Paper

Development of Simple Optical Sensor for Measurement of Sugar Content Using Bent Optical Fiber, Light Emitting Diode and Photo Diode

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ABSTRACT

To construct a simple optical sensor for measurement of the sugar content in an aqueous solution, a bent optical fiber was used as the sensor. The attenuation ratio, i.e., the differences in the light intensity through the bent optical fiber when the sensitive part (bent part) was soaked in a reference material and sample solution, was closely related of the solute content in the samples. However, slightly difference in the light intensity were observed in the practical application because the sugar content in foods and other bio-systems was much lower than that in the tested samples. Therefore, we constructed a sensor system using a light emitting diode (LED), photo diode (PD) and simple electronic circuit as a differential amplifier. As a result, measurement of the sugar content ranging from 0 to 30 g/dm³ was successful and excellent agreement between the FT-IR method as the conventional method and the sensor method using an operational amplifier (OP-amp) was observed. In addition, the time courses of the sugar content and the uptake rate of plant cells were able to be calculated during the cultivation process. The process monitoring for the fermentation process management will be possible by using this sensor. These results suggested that the developed sensor using a bent optical fiber is applicable as a simple and disposable optical sensor.

KEYWORDS: bent optical fiber, optical sensing, optical sensor, LED, sugar content

1. Introduction

Optical sensing, such as a spectroscopic analysis and colorimetric method, is widely used in various fields, for example, management of agricultural and food processing¹⁾, environmental measurement^{2, 3)}, bioprocess measurement and control⁴⁾, medical monitoring^{5, 6)}, etc. The optical sensing is advantageous in that a simple, rapid, chemical free, non-invasive, and non-destructive measurement is possible. In addition, the obtained data have a high affinity with Information Communication Technology (ICT) because the optical sensing data are recorded as digital information. In the application of new digital technologies, such as the concept of Internet of Things (IoT), vast amounts of various types of digital data are produced and the aggregate of the data is called Big Data⁷⁾. The optical sensing method will be one of the important measurement tools for the IoT concept with the Big Data application in the field of agricultural and food processing⁸⁾.

The optical fiber is an essential intelligent technology for optical communications in the ICT field. The

underground optical fiber is useful as a sensor for the detection of ground strain⁹⁾ and displacement of ground positions¹⁰⁾. The optical fiber, which was specifically processed, is used to measure physical and chemical properties such as refractive index¹¹⁾, temperature¹²⁾ and humidity¹³⁾. The clad of the optical fiber was removed and the sensing materials, such as a fluorescence enhancer for specific materials and polymer film containing an organic pigment for humidity detection, were coated on the naked fiber core¹¹⁾. In the case of the refractive index sensor, gold (or platinum) was coated on the fiber core. For the other cases, the fiber shape was precisely processed^{10, 13)}. In these sensor systems, complicated signal processing is generally required for the detection of the target substrates or the properties. In our previous study, an air-oil and oil-water interface detector composed of a plastic optical fiber, light emitting diode (LED), photo diode (PD) and five analog electric circuits, was developed and the sensor was applied to measurement of the oil layer thickness of kitchen wastewater¹⁴⁾. The plastic optical fiber, which is not processed, was

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used to create the sensitive part of the sensor except that the fiber was bent. In other words, forming of the sensitive part is very simplified and the sensitive part of the sensor can be more easily supplied at a low price and in large quantities. In addition, signal processing for the sensor was able to be considerably simplified because the air-oil and oil-water interfaces are able to be detected by the light intensity changing through the bent optical fiber¹⁴⁾. If the measurement of the consecutive content of a solute in the solution is possible using the developed sensor in our previous study¹⁴⁾, a simple and disposable optical sensor will be applicable for foods and other bio-systems including environmental measurement in the IoT concept.

The light emitting diode (LED) is widely used in the field of applied optical radiation such as general lighting, flat-panel displays, light source for evaluation of color appearance of objects¹⁵⁾, indicator and traffic light¹⁶⁾. The LED is also used in a plant factory which requires precision control of the light environment¹⁷⁾. The application of LEDs as a stable light source is very useful because LEDs may have a very long life expectancy and the light-emitting intensity is able to be controlled only by amperage regulation of the current supplied to the LED. Therefore, the combination of the LED as the light source for the optical sensor and PD as the detector has recently attracted interest to construct various optical sensors^{18–20)}.

