한국생물공학회지 제4권 제3호
*Korean J. Biotechnol. Bioeng.*
Vol. 4. No. 3. 197-202(1989)

# On-Line Induction of Fermentation with Reconbinant Cells : Part I. Construction of Control Hardware

Choul-Gyun Lee and Cha Yong Choi
*Laboratory of Biotechnology and Bioengineering*
*Department of Chemical Technology, College of Engineering*
*Seoul National Univerisity, Seoul 151-742, Korea.*

# 유전자 재조합 세포 발효의 온·라인 유도 : 제 1부. 자동 제어 하드웨어 제작

이 철 균 · 최 차 용
서울대학교 공과대학 공업화학과

## ABSTRACT

The existing analog controllers for typical fermentation variables such as rpm and temperature were properly modified in order to be interfaced with microcomputers. Other necessary corrections like the impedance matching, the ground isolation, and the signal range adjustment were done for the successful interfacing between fermentor detectors and computers and for the eventual on-line feedback control of temperature and DO and the induction of the fermentation process with recombinant cells.

## INTRODUCTION

The use of computers in fermentation processes for data acquisition and analysis, and fermentor optimization and control increased significantly during the last decade(1-10). This was accompanied by further developments in the areas of biosensor(11-16), modeling(17-18), and identification(19).

The following advantages have been claimed for computer-controlled fermentation systems. :(a) Improved reliability and accuracy obtained by statistical methods or digital filtering enhance data acquisition function greatly. (b) Combination of several measurements and instant calculation make data analysis and interpretation powerful. (c) By introducing more sophisticated multivariable control methods, these systems expand opportunities tremendously for improved process control and optimization. (d) Improved productivity is achieved by rapid response or pre-programmed feed. (e) Finally, as a result of their analytical capabilities these systems can be used for the continuous evaluation and diagnosis of the status of fermentations.

Thanks to the recent advances in fermentation control the computer control of fermentation processes using microbial, mammalian, or plant cells, regardless of whether they are conventional or recombinant cells, is now a reality(20-23).

In this first part of a series of papers where the technical details of constructing hardwares for the computer control system and the application to the fermentation with recombinant cells are studied descriptions are made on the design of interfaces to connect computer with fermetor or other instruments and the modifications of already existing analogue controllers to accept more sophisticated digital control signals from computer.

## MATERIALS AND METHODS

### Computers

One unit each of IBM XT and PDP-11/03 respectively together with necessary peripherals such as CRT, keyboards, and a 24 pin printer(LQ 1000, Epson) were used.

#### Analogue Controllers

The existing analogue controllers from Marubishi for pH, temperature, and rpm were employed for interfacing.

#### DO Meter

As the analogue controller for DO was not available in our laboratory the existing DO meter was hooked up to the A / D converter for on-line digital feedback control.

#### Detector Probes

The typically known probes for pH, temperature, and DO were used. The infrared type $CO_2$ analyzer and the paramagnetic type $O_2$ analyzer, both from Fuji Denki, were used for gas analysis and also interfaced to computer. Although it is expected that the mass-spectrometer type off-gas analysis system may give more accurate, more stable, and more sensitive signals(11) it is hoped that the major information may be extracted by using the existing system. More accurate data will be reported later on when the mass-spectrometer system becomes available in a couple of years.

#### Fermentor

Marubishi fermentor of 2 liter or 5 liter volume is used.

#### Gas Pumps

Diaphragm type pumps from Enomoto Micro Pump Manufacturing Co., Ltd., were employed for sample gas delivery.

