Infrared spectral feature of plant-cell culture media

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Introduction

Recently, plant-cell cultivation technology has been developed and plays a significant role in various fields, from plant physiology to bioprocess engineering. Precision control of the cultivation process is one of the key technologies in both the scientific and engineering fields. Spectroscopic methods such as infrared spectroscopy provide significant potential for bioprocess monitoring. However, mid and near infrared spectroscopic methods have different advantages.

Fourier transform infrared spectroscopic method using an attenuated total reflection (FT-IR/ATR) technique has high potential as a quantitative analytical tool for liquids. The mid infrared (MIR) spectrum using the FT-IR/ATR method has shown that spectral additivity was applicable for sucrose, glucose and fructose in the plant-cell culture media, experimentally. We have examined the potential of sugar content determination in culture media of suspended *Nicotiana tabacum* cv. Bright Yellow No.2 (TBY-2) cells using the FT-IR/ATR method, by comparison with the high-performance liquid chromatography (HPLC) method, to analyse the sugar uptake rate by the suspension of TBY-2 cells.[1] Near infrared (NIR) spectroscopy is widely used for rapid and nondestructive analysis in industries such as agriculture, food, pharmaceuticals, textiles, cosmetics and polymer production. A larger number of NIR instruments have been developed because the NIR region is more flexible and easier to use more than the MIR region. However, light absorption of organic compounds is generally very weak and much overlap of absorption peaks is observed in the NIR region.

In this study, we investigated the spectral feature in the infrared region of plant-cell culture media, and studied how to apply the infrared spectroscopic method for analysis of the plant-cell cultivation process.

Materials and Methods

Plant-cell and culture condition

Arabidopsis thaliana MM2d cells were cultured in a Murashige and Skoog basal medium containing 3%(w/v) sucrose, 0.5 mgL^{-1} α-naphthalene acetic acid and 0.05 mgL^{-1} kinetin. Cells sub-cultured for 7 d were washed by the MS medium and inoculated into 40 mL of each fresh medium in 100 mL Erlenmeyer flasks. The cells were grown in the dark under agitation at 130 rpm at 27°C.

Measurement of infrared spectra

All the culture media in the flask were sampled at each sampling time, and filtered to enable the measurement of the MIR and NIR spectra. In order to determine the wave-number regions of the main sugars, aqueous solutions of sugar (sucrose, glucose, fructose, and a mixture of the three kind of sugar) were measured. To measure the MIR spectra, an FT-IR spectrometer (Nicolet, Magna 750) equipped with a KBr beam splitter and a deuterated triglycine sulfate KBr detector was used. The ATR spectra ranging from 4000 to 800 cm^{-1} at 4 cm^{-1} intervals were obtained with a horizontal zinc selenide ATR sampling accessory (Graseby Specac, SPECACLAMP ATR 11080). To measure the NIR spectra, the same spectrometer equipped with a cuvette holder was used. After filling the cuvette with a 0.1 mm or 2 mm path-length with the sample, the absorbance from 8000 to 4000 cm^{-1} at 4 cm^{-1} intervals was measured. The spectra were obtained by taking the average reading of 64 scans. Second derivative spectra were calculated by using the Savitzky–Golay method.

Results and discussion

Spectral feature of culture media during cultivation

Figure 1 shows the main absorptions observed on the infrared spectra of the culture media, and aqueous solutions of the mixture of three kinds of sugar.