The propose of this study is to develop a simple optical sensor using an LED, PD and bent optical fiber to measure the content of a bio-related substance in an aqueous solution which is considered to be in foods and other bio-reaction systems. In particular, sugar is the most essential bio-related substance for living organisms including agricultural products, cells, and the other living things because sugar is the main material for the starting point of the metabolism reaction. During the fermentation (cell cultivation) process, sugar is mainly used as the carbon source for cell growth, and maintains and allows the metabolic process in cells to continue. In general, sugar content in the fermented culture broth is measured by enzymatic²¹⁾ or by electrochemical detection²²⁾. These methods have a high detection sensitivity (micro molar order). However, it is one of the serious problems for the continuous monitoring that the activity is not stable in foods and the other biological fluids. In addition, the sensor should be sterilized to avoid the microbial contamination. Although the non-enzymatic method was developed^{23–25)} in recent study, stability of the sensitive materials is also serious problem because these methods are detected based on the chemical or physical interaction between sugar and the sensitive material.

Optical sensing is one of the suitable techniques for measuring the content of a bio-related substance such

as the sugar during the bioprocess progression because a simple, rapid, and non-destructive measurement is possible. In addition, the target substance might be able to be detected by an optical sensor without using the sensing materials. Therefore, we tried to develop an optical sensor to measure the sugar content in an aqueous solution base with the interface detector which we developed in a previous study¹⁴⁾. A fundamental performance test of the bent optical fiber used as the sensor was first carried out using an aqueous solution of sucrose, sodium chloride and mixture of sucrose and sodium chloride. A prototype sensor system was then constructed using an LED, PD and simple electronic circuit (differential amplifier) using an operational amplifier (OP-amp) and was applied to the monitoring of the sugar content in a plant cell culture broth which is one of the typical multi-component samples.

2. Materials and methods

2.1 Materials

Aqueous solutions of sucrose, sodium chloride (NaCl) and a mixture of sucrose and NaCl were used as the samples. Special grade sucrose and NaCl were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The contents of the sucrose and NaCl of the prepared aqueous solutions as the samples are summarized in Table 1. Sugar and salt are the main components in the bio-materials such as food and culture media. In addition, the interaction between the sugar and salt in the sugar-salt aqueous solution might be observed in the optical sensing data²⁶⁾. Therefore, the mixing ratio was changed dynamically as shown in Table 1 for the fundamental performance test of the optical sensor using bent optical fiber.

Arabidopsis thaliana MM2d was used as the plant cell cultivation²⁷⁾. The cells were sub-cultured in the Murashige and Skoog (MS) basal salts medium²⁸⁾ containing 30 g/dm³ sucrose, 0.5 mg/dm³ a-naphthalene acetic acid and 0.05 mg/dm³ kinetin. For the sub-cultivation, 4.0 cm³ of the 7-day-old inoculums in the MS medium was inoculated in 100 cm³ of separate fresh mediums in 300 cm³ flasks, and the pre-cultivation was carried out at 300 K using a rotary shaker (130 rpm) under dark conditions. For the cultivation, cells sub-cultured for 7 days were washed using the MS medium without containing sugar, and 1.6 cm³ of the suspension was inoculated in 40 cm³ of separate fresh medium in 100 cm³ Erlenmeyer flasks. The cells were grown in the dark under agitation at 130 rpm and 300 K. To get the various states of the culture broth, the broth in the flask was diachronically sampled.

2.2 Experimental apparatus

A plastic optical fiber (SK60, Mitsubishi Rayon Co., Ltd., Tokyo) was used as the sensor, and the optical

Table 1 Constituents of sugar-salt aqueous solutions.

