한국생물공학회지 제4권 제3호
Korean J. Biotechnol. Bioeng.
Vol. 4. No. 3. 203-207(1989)

On-Line Induction of Fermentation with Recombinant Cells: Part II. Control Algorithm and Software Development

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

유전자 재조합 세포 발효의 온·라인 유도 : 제 2부. 제어 알고리즘 및 소프트웨어 개발

이 철 균 · 최 차 용
서울대학교 공과대학 공업화학과

ABSTRACT

Software for the on-line feedback control of such variables as DO and temperature was developed and tested successfully for a real fermentation system. Several aspects like PI, PID, DSC, and DDC were incorporated into the algorithm. Any kind of on-line computer control system can be successfully implemented without much difficulty.

INTRODUCTION

To achieve fully automated and optimized fermentor processs control, it is necessary to design control algorithm and develop the software after setting up the control hardware. However, the lack of reliable on-line sensors in monitoring the physical and biological parameters inside a fermentor, and also the lack of adequate mathematical models to formulate a meaningful process control and optimization have inhibited the advancement in fermentor control. In order to circumvent these problems estimated values from indirect measurements were used in numerous works, which inhibits successful application of computer control in fermentation processes.

Direct digital control(DDC)was introduced to chemical processes in the later part of 1950's. In the case of fermentation processes, however, DDC began to be attempted from late 1960's. Applications of various theories like maximum principle, energy and / or mass balances etc. were published, but limitation of reliable sensors and adequate models made the optimal fermentation control strategy inevitably heuristic or adaptive.

Dissolved oxygen tension is one of the most important parameters. However, DO is not considered a routinely controlled parameter like pH or temperature. For the control of DO, there must be some data about the dependence of DO upon media composition, pressure and temperature, the characteristics of DO electrode, and the volumetric oxygen transfer coefficient. The effects of pressure (1) and temperature (2) on dissolved oxygen tension can be found easily in early works. Almost all the principal parameters, e.g. the effect of electrolyte by Schumpe (3, 4), the time constant of DO electrode by Turner(5), and $k_L \cdot a$(6-12), were examined. Based on these fundamental studies numerous algorithms were proposed(13-17). No algorithm can regulate DO properly when the cell concentration exceeds certain level even if pure oxygen, low temperature, and high pressure are applied.

MATERIALS AND METHODS

The equipments used to perform laboratory tests to confirm the control software are the same as those listed in Part I.

RESULTS AND DISCUSSION

Temperature Control Algorithm

The original temperature controller(fermentor, MD-300, Marubishi, Japan)has high hysteresis which makes fluctuation of about ± 2℃ from the set point. This deviation can be eliminated by introducing digital setpoint control(DSC) having PI control action. The actuating signal is produced by the following equation.

$$\text{New Set Point} = \text{Old Set Point} + K\,\varepsilon$$

where K is controller gain and ε is the observed deviation from the desired temperature. This newly calculated set point becomes the temporary set point for the modified controller during the next control cycle. In the case of thermal induction, however, this integration action causes overshoot. In other words, when one attempts to elevate the temperature to 42℃ from 32℃ for thermal induction, temperature can reach as high as 45℃. Although temperature may return to 42℃ soon, the debilitation effect on cell growth can not be ignored. This debilitation effect can be precluded by using the characteristics of digital control which stops PI control action during the course of temperature change. Figure 1 represents the flow chart for temperature control algorithm.

Control Algorithm for Dissolved Oxygen Tension

Effect of Pressure on DO

Like other gases, the solubility of oxygen depends on pressure. Fortunately this solubility is so small that correction can be made easily by Henry's Law.

In this study, the guage pressure in the fermentor vessel is always 0.1kg / m^2 and the unit of DO is expressed

