Journal of Industrial Microbiology, 2 (1987) 305–317 Elsevier SIM 00095

Data acquisition and control of a continuous fermentation unit

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Received 8 April 1986 Revised 2 September 1987 Accepted 15 September 1987

Key words: Data acquisition; Computer control; Continuous fermentation; Fermentation software

SUMMARY

Data acquisition and computer control of Zymomonas mobilis fermentation for ethanol production has been studied. An HP 200 series microcomputer system was used in conjunction with an HP 3497A data acquisition unit. On-line ethanol, glucose and cell mass were measured for use as possible control variables. Dilution rate was used as the manipulative variable. A versatile, user-friendly data acquisition program was written to gather, control and analyze data from the continuous fermentation. The program allows user-given control and calibration algorithms so that sophisticated control policies, e.g., self-tuning regulator (STR) and instrumentation, can be implemented with relative ease.

INTRODUCTION

The current work is a part of on-going research activities at Colorado State University in the area of computer process control and automation of biochemical processes. This work, in particular, describes the interfacing of a microcomputer system with the appropriate instruments and fermentation unit. Emphasis in this work was placed on the development of versatile software for data acquisition, analysis and control of fermentation processes. The software has been written so that it can be used for different fermentation systems. The program allows user-defined control and calibration algorithms. The graphical output of the on-line fermentation activities is in the process of being developed. In what follows is a description of the process and instrumentation, computer system, software design, and some experimental results of the real-time application of the computer-control methods.

FERMENTATION SYSTEM

The system under study consisted of a Chemap fermenter, model GF-0014. The fermentation system was set up for continuous operation as shown in Fig. 1. The feed tanks consist of two 50-liter carboys with separate inlet lines to the fermenter. Feed and exit streams were drawn using computer-driven peristaltic pumps. The exit stream was drawn from the interior of the fermenter broth, and the level was maintained using a level sensor in hydraulic

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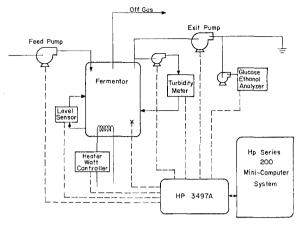


Fig. 1. Fermentation system.

equilibrium with the fermenter. A semi-continuous stream of broth was recycled through the nephalometer for cell mass determination by means of an additional peristaltic pump. A sample was withdrawn from the exit stream by the enzyme analyzer to continuously monitor the glucose and ethanol concentrations.

The fermenter has built-in pH and temperature controllers. The pH controller is used for all studies and is not interfaced with the computer system. The pH was maintained at 6.2 for this study by the addition of sterile 2 N NaOH [3].

The fermenter temperature controller is used when the computer is off-line. This consists of a contact thermometer with a variable set point, a 240 V/1000 W heater and a heat exchanger within the fermenter. An incoming stream of city water is passed through the heater and heat exchanger and then sewered. If the temperature is below the set point, the controller provides full power to the heater, otherwise no heating takes place. The computer-driven heater controller made by Research Inc. is a single-phase power controller (model 661D). It is calibrated for our purposes to accept a 0-6 V input from the HP 3497A; this corresponds linearly from zero to full output of the line voltage to the Chemap heater. A latch relay is used to select between the computer and fermenter heater controllers. In the event of a power shortage, the computer program will stop. This relay selects the fermenter controller when the power comes back, and the temperature will be maintained.

Temperature measurements are made by the computer on four process streams. These are as follows: inlet to heater, outlet from heater, outlet from heat-exchanger, and the actual fermentation broth. This provides enough information on an enthalpy balance. These measurements are made by J-type thermocouples and the HP 3497A provides accurate temperatures by electronically compensating for the iron-copper and constantin-copper junctions in the 44422 A board on the 3497A system.

The feed and exit pumps are manufactured by Watson Marlow. They are both 60 rpm peristaltic computer-controlled pumps (model 501U). They are calibrated for this study to accept to 0–10 V signal from the HP 3497A. This signal corresponds linearly from zero to full pump speed.

A level sensor, developed in this laboratory [4], consists of a sight glass, light source and a photosensitive transistor. A float in the sight glass is in hydraulic equilibrium with the fermenter at all times. The light source and transistor are set at the desired fermenter level. When the level is too high, the light beam is broken by the float and the output is less than 1 V, otherwise the output is 10 V. The exit pump is controlled by this signal. The feed pump signal is provided by the computer control algorithm.

