환경위생공학, 제23권 2호(2008)
Journal of Environmental and Sanitary Engineering, Vol. 23, No. 2, pp.19~25 (2008)

Application for Measuring the Glucose, Ammonia nitrogen, and Tylosin Concentration using Near Infrared Spectroscopy

Jong-Soo Kim[1] · Hoon Cho[2*]

[1]*Division of Chemical Engineering, College of Engineering, Chonbuk national university, Chonbuk 561-756, Korea.*
[2]*Department of Polymer Science & Engineering, Chosun University, Gwangju 501-759, Korea*

Abstract

For measurement of tylosin, ammonia nitrogen, and glucose concentration during the culture of *Streptomyces fradiae* using Near Infrared Spectroscopy, the calibration using various mathematical models was performed and then, based on the linear model, the validation was carried out. In the case of sucrose concentration using the MLR method, the Standard Error of Prediction and Multiple correlation coefficient were 1.97, and 0.991, respectively. In the case of ammonia nitrogen concentration using the PLSR method, the Standard Error of Prediction and Multiple correlation coefficient were 0.13, and 0.990, respectively. In the case of tylosin concentration using the PLSR method, the standard Error of Prediction and Multiple correlation coefficient were 0.54, and 0.984, respectively.

Key words : Glucose, Ammonia nitrogen, Tylosin, Near Infrared Spectroscopy.

* Corresponding author E-mail : hcho@chosun.ac.kr

Ⅰ. INTRODUCTION

Generally, for the measurement of sugar and ammonia nitrogen concentration in the fermentation broth, somogyi, HPLC, and kjeldahl analyzer methods requires that samples be taken from the fermentor were used. These steps can create concerns for contamination, can restrict the number of samples taken from the fermentor, and can complicate the automation schemes during the culture. In addition, the operation of these methods was not simple and required time for measurement. In order to overcome these problems, the noninvasive and nondestructive method has been applied. Spectroscopic methods provided a promising alternative for the monitoring of the fermenter. Such methods may be used to rapidly quantify the concentration of various species in aqueous system in a noninvasive and nondestructive method. Spectroscopic quantifications are based on introducing a beam of Near-infrared radiation to a sample, collecting transmittanted or reflected light, and correlating the amount and frequencies of light absorbed to the composition of the sample. This quantification process is rapid, requires no sample preparation, and can be used to quantify the concentration of multiple species simultaneously[1].

These spectroscopic methods have recently been applied to monitor a number of biological reactors. Near-Infrared (NIR) Spectroscopy was especially used for rapid analysis in industries, such as agriculture, food, pharmaceuticals, textiles, cosmetics, and polymer production industries. In the fermentation, Near-Infrared (NIR) Spectroscopy has been applied to measure the concentrations of ethanol[2] fructose, glycerol, glucose, and ethanol[3] the cell density[4] in the culture of *saccharomyces cerevisiae* and the concentrations of heterologous protein, glycerol, and methanol in the recombinant *Pichia pastoris* fed-batch process. [10] In a number of bacterial fermentations, the concentrations of lactate, glucose, and cell using *Lactobacillus casei* [5] the concentration of acetate,ammonia, glycerol, and cell[6] and the concentrations of cell, acetate, glycerol, and ammonium ion[7] using *Escherichia coli* have beensimilarly quantified by NIR spectroscopy. In the culture of mouse-mouse hybridoma cell produced by fusion of the mouse myeloma P3-NS1/1Ag4-1 with spleen cells from BALB/c mice, the concentrations of glucose, glutamine, ammonia, and lactate[8] and in theculture samples of Sf-900II growth medium of insect cell, the concentrations of glucose and glutamine[9] have been simultaneously quantified by NIR Spectroscopy. The NIR spectroscopy has been also applied to the measurement of cell mass for mushroom cultivation in solid media. However, there were no reports for the on-line measurement of the sucrose concentration using NIR spectroscopy in the L-lysine fermentation process when molasses was used as the carbon sources.

In a recent, for on-line measurement of the concentration of glucose, ammonia nitrogen source, and tylosin in the culture of *Streptomyces fradiae* when molasses was used as the carbon source, we

developed the system comprising a fermentor with NIR Spectroscopy.