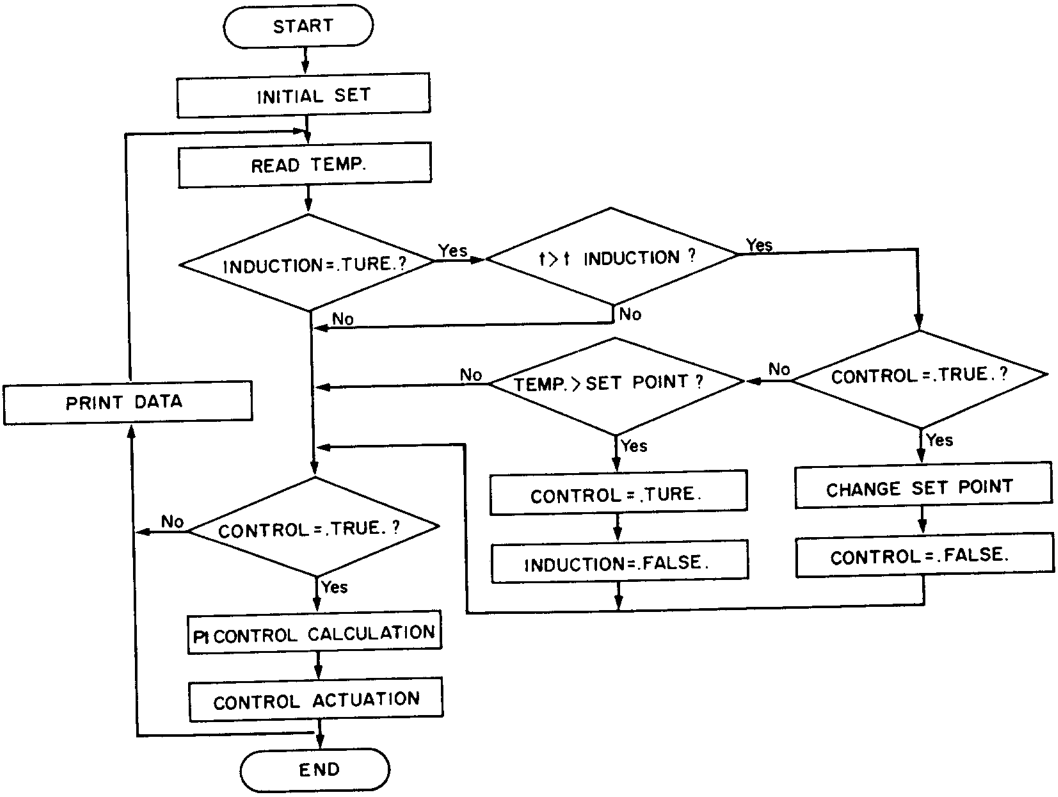

Fig. 1. The Flow Chart for Temperature Control Algorithm.

in terms of %. By measuring the saturated dissolved oxygen tension (DOT*)at the pressure, the correction can be done simply.

The used DO meter is manufactured by Marubishi (MDO-1P, Japan)

Effect of temperature and its correction

A number of works on the dependence of DO upon temperature were reported. In most fermentation processes, the temperature will be within the range of 0-50 ℃ and in this range Hitchman's relation (2) is widely adopted. The relation is as follows.:

$$\alpha (O_2) = 4.900 \times 10^{-2} - 1.335 \times 10^{-3} \times T + 2.759 \times 10^{-5} \times T^2 - 3.235 \times 10^{-7} \times T^3 + 1.614 \times 10^{-9} \times T^4$$

where the unit of T is centigrade. and α is the oxygen solubility.

This holds for pure water and hence this correction will reduce the effect of thermal induction only to some extent.

Control algorithm

At constant DOT, oxygen uptake rate(OUR) equals oxygen transfer rate(OTR). OTR can be expressed as follows.:

$$OTR = k_L{\cdot}a(pO_2^* - DOC)$$

where $k_L{\cdot}a$ is volumetric mass transfer coefficient, pO_2^* is partial pressure of oxygen at equilibrium and DOC is dissolved oxygen concentration which has same unit as pO_2^*.

Then continuous adjustment of the OTR value to the value of measured OUR by changing the $k_L{\cdot}$ a value is DO control itself. For pure water $k_L{\cdot}a$ is proportional to $N^{2.1}$(18) where N is the agitation rate in rpm. In the present study, however, cell density, ionic strength and composition of media vary during the course of fermentation. A rough control was made in order to know the relationship between rpm and OUR(Figure 2.). From this data at least in this case $k_L \cdot a$ is proportional to fourth power of N.

To adjust OTR to OUR, the required rpm is

$$N = \left(\frac{OUR}{7.714 \times 10^{-3}(pO_2^* - DOC)}\right)^{\frac{1}{4}}$$

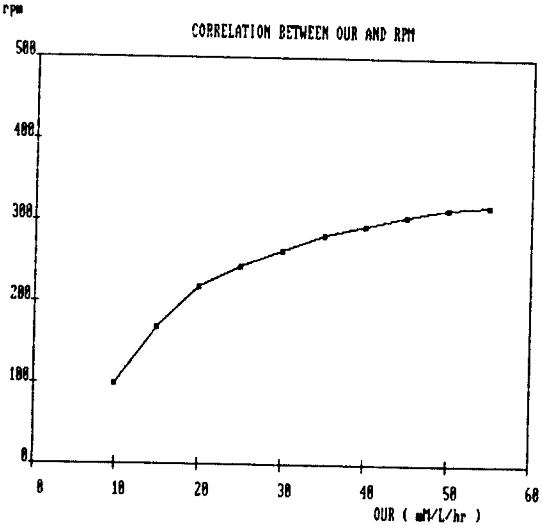

Fig. 2. The Calibration Curve between OUR and RPM

This is not exact solution because this stems from rough experiment and least square fit. Now by introducing PID control action, elimination of deviation error caused by this approximate estimation is achieved. That is, base rpm is calculated from measured OUR by above equation followed by PID action which has very small controller gain just enough to correct estimation error. The correction rpm is as follows.:

$$\text{New } n = \text{Old } n + K_1 \times \varepsilon + K_2 \times \tau_D \times \left(\frac{d\varepsilon}{dt}\right)$$

where K_1 and K_2 are controller gains, τ_D is time constant for derivative action, and ε is the error. Intergral action is included by iteration like temperature control algorithm. The applied rpm becomes N + New n. PID controller gains turned out to be too small to change the rpm profile any significantly. Figure 3 obviously shows the fact that rpm(upper line)mainly depends on OUR (lower line)except that minimum rpm is set to 125 for preventing settling of cells.

Software Development

FORTRAN IV is used as programming language bacause only this language supports the basic control subroutines (FORTRAN IV subroutine library, LSILIB, is supplied from DEC) for environments [e.g., Analog-to-Digital Converter (ADV-11, DEC), Digital-to-Analog Converter(AAV-11, DEC), Real Time Clock(KWV-11, DEC)] of the host computer (PDP-11/03, Digital

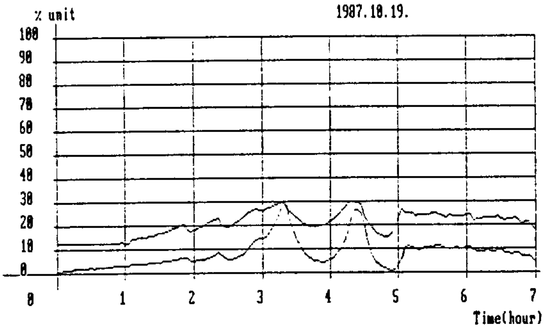

Fig. 3. The Profiles of OUR and RPM for 30% saturated DO Control

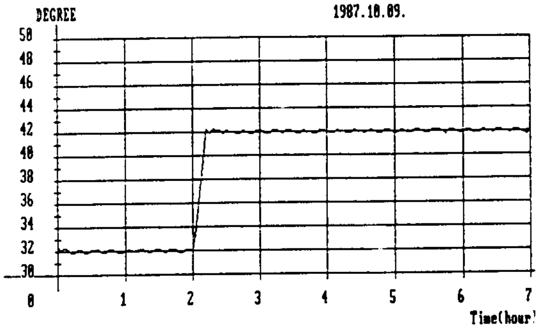

Fig. 4. The Controlled Profiles of Temperature

Equipment Co., U.S.A.). For some results of A / D conversion exhibit nonlinear behavior, the software contains experimentally pre-determined calibration curves. Using these curves pH, temperature, rpm, DO, CER and OUR are calculated and stored for next processing. To reduce the noise, all these input parameters are measured 600 times with the interval of 2 msec and averaged because the noise level of n measurements decreases by a factor of $1/\sqrt{n}$ (19).

The computer can control 4 independent parameters, namely, it has 4 channels of Digital-to-Analog Converter (DAC). In this software two channels are used to control the temperature including thermal induction and the dissolved oxygen, respectively. Heating band for rapid temperature elevation during thermal induction occupies another channel. The remaining channel actuates peristaltic pump for chemical induction or media feeding.

This program also has the capabilities to report processed data, to draw graphics from these data and to change operation parameters.

Applications of the Developed Software

With modified temperature controller which can accept set point from computer DAC, it becomes possible to introduce DSC by traditional PI algorithm and this eliminates the oscillation in the existing controller caused by hysteresis. Furthermore a little improvement using digital control technique removes typical overshoot of PI controller during thermal induction. Figure 4 demonstrates that this control algorithm is extremely suitable for on-line thermal induction and the oscillation reduces to a satisfactory extent. Original oscillation of ±2℃ decreased to give a slight fluctuation of only ±0.1℃. Also the typical overshoot resulting from conventional PI control disappeared perfectly.

The built-in rpm controller is also modified to be able to process the actuating signal. This signal is calculated by above DO control algorithm as follows: First, it estimates $k_L a$ value from OUR after correcting for the effect of DO change and from this it gives out proper rpm to give roughly same OTR as corrected OUR. Finally it pursues the fine tuning of rpm for getting rid of estimation error existing in the base rpm by PID control with small controller gain. This PID action also plays an important role in removing gas analyzer lag time. As can be seen in Figure 3, rpm peaks shift slightly to the left as compared with OUR peaks. This results from the difference of lag times between estimation and correction, i.e. base rpm is estimated by OUR or paramagnetic O_2 gas analyzer (Oxymat II, Fuji Electric, Japan) whose dead time is about 20 seconds but tuning rpm by PID control is calculated on the basis of deviation of DO from its set point. This deviation is measured by DO electrode whose dead time is much shorter than that of gas analyzer. Consequently the estimation error caused by long lag time in measuring OUR can be overcome by coupling with PID control having shorter lag time. The resulting DO profile is satisfactory enough to serve our purpose as shown in Figure 5.