ON-LINE INSTRUMENTS

Cell mass is measured by a Ratio 2000 Process Turbidimeter manufactured by Hach Co. (model 42100-00). Output to the computer is provided by a Ratio turbidity meter (model 42200-00). The output is 0–0.1 V and the actual value of turbidity (in nephalometric turbidity units (NTU)) is dependent upon the scale used (for our applications 0–2000 NTU units). Cell mass is obtained from previous batch studies which provide cell concentration as a function of NTU units. The measure of NTU is determined optically. A peristaltic pump is used to recirculate the fermenter broth from the fermenter to the turbidity meter. The power source for this pump is controlled by the HP 3497A.

Glucose and ethanol concentrations are measured by means of immobilized enzymes and oxidases. The instrument was manufactured for this project by Analytical Research Inc. of Yellow Springs, OH. On-line direct measurement of both glucose and ethanol is provided by directly accessing the exit stream of the fermenter. This sample stream is continuously diluted 625-fold by means of a Sage peristaltic pump (model 375A). The diluted stream is passed over two probes; the first probe contains an immobilized alcohol oxidase and the second contains a glucose oxidase. The enzymes react with the sample and generate peroxide which further diffuses through the membrane layers to the Clark-type electrodes. The electrodes convert the concentration of peroxide into nA. These signals are then converted to two outputs of 0-10 V. The enzymes are readily available from Fisher Scientific and are identical to those used in the Yellow Springs Instruments (model 27) industrial analyzer. The buffer used for dilution of the sampled stream consists of the following compounds per U.S. gal-

3	0	7
~	v	1

lon: 130 g of disodium phosphate, 17 g of monosodium phosphate, 2 g of tetrasodium EDTA, 1 g of sodium chloride, and 0.2 g of chlorhexidine diacetate.

COMPUTER SYSTEM

The computer system consists of a Hewlett-Packard series 200 computer (model 220) and the following HP peripheral device: video monitor (model 82913A), flexible disk drive (model 9121), printer (model 82906A), and data acquisition/control unit (model 3497A). The data acquisition/control unit consists of a real-time clock, digital volt meter, 20-channel J-type thermocouple compensated multiplexer (model 44422A), 20-channel multiplexer (model 44421A), two dual-output 0–10 V digital to analog converters (model 44429A), and a high-voltage actuator assembly (model 44431A). The 3497A is the software-driven interface between the fermenter and the computer system. The configuration is given in Table 1.

Slot	Board ID	Position	Instrument/Signal	
0	44422A	Al	Fermenter thermocouple:	J type
		A2	Cold water thermocouple:	J type
		A3	Hot water thermocouple:	J type
		A4		
1 44421A	44421A	A1	Turbidity meter:	0-0.1 V
		A2	Glucose analyzer:	0–10 V
		A3	Ethanol analyzer:	0-10 V
		A4	CO_2 analyzer:	0-0.01 V
		A5	pH meter:	V
			Level sensor:	0–10 V
		A6		
2 44419A	44419A	0	Feed pump:	0-10 V
		1	Exit pump:	0–10 V
3	44429A	0	Heater controller:	0-5 V
4	44431A	0	Turbidity pump:	115 V
		1	Enzyme analyzer blank solenoid	
		2	Enzyme analyzer calibration solution solenoid	

 Table 1

 HP 3497A data acquisition/control unit configuration

MATERIALS AND ANALYTICAL METHODS

The Zymomonas mobilis used in this study was strain ATCC 10988. It was obtained from the American Type Culture Collection of Rockville, MD. The Z. mobilis was received in pellet form and stored in the freezer until used. Each pellet was suspended in 1 ml of the following medium: 50 g/l of glucose, 3 g/l of yeast extract, 1 g/l of KH₂PO₄, 1 g/l of $(NH_4)_2SO_4$ and 0.5 g/l of MgSO₄ · 7H₂O. All compounds were of reagent grade quality. This medium was used throughout the course of the study. The suspension was immediately transferred to an additional 9 ml of the medium. During log growth phase of the organisms, the inoculum was transferred to ten 10-ml tubes of the medium in an anaerobic chamber maintained at 30°C. There was no agitation except for occasional shaking of the tubes. Typical time of growth from pellet to 10-ml tube inoculum was about 24 h. After these cultures completed growth, the tubes were kept in the refrigerator and used as the initial inoculum in future studies. All transfers and inoculum build-up were carried out in an anaerobic chamber.

