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On Energy Saving and Quality Improvement of Food Process (1). Applications of Hotwire Monitoring System for Food Biotechnology

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食品工程의 에너지 節減과 品質向上에 關한 硏究(1). 細線 加熱法의 食品 生物工學에의 應用

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ABSTRACT

Application of the Rheocatch Hotwire Monitoring System for food biotechnology process was evaluated. The growth of microogranism, *E. coli* (JM 83 and Sigma) and *Corynebacterium glutamicum*, were monitored. in the fermentor. The cell growth could not be detected the temperature differences between the hotwire and samples(ΔT) as indicated by the monitoring system during the fermentation processes. The cell concentration of less than 2g / dl was not sufficient to generate the measurable temperature difference in the fermentor.

In order to calibrate the Rheocatch Monitoring System, the temperature difference as a function of solute concentration (microbial cells, sodium cholide, sucrose and dextran) was studied. The relationship between ΔT and the concentration of microbial cells, sucrose and dextran can be expressed in a power series. Further studied with dextran indicated that viscosity and / or kinematic viscosity increase exponentially with an increase in ΔT This is regardless of the concentration and molecular weight of dextran. ΔT linearly increases with the logarithm of molecular weight, while the logarithm of viscosity and the logarithm of kinematic viscosity increase with the logarithm of molecular weight.

INTRODUCTION

Online monitoring systems are the instruments probably in greatest demand for process and quality control in food biotechnology. The objective of this study is to evaluate the Rheocatch Hotwire Monitoring system for applications in biotechnology processes; specifically, monitoring the growth of cells and yield of biological products during fermentation in the bioreactor. For initial evaluation, ΔT, at constant applied current density, was used to monitor the growth of *E. coli* and *Corynebacterium glutamicum* fermentation in the fermentor. In addition, experiments necessary to evaluate the relationship between ΔT(the temperature difference between the hotwire and the sam-

ples) and the type and concentration of biological products were carried out. These experiments also gave us preliminary indications of the sensitivity of the Hotwire system under various solutions and processing conditions. This report presents the change in ΔT, at a constant applied current density, as a function of concentration of microbial cells, sodium chloride, sucrose and dextran in the absence of convective heat transfer.

MATERIALS AND METHODS

Fermentation Monitor

In the MBR Bio Reactor (Biotech System, Woodbury, N. Y.), the fermention of 1) *E. coli* (JM83) containing the pUCB plasmid pS18 in 11 liters of LB medium at 30℃, pH 7 and 1000 rpm was monitored with 0.2 amp applied current density, 2) *E. coli* (Sigma, St. Louis, MO) in 11 liters of medium (containing 16 g trypton, 10 g yeast extract and 5 g NaCl per liter) at 37℃, pH7 and 1000 rpm was monitored with 0.3 amp applied current density, and 3) *Corynebacterium glutamicum* in 11 liters of minimal MCGC medium (containing 10 g glucose, 1 g citrate, 6 g Na_2HPO_4, 3 g KH_2HPO_4, 1 g NaCl, 4 g NH_4SO_4 and trace elements per liter) at 30℃, pH7 and 1000 rpm was monitored with 0.3 amp applied current density with the Rheocatch Hotwire Monitoring System (Snowbrand Milk Products Co., Ltd., Saitama, Japan). The concentration of the cell in the fermentor was determined by spectroscopy every hour. Solutions of *E. coli* (Sigma) were collected every hour to measure ΔT at 37℃ with 0.3 amp applied current density and in the absence of convective heat transfer.

Effect of Microbial Cell Concentration

Dry E. coli (Sigma, St. Louis, MO)was washed in deionized water and centrifuged at 1000 g for 1 hour. The procedure was repeated three times. The dry cell content was determined by drying 1 ml of concentrated cell solution in an oven at 90℃ until the weight was constant. The rest of the concentrated cell solution was suspended in the medium (containing 16 g trypton, 10 g yeast extract and 5 g NaCl per liter)to obtain various cell concentrations.