The whole software is also satisfactory in the facts that is performs input of measurement variables without serious noises and mutual interferences and it can carry out output of controlled variable successfully including the temperature control whose profile may be determined any way one wants and the DO control with new algorithm. On-line thermal or chemical induction is possible with the system developed in the current study and the determination of induction point can be done on-line with any one of time, estimated biomass and etc. All

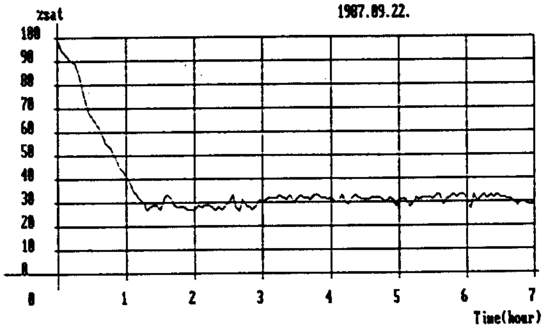

Fig. 5. The Controlled Profile of DOT for 30% saturated DO Control

experiments in Part III are done with the aids of the hardware of Part I and the software developed here.

ACKNOWLEDGEMENT

This study was made possible by the financial support from Ministry of Education and KOSEF, for which authors are very grateful.

요 약

용존 산소와 온도 같은 변수들을 온·라인 휘드백 제어할 수 있는 소프트웨어를 개발하여 실제 발효 공정에 성공적으로 적용하였다. PI, PID, DSC 그리고 DDC 같은 여러가지 면을 알고리즘에 포함시켰다. 발효공정을 위한 어떠한 형태의 온·라인 콤퓨터 자동제어도 별어려움 없이 성공적으로 실현 시킬 수 있었다.

REFERENCES

1. S. Glasston(1947), *in Thermodynamics for Chemist*, van Nostrand Co., N. J.
2. M. L. Hitchman(1966), in *Measurement of Dissoved Oxygen*, John Wiley & Sons, N. Y.
3. A. Schumpe and W. D. Deckwar(1984), ***Biotchnol. Bioeng.*, 21**, 1075.
4. A. Schumpe, G. Quicker, and W. D. Deckwar(1982), *Adv. Biochem. Eng.*, **24**, 1.
5. C. Turner, and J. A. Howell(1984), ***Biotechnol. Letters.*,6(4)**, 215.
6. L. Ju, and C. S. Ho(1984), ***Biotechnol. Bioeng.*, 27**, 1495.
7. C. S. Ho, L. Ju, and C. -T. Ho(1986), ***Biotechnol. Bioeng.*, 28**, 1086.
8. J. Vortruba, and R. Guthke(1985), ***Biotechnol. Letters.*, 7(4)**,267.
9. J. A. Spriet, J. Botterman, D. R. Debuyser, P. L. DeVisscher, and E. J. Vandamme(1982), ***Biotechnol. Bioeng.*, 24**, 1605.
10. J. G. Mooyman(1987), ***Biotechnol. Bioeng.*,29**, 180.
11. F. Vardar-Sukan(1985), ***Process Biochem.*, 20**, 181.
12. F. Vardar-Sukan(1986), ***Process Biochem.*, 21**, 40.
13. J. Chen, A. L. Tannahill and M. L. Shuler(1985), ***Biotechnol. Bioeng.*, 27**.
14. T. A. Clark, T. Hesketh and T. Seddon(1985), ***Biotechnol. Bioeng.*, 27**, 1507.
15. T. Yano, T. Kobayashi, and S. Shimizu(1981), ***J. Ferment. Technol.*, 59(4)**, 295.
16. D. Williams, P. Yousefpour, and E. M.H. Wellington(1986), ***Biotechnol. Bioeng.*, 28**, 631.
17. T. Suzuki, T. Yamane and S. Shimizu(1986), ***J. Ferment. Technol.*, 64**, 317.
18. H. Brauer, (ed.)(1984), in *Fundamentals of Biochemical Engineering, Biotechnology*, **2**, 587, Verlag-Chemie.
19. N. S. Wang, and G. N. Stephanopoulos(1984), in *Computer Applications to Fermentation Processes, CRC Critical Review*, **2(1)**, 51.

(Received September 22, 1989)