Mycobiology 31(4): 226-228 (2003)

Estimation of Cell Concentration by Light Transmitter During the Culture of Methylotrophic Yeast *Pichia pastoris*

Du Bok Choi*, Enoch YS Park[1], Yong Bo Lee[2], Young Hee Na[2] and Chae-Kyu Lim[3]

Department of Environmental Engineering, Cho-dang University, 419, Songnam-ri, Muan-up, Muan-kun, Chonnam 534-800, Korea
[1]Biochemical Engineering Lab, Applied Biological Chemistry, Shizuoka University, 836 Ohya, Shizuoka City, 422, Japan
[2]Division of Science Education, College of Education, Chosun University, Gwangju 501-759, Korea
[3]Department of Herbal Medicine Resources Development, Naju College 520-713, Korea

(Received June 30, 2003)

The multiple correlation coefficient between the values determined by dry weight and those determined by fluorometer was observed with r = 0.96 and the standard error of calibration was 0.034. Using the best calibration data, in order to reconfirm the reliability of the fluorometer results in comparison with those obtained by dry weight on the cell concentration, fed-batch cultures were carried out. The results obtained by fluorometer measurements were in good agreement with those obtained by dry weight. The on-line monitoring of cell concentration by the fermentor system linked to a computer equipped with fluorometer was successfully carried out.

KEYWORDS: Fluorometer system, Light transmitter, *Pichia pastoris*

There are various methods for monitoring the cell concentration by on-line or off-line, for example, the control of carbon source concentration using an independent parameter such as the amount of oxygen consumed, amount of carbon dioxide exhausted, amount of the by-products produced, and amount of ammonia used while using carbon source in an amount obtained by multiplying them with a predetermined proportional coefficient (Armiger *et al.*, 1985). However, microbial activity by these methods during the culture were difficult to be measured with high accuracy so that in some instances the concentration can not be measured effectively when the activity has abnormally changed. Recently, in order to overcome these problems, the noninvasive and nondestructive methods have been applied (Stark *et al.*, 1986). The spectroscopic methods provided a promising alternative for the monitoring of the reactor. Such methods were used to rapidly quantify the concentration of various species in aqueous systems. The spectroscopy quantifications are based on introducing a beam of Near-infrared radiation to a sample, collecting transmittaned or reflected light, and correlating the amount and frequencies of light absorbed to the composition of sample. This quantification process is rapid, requires no sample preparation, and can be used to quantify the concentration of sample species simultaneously (Osborne *et al.*, 1986). In a number of bacterial cultures, the cell concentration using *Lactobacillus casei* (Ge *et al.*, 1994) and *Escherichia coli* have been similarly quantified by NIR spectroscopy. The NIR spectroscopy has been also applied to the measurement of the centent of water and rice bran (Yano *et al.*, 1999) and management of mixing process in preparation of solid media used for mushroom cultivation (Yano *et al.*, 2000).

Generally, for the measurement of cell concentration in the culture broth, dry weight, packed cell volume, DNA, and optical density methods using spectroscopy requiring that samples be taken from the reactor have been used for a long time. We previously used the spectroscopy and dry weight for the measurement of cell concentration for high cell culture of methylotrophic yeast *Pichia pastoris* using Jar fermentor by off-line (Uehara *et al.*, 2000). This method can create concerns for contamination, can restrict the number of samples taken from the reactor, and can complicate the automation schemes during the culture.

In this study, in order to measure the cell concentration using the fluorometer during the culture of methylotrophic yeast *Pichia pastoris* at the on-line monitoring system, the multiple correlation coefficients between the values determined by dry weight and those determined by fluorometer was investigated. Using the best calibration data, fed-batch cultures were also carried out.

Materials and Methods

Microorganisms, medium, and culture conditions. The strain used in this study was *Pichia pastoris* Mut s G2-66319. The composition of the agar slant medium used was as follows (g/l): glucose, 50; peptone, 20; $NaNO_3$, 2; $MgSO_4 \cdot 7H_2O$, 1; yeast extract, 3. The composition of the seed medium used in the seed culture was as follows (g/l): glycerol, 10; yeast nitrogen base w/o amino

*Corresponding author <E-mail: choidubok@hotmail.com>

acids, 6.7; yeast extract, 6.7; polypeptone, 20; and biotin 0.4 mg (Uhehara *et al.*, 2000). All the media components were sterilized at 121°C and 1.2 atm for 20 min. The pH of the media was adjusted to 6.0 before sterilization. One looful of *Pichia pastoris* 66319 was transferred to the slant medium and cultured at 30°C for 5 d. Then, one looful of the slant culture of *Pichia pastoris* Mut s G2-66319 was inoculated into a 500 *ml* Erlenmeyer flask containing 50 *ml* of the seed medium and cultured at 28°C for 1 d on a reciprocating shaker at 150 rpm. The dissolved oxygen concentration in the culture broth was measured by a DO sensor (Marubishi MDL-300). In order to maintain DO concentration at 20~30%, oxygen-enriched air (30%) was supplied throughout the experiment and the oxygen concentration was controlled by oxygen concentrator.