Concentrated *E. coli* (JM83), 23.2% dry cell weight was also obtained from the fermentation in the LB medium (containing 10 g trypton, 5 g yeast extract and 10 g NaCl per liter). The final dry cell concentration in the fermentor was determined by spectroscopy to be 0.8 g dry cell per liter. The cells were re-suspended in the medium to obtain various cell concentrations. Then, ΔT of various cell concentrations was measured by the Rheocatch Hotwire Monitoring System at 25℃, with 0.3 amp applied current density and in the absence of convective heat transfer.

Effect of Salt and Sucrose Concentration

The ΔT of different solute concentrations, 0.003M to 1 M NaCl(Sigma, St. Louis, MO)in deionized water and 0.1 g / dl to 17 g / dl sucrose (Amstar Sugar Corp., New York, N. Y) in deionized water, was measured by the Rheocatch Hotwire Monitoring System at 25℃, with a 0.3 amp applied current density and in the absence of convective heat transfer.

Effect of Concentration and Molecular Weight of Polymer

The ΔT of different concentations of dextran(Sigma, St. Louis, MO) of molecular weight 2,000,000, ranging from 0.1 g / dl to 5 g / dl in 0.2M NaCl, was measured by the Rheocatch Hotwire Monitoring System at 25℃, with a 0.3 amp applied current density and in the absence of convective heat transfer. The ΔT of dextrans of various molecular weights (9,000, 35,600, 73,400, 48,000 and 2,000,000) was measured by the Rheocatch Hotwire Monitoring System at 25℃, with 0.2, 0.3 and 0.4 amp applied current density and in the absence of convective heat transfer. The viscosity of dextran solutions was determined using shear rate ranging from 1 to 300 sec^{-1} with the Bohlin Rheometer System(Bohlin AB, Lund, Sweden) at 25℃. The density of the dextran solutions was determined with a pycnometer using toluene as the standard. The kinematic viscosity of the dextran solutions was calculated from the ratio of the lower Newtonian viscosity and the densit. Then, the relationship between ΔT and the viscosity of the lower Newtonian region or kinematic viscosity was evaluated.

RESULTS AND DISCUSSION

Fermentation Monitoring

The growth of microorganisms, *E. coli*(JM 83 and

Sigma) and *Corynebacterium glutamicum*, reached was the concentration less than 2 g / l in the fermentor. This concentration of cells is not high enough to be detected by the change in ΔT (Figures 1, 2 and 3). Furthermore, the mixing and other conditions which are not well defined in the fermentor made it more difficult to obtain ΔT consistent and significant ΔT from which the difference could be measured. For an example, the mixing in the fermentor, convective heat transfer takes place and ΔT decreases 0.4℃ as rpm increases from 0 to 1000 at 0.3 amp applied current density due to the increase in heat transfer (Figure 2). Such a decrease will counter act the increase, if any, of ΔT due to the increase in the concentration of the cells.

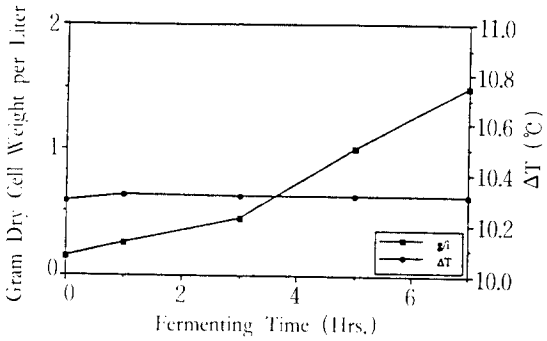

Fig. 1. The growth of *E. coli*(JM 83) as measured by ΔT (Current Density=0.2 amp, Mixing Speed=1000rpm).

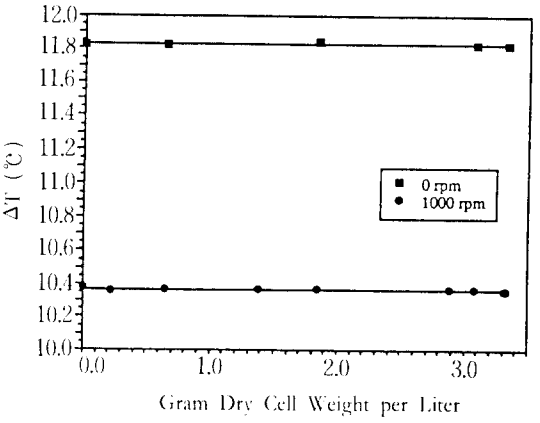

Fig. 2. The growth of *E. coli* (Sigma) as measured by ΔT(Current Density=0.3 amp).