The fermentations studied were carried out in a working volume of 7 liters. The initial inoculum was obtained by transferring the starter tubes to 100 ml of the medium. This suspension was transferred to an additional 600 ml of medium during exponential growth which provided a final 700 ml inoculum. This final inoculum was transferred to 6.3 liters of medium in the fermenter during exponential growth.

The fermenter was maintained at a temperature of 31°C and a pH of 6.2. The medium used for continuous studies was of the same composition as described previously. The medium was filter-sterilized using two filters in series with pore sizes of 0.22μ . Prior to inoculation, the fermenter was initially sterilized in an autoclave for 30 min at 121°C. Additional sterilization was performed by pressurizing the fermenter with the steam system as defined in the Chemap manual. All connections to the fermenter were made under flame to insure aseptic conditions. The fermenter was flushed with filtersterilized nitrogen prior to inoculation to insure anaerobic conditions. Cell mass was determined by centrifuging 25 ml samples of the fermentation broth for 10 min at 10000 rpm. The supernatant was discarded and the pellet was resuspended in an additional 25 ml of water and recentrifuged. The final pellet was transferred by washing into aluminum pans and dried at 90°C for 24 h. Off-line analysis for glucose and ethanol was made using a Waters Associates HPLC system. A Bio-Rad fast acid analysis column (100 \times 7.8 mm, model No. 125-0100, serial No. 3042) was used. The column effluent composed of 0.01 N H₂SO₄ was analyzed using a differential refractometer.

DATA ACQUISITION PROGRAM

This section will briefly describe the software programmed to interface with the Chemap fermenter and the associated instruments. Details of the software package are given in Ref. 5.

The program is written in an interactive menudriven format in which the user is asked to specify all operating information. All of the information specified by the user is immediately interpreted by the computer and displayed back to the user. This allows for incorrect system specifications and typographical errors to be easily corrected. To prevent many program failures, the program is designed to trap any user I/O errors, identify the error and allow for a correction. No computer experience is needed to run this program.

The program is divided into two parts: data acquisition/control and reports. Reports allow for output from data collected from past studies. There are three reports available: operating parameters, data and history.

The data acquisition and control section of the program prompts the user for all necessary operating parameters. These include: describing the study, identifying which instruments are on-line, specifying control loops and their necessary parameters, providing a complete list of set points and instrument calibration constants.

The program then begins on-line data acquisition and control. The HP 3497A is then the main controller; data are taken from each instrument every minute and written to the disk as often as the user specifies. The system can be interrupted by pressing the SRQ button on the HP 3497A. This brings the program back into the interactive mode to allow for any system changes needed. During the course of the study, any operating changes or system changes are identified by messages written to the printer and the history file.

This program is implemented on an HP series 200 computer using Pascal programming language. The HP procedure library is used extensively for the input/output needs of the program. The program is resident in a code library on disk and is structured in modules which can interact with other sections of code as needed.

The program is written to accept calibration and control algorithms written by the user independent of this program. These algorithms must be developed and compiled before starting this program. Use of these files allows for testing of more than one control algorithm during a study and bringing equipment on-line that has not been calibrated in the main program. Use of these user-specified algorithms and controllers is very powerful. These modules directly access the primary record and data array. A text file for each of these modules has been developed for the user. These text files contain all the necessary Pascal codes with correct procedure arguments and instructions for the development of user calibration algorithms.

ENZYME ANALYZER

The glucose and ethanol analyzer is operated at a dilution factor of 625. This is achieved with tubing sizes of 0.5 and 2.5 mm in the pump. The pump setting of 400 corresponds to a flow rate of 6 ml/min through the analyzer. The process sample is diluted with a buffer to maintain the proper pH and provide the necessary electrolytes for the probes. The peristaltic pump causes a cyclic nature in the output signal due to uneven line pressure. It is found that the period of this cycle is 20 s at a pump setting of 400. In order to overcome this error, five readings (5 s apart) are taken during every 1-min sample interval. The final value is the average of these readings. The transfer function (input and output relationship in terms of Laplace operators) for this measuring device is as follows (units are $g/l \cdot min$):

$$T(s) = \frac{\exp(-3.34 \ s)}{(0.78 \ s + 1)} \tag{1}$$

The time lag of 3.34 min is due to transport of the sample. Approximately 2.5 min are due to the exit line from the fermenter to the instrument and the remaining time is due to the transport within the instrument and sample dilution.