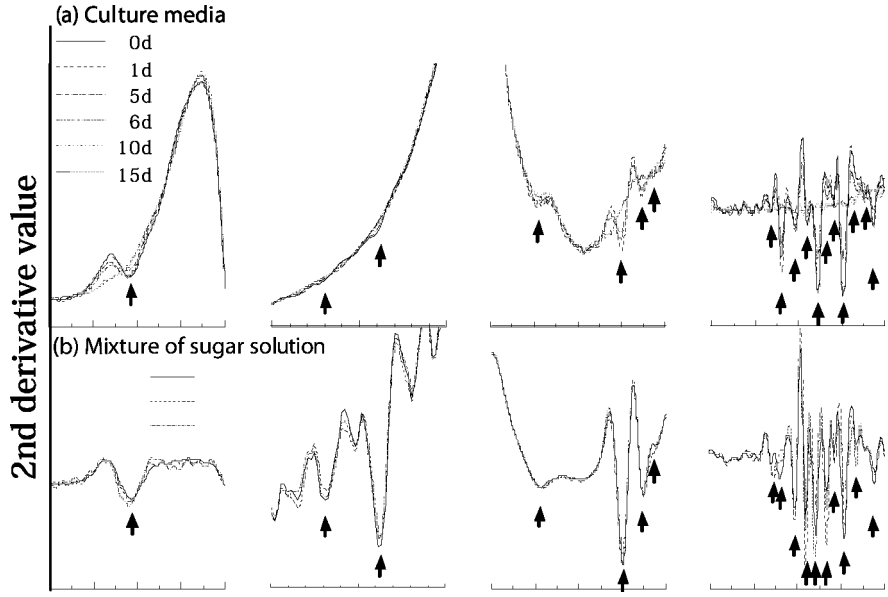

Figure 1. Main absorption peaks observed in the infrared spectra of (a) the culture media and (b) aqueous solution of sugar mixtures.

The mixture ratio was changed in same total sugar concentration. The most suitable conditions for calculation of the second derivative spectra were determined for focused wave-number regions. Second derivative spectra ranging from 1300 to 900, from 5000 to 4200, from 5500 to 5400 and from 6100 to 5700 cm^{-1} were calculated using 9, 41, 21 and 51 data points, respectively.

Absorption peaks caused by each sugar were observed on the lower wave-number region, MIR. Although some peaks overlapped, a detailed analysis would be possible even if the culture media of the plant-cells contained complex metabolic materials. On the higher wave-number region, NIR, several broad peaks were observed. The spectral correlation of the MIR region and NIR region was investigated with two-dimensional correlation spectroscopy,[2] by using the 2Dshige software that was programmed by Morita *et al.* These broad peaks had positive correlation with the peaks observed in lower wave-number region. However, although the mixture ratios of the sugar concentration were different, these lines were overlapped on the spectra of the sugar mixture solutions. Total sugar concentration will be measured by calibration equation using only one wave-number.

Time courses of sugar concentrations during cultivation

In our previous study, more detailed analysis data for sugar concentrations such as sucrose, glucose, fructose, mannose, galactose, treharose, mannose and lactose of culture media were reported, using MIR spectroscopy.[1,3,4] Figure 2 shows the time courses of the cell, sucrose, glucose, fructose and total sugar concentrations in the culture media during cultivation. In this figure, each sugar was measured by MIR and the total sugar concentration was calculated by summing up each sugar concentration.

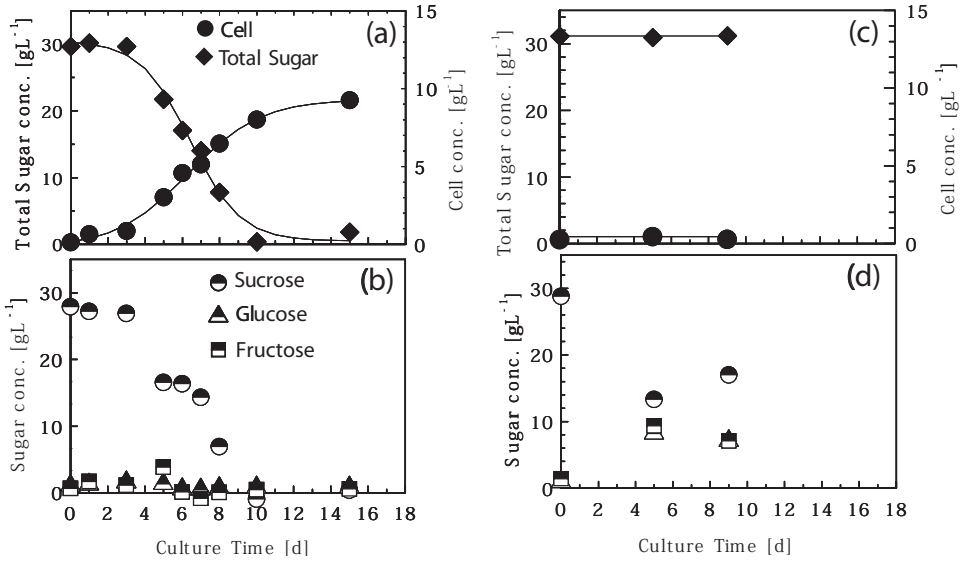

Figure 2. Prediction results of each sugar concentration using MIR spectral values during cultivation; Cell growth was observed (a) and (b) and not observed (c) and (d) in the processes.