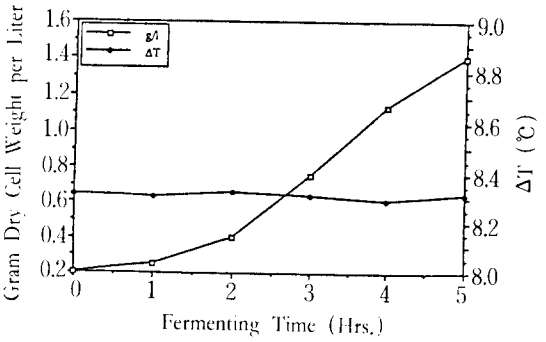

Fig. 3. The growth of *Corynebacterium glutamicum* as measured by ΔT (Current Density=0.3 amp, Mixing Speed=1000 rpm).

Effect of Microbial Cell Concentration

The increase in microbial cell concentration results in an increase in ΔT (Figures 4 and 5). The relationship between ΔT and microbial cell concentration can be expressed as:

for E. coli(Sigma) at the concentration ranging from 40 to 70 g dry cell per liter (Figure 4)

$$\Delta T_c = 11.880(1+0.00003C+0.00002C^2) = 11.880 + 0.0004C + 0.0002C^2, \text{ and}$$

For *E. coli*(JM83) at the concentration ranging from 0.8 to 22 g dry cell per liter (Figure 5)

$$\Delta T_c = 11.832(1+0.004C) = 11.832 + 0.0473C$$

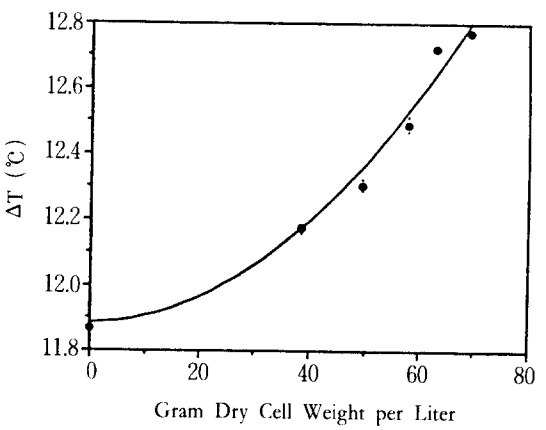

Fig. 4. Concentration of *E. coli* (Sigma) as measured by ΔT (Current Density=0.3 amp).

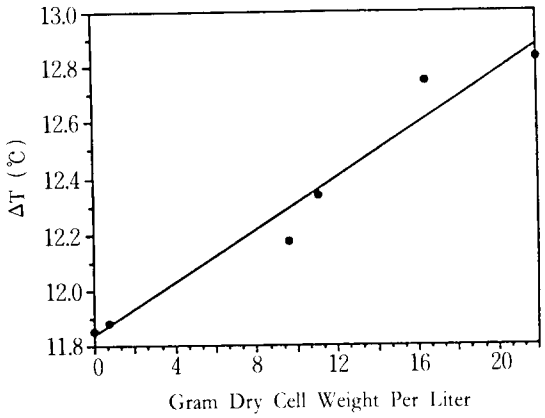

Fig. 5. Concentration of *E. coli* (JM 83) as measured by ΔT (Current Density= 0.3 amp).

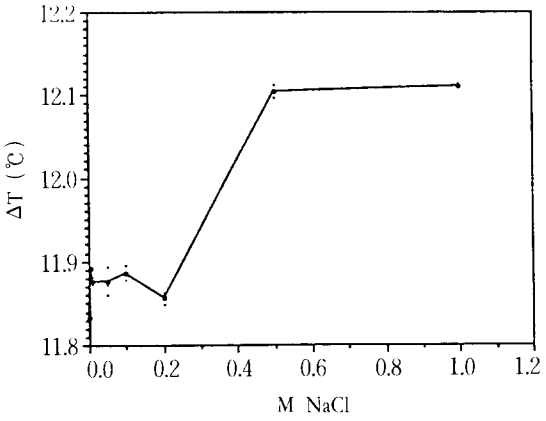

Fig. 6. Concentration of NaCl in deionized water as measured by ΔT (Current Density=0.3 amp).

