Estimation of Biomass Concentration for Palm Oil Fermentation

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Palm oil 醱酵중의 微生物 菌體濃度의 測定方法

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Summary

Several methods were investigated to estimate the biomass concentration of the culture broth rapidly and accurately in palm oil fermentation. Among the methods used in this study, the measurement of optical density of cell suspensions had a good result, and the procedure was as follows. After centrifuged the culture broth with the addition of solvent system (1,4-dioxane : ethylacetate : broth=3 : 2 : 1, by volume), and resuspended the pellet with deionized water to a suitable concentration, the optical density was measured at a wavelength of 560nm.The determination of cellular protein content in the culture broth, cumulative alkali consumption during cultivation, and dried weight measurement were also showed good results.

Introduction

Biomass concentration is one of the most fundamental fermentation process variables. Methods employing the optical properties of cell suspensions have rapid response times, but suffer from numerous interferences such as medium solids, dense medium coloration, mycelial culture morphology, and separatin of substrate from culture broth such as hydrocarbon and palm oil fermentation, which are characteristic of many practical fermentations (Zabriskie and Humphrey 1978). The standard method of monitoring cell concentration is direct determination of cell weight after separation from culture fluid and drying at temperatures of about 100°C, this is rather time-consuming and impractical for fermentation media containing insoluble material (Koliander et al. 1984). In the last decade, attention has been focused on procedures providing indirect estimates by measuring process variables associated with culture growth, such as culture viscosity (Shimmons et al. 1976), heat (Wang et al. 1978), carbon dioxide evolution (Harima and Humphrey 1980, Park et al. 1983), oxygen uptake

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(Zabriskie and Humphrey 1978), mass balancing (Wang et al. 1977, Mou and Cooney 1983) and alkali consumption (Verkooyen and Rietema 1980). Besides, indirect methods using quantification of typical cellular compounds such as proteins, nucleotides (ATP, NAD) or nucleic acids may be alternative techniques (Hysert et al. 1979, Johnson-Wint and Hollis 1982, Koliander et al. 1984).

Among these, the mesurement of optical density of cell suspension has been employed widely in the estimation of biomass concentration. However, it has not been established to now in water-insoluble substrates based fermentation. The methods of cell mass determination with optical density used in hydrocarbon fermentation (Nakahara et al. 1968) were inadquate in palm oil fermentation because of the properties of the substrates. Furthermore, in the determination of cell growth with optical density, the values would be different according to the species of strains such as size, density and physiological characteristics, and properties of culture broth. In this study, several methods were investigated to determine the biomass concentration rapidly and acccurately, and was compared with each other and discussed in palm oil fermentation.

Materials and Methods

Microorganisms

Microorganisms used in this study were *Torulopsis candida* Y-128 and *Acinetobacter calcoaceticus* KB-2, SCP-producing yeast and bacterium strains. Taxonomic characteristics and procedures for maintaining stock cultures were described previously (Koh et al. 1983, Koh et al. 1985b).

Media and Culture conditions

Crude palm oil was supplied by Kao Soap Co. Ltd., and refined palm oil was commercial basis (Nippon Oil and Fats Co. Ltd.). Media and culture conditions for *T. candida* Y-128 and *A. calcoaceticus* KB-2 were described previously (Koh et al. 1983, Koh et al. 1985a, Koh et al. 1985b).

Assay procedure

In screening of yeast strains capable of assimilating palm oil, assay procedure was carried out by the determination of protein content in the culture broth (Koh et al. 1983). For the determining of cultural conditions of *T. candida* Y-128, cell growth was monitored by measuring the optical density of the culture broth with the addition of organic solvent system (1, 4-dioxane : ethylacetate=3 : 2, by volume). The optical density of the cell suspension was measured directly with a Spectronic-20 (Bauch & Lomb) in a shake culture (Koh et al. 1983), and was measured after centrifugation and resuspension of precipitated cells in a jar fermentor culture (Koh et al. 1985a). For the strain of *A. calcoaceticus* KB-2, the optical density of cell suspension was measured with a double beam spectrophotometer (Hitachi, model-124). Dry cell weight measurements in continuous culture of *A. calcoaceticus* KB-2 was as follows. Two ml of culture broth was transferred into a tared centrifuge tube, and added 5 ml of acetone. The biomass was sedimented by centrifugation (3,000 rpm, 10 min), and the supernatant fluid was discarded. The pellet was resuspended in 5 ml of acetone, sedimented, and washed with 5 ml of ethylether. The pellet was dried at 105°C, cooled and weighed. The dry weight mesurement was performed in triplicate, and the averages are reported.