The enzyme analyzer was checked for linearity over the concentrations of interest for this study. A calibration standard of 30 g/l of ethanol and 5 g/l of glucose was prepared. An additional standard solution containing 50 g/l of ethanol and 10 g/l of glucose was also prepared. This second standard was then used to make nine additional samples by dilution. Each of the ten samples was then measured using the enzyme analyzer with calibration between each reading. As shown in Figs. 2 and 3, both ethanol and glucose enzyme analyzers were linear over the regions of interest. The sample coefficient of determination or goodness of fit, r^2 , for

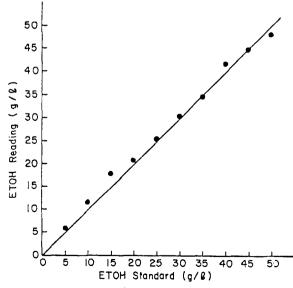


Fig. 2. Linearity of on-line alcohol measurements.

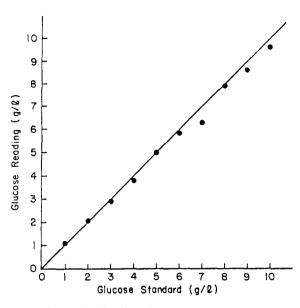


Fig. 3. Linearity of on-line glucose measurements.

the glucose and ethanol analyzer is 0.815 and 0.973, respectively.

Computer calibration of the enzyme analyzer is required to assure accurate analysis of the process effluent stream. The solenoids which select the sampled stream are actuated by the HP 3497A.

When the autocalibration routine is turned on by the user, the program calibrates both of the analyzers every 20 min. The calibration cycle is shown in Fig. 4. It takes 5 min to flush the system of the process effluent and take a blank reading. The calibration solution then flows through the system for 4 min before the analyzers reach steady state again. Finally the process effluent flows through the system for 11 min, although the first 5 min are used to flush out the previous calibration solution. The process is analyzed every minute for the remaining 7 min and then the calibration cycle restarts. During calibration, the program uses a linear regression routine to predict the glucose and ethanol concentrations in the fermenter from the last seven actual process readings.

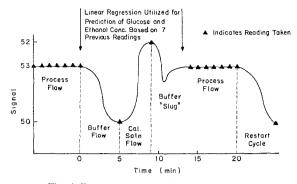


Fig. 4. Enzyme analyzer calibration sequence.

NEPHALOMETER

The turbidity meter measures the turbidity of a semi-continuous recycled stream from the fermenter. The pump used to recycle the fermenter fluid is controlled by the HP 3497A.

The carbon dioxide produced during the fermentation tends to degas in the recirculation line and the sample cell. The resulting bubbles tend to scatter the light and cause erratic readings. It was determined that the samples needed to stop flowing for 15 s in order to 'off-gas.' The pumping speed and the tubing length were chosen such that the sample cell would be completely rinsed with the incoming solution in 45 s.

The transfer function for the nephalometer is as follows (units are NTU/min):

$$T(s) = \frac{\exp(-0.2 s)}{(0.16 s + 1)}$$
(2)

The correlation between cell mass and NTU was made by comparing off-line cell mass concentrations with the nephalometer readings. The NTU measurements are shown to be a linear function of cell concentration over the range of 0.9–1.9 g/l with a correlation coefficient of 0.995. The correlation between NTU and cell concentration is given as:

cell mass concentration (g/l) = $0.000935 \cdot \text{NTU} + 0.618$

HEATING SYSTEM

The Chemap fermenter was updated with a 240 V, 1000 W resistance heater. The line voltage to the heater was 195 V; therefore only 815 W of power were available. The temperature of the fermenter broth was measured using the existing J-type thermocouple and the HP computer system. Any transfer function due to the measuring device was inherent in the overall process transfer function. The transfer function was as follows (units are degree C, W, min):

$$T(s) = \frac{1.2 \left(\exp\left(-2 s\right)\right)}{(32.4s+1)}$$
(3)

LEVEL SENSOR

The fermenter level is set by adjusting the height of the sensing beam. The level is controlled by maintaining the exit flow rate at 90% of the feed flow rate when the level is less than or equal to the set point. As the level rises, the beam is interrupted and the output signal to the computer is less than 1 V. The exit flow rate is then increased to twice that of the feed rate until the time that the beam is again uninterrupted by the float.