## RESULTS AND DISCUSSION

#### An Overview of System Construction

The instruments that send electrical signals of fermentation parameters are pH controller, DO meter, $O_2$ analyzer, $CO_2$ analyzer and built-in temperature controller. The built-in rpm controller need modification to be able to send electrical signal to computer and both of the built-in controllers, namely rpm controller and temper-

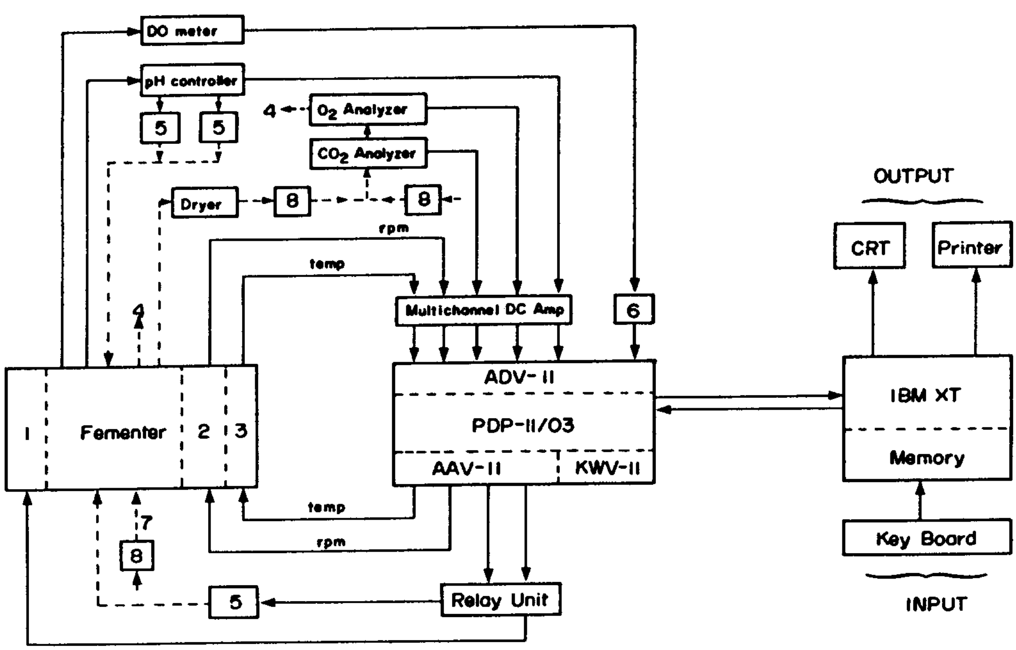


**Fig. 1. The Schematic Diagram of the Computer Controlled Fermentation System**

1. Heating band
2. Modified rpm controller
3. Modified temperature controller
4. Air vent
5. Peristaltic pump (acid, base, media, chemicals)
6. Isolation amplifier
7. Air inlet
8. Air pump (diaphragm pump)

**Table 1. The Instruments and Their Models**

| Instruments | Company | Model |
|---|---|---|
| Fermentor | Marubishi | MD-300 |
| pH Controller | Marubishi | MpH-1MC |
| DO Meter | Marubishi | MDO-1P |
| Antifoamer | Marubishi | MAF-1P |
| $O_2$ Analyzer | Fuji | Oxymat II |
| $CO_2$ Analyzer | Fuji | ZFD |
| Air Pump | Enomoto | GA-380 |
| Dryer | Perma Pure | MD-250 |
| Air Filters | Gelman | Acro 50 |
| Interfaces | Self - made | |
| Computers | DEC | PDP-11/03 |
| | IBM | XT |
| A/D Converter | DEC | ADV-11 |
| D/A Converter | DEC | AAV-11 |
| Line Time Clock | DEC | KWV-11 |

ature controller are modified to receive actuating signal from computer. All input signals must be conditioned to match impedance between computer and instruments and to adjust signal range for obtaining maximum resolution. When DO meter uses the same ground as pH controller, their mutual interference results in a serious complication, necessitating the isolation of the ground for one probe from that of the other. This problem is solved by using ground isolation amplifier. For actuating the peristaltic pumps for chemical induction or media feeding and the heating band for rapid thermal induction, additional interface such as relay unit is necessary.

