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ANALYSIS OF CULTURE FLUORESCENCE BY A FIBER-OPTIC SENSOR IN *NICOTIANA TABACUM* PLANT CELL CULTURE

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Abstract—The on-line sensing of viable cell weight in plant cell culture process is applied to analysis and control of process. The fiber-optic fluorescence sensor was constructed to measure the NADH-dependent fluorescence in *Nicotiana tabacum* plant cell culture and the analysis of fluorescence signal was done to be correlated with the viable cell weight. The structured kinetic model for cell growth was proposed to estimate the theoretical viable cell weight. The dimensional analysis was proposed for the interpretation of fluorescence signal, in which the path length, the inner filter effect and the hydrodynamic conditions were considered as the key factors on fluorescence signal. The dimensional analysis and empirical correlation of fluorescence signal to viable cell weight was applied to the interpretation of the detected fluorescence signal during cultivation. The proposed interpretation of fluorescence signal using dimensional analysis was well correlated with the viable cell weight estimated by the structured kinetic model as well as by empirical correlation.

Key words: *Nicotiana tabacum, Fiber-Optic Sensor, NADH Fluorescence, Dimensional Analysis, Structured Kinetic Model*

INTRODUCTION

In recent years, it has been apparent that the measurement of biological parameters is required for efficient cultivation, monitoring, control and modeling in biotechnology. Until now, there was lack of reliable sensors for the measurement of biological parameters, which could be used routinely and easily in monitoring systems for biotechnological process [Humphrey et al., 1989]. Since on-line sensing of viable cell mass could be applied to the analysis and control of the bioprocess, various detection systems and signal interpretations have been developed to detect viable cell mass [Humphrey et al., 1989; Wang and Simmons, 1991]. Detection of viable cell mass could be applied to the prediction of cell growth and construction of strategies to produce a useful metabolite. To measure NADH-dependent fluorescence inside cells has been widely used as basis of construct an spectroscopic detection system for the measurement of viable cell mass since viable cells have NADH which commonly participates in various intercellular redox reaction [Copella and Rao, 1990; Li et al., 1991]. Intercellular NADH can be measured by using its fluorescence property which it emits 460 nm light at the excitation of 340 nm [Duysens and Amesz, 1957; Baeyens et al., 1991]. NADH-dependent fluorescence can be correlated with viable cell mass and applied to the real-time detection of viable cell mass in cell culture. This was the basis of the two fluorometric instruments (BioChem Technology and Ingold Co.) presently available commercially [Wang and Simmons, 1991]. Since its first application in a bioreactor, fluorescence sensor has been used for the measurement of cell mass in process analysis because it gives on-line *in situ* information without damaging to cells.

Recently, optical fiber has been widely applied to optical sensors as the light guide in various industrial fields due to its outstanding advantages [Abolel-Latif and Guidbault, 1990]. Fiber-optic sensor is easy to be miniaturized, which can lead to the development of very small and flexible instrumentation for the easy installation in bioreactor. Furthermore, analyses can generally be performed in real time and the coupling of the sensors for different target materials may allow to monitor many kinds of analytes simultaneously. Instead of the commercially available large sensors consisted of sets of optical parts, the fiber-optic fluorescence sensor has been developed for the reduction of size, easy installation, multi-point detection and simultaneous measurement of other analytes in bioreactor [Junker et al., 1988; Abolel-Latif and Guidbault, 1990; Anders et al., 1990].

On-line measurement of culture fluorescence during cultivation of various microorganisms has been investigated by a number of researchers [Ristroph et al., 1977; Groom et al., 1988; Peck and Chynoweth, 1990]. When the intracellular NAD(P)H-dependence fluorescence measurement was interpreted with the help of the pathway rate analysis, additional information on metabolic function was obtained in suspended [Reardon et al., 1977] and immobilized cell [Reardon and Bailey, 1989]. The fluorescence probe could be used to detect differences in the metabolic demands made on an over-producing recombinant organism [Walker and Dhurijati, 1989]. Control strategies for continuous bioprocesses based on biological activity was investigated with on-line measurement of intracellular NADH-dependent fluorescence as an immediate indication of onset of glucose repression [Meyer and Beyeler, 1984]. Though the application of fluorescence sensor to estimate cell mass has been examined for many organisms, the estimation of viable cell weight for plant cell culture by fluorescence sensor has not been reported.