FEED AND EXIT PUMPS

The feed and exit pumps have been calibrated to accept an input signal of 1-10 V. The actual flow rate is dependent upon the tubing used in the pump. Experiments have been done in order to provide algorithms for the most common tubing sizes. The data from these experiments were then analyzed using linear regression to determine a default algorithm for odd tubing size. The program uses the algorithm for the specified tubing inside diameter. If the tube i.d. does not match one of the four tested tube sizes, the default algorithm is used. The specific algorithms are accurate to 5%. The default algorithm is accurate to 15%.

RESULTS AND DISCUSSION

Use of the data acquisition program for the fermentation studies has provided timely chemical analysis of the exit stream and excellent datagathering operations. A fermentation was run with hand sampling of cell mass and manual flow rate checks. Off-line chemical analysis and cell mass determinations were used to validate the data collected by the computer. The flow rates were always within 5% of that specified by the computer. The accuracy of the pumps is very dependent upon using tubing recommended by the manufacturer in the pump head.

ENZYME ANALYZER

The enzyme analyzer was first operated with manual calibration every hour. This proved to be a difficult task and the results during the initial tests were not good. These results are shown for the alcohol and glucose analyzers in Figs. 5 and 6, respectively. The results indicate that the alcohol analyzer is the least stable, especially the alcohol oxidase. Carr and Bowers [2] discussed this phenomenon in their text. It is suggested that the enzyme lost its ability to generate peroxide but did catalyze a reaction between the substrate and oxygen. The resulting reaction produces a larger signal than the peroxide reaction. The electrode retained its sensitivity for approximately 4 months.

CONTROL STUDIES

Temperature control

A PID (proportional + integral + derivative) controller was realized in discrete form to maintain the fermenter temperature. The manipulated variable is the wattage to the heater. The control variable is the temperature of the fermenter broth. Disturbances of the system include the water flow rate, the water temperature and the line voltage to the heater.

The microbial population is very sensitive to

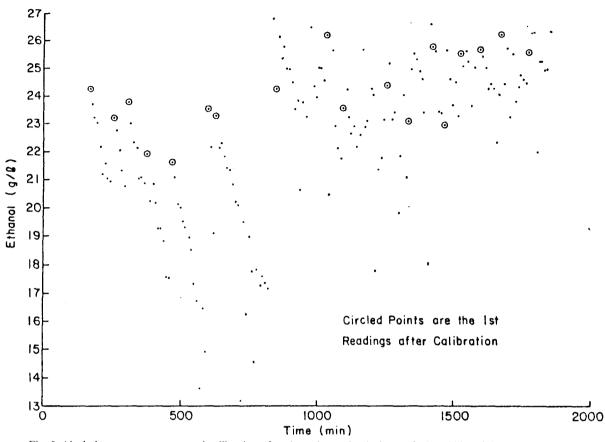


Fig. 5. Alcohol measurements: manual calibration of analyzer (scattering is due to the instability of the enzyme system).

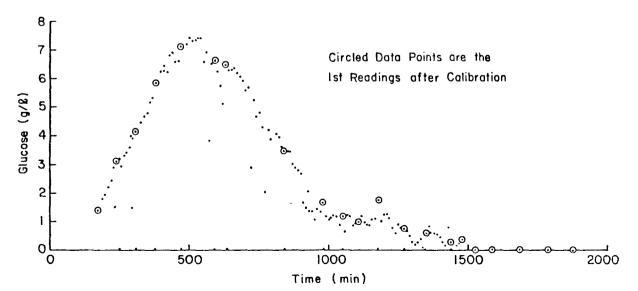


Fig. 6. Glucose measurements: manual calibration (glucose oxidase is more stable and readings are reliable).

high temperatures. This was accounted for in the initial design by providing a low-capacity heater. A simple mass and energy balance shows that an incoming water flow of 10 ml/s at 10°C can be heated to approximately 35°C at full power. This low-capacity heater put a severe constraint on the upper limit of the control action.