The computer(PDP-11 / 03) has 32k words RAM, two 8" floppy disk drivers, 16 channels of analog-to-digital converter(ADC), 4 channels of digital-to-analog converter(DAC) and line time clock(LTC)for real time sampling. However, it does not have its own monitor system. Although it can execute input / output tasks, it is impossible to draw graphs or to display menu screen because PDP-11 / 03 supports only the line printer not the cathode ray tube. The inconvenience caused by this fact disappears when PDP-11 / 03 is connected to IBM XT by communicating with RS-232C serial interface card.

By using communication parameters of 4800 baud rate, even parity, 7 data bits and 1 stop bit, IBM XT is able to respond to all inputs and outputs. The results of fermentation is sent to IBM XT for such analytical jobs as graphics and every input action to PDP-11 / 03 is pursued through the key board of IBM XT. The schematic diagram of this construction is shown in Figure 1 and the used instruments in Table 1.

## Construction of Interfaces

### DC voltage amplifiers

The resolution of ADV-11 is 12 bits, i.e. the dc voltage ranging from 0V to 5.12V is converted to digital value between 0 to 4095.

Also this ADC requires a dynamic resistance of more than 100 M Ω and an input bias current below 50 nA. Since most instruments send dc voltage signals of different ranges and impedances signal conditioning is essential to secure maximum resolution and for optimal matching between ADC and instruments.

The signal from $CO_2$ analyzer is in the form of current between 0 and 1 mA and is converted to the dc voltage signal, 0-10 mV, using standard resistance. Both the pH controller and built-in temperature controller produce dc voltage signals of 0-10mV. Even though the DO meter produces a dc voltage output of 0-10 mV it interferes seriously with pH controller and must be processed by other amplifier as explained in the next section. $O_2$ analyzer also produces dc voltage yet with a range of 0 to 1V different from those of others. Consequently $CO_2$ analyzer, pH controller, and temperature controller use the same circuit(Figure 2) for singnal conditioning to have desired ranges, resistances, and currents. The voltage source from $O_2$ analyzer goes through a similar circuit yet with different amplification ratio. Every variable resistance is 10-turn potentiometer to adjust gain and output precisely.

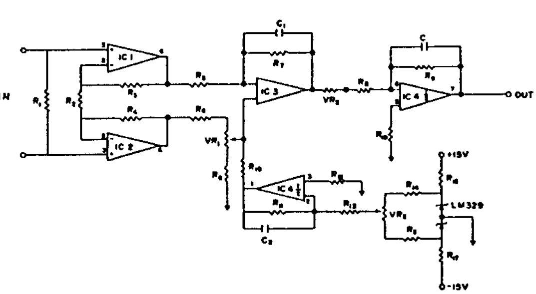


**Fig. 2. The Circuit Diagram of DC Amplifier used as Interface between Fermentor and ADC in Computer for High Amplification Ratio (500:1)**

#### Ground isolation amplifier

DO meter affects pH measurements seriously when both electrodes are submerged in the same broth and both instruments are earthed to the same ground.

Because ADV-11 provides only one ground the ground isolation between pH controller and DO meter is inevitable. The situation requires ground isolation by supplying two totally different power sources and grounds and also necessitates the matching of dynamic resistance, input current and voltage range. The proper applicable circuit diagram can be found easily in many electronic circuit books and the circuit used in the present study is shown in Figure 3.

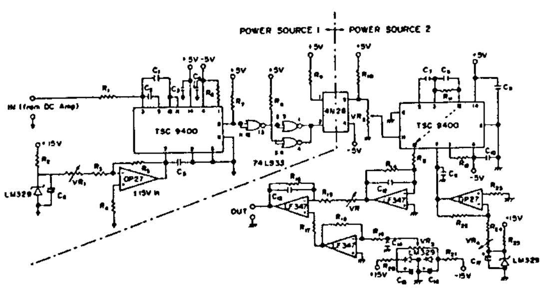

**Fig. 3. The Circuit Diagram of Isolation Amplifier used as Interface between DO meter and ADC of Computer (⏚ and ⏚ are different grounds and both isolated from chassis ground.)**

#### Modification of the built-in rpm controller

The value of rpm can be obtained form small generator attached coaxially to the driving induction motor for agitation.: the ac source obtained from the generator is rectified after transformation to proper range(Figure 4-A). The resulting dc voltage source is suitable for connection to ADC.