	Sucrose content		NaCl content		Density [g/cm ³]	Brix% [%]	EC [S/m]	Factor [—]
	[mol/dm ³]	[wt%]	[mol/dm ³]	[wt%]				
Water	0.000	0.00	0.00	0.00				1.000
Sucrose	0.125	4.20	0.00	0.00	1.01	4.20		0.9689
	0.250	8.30	0.00	0.00	1.03	8.30		0.9425
	0.375	12.3	0.00	0.00	1.04	12.3		0.9159
	0.500	16.1	0.00	0.00	1.06	16.1		0.8901
NaCl	0.000	0.00	0.50	2.90	1.02	3.60	4.39	0.9861
	0.000	0.00	1.00	5.60	1.04	6.70	7.70	0.9774
	0.000	0.00	2.00	10.9	1.07	12.7	13.70	0.9562
	0.000	0.00	4.00	20.4	1.15	23.1	19.51	0.9134
Sucrose+NaCl	0.125	4.10	0.50	2.90	1.03	7.70	3.98	0.9604
	0.125	4.10	1.00	5.60	1.05	10.7	7.10	0.9512
	0.125	3.90	2.00	10.7	1.09	16.4	11.96	0.9312
	0.125	3.70	4.00	20.1	1.16	26.5	17.90	0.8865
	0.250	8.20	0.50	2.80	1.05	11.5	3.75	0.9327
	0.250	8.00	1.00	5.50	1.07	14.5	6.74	0.9238
	0.250	7.70	2.00	10.6	1.11	20.0	11.34	0.9038
	0.250	7.30	4.00	19.8	1.18	29.8	16.44	0.8590
	0.375	12.1	0.50	2.80	1.06	15.4	3.43	0.9065
	0.375	11.8	1.00	5.40	1.08	18.3	6.10	0.8968
	0.375	11.5	2.00	10.4	1.12	23.6	10.2	0.8759
	0.375	10.8	4.00	19.6	1.19	33.0	14.8	0.8314
	0.500	4.10	0.50	2.90	1.05	19.2	3.13	0.8806
	0.500	4.10	1.00	5.60	1.10	21.9	5.52	0.8708
	0.500	3.90	2.00	10.7	1.14	27.0	9.29	0.8492
	0.500	3.70	4.00	20.1	1.21	36.1	13.3	0.8057

Figure 1 Overview and schematic diagram of experimental apparatus.

fiber was bent for creating the sensitive part. To check the fundamental performance of the sensor, the experimental apparatus was constructed as shown in Figure 1. Both ends of the bent optical fiber were connected to a light source (L10671, Hamamatsu Photonics K. K., Hamamatsu, Japan) and spectrometer equipped with a CCD image sensor with a spectral response from 320 to 1000 nm (C10083CAH, Hamamatsu Photonics K. K., Hamamatsu, Japan) through the quartz optical fiber. The output signal from the spectrometer was pro-

cessed using attached software and the light intensity spectrum was recorded by the AD count value scaled in 16 digital bits.

2.3 Conventional analysis of sugar concentration in culture broth

The sugar contents of the sampled culture broth were determined by the FT-IR/ATR (Attenuated Total Reflection) method as previously described²⁹⁾. For the analysis, an FT-IR spectrometer (Nicolet Magna 750,

Thermo Fisher Scientific Inc., USA) was equipped with a KBr beam splitter, and a deuterated triglycine sulfate KBr detector was used to obtain the spectra. The ATR spectra were obtained using a horizontal zinc selenide ATR sampling accessory (SPECACLAMP ATR 11080, SPECAC Ltd., UK). The dry cell weight of the culture broth was gravimetrically measured after collection by centrifugation, washed and the excess water removed.

The logistic function expressed by Eq. (1) was applied to the plots of the dry cell weight and sugar uptake versus the cultivation time described in our previous report³⁰⁾

$$W = \frac{W_{ini} - W_{fin}}{1 + e^{((t-t_0)/w)}} + W_{fin} \quad (1)$$

where W [g/dm³] is the dry cell weight or the total sugar content. t [d] is the cultivation time. The parameters t_0 and w , respectively, denote the inflection point of the time course and the time intervals that define its curve width. Moreover the subscripts *ini* and *fin* indicate the value at the initial and final stages, respectively. The growth and sugar uptake rates of the cells were calculated by differentiating Eq. (1) with the cultivation time, t .

Figure 2 Spectra of light intensity passing through the bent optical fiber (a) and calculated attenuation ratio, AR , of the light intensity (b).