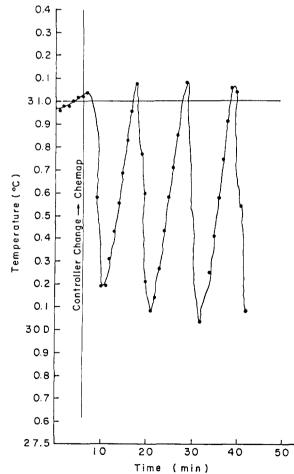
The PID controller was realized in the discrete form by the Local Input Approximation method. The equation is given by:

$$WATT = F(k - 1)T + K_{c} (1.5 E(k)T - E(k - 1)T) + 0.5 E (k - 2)T) + K_{c} (T_{d}/T) (2E(k)T) - 3 E(k - 1)T + E(k - 2)T) + K_{c} (T/T_{i}) (0.667 E(k)T + 0.167 E(k - 1)T) + 0.167 E(k - 2)T)$$
(4)

where K_c is the proportional gain in W/°C; T_i is the integral time constant in min; T_d is the derivative time constant in min; F(k - 1)T is the last wattage output; E(k - 1)T is the set point minus the last temperature; E(k)T is the set point minus the present temperature; E(k - 2)T is the set point minus the temperature, two sampling rates past; T is the sampling rate; and WATT is the final control action.

The sampling period for control action is 1 min. On-line tuning of this controller was achieved with a water flow rate of 8 ml/s at 10°C. The set point was 31°C and the final PID settings used were: K_c = 75, $T_i = 2.0$, and $T_d = 4.0$.

The response of this controller to a set point change is shown in Fig. 7. The overshoot for this



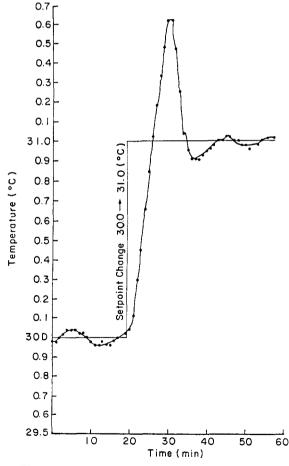


Fig. 7. Control of fermenter temperature: PID algorithm.

Fig. 8. Control of fermenter temperature: Chemap controller.

example is 60%, the decay ratio is very small and the rise time is quite rapid – 7 min. This is not an ideal response but in larger set point changes the absolute value of the overshoot remained the same – 0.6° C.

Fig. 8 shows the action of the existing Chemap controller, a contact thermometer. The system was not undergoing a step change and the process disturbances were at a minimum. This test immediately followed the previous test in the set point change of the PID controller. The computer-driven PID controller was in effect during the first 6 min in Fig. 8.

PRODUCT COMPOSITION CONTROL

PID controller

A PID controller was realized in discrete form to maintain the product concentration. The manipulated variable is the feed flow rate. The control variable is the ethanol concentration in the exit stream. Disturbances to the system include the effects of pH and temperature on the microbial population as well as those which can interfere with the enzyme analyzer.

The maximum dilution factor allowed for a Z. mobilis fermentation is 0.33 h^{-1} . This corresponds to the maximum growth rate. Any flow rates greater than this result in washing the microbial population out of the fermenter. The maximum allowable flow rate for this algorithm was 37.33 ml/min. This corresponded to a dilution factor of 0.32^{-1} . This is seen as a constraint on the upper limit of the control action.

This process is chosen to be represented by a second-order linear system with a time lag [4]. The transfer function is different for either a positive or negative step change in feed. These studies were carried out at 5% glucose concentration in the feed. The transfer function for an increase in feed rate is given by (units are g/l, l/h, h):

$$\frac{\text{ethanol}}{\text{feed rate}} = \frac{-1.62 \exp(-0.52s)}{(s + 0.27)(s + 1.38)}$$
(5)

The transfer function for a decrease in feed rate is given by:

$$\frac{\text{ethanol}}{\text{feed rate}} = \frac{-0.81 \exp(-0.28s)}{(s + 0.87)(s + 0.96)}$$
(6)

The transfer function used for the study was the average of Eqns. 5 and 6 and is:

$$\frac{\text{ethanol}}{\text{freed rate}} = \frac{-2.65 \exp(-0.40s)}{(2.4s+1)(0.88s+1)}$$
(7)

The transfer function of the process representing the time lag due to the enzyme analyzer is:

$$\frac{\text{ethanol}}{\text{feed rate}} = \frac{-2.65 \exp\left(-0.48s\right)}{(2.4s+1)(0.88s+1)}$$
(8)

The closed-loop block diagram for this control loop is shown in Fig. 9. Bode stability criteria were used to evaluate the open loop response of Eqn. 8. The crossover frequency was found to be 1.72 rad/h with an amplitude ratio of 3.65. PID controller settings for this transfer function are found by the Ziegler-Nichols technique to be (units are g/l, ml/min, min): $K_c = -28$, $T_i = 109$ and $T_d = 27$.