Utilizing the fact that the speed of induction motor can be controlled by changing the waveform of supplied ac power using triac, a new rpm controller which can accept actuating signal and produce signal for rpm value was designed. However, resonance which causes rpm oscillation made the new circuit unusable. Finding that elimination of this resonance throughout the whole rpm range was very difficult, modification of the originally built-in rpm controller was attempted.

For rpm control the second plan is to mechanically connect the rpm control knob in the built-in controller to servo motor balanced in the potentiometer circuit which accepts signal from DAC. However due to the characteristics of dc servo motor it needs different driving force for each direction. This causes other difficult problems to solve and this idea was also abandoned.

In the final attempt the variable resistance driven by the controller knob in the original built-in controller was replaced by CdS, a kind of photoconductive cells. At the expense of linearity the CdS in this modified controller was coupled photoelectrically to a light emitting diode(LED) whose light intensity varies with the voltage signal from DAC after proper current amplification. A simple experiment using tachometer gives a calibration curve to compensate for the non-linearity.

As in Figure 4-B the original built-in controller was not totally abandoned but instead a toggle switch was installed in order to use either of the two alternate controllers in case when such need occurs.

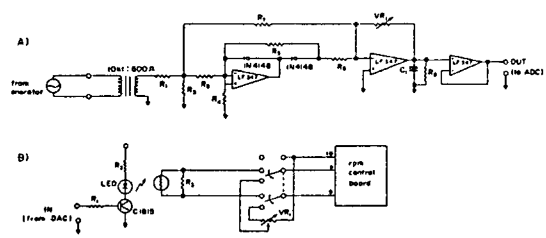

**Fig. 4. The Circuit Diagram of rpm Interfaces**
**(A) Interface between Fermentor and ADC for Data Acquisition**
**(B) Interface between DAC and rpm Control Board**

#### Modification of the built-in temperature controller

The built-in DAC, AAV-11, also has 12-bits resolution and it converts the number 0 and 4095 to a dc voltage between − 5.12 V and + 5.12V. Original control action remains unchanged, only small modification is introduced to accept actuating signal from DAC. The main purpose of this modification is the replacement of the existing variable resistance in the control knob with simple circuit that can convert the actuating signal to resistance in proportion to the magnitude of the signal. As in the case of the rpm controller the original built-in temperature controller can be used as it is with the temperature set point determined by control knob. Modified circuit diagram is shown in Figure 5.

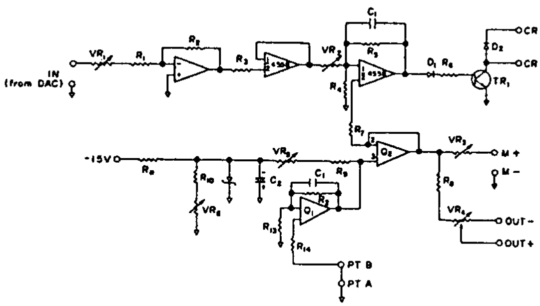


**Fig. 5. The Circuit Diagram of Modified Tempe-rature Controller Board**

#### Relay unit

Like the above two modified controllers, the use of DAC signal to actuate controllers needs careful consideration to match DAC and control unit. Major problem is current amplification because the drive capability of AAV-11 is only 50 nA which is insufficient to light LED as in modified rpm controller or to actuate relays. Relays are responsible for operating peristaltic pumps for chemical induction. For lighting LED the necessary current amplification can be obtained in one step as in Figure 4-A but relays need more current. Open collector type current amplification using TTL is suitable for this purpose if used in multi-steps as in Figure 6. The relay is from Matsushida(model DS 2V-DC 3V).