Cell concentration. The cell concentration was measured by dry weight as follows; a sample of culture broth has centrifuged at 3000 rpm for 15 min, removed the supernatant, and filtrated by the filter paper (Sartorius 0. 8 micro). The filtrated cell was dried at 80°C for 12 hr and the dried cell was weighed.

On-line systems using the light transmitter. Schematic diagram for on-line monitoring of the cell concentration during the culture of *Pichia pastoris* Mut s G2-66319was shown in Fig. 1. The on-line systems were consisted of reactor, computer, and interface. The fluorometer was set into the reactor directly. It was consisted of optical filter passing 366 nm and 460 nm light fluorometer, photo mutiplier, lamp supply and detecter. It measures the amount of light that passes through a process fluid and has a little effect through microorganism itself and radiation materials remained at the culture broth by intercepting below 366 nm of wavelength using infrared filter.

The measurement of cell concentration by the fluorometer was carried out under follow conditions: The cell concentration was measured by the transmitted light at 20 min intervals. The culture was irradiated with light near 366 nm by using a fluorometer ultraviolet lamp and an optical filter (flurometer CT corning optical products, Inc., USA). This light excited the culture, causing it to fluoroesce near 460 nm. The fluoresent light was measured using a photomultiplier that was filtered to screen out.

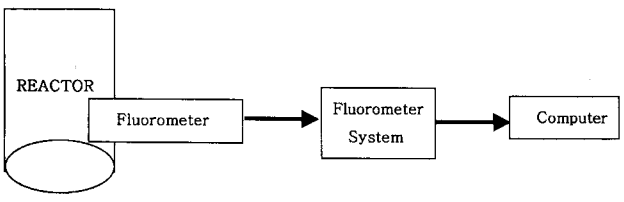

Fig. 1. On-line monitoring system for measuring the cell concentration.

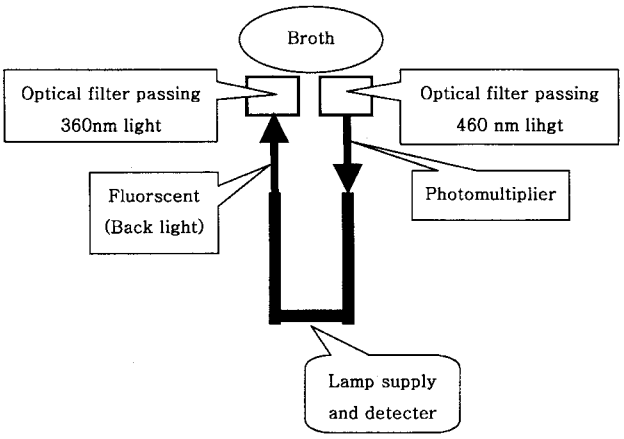

Fig. 2. Fluorometer system for measuring the cell concentration.

Result and Discussion

Calibration of cell concentration. In order to perform the calibration using dry weight and fluorometer, various samples were measured. The cell concentrations in the calibration sample set were determined and compared with the actual values. All samples were taken from the reactor at irregular intervals. The cell concentration was measured by dry weight.

The results are shown in Fig. 3 and Table 1. In Fig. 3, the values determined by the fluorometer were plotted on the vertical axis and the dry weight, on the horizontal

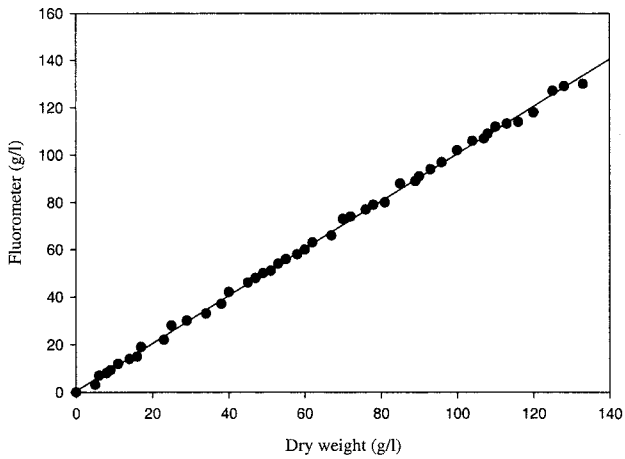

Fig. 3. Relationship between values by light transmitter and dry weight.

Table 1. Summary of calibration results for cell concentration using culture broth

N[a]	SD	R	SEC	B
45	1.25	0.96	0.034	−0.25

[a]N, Number of samples; SD, Standard Deviation; R, Multiple Correlation Coefficient; SEC, Standard Error of Calibration; and B, bias.