Results and Discussion

In screening of yeasts capable of assimilating palm oil for single cell protein poduction, the

determination of protein content of the culture broth was used. The modified Lowry method(1951) had a good result in the determination of cell mass of *T. candida* Y-128 as shown in Figure 1. However, this method was time-consuming, and cell mass was not known exactly by the strains and culture conditions, but other methods such as the measurement of optical density or dry weight of cell mass were also inadequate in screening of the strains for single cell protein production. The method of measuring protein content would be useful for this purpose.

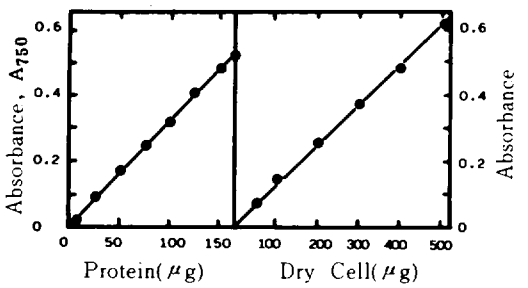

Fig. 1. Determination of protein by the method of Lowry et al. (1951). Proceeded by heating in 1 N NaOH for 10min at 100°C, was applied to standard solutions of bovine serum albumin(left), and a dried cell of *T. candida* Y-128 (right).

After screening and selection of the strain, various organic solvents were employed to determine the cell growth with optical density directly or indirectly. Palm oil was not solubilized easily in polar solvents such as alcohols and acetone, but was solubilized easily in some kinds of nonpolar solvents such as ethylacetate, 1,4-dioxane, chloroform, n-hexane and cyclohexane. Solvent systems used in hydrocarbon fermentation (Nakahara et al. 1968) were not adapted in palm oil fermentation for measuring the cell growth as shown in Table 1. Therefore, new solvent system was needed in palm oil fermentation. After employing of various organic solvent systems, the solvent system (1,4-dioxane : ethylacetate : water = 3 : 2 : 1) was found to be the best to determine the cell mass and cell concentration with optical density.

Although experimental errors were arised in the initial stages of cultivation, these are probably to come from the sampling techniques in a heterogenous phase of culture broth. When the solvent system was mixed with culture broth, it was possible to measure the optical density directly in low concentrations of cell mass(Koh et al. 1983). However, cells were precipitated easily in high concentrations of cell mass. Optical density was

Table 1. Selection of solvent systems in palm oil fermentation

Solvent System	Solubility of 5% Palm Oil (Water/Solvent = 1/5)*	Remark
Ethanol : Ethylacetate: Cyclohexane (50:50:1)**	+	Milky and turbid
Ethanol : Butanol : Chloroform (10:10:1)**	+	〃
Butanol : Dioxane (1:1)**	++	Milky
Butanol : Ethylacetate (1:1)**	++	〃
Dioxane : Ethylacetate (3:2)	+++	Transparent
Dioxane : Butylacetate (1:1)	+	

* + a little soluble, ++ soluble well, +++ soluble homogeneously.
** Solvent systems used in hydrocarbon fermentatin (Nakahara et al. 1968).

measured directly after the addition of solvent system in a shake culture of palm oil fermentation, and the calibration curve shows a good linear correlation between optical density and dried cell weight as shown in Figure 2.