3. Experimental results

3.1 Determination of optimal bending width of the plastic optical fiber

To create the sensitive part of the sensor, the plastic optical fiber was gradually bent. Figure 2(a) shows the light intensity spectra passing through the bent plastic optical fiber which is the sensitive part that was soaked in air and water. The vertical axis of Figure 2(a) is the AD count values obtained from the spectrometer which recorded the light intensity scaled in 16 digital bits. The spectral pattern is almost the same as the light source except at around 900 nm. The absorption at around 900 nm might be caused by the plastic as the fiber material.

The differences in the light intensity when the sensitive part was soaked in air and water are also displayed in Figure 2(b). In the figure, the attenuation ratio, AR , of the light intensity in the case of air and water was defined by the following equation

$$AR = \frac{I_r - I_s}{I_r} \quad (2)$$

where I_r and I_s are the light intensity through the bent plastic optical fiber when the sensitive part was soaked in the reference (air) and sample (water) materials, respectively. To determine the optimal bending width of the sensitive part, the relationship between the bending width and the attenuation ratio at 707 nm was plotted (Figure 3). As a result, it was determined that the plastic optical fiber with bent a width of 5 mm maximized the AR value.

3.2 Fundamental performance test using sugar-salt aqueous solutions

Figure 4 shows the relationship between the AR values and the mass percentage, density or Brix% of the sucrose aqueous solutions. The AR value is closely related to the sucrose content in the aqueous solution.

Figure 3 Relationship between bending width of optical fiber and AR .

Figure 4 Relationship between AR and mass percentage, density or Brix% of sucrose aqueous solutions.

Figure 5 Relationship between AR and sucrose or NaCl contents in mixtures of sugar-salt aqueous solutions.

This result suggested that the bent optical fiber could be used as the sensitive part of the sensor to measure the solute content in aqueous solutions. It is considered that the intensity of light through the bent optical fiber depends on the refractive index of the sample touching the sensitive part. In general, foods and the other bio-systems have a complicated component structure such as a mixture of organic components and metal ions. Therefore, we checked the sensor performance using sucrose–NaCl aqueous solutions as the sample.

Figure 5 shows the measurement results of the AR values when the sensitive part was soaked in the sucrose–NaCl aqueous solutions. The AR value increased when the sucrose was added to the NaCl aqueous solutions (Figure 5(a)) and when the NaCl was added to the sucrose aqueous solutions (Figure 5(b)). The AR value is indicated by a logarithmic scale in the figure and a good linearity was obtained. It seems that the AR value exponentially increased with the content of the sucrose or NaCl.

3.3 Measurement of sugar content of culture broth during plant cell cultivation

We used the developed sensor to measure the sugar content in the culture broth during plant cell cultivation which is one of the typical complicated composition samples as a mixture of sugar and salts. However, the plant cell cultivation broth only contains under 30 g/dm^3 sugar. Although it was confirmed that the constructed sensor and the experimental apparatus can measure the solute content in an aqueous solution, the content of

the used samples for the fundamental performance test (the sugar–salt aqueous solutions) is higher than that in the actual foods and bio-systems. The detection of slight differences in the light intensity would be required for the bent optical fiber sensor. Therefore, we constructed a prototype sensor system using an LED (NSPL570DS, NICHIA Co., Tokushima, Japan), PD (OSD1-5T, Centronic Ltd., UK) and OP-amp (LM248N, STMicroelectronics, N. V., Geneva, Switzerland) for differential amplification (Figure 6). Although the constructed circuit is common differential amplifier, however, it was improved that the weak current from the photodiode is converted into the higher voltage. The main circuit was also constructed using a general purpose electric parts.

Figure 7 shows the correlation between the sugar contents of the culture broth measured by the FT-IR method and the sensor system using the experimental apparatus (gray triangles) or using the differential amplification circuit (black circles). The calculated results of the sugar contents using the experimental apparatus were not suitable for the culture broth of the plant cells. On the other hand, an excellent agreement between the FT-IR method and the sensor method using the OP-amp was observed. As the results, the time courses of the sugar content and the consumption rate were able to be calculated during the plant cell cultivation process (Figure 8). Good agreement of the calculated results was obtained between the FT-IR method (gray squares and dotted line) and the constructed sensor (black circles and line).