However, interpretation of culture fluorescence is not straightforward since culture fluorescence is a function of cell concentra-

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tion, physiological state and also is confounded by the high background fluorescence of media typically used in bioreactors [Li and Humphrey, 1989; Li et al., 1991; Wang and Simmons, 1991]. So the fluorescence sensor could not be used widely in cell culture because of no simple and accurate interpretation method of fluorescence signal though culture fluorescence based on NADH has great potential as a tool with which to analyze and control the cell culture process. Optical interferences such as scattering, absorption or transmittance are involved in detecting viable cell mass, which may cause intrinsic nonlinearity in the correlation with fluorophore concentration. Therefore, the proper interpretation of fluorescence signal should be introduced to increase the performance of fluorescence senor. The fluorescence signal is significantly affected by a number of factors which are the absorption of excitation and emission light by the solvent and other absorbing species (inner-filter effect), the background signal, the light path length in the bioreactor, the fluorescence yield and so on [Humphrey et al., 1989; Wang and Simmons, 1991]. To investigate the above effects, the interpretation models of fluorescence signal in cell culture have been proposed for commercialized non-fiber optic fluorometer [Li and Humphrey, 1989; Li et al., 1989; Wang and Simmons, 1991] but the model in plant cell culture has never been reported for a fiber-optic sensor.

In this study, a fiber-optic sensor using the bifurcated optical fiber is constructed to measure NADH-dependent fluorescence and is applied to the monitoring of culture fluorescence of *Nicotiana tabacum* plant cells. The structured kinetic model is proposed to estimate the theoretical viable cell mass. The interpretation technique using the dimensional analysis is proposed based on three important factors which are inner-filter effect, path length and hydrodynamic condition. It is investigated that the interpretation of fluorecence signal using dimensional analysis is related with the viable cell weight estimated by the structured model as well as empirical correlation.

THEORY

1. Structured Kinetic Model

Structured kinetic model for *Nicotiana tabacum* cell was developed to estimate the theoretical value of viable cell mass. It was based on the structural classification of cells to viable cells and nonviable cells. Viable cells are further divided into two types, active-viable (dividable) cells and nonactive-viable (resting) cells. Active-viable cells can become nonactive viable cells which then can further degenerate to dead cells when the depletion of sugar occurs. Variables are described as active-viable cell dry weight X_{ad}, nonactive-viable cell dry weight X_{nd}, viable cell dry weight X_{vd}, nonviable cell dry weight X_{dd}, total cell dry weight X_d, fresh cell weight X_f, activity A and viability, v. Activity is defined as the fraction of the active-viable cell weight to viable cell weight and viability is defined as the fraction of the viable cell weight to total dry cell weight.

1-1. Active-viable Dry Weight Equation

After the inoculation, lag phase is observed, which is required for cells to adapt change of environment. In cell culture of *Nicotiana tabacum*, lag-time, t_L, is about one day and the following equation is used for μ_s;

$$\mu_s = \frac{\mu_{max} S}{K_s + S} \tag{1}$$

where K_s is a Monod type constant, μ_{max} is the maximum growth rate. It is assumed that the rate of loss of cell activity is proportional to viable cell dry weight and the nonactive-viable cells do not consume substrates for growth. Then the quantity of active-viable cell is determined based on substrate consumption rate.

Mass balance for active-viable cell dry weight is as;

$$\frac{dX_{ad}}{dt} = \left[1 - \exp\left(-\frac{t}{t_L}\right)\right] \frac{\mu_{max} S}{K_s + S} X_{ad} - k\phi X_{vd} \tag{2}$$

where

$$\phi = \frac{1}{A} \frac{X_f}{X_d} \frac{1}{1 + S/X_d} \tag{3}$$

The first term represents the growth of glucose utilization and the second term represents the activity loss. The exponential term is used to express the lag time [Moser, 1988]. The variable, ϕ, represents the loss of activity due to susceptibility of shear, osmotic effect and sugar depletion effect since the total sugar concentration, S, and ratio of X_f to X_d means cell expansion.