where ΔT_C=the temperature difference between the hotwire and the microbial cell solution of C concentration(℃)

C=concentration (g dry cell / l)

Figure 5 shows that ΔT of *E. coli* (JM83) concentration obtained in the fermentor, 0.8 g dry cell / l, gives ΔT not significantly higher than that of the medium. The actual cell contains 80% water; 0.8 g dry cell / l corresponds to 4 g cell / l. *E. coli* has a spherical shape, of which the shape factor is 2.5. The viscosity can be estimated from Einstein's equation(Einstein, 1911) as:

$$\eta=\eta_0(1+2.5c)$$

where η=the viscosity of microbial cell solution of C concentration (mPa · s)

η_0=the viscosity of medium (mPa · s)

C = microbial cell concentration (g cell / ml)

The relative viscosity, η / η_0, of 0.8 g dry cell / l or 4 g cell / l is estimated to be 1.01. In the fermentation experiment, the growth of the cells was lower than 2 g dry cell / *l*, which gives relative viscosity of the cell solution lower than 1.03. Therefore, the increase in viscosity, lower than 3% from the viscosity of the medium, may be not high enough to affect an increase in ΔT, and especially so when a nember of other variables, such as mixing, aeration, composition of medium etc., affect Δt.

Effect of Salt and Sucrose Concentration

A large variation in the value of ΔT_C was obtained for

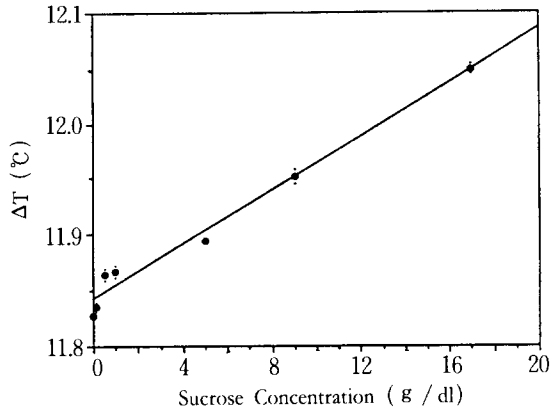

Fig. 7. Concentration of sucrose in deionized water as measured by ΔT (Current Density=0.3 amp).

NaCl solutions at the low concentration of 0.003 to 0. 2 M (Figure 6). The change in ΔT_C with concentration in this range does not always increase with concentration as expected, since the kinematic viscosity increases and the thermal conductivity decreases with an increase in concentration: see Appendice (Bingham and Jackson, 1 917 and Perry, 1950).

For sucrose, ΔT increases linearly with the concentration in the concentration ranging from 0.1 to 17 g / dl, and ΔT can be expressed as a function of concentration as Figure 7):

$\Delta T_C = 11.841(1+0.001C) = 11.841+0.012C$)

where ΔT_C= the temperature difference between the hotwire and the sucrose solution of C concentration(℃)

C= concentration (g / dl)

Effect of Concentration and Molecular Weight of Dextran

The increase in concentration of dextran, at M.W. of 2,000,000 and in the range of 0.1 to 5 g / dl in 0.2 M NaCl, cause an increase in ΔT in the same way as microbial cells and sucrose(Figure 8) and the expression is

$\Delta T_C = 11.838(1+0.013C) = 11.838+0.149C$

where ΔT_C=the temperature difference between the hotwire and the dextran solution of C concentration (℃)

C=concentration(g / dl)

The increase in ΔT with molecular weight of dextran, 5 g / dl in 0.2M NaCl, can be expressed as (Figure 9):

$M = 2.8\times10^{-31}\exp(78.2\Delta T_M / 5.20) = 2.8\times10^{-31}\exp(15.05\Delta T_M)$ for 0.2 amp

$M = 2.4\times10^{-43}\exp(105.9\Delta T_M / 11.81) = 2.4\times10^{-43}\exp(8.97\Delta T_M)$ for 0.3 amp