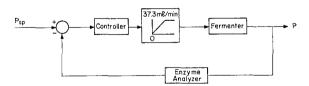


Fig. 9. Block diagram: product composition control.

The PID controller was realized in discrete form as before by the Local Input Approximation method. The equation is:

$$FLOW = F(k - 1)T + K_{c} (1.5 E(k)T - E(k - 1)T) + 0.5 E(k - 2)T) + K_{c} (T_{d}/T) (2E(k)T) - 3 E(k - 1)T + E(k - 2)T + K_{c} (T/T_{i}) (0.667 E(k)T + 0.167 E(k - 1)T) + 0.167 E(k - 2)T$$

where K_c is the proportional gain in (ml/min)/(g product/l); T_i is the integral constant in minutes; T_d is the derivative constant in minutes; F(k - 1)T is the last flow rate; E(k - 1)T is the set point minus the last product concentration; E(k)T is the set point minus the present product concentration; E(k - 2)T is the set point minus the product concentration two sampling rates past; T is the sampling rate; and FLOW is the final control action.

The sampling period for control action is 20 min. This period is due to the calibration requirements of the enzyme analyzer. As the control action is limited by the constraints imposed on the maximum flow rate by the washout condition, controller parameters found by Ziegler-Nichols settings needed to be changed. On-line tuning of this controller was achieved with an initial glucose concentration of 5%. The final PID settings used are: $K_c = -2.5$, $T_i = 2.0$ and $T_d = 0.5$.

The responses of product concentration, substrate concentration and feed rate to a set point change in product concentration are shown in Figs. 10, 11 and 12, respectively. After the set point change, the resulting final product concentration is within the accuracy of the enzyme analyzer: 5-10%. The final product concentration is reached within 8-9 h. This is a reasonably fast response; open loop responses usually take 24 h to come to steady state. The substrate concentration is also stabilized within

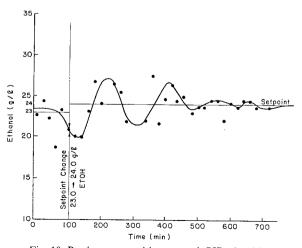


Fig. 10. Product composition control: PID algorithm.



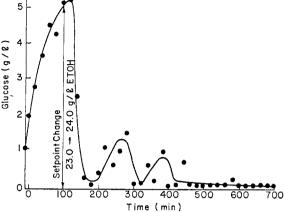


Fig. 11. Substrate concentration (glucose): PID control of product.

8–9 h. This confirms that the ethanol enzyme is behaving well.

Self-tuning controller

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The nonlinear and nonstationary nature of the biochemical process leads to a change in its dy-

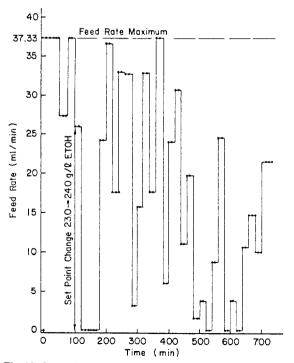


Fig. 12. Control action (feed flow rate): PID control of product.

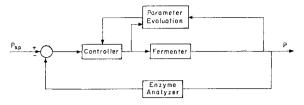


Fig. 13. Block diagram: STR algorithm.

namic characteristics during operation. To cope with this situation, a controller should be able to adjust its parameters as the process changes. Self-Tuning Regulator (STR) is such a controller [1].

A self-tuning regulator was proposed to control the process. A block diagram for this controller design is shown in Fig. 13. This algorithm was designed to determine the coefficients of the process model by first achieving steady state at a constant feed rate and then initiating a step change in the feed rate for a prescribed length of time. The process was proposed to be described by a third-order linear difference equation without time delays given by:

$$Y_n = a_1 (Y_{n-1}) + a_2 (Y_{n-2}) + a_3 (Y_{n-3})$$
(9)
+ $b_1 (M_{n-1}) + b_2 (M_{n-2}) + b_3 (M_{n-3})$

where Y_i and M_i are the process input and output variables at the *i*th sampling instant. Y is the ethanol concentration and M is the feed rate. Though time delays as a function of sampling time, T can be easily incorporated in the STR. The model (Eqn. 8) indicates that the dead time is not an exact integer function of the sampling period (20 min). A third-order model (second-order process with a first-order Pade' approximation for the dead time) should be accurate enough for the biochemical process.