1-2. Nonactive-viable Dry Weight Equation

It is assumed that the nonactive-viable dry weight increases at a rate that is indirect proportional to the cell viability.

$$\frac{dX_{nd}}{dt} = k\phi X_{vd} - k_d \frac{X_f}{v} \tag{4}$$

The first term represents the loss of cell activity from Eq. (2) and the second term represents the loss of cell viability that means the transfer from viable dry weight to dead dry weight.

1-3. Viable Dry Weight Equation

The viable cell dry weight is the sum of the active- and nonactive-viable dry weight:

$$X_{vd} = X_{ad} + X_{nd} \tag{5}$$

Differentiating Eq. (5) and substituting Eqs. (2) and (4) on the right side yields

$$\frac{dX_{vd}}{dt} = \left[1 - \exp\left(-\frac{t}{t_L}\right)\right] \frac{\mu_{max} S}{K_s + S} X_{ad} - \frac{k_d X_f}{v} \tag{6}$$

1-4. Nonviable Dry Weight Equation

It is assumed that the rate of cell lysis is first order in dead dry weight and viable dry weight decays with time coefficient of k_L,

$$\frac{dX_{dd}}{dt} = k_d \frac{X_f}{v} - k_L X_{dd} \tag{7}$$

The first term represents the transfer from viable dry weight to dead dry weight and the second term represents the decay of cell mass as cell lysis.

1-5. Dry Weight Equation

Dry cell weight is the sum of viable and nonviable cell dry weight:

$$X_d = X_{vd} + X_{dd} \tag{8}$$

Differentiating Eq. (8) and substituting Eqs. (6) and (7) on the right side yields

$$\frac{dX_d}{dt} = \left[1 - \exp\left(-\frac{t}{t_L}\right)\right] \frac{\mu_{max} S}{K_s + S} X_{ad} - k_L X_{dd} \tag{10}$$

1-6. Fresh Weight Equation

Fresh cell weight is influenced by dry cell weight as well as sugar concentration. Fresh cell weight increases as dry cell weight increases and decreases as cell ruptures.

$$\frac{dX_l}{dt}=\left[1-\exp\left(-\frac{t}{t_L}\right)\right]k_1X_{ad}-k_2k_LX_{dd}-\theta(s)X_l \tag{11}$$

where,

$$\theta(s)=\kappa\exp\left(1-\frac{S}{S^o}\right) \tag{12}$$

In Eq. (11), the first term represents effect of cell growth, the second term represents cell disruption and the third term represents cell expansion due to osmotic pressure. Eq. (12) is used for the mathematical representation of cell expansion due to sugar concentration in the experimental result.

The above proposed structured kinetic model for *Nicotiana tabacum* cell could be applied to predict the changing trend and theoretical estimate of viable cell mass during cultivation.

2. Dimensional Analysis

Microscopic phenomena of picking up the fluorescence at the probe tip would be very complex and affected by various factors. Dimensional analysis has been applied to interpret a complex system because of its advantages like the simple interpretation of a complex system and the easy application to a similar system. In this study, the dimensional analysis accounted with the important factors on the fluorescence signal is applied to the interpretation of fluorescence signal.

In the measured fluorescence signal in cell cultivation, four important factors that affect on the fluorescence signal are viable cell mass, path length, inner-filter effect and hydrodynamic condition. Path length is the distance to which the excitation light can reach in a sample. Inner-filter effect refers to the attenuation of fluorescence by absorption and scattering of excitation or emission light by absorbing materials of cells and medium components. The fluorescence signal would be directly proportional to viable cell mass and path length, and be inversely proportional to the concentration of the absorbing material such as suspended cells and glucose which takes large portion in the culture medium. Hydrodynamic conditions were determined by the density and viscosity of the culture mixture, geometry of the detection vessel and agitation speed. The density and viscosity would affect the turbidity of the medium. Agitation of the medium could increase the collision of fluorescent materials which would cause the attenuation of fluorescence. Although the agitation speed was maintained at a constant value, the possibility of the collision could be changed according to the geometry of the detection vessel. Therefore, the geometry of the detection vessel as well as the agitation speed would affect the fluorescence signal.