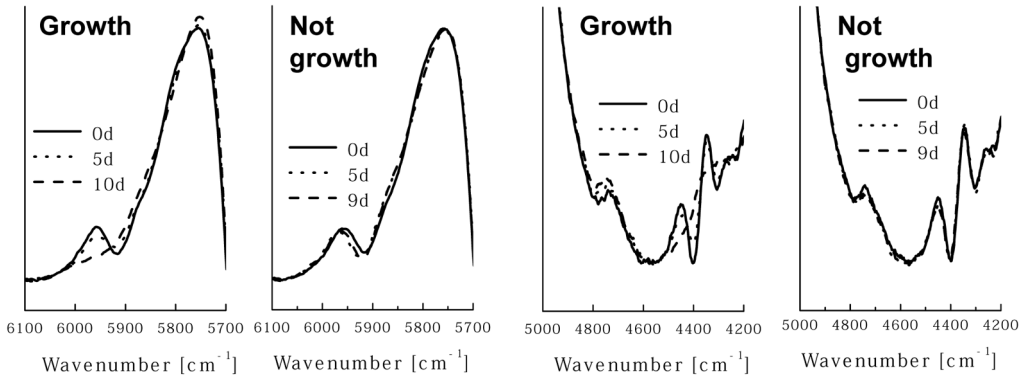

Figure 3. NIRspectral features of the culture media during cultivation.

In the culture media, sucrose was hydrolysed to glucose and fructose by the enzymes of the plant-cells, and the resulting glucose and fructose were consumed for cell growth [Figure 2(b)]. As a result, the total sugar consumption was closely related to cell growth [Figure 2(a)]. Practical bioprocess monitoring and simulation will be possible if total sugar concentration can be measured by a simple method.

In the case of the cultivation where cell growth was not observed, total sugar concentration did not change during cultivation [Figure 2(c)]. Even if the sucrose was hydrolysed and glucose and fructose were formed [Figure 2(d)]. The cause of this accident of cultivation was made clear by the detailed analysis using MIR.

Process management by observing NIR spectral pattern

NIR spectral feature of culture media was investigated in two processes (Figure 3).

The spectra of the culture media where cell growth was not observed were not changed during cultivation process. This could be detected by observing the NIR spectral pattern. A simple and cheap NIR analyser will be useful, if it is to work with a small-scale bioreactor. In addition, monitoring of total sugar concentration in culture media will be possible. It is very important because biological reactions such as sugar uptake and metabolism of the plant-cells can be described by kinetic models, and monitored using the NIR instrument.

Conclusion

In the infrared spectroscopic region, absorption peaks of sugars such as sucrose, glucose and fructose were observed in the MIR region and the peaks caused by these sugars were duplicated in the NIR region. The MIR region was suitable for detailed analysis, while the NIR region was suitable for rapid daily monitoring of the bioprocesses, and for determination of the total sugar concentrations more simply and easily. These results suggested that the NIR method has an advantage as a simple and practicable analyser in plant-cell cultivation.

Acknowledgment

This work was partly supported by a Grant-in-Aid for Scientific Research (C) 20560729 from the JSPS KAKENHI.

References

1. A. Hashimoto and T. Kameoka, *Appl. Spectrosc.* **54,** 1005 (2000).
2. I. Noda and Y. Ozaki, *Two-Dimensional Correlation Spectroscopy.* John Wiley & Sons Ltd, Chichester, UK (2004).
3. A. Hashimoto, A. Yamanaka, M. Kanou, K. Nakanishi and T. Kameoka, *Bioprocess Biosyst. Eng.* **27,** 115 (2005)
4. A. Yamanaka, A. Hashimoto, T. Matsuo, M. Kanou, K. Suehara and T. Kameoka, *Bioprocess Biosyst. Eng.* **30,** 457 (2007)