Ⅱ. MATERIALS AND METHODS

1. Strain, Media, and Culture

The tylosin producing strain used in this study was *Streptomyces fradiae*TM-224. The composition of the agar medium was as follows (g/L): glucose, 10; yeast extract, 5; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.5; and agar 10. The composition of the seed medium was as follows (g/l): glucose, 5: yeast extract, 1; soybean meal, 2; $NaNO_3$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5. For the production of tylosin, the basal medium used was as follows (g/l): K_2HPO_4, 0.25; $MgSO_4 \cdot 7H_2O$, 0.5; and a solution of trace elements, 3 mL. The trace element solution contained the following ingredients (ppm): $FeCl_3$, 500; $ZnCl_2$, 600; $MnCl_2$, 100; $CoCl_2$, 300. The pH of the media was adjusted to 7.0-7.5 using 2M-KOH prior to sterilization. All the media components were sterilized at 121℃and 1.2 atm for 30 min. In the case of corn starch, it was partially hydrolyzed by the addition of 0.2% alpha-amylase (HS, Nagase Biochem. Ind. Ltd., Kyoto) at 121℃for 30 min. One loopful of *Streptomyces fradiae* TM-224 was transferred to a slant of the medium and cultured for 5 days. One loopful of the slant culture of *Streptomyces fradiae* TM-224 was then inoculated into a 500 mL Erlenmeyer flask, containing 50 mLof the seed medium, and cultured for one day on a reciprocating shaker at 120 rpm. For the production of tylosin, 10% of the seed was inoculated into a 500 mL Erlenmeyer flask, containing 50 mLof the production medium, or to fermentor, and cultured at 30℃

2. Calibration

A calibration is necessary to establish a correction between variation in the sample spectra and the parameters of interest, i.e, quantifiable properties in a quantitative calibration, or the identity of sample in a qualitative calibration. Quantitative calibrations are used to determine quantifiable properties of a product. These can be chemical or physical parameters. As different quantities of these properties will be reflected in the sample spectra, it is possible to calculate the property values from the NIR spectra. In order to obtain a linear model for quantitative applications, the various mathematical models were used. The derivation and the regression analysis were carried out using BRAN-LUEBBE software.

Ⅲ. RESULT AND DISCUSSION

Multiple Linear Regression is a quantitative calibration model that allows to establish a relationship between NIR spectra and sample properties. The basis for an MLR calibration are two sets of data: the set of reflectance or transmittance values measured at the different wavelengths for the calibration set samples and the set of the reference property values for the corresponding samples. The basic procedures of line or transformed values at a given wavelength.

Then a least squares fit is performed to determine the regression line. To find the best combination of wavelengths, the combination search and the step-up search were investigated. In an MLR calibration using the combination search algorithm (MLR), the best wavelengths are determined by trying all possible combinations for the number of search wavelengths entered in the "create/Edit calibration" dialog box. For each combination, the multiple correlation coefficient R is calculated by comparing the reference values with the values calculated from the regression equation. The best wavelength combination to model the calibration set is then the one where R is highest. In an MLR calibration using the step-up search (MLR-S), this method is a faster way to determine a combination of wavelengths than the combination search. It is especially suitable for scanning instruments, which provides a larger number of wavelengths by testing all possible combinations. If this algorithm is used, only the first wavelengths are determined in the same as for the combination search: all possible combinations are tried, and the combination with the highest Multiple Correlation coefficients, R, is chosen. After that, additional wavelengths are determined in step-up search. To determine the next wavelength, the wavelength set found in combination search is held constant, and then the best third wavelength is searched. PLSR and PCR use Principal Component Analysis to reorder the spectral information. This is a special kind of factor analysis in which a user-defined number of factors is calculated which best describe the variance in the calibration set of spectra. The difference between PLSR and PCR is that PLSR takes into account the reference property values for the determination of the factors whereas PCR does not. PLSR may therefore give better results if there are large spectral variations, which are not related to the properties of interest. In PLSR, eachspectrum is then described by a set of factors and scores and weights for each factor. In PCR, each spectrum is described by a set of factors and just the scores for each factor. Before deciding the use of a calibration equation for the prediction of property values of unknown samples, the predictive ability should be evaluated. In order to find the optimum calibration obtained by various methods, the validations were carried out using validation samples set, respectively, which was not used for calibration,