Dimensional analysis based on the above four factors was performed to correlate the fluorescence signal with the viable cell mass. The fluorescence signal per volume of detection vessel (I/V) could be expressed by a function of seven variables as shown in Eq. (13). Since the detection vessel was a rectangular, the diameter of the detection vessel (D) was the hydrodynamic diameter. In the term of the concentration of absorbing species (C_a), glucose was regarded as the major absorbing species because glucose took a large portion in the culture medium.

$$\frac{I}{V}=f(D_p,\ \rho,\ D,\ u,\ \mu,\ C_v,\ C_a) \tag{13}$$

where I: fluorescence intensity (Arbitrary unit), V: volume of detection vessel (L^3), D_p: path length (L), ρ: density of medium (M/L^3), D: diameter of detection vessel (L), u: stirring speed (L/t), μ: viscosity of medium (M/Lt), C_v: concentration of viable cell (M/L^3), C_a: concentration of absorbing species (M/L^3).

By the arranging of Eq. (13), the final dimensionless formula from the dimensional analysis was obtained as shown in Eq. (14).

$$\frac{D^3I}{V}=A\left(\frac{D_p}{D}\right)^B\left(\frac{C_v}{\rho}\right)^C\left(\frac{C_a}{\rho}\right)^D(Re)^E \tag{14}$$

where A, B, C, D and E are parameters to be determined by a system analysis.

Four dimensionless groups shown are defined as Path length number (Pl: D_p/D), Viable cell number (V_c: C_v/ρ), Absorption number (Ab: C_a/ρ) and Reynolds number (Re: $Du\rho/\mu$). Pl means the path length of the excitation light in the given detection vessel, and V_c is defined as the viable cell mass with regard to the total mass in the medium. Ab indicates the amount of absorbing species, total cell mass and glucose to the total mass in the medium. Re is an well-known dimensionless group to define the hydrodynamic condition of fluid.

MATERIALS AND METHODS

1. Cell Culture

Nicotiana tabacum cells were supplied by Dr. D. I. Kim (Inha Univ., Korea). Cell suspension cultures have been maintained on Murashige and Skoog (MS) medium modified with 10.74 μM of naphthylacetic acid (NAA), 3.23 μM of 6-Benzyl amino purine (BAP), vitamin stock solution, and 30 g/L of glucose as carbon source. The medium pH value was adjusted at 5.8 with 1N NaOH solution. Suspended cells were cultivated on a rotary shaker at 180 rpm and 25℃ under normal room light.

2. Batch Experiment Procedure

For the batch experiment in shake flasks, cells in the late exponential growth phase, which are usually 5-6 days old, were used. To avoid heterogeneity of the innoculum, all the cells from different flasks were collected in a pre-autoclaved large flask and mixed well by shaking. The cells were filtered through Whatman No. 1 filter paper on a Buchner funnel under slight vacuum and washed with fresh medium which was prepared according to the purpose of experiment. 3 g of cells by fresh weight was inoculated into a 125 ml Erlenmeyer flask containing 50 ml of medium. The cultures were incubated at 25℃ on a shaker at 180 rpm. Two or three replicas of flasks were sacrificed for analysis of samples. After filtration, the cells were collected for cell mass measurement and intracellular product determination. The filtrates were usually stored in the refrigerator for extracellular product and sugar assays.

3. Cell Mass Measurement

For dry cell weight (DCW), suspension cells were filtered with dried and pre-weighted Whatman No. 1 filter paper under slight vacuum. Filtered cells were washed with distilled water and dried in a oven at 60℃ to constant weight.

4. Fiber-Optic Fluorescence Sensor System

The developed fluorescence sensor was composed of three parts which were light source, light transmit and light detection part as shown in Fig. 1. 150W Xenon lamp (Oriel Co., Stratford, CT) was used as light source and a 1/4 m monochromator (Oriel Co., Stratford, CT) was used to produce the narrow band excitation light of 340 nm. A bifurcated optical fiber (Oriel Co., Stratford, CT) was used to introduce the excitation light into the sample