$M = 1.2\times10^{-43}\exp(106.9\Delta T_M / 21.21) = 1.2\times10^{-43}\exp(5.04\Delta T_M)$ for 0.4 amp

where ΔT_M=the temperature difference between the hot wire and the solution of dextran of M molecular weight(℃)

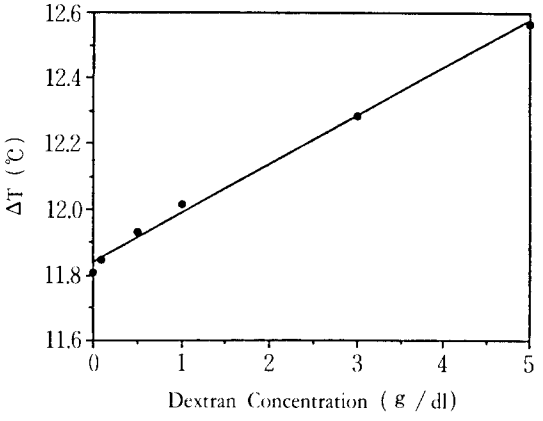

Fig. 8. Concentration of dextran (M.W. =2,000,000) in 0.2 M NaCl as measured by ΔT (Current Density = 0.3 amp).

M=molecular weight of dextran

In the presence of 0.1 g / dl dextran (M.W.=2,000,000), ΔT increases 0.04℃ from that of the solvent measured at 0.3 amp applied current density, while both viscosity and kinematic viscosity are the same as the solvent when measured by the rotational viscometer (Figures 8 and 10). This suggests that the Hotwire Monitoring System is more sensitive and can detect a smaller change in concentration of dextran than the commercial rotational viscometer considered acceptable. The concentration affects the viscosity or kinematic viscosity of dextran solutions up to 5 g / dl as (Figure 10):

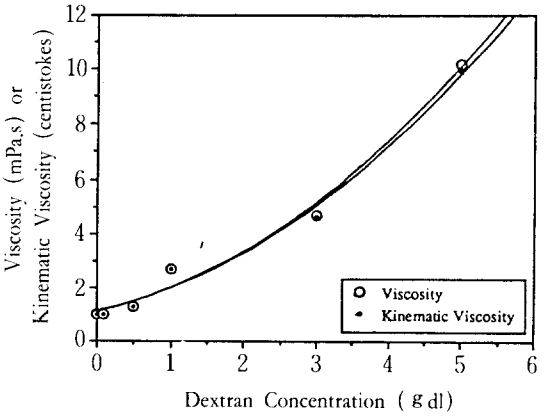

Fig. 9. Increase in viscosity and kinematic viscosity with concentration of dextran (M.W.=2,000,000) in 0.2M NaCl.

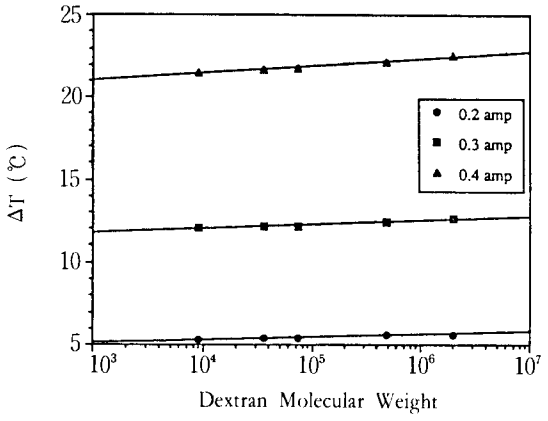

Fig. 10. Increase in ΔT with molecular weight of dextran (5.0 g / dl in 0.2M NaCl).

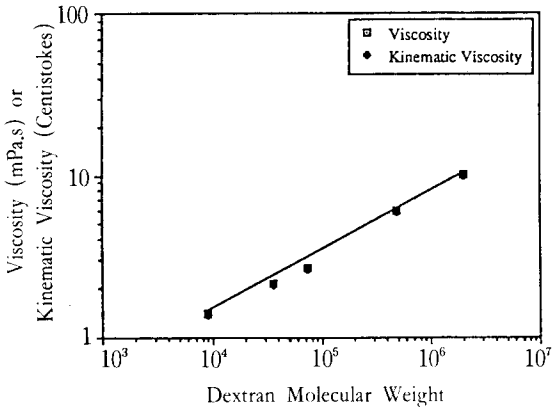

Fig. 11. Increase in viscosity and kinematic viscosity with molecular weight of dextran (5.0 g / dl in 0.2 M NaCl).