Figure 6 Prototype sensor system using LED, PD and differential amplifier (OP-amp.) with two bent optical fibers (for reference and sample).

Figure 7 Correlation between sugar contents of culture media measured by FT-IR method and the sensor system using the experimental apparatus (gray triangles) or differential amplifier electric circuit constructed with LED and PD (black circles).

Figure 8 Time courses of the sugar content (a) and the uptake rate (b) measured and calculated by FT-IR method and the sensor system.

4. Discussion

The bent optical fiber was used for measurement of the solute content in aqueous solutions (Figures 4 and 5). The differences in the light intensity when the reference material and the sample solution were contacted with the sensitive part was defined as the AR value expressed by Eq. (2). The relationship between the calculated $\log(AR)$ values and the solute contents in the aqueous solution is a linear correlation. These results suggested that the AR value obtained by the developed sensor is treated as the transmittance values using the Lambert–Beer law³¹⁾. The law is normally described by the following equation

$$A_{\lambda} = -\log \frac{I_{\lambda}}{I_{0,\lambda}} = -\log T_{\lambda} = \varepsilon_{\lambda} c L \quad (3)$$

where $I_{0,\lambda}$ and I_{λ} are the radiant intensity of light entering the sample and exiting the sample, respectively. The subscript λ is the wavelength of light. The ratio, $I_{\lambda}/I_{0,\lambda}$, is defined as the transmittance (T_{λ}) and A_{λ} is the absorbance of the sample. ε_{λ} , c , and L are the absorption coefficient, which is specific to the kind of material the content of the target material in the sample, solute content in the sample, and the light pass length of the sample, respectively. The kind of material can be identified by the spectrum pattern because ε_{λ} is different for every kind of material. At the same time, several materials in samples with a complicated composition can also be simultaneously measured because the absorbance A_{λ} is proportional to the content c .

In Figure 5(a) and 5(b), the fitting results of the linear approximation (slope and intercept of the straight line) are also displayed. The slope for the sugar contents is slightly difference for the salt contents. The sucrose and sodium chloride contents of the aqueous solution used in this fundamental performance test is higher than the contents that are assumed to be in actual foods or bio-systems, and the interaction between the sugar and salt will be more definitely observed in the optical sensing data²⁶⁾. The optical sensing data obtained by this bent optical fiber sensor will be used to recognize the kind of materials. In the case of the tested sample which is a transparent liquid, the obtained spectral information is an almost flat pattern because the light source for the visible radiation was used to construct the experimental apparatus. Figure 9 shows the observed spectra of two kinds of pigments (0.025 g/dm³ rhodamine B and 0.025 g/dm³ methylene blue) in aqueous solutions obtained by the experimental apparatus with the bent optical fiber sensor. Although the optical fiber of 3 mm in diameter was used to more strongly obtain the absorption the spectral information, which characterized the two kinds of pigment solutions, was obtained. These results suggested that the bent optical fiber could be used as an attenuate total reflection (ATR) immersion

Figure 9 Spectra of two kinds of pigment aqueous solutions obtained from experimental apparatus with bent optical fiber.

type sensor probe. The processing of the sensor probe is very easy because the optical fiber was only bent. If a sensing material, such as a fluorescence enhancer for specific materials, polymer film, and a pigment that turns color depending on the pH, is fixed to the bent optical fiber, various types of optical sensors, which were developed in the preliminary studies^{12, 13)}, are able to be developed. In addition, spectral information will be able to be simultaneously obtained. In general, the structure of the bio-related substrate may change depending on the environmental situations such as the pH and temperature³²⁾. The structure change in the bio-related substrate may influence the spectral information which is obtained by the optical sensing^{26, 32)}. Simultaneous measurement of the spectral information and the environmental situation will be tested in a future study.

The developed sensor and the experimental apparatus was used in an actual situation, such as a plant cell culture broth, and a slight difference in the light intensity between the reference (water) and the sample (culture broth) had to be detected because the sugar content in the culture broth were lower than that of the tested sample solutions. The prototype sensor using the OP-amp for differential amplification, two LEDs and two PDs was used to apply the bent optical fiber sensor in an actual situation. This prototype sensor can be constructed very cheaply because the sensor and the electronic parts are not expensive and the sensor system has a simple configuration. For the sensitive part of the sensor, the bent optical fiber, there is no processing to make the sensitive part except for the bending. The sensor system was able to be constructed only in a simple light detection circuit where the LED and PD were placed on both ends of the bent optical fiber.