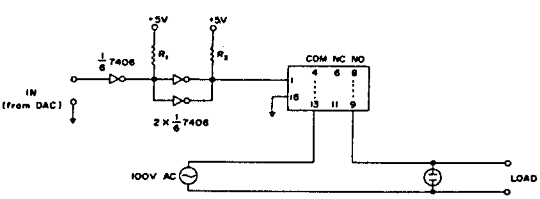


**Fig. 6. The Circuit Diagram of Relay Unit (represent only one channel)**

### ACKNOWLEDGEMENT

This study was financially supported by the generous grants from Ministry of Education and KOSEF, for which authors are deeply grateful.

### 요 약

rpm이나 온도와 같은 전형적인 발효 변수들을 위한 기존의 아나로그 제어장치들을 적절히 교정하여 마이크로콤퓨터에 연결시켰다. 임피던스 조정, 그라운드 격리, 및 시그날 범위 조정등의 여러가지 필요한 수정을 가하여 발효조계측기와 콤퓨터 사이의 인터페이싱을 성공적으로 수행할수 있었으며 결국 온도와 용존 산소를 온·라인 휘드·백 제어하고 유전자 재조합 발효공정을 온·라인 유도할 수 있었다.

### REFERENCES

1. C. Y. Choi(1979), *KOSEF Report*.
2. H. W. Park. and C. Y. Choi(1981), *K. I. Ch. E. J.*
3. T. Suzuki. T. Yasuda, T. Yamane, and S. Shimizu(1986), *J. Ferment. Technol.*, **64**, 63.
4. Y. H. Lee. M. Takashima, and R. E. Speece(1987), *Biotech. and Bioeng.*, **30**, 329.
5. M. R. Walach, M. J. Bazin, S. J. Pirt, and H. H. M. Balyuzi(1987), *Biotech. and Bioeng.*, **29**, 520.
6. M. Mota, J. M. Besle, P. Strehaiano, and G. Goma(1987), *Biotech. and Bioeng.*, **29**, 775.
7. T. Suzuki, T. Yasuda, T. Yamane and S. Shimizu(1986), *J. Ferment. Technol.*, **64**, 63.
8. J. M. Modak and H. C. Lim(1987), *Biotech. and Bioeng.*, **30**, 528.
9. J. Harmon. S. A. Svoronos, and G. Lyberatos(1987), *Biotech. and Bioeng.*, **30**, 335.
10. W. T. Wu, W. D. Jang, and S. C. Wu(1987), *The Chemical Engineering Journal.*, **36**, 81.
11. S. J. Coppella and P. Dhurjati(1987), *Biotech. and Bioeng.*, **29**, 679.
12. E. A. H. Hall(1986), *Enzyme Microb. Technol*, **8**, 651.
13. G. Davis, M. J. Green, and H. A. Hill(1986), *Enzyme Microb. Technol*, **8**, 349.
14. B. Birou, I. W. Marsion, and U. V. Stockar(1987), *Biotech. and Bioeng.*, **30**, 650.
15. D. J. Fink, and K. K. Chittur(1986), *Enzyme Micronb. Technol.*, **9**, 568.
16. M. A. N. Rahni, S. S. Kuan, and G. G. Guilbault(1986), *Enzyme Microb. Technol.*, **8**, 300.
17. B. O. Palsson, and A. Joshi(1987), *Biotech. and Bioeng.*, **29**, 789.
18. J. E. Bailey and D. F. Ollis(1986), *"Biochemical Engineering Fundamentals,"* McGraw Hill, New York.
19. G. Caminal, J. Lafuente, J. Lopez-Santin, M. Poch, and C. Solá(1987), *Biotech. and Bioeng.*, **29**, 366.

20. D. W. Zabriskie and E. J. Arcuri(1986), ***Enzyme Microb. Technol.***, **8**, 706.
21. T. V. Ramabhadran(1987), ***Trends in Biotechnology***, **5**, 175.
22. A. Stafford, P. Morris, and M. W. Fowler(1986), ***Enzyme Microb. Technol.***, **8**, 578.
23. H. E. Flores, M. W. Hoy, and J. J. Pickard(1987), ***Trends in Biotechnology***, **5**, 64.

**(Received September 22, 1989)**