$\eta_C=1+0.64C+0.23C^2$

$\eta_{kC}=1+0.65C+0.22C^2$

where η_C=viscosity of solution at C concentration (mPa · s)

η_{kC}=kinematic viscosity of solution at C concentration(centistokes)

The power law equation can be used to describe the increase in viscosity or kinematic viscosity of 5 g / dl dextran in 0.2M NaCl with molecular weight as(Figure 11):

$\eta=0.044M^{0.37}$, and

$\eta_k=0.042M^{0.37}$

where η=viscosity of solution (mPa · s)

η_k=kinematic viscosity of solution (centistokes)

M= molecular weight of dextran

Figure 12 shows that viscosity and / or kinematic viscosity increase exponentially with an increase in ΔT. This relationship holds regardless of the concentation and molecular weight of dextran and is expressed as:

$\eta=9.35\times10^{-17}\exp(3.12\Delta T)$, and

$\eta_k=1.44\times10^{-16}\exp(3.09\Delta T)$

In the presence of solute, ΔT increases due to an increase in viscosity and / or a decrease in thermal conductivity of the solution. The result of this study suggests that the relationship between ΔT and solute concentration may be expressed in a power series in the same way as the relationship between viscosity and concentration (Philippoff, 1942)as

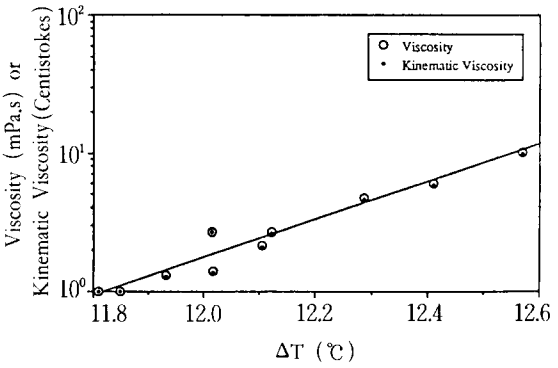

Fig. 12. Relationship between ΔT and viscosity and kinematic viscosity ofdextran in 0.2M NaCl.

$\Delta T_C=\Delta T_0(1+\alpha C+\beta C^2+\cdots\cdots)$

where ΔT_C=the temperature difference between the hotwire and the solution of C concentration (℃)

ΔT_0=the temperature difference between the hotwire and the solvent (℃)

C=concentration (g / dl for sucrose and dextran solutions, g / l for cell solution)

α,β,χ,δ=constants which may depend on solute, solvent and the hotwire probe

The constant α should represent the intrinsic property of the solute, and the constants β,χ, and δ should relate to the solute-solvent interaction and to the solute-solute interaction.

The relationship between ΔT and molecular weight of polymer at the constat concentration may be expressed as an exponential function as:

$M=a\exp(b\Delta T_M / \Delta T_0)$

where ΔT_M=the temperature difference between the hotwire and the solution of dextran of M molecular weight (℃)

ΔT_0=the temperature difference between the hotwire and the solvent (℃)

M=molecular weight of dextran

a and b=constants which may depend on solute, solvent, the hotwire probe and the applied current density

The values of "a" and "$b / \Delta T_0$" decrease, but the value of "b" increases with an increase in the applied current

density.

The concentration and molecular weight can be determined from ΔT using these basic relationships. The relationship between the constants in these expressions and the intrinsic properties of solute, such as size, shape and conformation, needs to be evaluated for application of the Hotwire Monitoring System to food biotechnology.

