

uptake [15]. Thus, by coupling glucose addition to ammonium addition, a steady concentration of glucose should be maintained in the fermentor. The addition of ammonium was measured by recording the weight of 15% (v/v) ammonium hydroxide solution added as a titratant to keep the pH at the required setpoint. The computer used a pre-determined ratio of glucose weight to base weight to calculate required glucose additions. The entry of measured glucose concentrations by the fermentor operators caused the computer to add sufficient glucose to increase the glucose concentration to the setpoint value, or to withhold glucose until a sufficient amount of base had been added to account for the excess glucose over the desired setpoint. Typical results for two such fermentations, in terms of OD and glucose concentration, are shown in Figure 6. A relatively steady concentration of glucose was maintained throughout the fermentation.

CO_2-coupled glucose feed: From considerations of cellular carbon mass balance, there should be a near constant relationship between glucose uptake and CO_2 evolution rate until there is a change in growth or metabolism. Therefore, coupling the glucose addition rate to the CO_2 evolution rate should allow a constant level of glucose to be maintained. However, experience has shown that the metabolism of the cells changes significantly during the fermentation, and that as a consequence, the number of CO_2 molecules produced from each molecule of glucose added increases once the cells cease exponential growth, ie more of the added glucose is oxidized to CO_2 after the exponential growth phase, leading to a decrease in the observed glucose/CO_2 yield as the fermentation progresses. If there is no adjustment to the glucose/CO_2 yield coefficient during this latter part of the fermentation, glucose accumulates to a significant extent (Figure 7). A similar change in CO_2/glucose yield was also observed by Kleman and Strohl [14]. Therefore, the measured glucose concentrations were manually entered by the fermentor operators into the computer and the computer added or withheld glu-

cose as for the ammonium-coupled system described above. In addition, if a significant excess of glucose was measured, the control program decreased the CO_2/glucose yield coefficient by 10% as well. Two typical fermentations using this feed control method are shown in Figure 8. In this case, the speed of the glucose addition pump was set by the computer, based on the rate of CO_2 evolution multiplied by the glucose/CO_2 yield coefficient. Again, a relatively steady glucose concentration was maintained throughout the fermentation.

Discussion

The computer system described above has been used to collect fermentation data simultaneously from a variety of instruments commonly used in fermentation process development and from a number of fermentors of very different scale, design and operation. In addition, it has been used to perform low level control of dissolved oxygen, pH, air mass flow and oxygen mass flow. A number of glucose feed strategies have been successfully run to give both glucose-limited and non-limited growth. The combination of distributed I/O hardware for analog communication and the commercially available, easily configurable PC based monitoring and control software has proved extremely flexible. Not only is it possible to incorporate the standard fermentation instruments into the monitoring and control system, but by using custom written device drivers, an array of computer-controlled laboratory instruments can also be directly incorporated. More recently, we have replaced all the local fermentor controllers for pH, temperature, dissolved oxygen and agitation speed with a single GE Genius 90/70 Programmable Logic Controller (PLC), directly integrated into the GE Genius LAN. This has significantly improved the performance of low level control on these fermentors as compared to the analog controllers supplied with the fermentation equipment. A much higher degree of low-level control uniformity was also obtained using this single PLC to control multiple fermentors with the same digital algorithm.

In the next stage of development we plan to explore further how more sophisticated on-line and off-line instruments can be incorporated into this integrated monitoring and control system to extend our understanding of the fermentation process. By adding computer process monitoring and control and by combining this with more advanced process monitoring instruments, a deeper understanding of the fermentation process, along with clearer insights into the consequences of genetic changes deliberately introduced into the organism, should be available. We expect the benefits to include more predictable fermentation outcomes and more predictable fermentation scale-up.

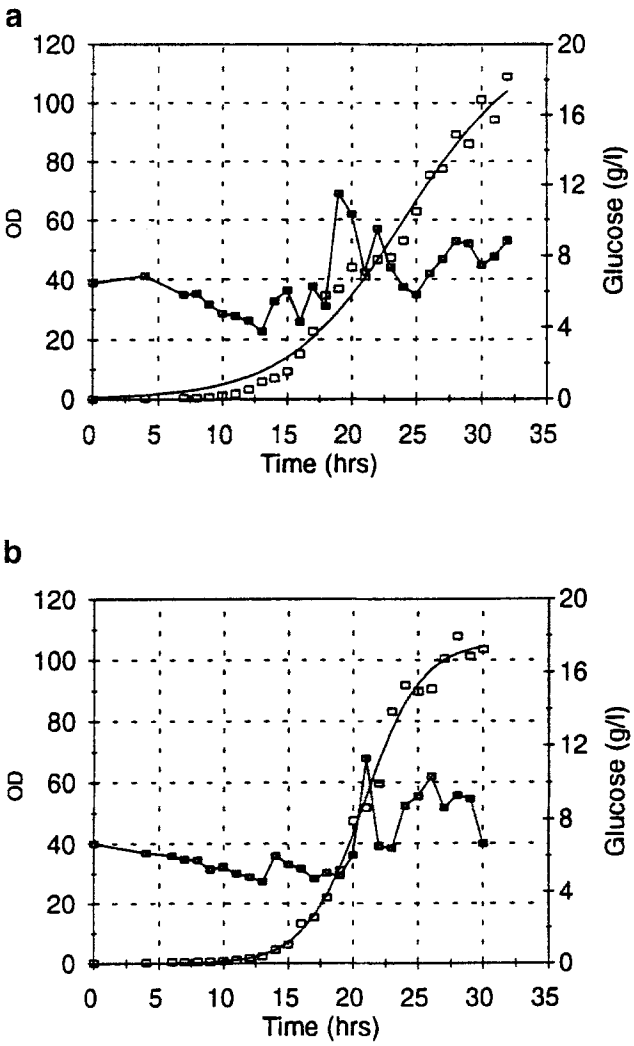

Figure 8 OD and glucose profiles observed for two fermentations with the CER-coupled glucose feed system run with correction for the increasing CO_2/glucose yield coefficient. Filled symbols (■) indicate glucose concentration: open symbols (□) indicate OD at 600 nm; the solid lines show the fit of a logistic growth equation to all the OD data. Glucose setpoint was 6 g L^{-1} and a 15-L fermentor was used in this study. The growth rate was 0.22 h^{-1} for (a) and 0.46 h^{-1} for (b).

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