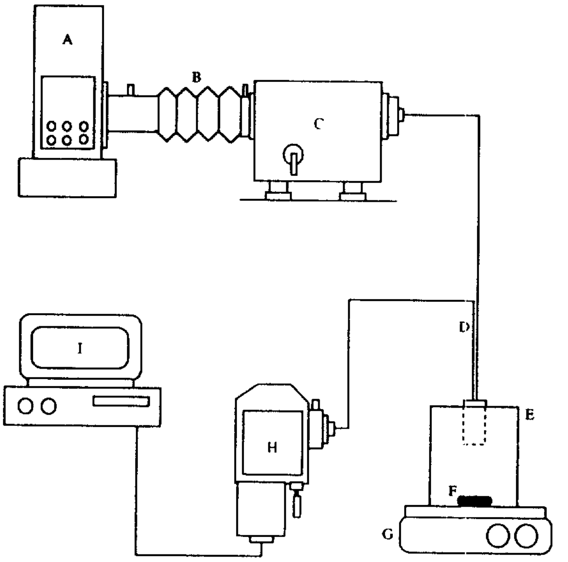

Fig. 1. Schematic diagram of fiber-optic fluorescence sensor system.

A. 150W xenon lamp
B. Light shield
C. Monochromator
D. Bifurcated light guide
E. Detection vessel
F. Magnetic bar
G. Stirrer
H. Detector
I. Computer

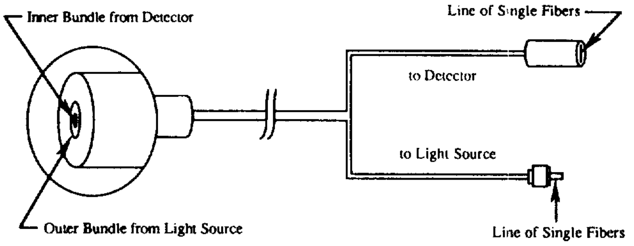

Fig. 2. Schematic diagram of bifurcated fiber bundle.

and pick up the fluorescence. As shown in Fig. 2, the bifurcated optical fiber has the special geometry which makes it possible to pick up the fluorescence effectively at the nearest region to the tip. Spectrograph and photodiode array (Multispec II system, Oriel Co., Stratford, CT) were used to detect the fluorescence signal, which was connected to an IBM compatible P.C.. To obtain the stable light source, the fluorescence detection was started after 30min from the turn-on of the lamp.

Since the sensing by optical fiber could be interfered by ambient light, which might make some serious problems, a rectangular detection vessel (20 ml) coated with a black non-fluorescent material was designed to eliminate the background signal due to ambient light as shown in Fig. 1. The bifurcated optical fiber was placed at the top of the detection vessel and the tip was dipped into a sample. Agitation of sample was performed by a magnetic stirrer to prevent aggregated cells from settling down. The detection of NADH fluorescence was performed for 11 days with the designed detection vessel. The intensity of the emitted light of 460 nm was detected since the emitted wavelength of intracellular-NADH is 460 nm with the excitation wavelength of 340 nm. The sensor signal was analyzed with a Multispec II software (Oriel Co., Stratford, CT) including filtering, averaging and integration technique. The raw data was filtered and averaged on the stable region of sensor signal. And the previous signal was corrected by background signal to obtain the pure signal due to the NADH-dependent fluorescence. Finally, integration technique could provide the amplification of weak sensor signal. The typical settings of detection system for a data acquisition are given as follows: Detection mode: chart mode (fluorescence intensity vs time), Exposure time: 1 sec, No. of integrations: 5, Detection time: 18.6 min, Detection head temperature: 10℃.

RESULTS AND DISCUSSION

1. Cell Growth and Fluorescence Measurement

For accurate analysis of the kinetic behavior of cell growth in *Nicotia tabacum* cell suspension culture, a batch experiment was carried out in shake flasks and samples were taken every day. Fig. 3 shows the time course changes of dry cell weight. The lag phase for cell growth existed to about the first day of cultivation. Approximately exponential growth occurred after about one day, the time at which glucose disappeared from the media. Dry cell weight reached a maximum at the eighth day after inoculation and then decreased slowly. In the late death phase, cell becomes dead and disrupted rapidly. But macromolecules in the cells were not completely decomposed and then may be included in the observed value of dry cell weight.