In order to validate the calibration equation using the MLR, PLSR, MLR-S, and PCR method, various samples were scanned. The concentrations of sucrose, ammonia nitrogen, and L-lysine in the prediction sample set were determined using the calibration equation and compared with the actual values. All samples were taken from the fermentor at irregular intervals. The concentrations of tylosin and sucrose were determined by HPLC method and the concentrations of the ammonia nitrogen, by kjeldahl. The results are shown in Fig. 1 and Table 1. In the Fig. 1, the NIR spectroscopy values were plotted on the vertical axis and the actual values, on the horizontal

axis.

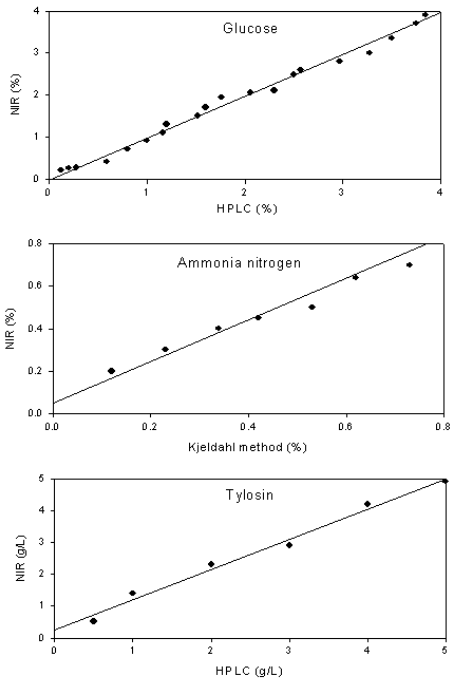

Fig. 1. Validation curves of glucose, ammoni nitrogen, and tylosin concentration.

Table. 1 Summary of validation results using fermentation broth.

Constituent	Range	N	SD	R	SEP	B
Glucose (%)	0.1-4.0	25	1.63	0.991	1.97	-0.033
Ammonia nitrogen (%)	0.12-0.8	27	0.32	0.990	0.13	-0.033
Tylosin (g/L)	0.5-5.0	17	4.15	0.984	0.54	-0.016

In the case of sucrose concentration using the MLR method, the validation regression equation was y = 1.016 x - 0.0413. The Standard Error of Prediction, Standard Deviation, and Bias were 1.97, 1.63, and - 0.03, respectively. Multiple correlation coefficient between the HPLC method and NIR spectroscopy method was observed with r = 0.991. In the case of ammonia nitrogen concentration using the PLSR method, the validation regression equation was y = 1.0016x + 0.026. The Standard Error of Prediction, Standard Deviation, and Bias were 0.13, 0.32, and -0.033, respectively. Multiple correlation coefficient between the Kjeldahl method and NIR spectroscopy method was observed with r = 0.990. In the case of L-lysine concentration using the PLSR method, the validation regression equation was y = 1.00 x + 0.013. The Standard Error of Prediction, Standard Deviation, and Bias were 0.54, 4.15, and -0.0163, respectively. Multiple correlation coefficient between the HPLC method and NIR spectroscopy method was observed with r = 0.984.

In order to reconfirm the reliability of the Near-Infrared spectroscopy results in comparison with those obtained by conventional methods on the concentration of glucose, ammonia nitrogen, and tylosin, batch cultures were carried out in a 5L fermentor containing 1.3 of initial volume comprising 40 g/L of glucose. The culture temperature was maintained at 30℃ Agitation rate was fixed at 300 rpm and aeration was also fixed at 1.0 vvm. The results are shown in Fig. 2 The results obtained by NIR Spectroscopy measurements were not showed any significant differences in comparison with those obtained by conventional methods and the variations in the concentration of the two components studied were successfully monitored by the NIR spectroscopy readings. There

were no contamination problems coming from the employment of NIR spectroscopy and the flow cell did not become fouled for tylosin fermentation.