Although the maximum values of the sugar content in the culture broth is under 30 g/dm³, a good agreement between the calculated results using the sensor

system and using the FT-IR method^{27, 29)} was obtained (Figures 7 and 8). In both methods, the curve fitting for obtaining the time courses of the sugar content were possible using the logistic function, Eq. (1). In addition, the calculated results of the sugar uptake rate using the sensor system also corresponded to the calculated result using the FT-IR method³⁰⁾. However, a slight error was observed in the case of the measurement results using the developed sensor system. The reason for the error will be the influence of the small amount of salts and the trace elements (mineral sources) in the culture broth. The salt and the trace element contents of the culture broth could be estimated by measurement of the electric conductivity (EC) values, and the cell content in the culture broth is also able to be estimated by the EC values³³⁾. The observed error may be corrected using the EC values of the culture broth. On the other hand, the sugar uptake rate is one of the important results for the bioprocess management of the cultivation. The monitoring of the sugar contents and the uptake rate are very important in order to analyze the sugar metabolism of the plant cells^{27, 30)}. In our future plans, we intend to control the growth and metabolism reaction of the plant cells by changing the carbon source (sugar) type in the middle of the cultivation stage without using the gene recombination technique. In other words, we consider this application as a simple sensing tool to regulate the growth and the metabolic reaction rate of the cells by controlling the environment during the plant cell cultivation. It is very important results that the time courses of the metabolic material contents could be expressed quantitatively for the bioprocess management. In general, the time courses of the content of the cells and the metabolic materials, such as cell growth, sugar uptake, and production of the metabolic products, during the fermentation process is able to be explained by the logistic function curves. In the results of the curve fitting using the Boltzmann equation (Eq. 1), good agreement was obtained between the calculated curve obtained by the sensor system and by the FT-IR method because the correlation coefficient of the sugar contents and the sugar uptake rate curves were 0.9994 and 0.9976, respectively (Figure 8). As shown in Figure 8(b), slight error of the maximum sugar uptake rate and the time were observed, however, the relative error were about 2.5% for the maximum rate and 1.3% for the culture period of the peak time, respectively. These results suggested that the optical sensor system constructed by the bent optical fiber is applicable for the bioprocess monitoring of the sugar content.

The constructed simple optical sensor is one of the powerful tools for a simpler and easier bioprocess management. In addition, the structure of the proposed sensor system in this study is very simple, and a price reduction and disposable use of the sensor will be

realized. The application to optical sensing under the condition of wide use such as at an agricultural production site and environmental measurement site using the characteristics of the optical fiber will be possible. The simple optical sensor is applicable as one of the disposable sensing tools for the sensor network in the IoT concept such as in a smart agriculture setting and smart factory.

5. Concluding remarks

A simple optical sensor using the bent optical fiber was developed for the optical sensing of the solute content in an aqueous solution without precise processing of the sensitive part. The attenuation ratio of the light intensity obtained by the constructed sensor was treated as the transmittance values of the optical sensing. In fact, the spectral information of two kinds of pigment aqueous solutions was able to be acquired using the bent optical fiber. However, a slight change in the attenuation ratio must be detected in a practical application such as the differences in the light intensity slightly changed between the reference materials (water) and the samples (culture broth). Therefore, we constructed a sensor system using an LED, PD and OP-amp for the differential amplification. As a result, measurement of the time courses of the sugar content during plant cell cultivation ranging from 0 to 30 g/dm³ was possible. In addition, the sugar uptake rate of the plant cell during cultivation was able to be calculated and excellent agreement between the FT-IR method and the sensor method using an electric circuit was observed. These results suggested that the developed prototype sensor using a bent optical fiber is applicable as a simple and disposable optical sensor. The continuous measurement and the precious control of the sugar content in the fermentation process (plant cell cultivation) using developed sensor system will be carried out in the future.

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