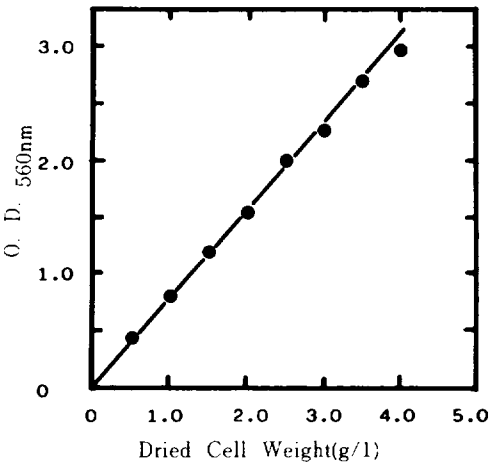

Fig. 2. Relation between optical density and dried cell weight of *T. candida* Y—128 in a shake culture.

In jar fermentor cultures, cell growth was monitored by measuring the optical density of the culture broth as following procedure: centrifugation after the addition of solvent system to culture broth, dilution of the precipitate with deionized water to a suitable concentration and measuring the optical density at a wavelength of 560 nm.

carotenoids contained in palm oil, especially crude palm oil, also affected the absorbance wavelength between 320 and 540 nm as shown in Figure 3, and samples containing cells showed maximal peak between wavelength 530 and 595 nm. Therefore, optical density used in this study was measured at a wavelength of 560 nm, although the values of optical density were different according to the spectrophotometers, the calibration curves show good linear correlations between optical density and dried cell weight as shown in Figure 4.

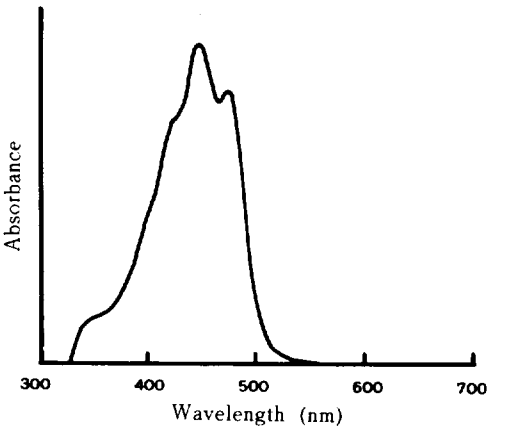

Fig. 3. Change in visible absorption spectra. Crude palm oil (0.4%) was dissolved in n-hexane.

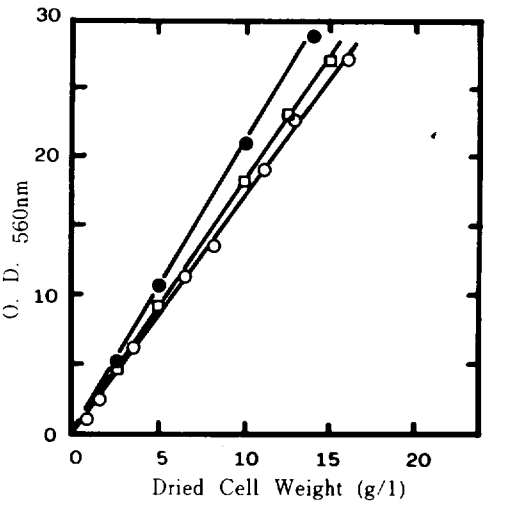

Fig. 4. Relation between optical density and dried cell weight of *T. candida* Y—128 (○), and *A. calcoaceticus* KB—2 with Spectronic—20(●) and with double beam spectrophotometer(□) in a jar fermentor culture.

The cumulative alkali consumption showed also a good correlation to the yeast cell growth (Koh et al. 1980, Verkooyen and Rietma 1980), and this method was very simple and convenient, but this

method could be used to determine the relative cell growth only when the chemical compositions of the medium is defined in a jar fermentor culture.

The cells of *A. calcoaceticus* KB-2 were not separated easily with low-speed centrifugation when the culture broth contained lipid materials. When acetone was added to the culture broth at the ratio of acetone: broth=2:1, the cells were precipitated easily. Therefore, cell growth in continuous culture of *A. calcoaceticus* KB-2 was monitored by measuring the dried cell weight. When the strain used in the experiments produce a surface active agent, or lipid materials is emulsified into the culture broth by the strain during cultivation, direct determination of cell weight with only centrifugation would cause the experimental errors because of residual oils. The use of acetone would be proper to remove the residual oils in the culture broth in this respect.