The culture fluorescence has been considered for use as a measurement of *in situ* information in bioreactor without damaging to cells. In order to clarify the estimation of cell mass by culture fluorescence, the time course change of NADH-dependent culture fluorescence was detected by the constructed fiber optic sensor as shown in Fig. 3. The changing pattern of fluorescence in the *Nicotiana tabacum* cell culture had a good agreement with the change of dry cell weight until the exponential phase. But the correlation of culture fluorescence with dry cell weight has discrepancy remarkably after about 8 days, the time at which dead phase began. The reason for discrepancy might be that dry cell weight includes the dry weight of living cell and cell debris. Since only viable cell has NAD-NADH conversion mechanism, NADH-dependent fluorescence can be interpreted as a marker for cell viability.

The constructed fiber-optic sensor could detect the culture fluorescence accurately without damaging to cells. Also the proposed sensor could be applied to do on-line sensing of cell mass in bioreactor with the modification of input port of bioreactor and the reduction of detection system size.

2. Validation of Kinetic Model

Fig. 4 presented the observed and model-predicted cell growth behavior. The complete model comprised with differential equations; Eqs. (1)-(12). The parameters which are involved in model equations were estimated by using nonlinear parameter estimation technique [Metzler et al., 1974]. While the parameter estimation was done, model equations were being solved simultaneously with numerical integration of differential equations using Runge-Kutta method. The experimental data are compared to the model predictions by choosing parameters that give a best fit of the model to the data. The estimated values of parameter are shown in Table 1. It can be concluded that the solution of the model equations represents the experimental data fairly accurately given the initial condition for cell mass.

3. Interpretation of Fluorescence Signal

In Fig. 3, dependence of the fluorescence signal on dry cell

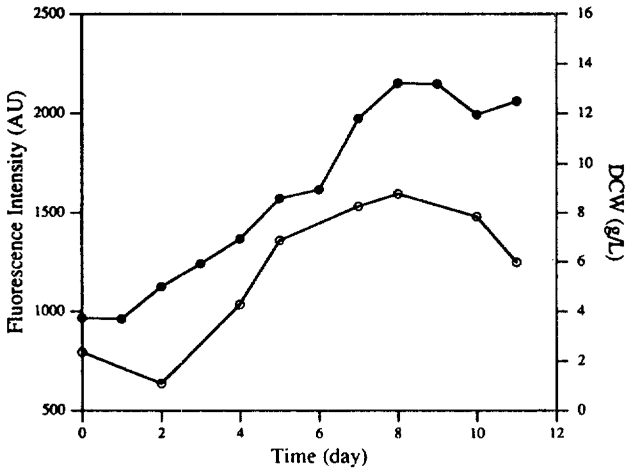

Fig. 3. Time course change of dry cell weight and culture fluorescence in batch culture.

(● : dry cell weight, ○ : fluorescence intensity)

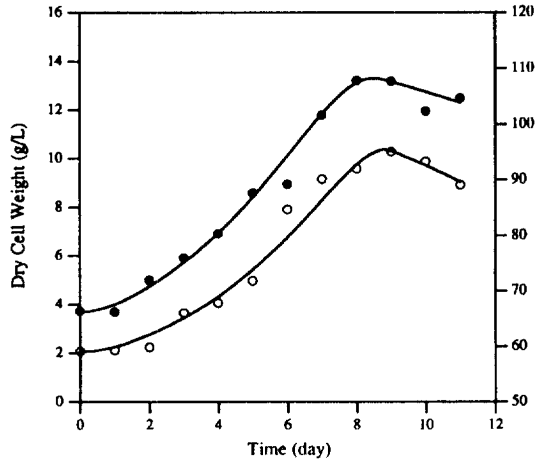

Fig. 4. The kinetic model results of the dry cell weight and fresh weight in batch culture. The symbols are experimental results.

(● : dry cell weight, ○ : fresh weight) and the lines are model results.