The coefficients were determined by minimizing the mean square error

$$P = \frac{1}{\dot{x}} \sum_{N=1}^{x} (Y_{n-}a_1 (Y_{n-1}) - a_2 (Y_{n-2}) - a_3 (Y_{n-3})) - b_1 (M_{n-1}) - b_2 (M_{n-2}) - b_3 (M_{n-3}))^2$$
(10)

The necessary conditions for the minimum point are:

$$\frac{\delta P}{\delta a_1} = \frac{\delta P}{\delta a_2} = \frac{\delta P}{\delta a_3} = \frac{\delta P}{\delta b_1} = \frac{\delta P}{\delta b_2} = \frac{\delta P}{\delta b_3} = 0 \tag{11}$$

A 7 \times 6 matrix, as defined by Eqn. 11, was solved using Gaussian elimination. The resulting coefficients a_1 , a_2 , a_3 , b_1 , b_2 and b_3 were then used in Eqn. 12 until the model parameters were updated by affecting a disturbance in the set point and tracking the values of Y and M for the x sampling periods and again solving Eqn. 11. The overall sequence of events during the STR study are shown graphically in Fig. 14.

After the coefficients have been determined, the control action which minimizes the square error at the next sampling period is given by:

FLOW =
$$(Y_{sp} - a_1Y_n - a_2Y_{n-1} - a_3Y_{n-2} - b_2M_{n-1} - b_3M_{n-2})/b_1$$
 (12)

where Y_{sp} is the desired set point.

The STR was shown to function properly. It effectively disturbed the process, evaluated the coefficients and updated the model at the prescribed time by slightly changing the set point and tracking the response of the system. However, the STR studies suffered from repeated contamination problems, and hence are not shown in a quantitative manner.

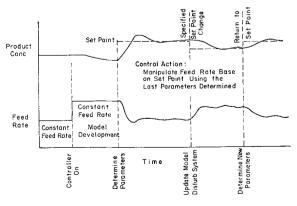


Fig. 14. STR sequence.

CONCLUSIONS

The application of on-line instruments and data acquisition coupled with computer calibration schemes is essential for computer process control. If implemented correctly, the on-line instruments have the ability to extract representative samples without disturbing the system.

The data acquisition and control program written for this study is very versatile. It gathers data in an accurate and timely manner and performs the necessary control functions within the limits of the algorithms provided.

The enzyme analyzer proved to be a difficult instrument to keep in good working order. The instrument proved to be a source of timely data for glucose concentration. With more experience, it is believed that the ethanol measurements would prove to be equally as valid. The greatest difficulty is stability of the enzymes. The glucose oxidase stabilizes in a matter of hours. The alcohol oxidase stabilizes in a matter of days. With advances in enzyme technology, the available enzymes can only improve. With more experience, the additional probes and the upgraded hydraulic system, this instrument will be a valuable asset for the analysis and control of biochemical processes.

The PID algorithm for the heater controller proved to be very effective in maintaining accurate temperature control of the fermenter. This algorithm was implemented with default settings which operated well with a set point of 31° C. The temperature deviation was generally less than 0.1° C. The contact thermometer controller on the Chemap had a deviation of 1° C.

The PID algorithm for product composition control based upon the ethanol concentration was shown to be reasonable when coupled with computer calibration of the enzyme analyzer. The controller varied the feed rate within the constraints of the process and the ethanol concentration was shown to stay within 5-10% of the set point. This is within the accuracy of the measuring device.

Repeated contamination of the fermenter resulted in three failed attempts to evaluate the performance of the self-tuning algorithm (STR). The algorithm was shown to function properly by evaluating the process and updating the control parameters. The actual parameters were not suitable for control and the contaminated fermentations were stopped.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Drs. J.L. Huntington and J.C Linden for their help during the course of this research. This study was funded in part by NSF Grant CPE-8214454 and Colorado State University Agricultural Experiment Station Project 383.

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