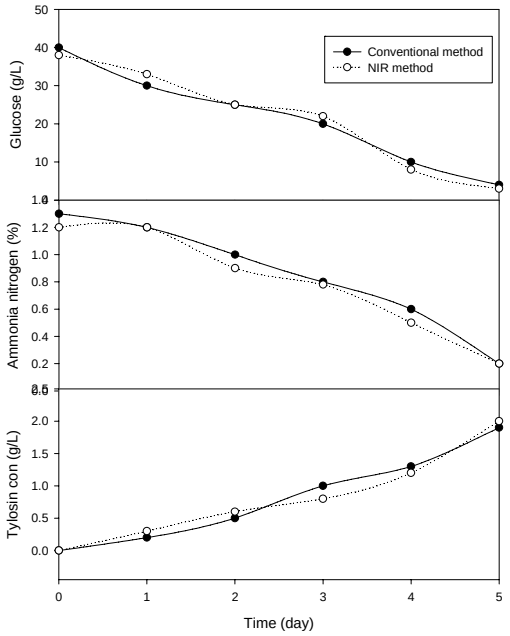

Fig. 2. Comparison of conventional method and NIR on the concentration of glucose, ammonia nitrogen, and tylosin,

Ⅳ. CONCLUSION

In order to reconfirm the reliability of the NIR Spectroscopy results in comparison with those obtained by conventional methods on the concentration of glucose, ammonia nitrogen, and tylosin in the culture of *Streptomyces fradiae*, batch cultures were carried out. The results obtained by NIR Spectroscopy measurements were not showed any significant differences in comparison with those obtained from conventional methods, and the variations in the concentration of the three components studied were successfully monitored by the NIR spectroscopy reading.

REFERENCES

1. Osborne, G. B. and Fearn. T.: Near Infrared Spectroscopy in food analysis, Longman Scientific and technical, Lonman Group UK Limited, 1986.
2. Cavinato, A.G., Mayes, D.M., Ge, Z., Callis, J. B.: noninvasive method for monitoring ethanol in fermentation processes using fiber-optic near-infrared spectroscopy, *Anal. Chem.*, 62, 1977-1982, 1990.
3. Fayolle, P., Picque, D., Perret, B., Latrille, E., corrieu, G.: Determination of major compounds of alcoholic fermentation by middle-infrared spectroscopy: study of temperture effects and calibration methods, *Appl. Spectrosc*, 50-1325-1330, 1996.
4. Ge, Z., Cavinato, A. G., Calis, J. B.: Noninvasive spectroscopy for monitoring cell density in a fermentation process, *Anal. Chem.*, 66, 1354-1362, 1994.
5. Vaccari, G., Dosi, E., Campi, A. L, Gonzalez-varay R, A., Matteuzzi, D., Mantovani, G.: A near-infrared spectroscopy technique for the control of fermentation processes: application to lactic acid fermentation, *Biotechnol. Bioeng.*, 43, 913-917, 1994.
6. Hall, J. W., Mcneil, B., Rollins, M. J., Draper, I., Thompson, B. G., Macaloney, G.: Near-infrared spectroscopic determination of acetate, ammonium, biomass, and glycerol in an industrial *Escherichia coli* fermentation, *Appl. Spectrosc*, 50, 102-108 (1996).
7. Macaloney, G., Draper, I., Preston,

J., Anderson, K. B., Rollins, M. J., Thompson, B. G., Hall, J. W., and McNeil, B.,: At-line controls and fault analysis in an industrial high cell density *Escherichia coli* fermentation, using NIR spectroscopy, *Food bioprod. Test.*, 74, 212-220, 1997.

8. Yano, T. and Harata, M.: Prediction of the concentration of several constituents in a mouse-mouse hybridoma culture by near-infrared spectroscopy, *J. Ferment. Bioeng.*, 77-659-662, 1994.
9. Riley, M. R., Rhiel, M., Zhou, X., Arnold, M. A., Murhammer, D. W.: simultaneous monitoring of glucose and glutamine in insect cell culture media by NIR spectroscopy. *Biotechnol. Bioeng.*, 55, 11-15, 1997.
10. Crowley, J., McCarthy, B., Nunn, N. S., Harvey, L. M., and McNeil, B.: Monitoring a recombinant *Pichia pastoris* fed batch process using fourier transform mid-infrared spectroscopy, Biotechnology letters, 22, 1907-1912, 2000.