Table 1. List of the estimated parameters in structured kinetic model

Parameter	Dimension	Value
μ_{max}	day^{-1}	0.3675
K_s	g/L	3.0000
k	day^{-1}	0.415
k_d	day^{-1}	0.0059
k_L	day^{-1}	0.0797
k_1	day^{-1}	1.200
k_2	day^{-1}	8.360
κ	day^{-1}	0.025
t_L	day^{-1}	1.000

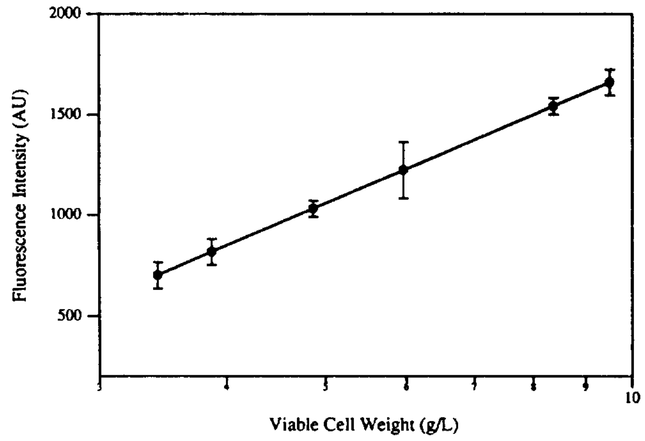

Fig. 5. Fluorescence intensity as a function of approximate viable cell weight.

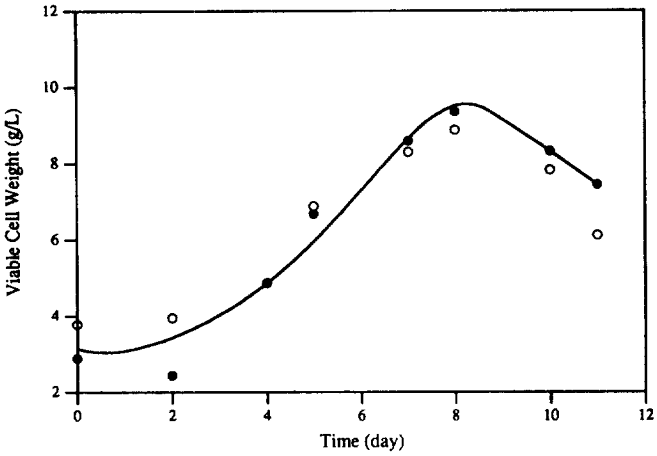

Fig. 6. Comparison of viable cell weight based on the empirical correlation and the dimensional analysis with the theoretical value based on structured kinetic model.

(● : dimensional analysis, ○ : empirical correlation, - : theoretical value).

weight appeared to be linear within the exponential cell growth phase while linearity was absent in the stationary phase since the fluorescence intensity is related with the viable cell mass. Thus it is easy to get the viable cell mass if pre-determined correlation is existed between fluorescence signal and viable cell mass. In this study, to make the correlation between the viable cell mass and fluorescence signal, the approximate viable cell mass is obtained by sieving the plant cells at the initial exponential growth phase through nylon meshes from 50 μm to 1 mm and then by using the tetrazolium chloride as a viability assay [Dixon, 1986]. Due to the large size of plant cell, the cell debris can be discarded by sieving through the 50 μm mesh. When the plant cells become aggregates and then makes very large aggregates, the most of cells inside the large aggregates are dead due to the diffusion limitation of nutrient. So by sieving through the 1 mm mesh, the large aggregates could be discarded. By sieving the freshly inoculated *Nicotiana tabacum* cells at the initial exponential phase, the approximate viable cell mass was obtained and then the related culture fluorescence was measured with the constructed sensor. Fig. 5 shows the plot of culture fluorescence as a function of approximate viable cell weight. The approximate viable cell weight could be correlated linearly with fluorescence intensity on the plot of log X_{vd} vs I (relative fluorescence inten-

Table 2. List of the estimated parameters for dimensional analysis in batch culture