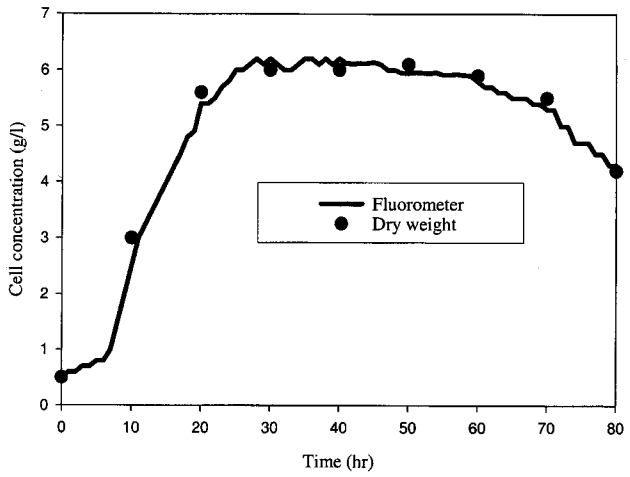

Fig. 4. Comparison of values measured by light transmitter and conventional method in batch culture.

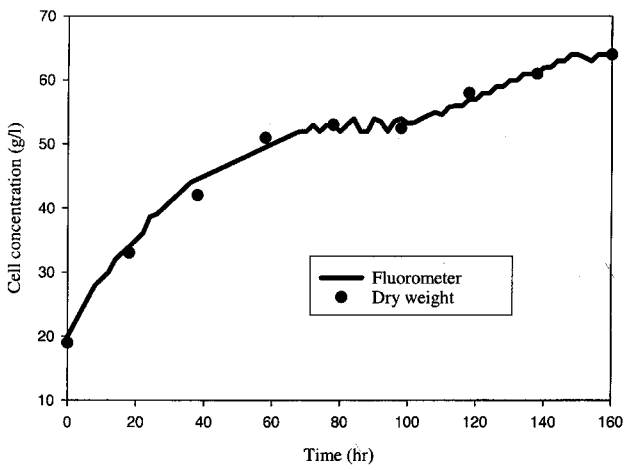

Fig. 5. On-line monitoring of cell concentration measured by fluorometer and conventional method in fed batch culture.

axis. The regression equation of calibration was Y = 0.96x −0.267. The multiple correlation coefficient between the dry weight and those determined by fluorometer was observed with r = 0.96 and the standard error of calibration was 0.034.

Comparison of cell concentration measured by fluorometer and conventional methods in batch culture. In order to reconfirm the reliability of the by fluorometer results in comparison with those obtained by conventional methods on the cell concentration, the batch cultures were carried out in a 5 *l* fermentor containing 3 *l* of initial volume comprising glycerol, 40; yeast nitrogen base w/o amino acids, 10; yeast extract, 6.7; polypeptone, 20; and biotin, 0.4 mg. Agitation rate was fixed at 600 rpm and aeration was also fixed at 1.0 vvm. The sensor direction was also fixed at 180°. The results are shown in Fig. 4. The results obtained by by fluorometer measurements were not showed any significant differences in comparison with those obtained by conventional methods.

On-line monitoring for measuring the cell concentration in fed batch culture. Using the best calibration data, the on-line monitoring system of cell concentration by the fermentor system linked to a computer equipped with fluorometer was developed. In order to reconfirm the reliability of the fluorometer results in comparison with dry weight on the cell concentration, fed-batch cultures were carried out. For fed culture in 5 *l* fermentor containing 2.5 *l* of initial working volume, the initial medium used was as follows (g/l): glycerol, 50; yeast nitrogen base w/o amino acids, 20; yeast extract, 20; polypeptone, 30; and biotin, 0.8 mg. Fifties of glycerol was added to fermentor every 24 h. The culture was maintained at 28°C for 160 hr. Agitation rate was fixed at 600 rpm and aeration was also fixed at 1.0 vvm. The sensor direction was also fixed at 180°. The results are shown in Fig. 5. The results obtained by fluorometer measurements were in good agreement with conventional method. The on-line monitoring system of cell concentration was successfully carried out.

References

Armiger, W. and Moo-young. 1985. In comprehensive biotechnology, pergamon press, NY. Vol. 1, 133-148.

Stark, E., Luchter, K. and Margoshed, M. 1986. Near-infrared analysis, App. Spectro, **22**(4): 335-399

Osborne, G. B. and Fearn, T. 1986. Near Infrared Spectroscopy in food analysis, Longman Scientific and technical, Lonman Group UK Limited.

Ge, Z., Cavinato, A. G. and Calis, J. B. 1994. Noninvasive spectroscopy for monitoring cell density in a fermentation process, *Anal. Chem.* **66**: 1354-1362.

Hiroyuki Uehara, Du Bok Choi, Enoch Y. Park, and Mitsuyasu Okabe. 2000. Expression of Mouse a-amylase gene in methylotrophic yeast *Pichia pastoris. Biotechnol. Bioprocess Eng.* **5**: 7-12.

Yano, T., Suehara, K. and Nakano, Y. 1998. Determination of the content of water and rice bran in solid media used for mushroom cultivation using near infrared spectroscopy, **86**(5): 472-476.

_____, _____ and _____. 2000. Management of mixing process in preation of solid media used for mushroom cultivation using near infrared spectroscopy, Near infrared spectroscopy (proceeding of the 19th intenational conference), 351-355.