The relationships between viscosity, kinematic viscosity, and molecular weight of dextran at a constant concentration are power law relationships as:

η or $\eta_k = hM^f$

where η=viscosity of solution (mPa · s)

η_k=kinematic viscosity of solution (centistokes)

M=molecular weight of dextran

hand f=constants dependent on polymer-solvent interaction

The relationship between ΔT and viscosity or kinematic viscosity of polymer is a exponential function as:

η or $\eta_k = k\ \exp(n\Delta T)$

where ΔT= the temperature difference between the hotwire and the solution of dextran (℃)

η=viscosity of solution (mPa · s)

η_k=kinematic viscosity of solution (centistokes)

kand n= constants which may depend on type of polymer, solvent, the hotwire probe and the applied current density (independent of molecualr weight and concentration for the same polymer)

Therefore it is possible to determine the viscosity of dextran solution from the temperature difference between the hotwire and the solution base on this relationship.

CONCLUSIONS

1. The growth of microorganism, *E. coli* (JM 83 and Sigma) and *Corynebacterium glutamicum*, cannot be monitored by the change in ΔT during the fermentation. That is mainly because the concentrations of the cells are too low (less than 2 g dry cell / *l*) in these particular experiment.
2. The relationships between ΔT and concentration of microbial cells, sucrose and dextran can be expressed in a power series as follows:

   $\Delta T_C = 11.880 + 0.0004C + 0.0002C^2$ for E. coli (Sigma) at 40−70 g dry cell per liter,

   $\Delta T_C = 11.832 + 0.0473C$ for *E. coli* (JM 83) at 0.8−2 2 g dry cell per liter,

   $\Delta T_C = 11.841 + 0.012C$ for sucrose solution in deionized water up to 17 g / dl and

   $\Delta T_C = 11.838 + 0.149C$ for dextran (M.W. 2,000,000) up to 5 g / dl in 0.2 M NaCl.
3. For 5 g / dl dextran solution in 0.2M NaCl, the relationship between molecular weight and ΔT are

   $M = 2.8\times10^{-31}\exp(15.05\Delta T_M)$ for 0.2 amp

   $M = 2.4\times10^{-43}\exp(8.97\Delta T_M)$ for 0.3 amp

   $M = 1.2\times10^{-43}\exp(5.04\Delta T_M)$ for 0.4 amp

For dextran in 0.2 M NaCl, viscosity or kinematic viscosity increase exponentially with an increase in ΔT as follws:

$\eta = 9.35\times10^{-17}\ \exp(3.12\Delta T)$, and

$\eta_k = 1.44\times10^{-16}\ \exp(3.09\Delta T)$

요 약

본 실험에서는 종래의 역학적 특성에 의한 점도계와 달리 열전달 현상을 이용하여 비파괴 측정 및 on line이 가능한 세선 가열장치를 식품 생물공학 공정에 응용하여 에너지 절감 및 품질향상을 위한 기초실험을 하였다.

발효공정 중의 *E. coli*(JM 83 and Sigma)와 *Corynebacterium glutamicum*의 세포 배양 농도는 이 장치에 나타난 열선과 시료의 온도차 (ΔT)로 검진할 수 없었다.

발효조에서 2 g / dl 이하의 세균의 농도는 측정가능한 온도차를 발생시키는데 충분하지 못하였다. 본 장치를 보정하기 위하여, 용질 농도(미생물 세포, NaCl, sucrose, dextran)의 기능으로서의 온도차 ΔT를 검토하였다. ΔT와 미생물 세포, sucrose와 dextran의 농도의 상관관계는 power series(멱수 계열)로 나타났다.

dextran의 경우, 점성계수(동점도계수)는 ΔT의 증가에 따라 지수적으로 증가하였는데, 이것은 dextran의 농도와 분자량과는 무관함을 나타내며, 점성계수와 동점도계수의 대수는 분자량의 대수에 비례한 반면, 온도차(ΔT)는 분자량의 대수에 따라 선형적으로 증가하였다.

감 사

본 연구는 1987년도 문교부 대학교수 해외 연수 계획에 의해 미국 MIT 응용 생물학과에 방문교수로 근무(1988~1990)중에 수행 되었음을 밝히며, 지원해 주신 문교부, MIT와 Snow Brand Milk Co. Ltd.에 감사 드립니다.

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APPENDICE

Kenematic Viscosity and Thermal Conductivity of NaCl (Brigham and Jackson, 1917 and Perry, 1950)