Parameter	Value
A	1.24×10^{-4}
B	0.61
C	1.28
D	−0.66
E	−1.64

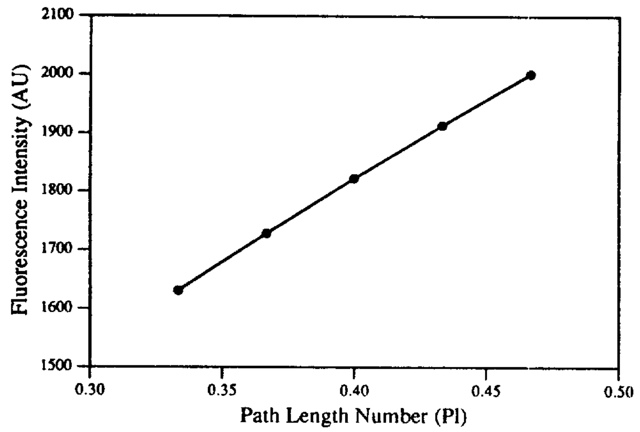

Fig. 7. The effect of Path length number on the fluorescence signal.

sity). Based on that empirical correlation, the viable cell mass during batch cultivation was plotted as shown in Fig. 6.

Four major factors on the fluorescence signal were interpreted by dimensional analysis, and four dimensionless groups (Pl, Vc, Ab and Re) were obtained from the dimensional analysis in Eq. (14). The parameters were calculated based on the experimental data during batch cultivation. The estimated parameters are shown in Table 2. Eq. (14) could be applied to not only the interpretation of the fluorescence signal, but also the determination of viable cell mass by the rearrangement with regard to viable cell mass. Viable cell weight was calculated based on Eq. (14) from the measured fluorescence signal by the fiber optic sensor and was shown in Fig. 6.

In Fig. 6, the theoretical viable cell weight based on the structured kinetic model was shown to evaluate the value of the empirical correlation and the dimensional analysis. The change of viable cell weight estimated by the dimensional analysis has the same trend compared with that based on the empirical correlation. Also the viable cell weight based on the dimensional analysis are well agreed with the theoretical value estimated by the structured kinetic model. The above results suggested that the proposed dimensional analysis could be used to interpret the fluorescence signal by a fiber optic sensor during the plant cell cultivation.

The effect of dimensionless group, Pl, on the fluorescence signal was shown in Fig. 7. Path length number was directly proportional to the fluorescence signal since the detectable cell density became higher as the path length of excitation light increased.

CONCLUSIONS

The fiber-optic fluorescence sensor was constructed to measure the NADH-dependent fluorescence in *Nicotiana tabacum* plant cell culture. The constructed sensor could measure the culture fluorescence rapidly and accurately. The structured kinetic model for cell growth was made to estimate the theoretical viable cell weight. The empirical correlation between fluorescence intensity and the viable cell weight was established as the linear relation on semi-log plot. The dimensional analysis was done for the interpretation of fluorescence signal, in which the path length, the inner filter effect and the hydrodynamic conditions were considered as the key factors on fluorescence intensity. The dimensional analysis and empirical correlation of fluorescence signal to viable cell weight was applied to the interpretation of the detected fluorescence signal during cultivation. The interpretation of fluorescence signal using dimensional analysis was well related with the viable cell weight estimated by the structured kinetic model as well as by empirical correlation.

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NOMENCLATURE

A : activity [g/g]
Ab : Absorption number (dimensionless group)
D : diameter of detection vessel [m]
D_p : light path length [L]
C_a : concentration of absorbing species [Kg/L]
C_v : concentration of viable cell [Kg/L]
K_s : Monod constant [g/L]
k : rate constant [day^{-1}]
k_d : viability loss constant
I : fluorescence intensity [Arbitrary unit]
Pl : Path length number (dimensionless group)
Re : Reynolds number (dimensionless group)
S : substrate concentration [g/L]
t : time [day]
u : stirring speed [m/sec]
V : volume of detection vessel [L]
V_c : Viable cell number (dimensionless group)
v : viability [g/g]
X : biomass concentration [g/L]

Greek Letters

ϕ : viability loss function
κ : cell expansion coefficient [day^{-1}]
μ : viscosity of medium [Kg/m sec]
μ_{max} : maximum specific growth rate [day^{-1}]
μ_s : specific growth rate [day^{-1}]
θ : cell expansion function
ρ : density of mixture [Kg/L]

Subscripts

ad : active-viable cell
d : dry weight or dead
dd : dead cell
f : fresh weight
L : lag phase
nd : nonactive-viable cell
vd : viable cell

Superscript

o : initial

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