In the determination of cell growth in palm oil fermentation, it is not easy to establish the method of measuring cell mass exactly without experimental errors. Palm oil was not solubilized easily in most of organic solvents, and that some amount of cellular lipids was extracted by nonpolar organic solvents such as ethylether and chloroform. Therefore, considering the strain used, medium compositions, and culture conditions, the method of determining cell mass should be selected. From the results of this study, it seemed to be reasonable in a degree to measure the optical density after removal of residual palm oil with proper organic solvents. The solvent system (1,4-dioxane: ethylacetate=3:2) is useful for measuring cell growth with optical density in palm oil fermentation, but toxicity of 1,4-dioxane is a problem for long-term uses.

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Literature cited

Harima, T. and Humphrey, A. E., 1980. Estimation of *Trichoderma* QM 9414 biomass and growth rate by indirect means. *Biotechnol. Bioengineer.*, 22: 821-831.

Hysert, D. W., Knudsen, F. B., Morrison, N. M. and Van Gheluwe, G., 1979. Application of a bioluminescene ATP assay in brewery wastewater treatment studies. *Biotechnol. Bioengineer.*, 21: 1,301-1,314.

Johnson-Wint, B. and Lollis, S., 1982. A rapid *in situ* deoxyribonucleic acid assay for determining cell number in culture and tissue. *Anal. Biochem.*, 122: 338-344.

Koh, J. S., Kodama, T., Yamakawa, T. and Minoda, Y., 1980. Microbial utilization of palm oil-screening of yeasts and cultural conditions of jar fermentor for cell production-. ICME *Ann. Rep.*, 3: 167-180.

Koh, J. S., Kodama, T. and Minoda, Y., 1983. Screening of yeasts and cultural conditions for cell production from palm oil. *Agric. Biol. Chem.*, 47: 1,207-1,212.

Koh, J. S., Yamakawa, T., Kodama, T. and Minoda, Y., 1985a. Cultural conditions for *Torulopsis candida* cell production from palm oil. *Agric. Biol. Chem.*, 49: 215-216.

Koh, J. S., Yamakawa, T., Kodama, T. and Minoda, Y., 1985b. Rapid and dense culture of *Acinetobacter calcoaceticus* on palm oil. *Agric. Biol. Chem.*, 49: 1,411−1,416.

Koliander, B., Hampel, W. and Roehr, M., 1984. Indirect estimation of biomass by rapid ribonucleic acid determination. *Appl. Microbiol. Biotechnol.*, 19: 272−276.

Lowry, O. H., Rosebrough, N. I., Farr, A. L. and Randall, R. J., 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.*, 193: 265−275.

Mou, D. G. and Cooney, C. L., 1983. Growth monitoring and control through computer-aided on-line mass balancing in a fed-batch penicillin fermentation. *Biotechnol. Bioengineer.*, 25: 225−255.

Nakahara, T., Hisatsuka, K. and Yamada, K., 1968. Determination of cell mass in hydrocarbon fermentation. *Hakkogyokaishi*, 26: 413−416.

Park, S. H., Hong, K. T., Lee, J. H. and Bae, J. C., 1983. On-line estimation of cell growth for glutamic acid fermentation system. *Eur. J. Appl. Microbiol. Biotechnol.*, 17: 168−172.

Shimmons, B. W., Svrcek, W. Y. and Zajic, J. E. 1976. Cell concentration control by viscosity. *Biotechnol. Bioengineer.*, 18: 1,793−1,805.

Verkooyen, A. H. M. and Rietma, K., 1980. Growth of yeast on n-alkanes. *Biotechnol. Bioengineer.*, 22: 615−637.

Wang, H. Y., Cooney, C. L. and Wang, D. I. C., 1977. Computer-aided baker's yeast fermentations. *Biotechnol. Bioengineer.*, 19: 69−86.

Wang, H., Wang, D. I. C. and Cooney,C. L., 1978. The application of dynamic calorimetry for monitoring growth of *Saccharomyces cerevisiae*. *Eur. J. Appl. Microbiol. Biotechnol.*, 5: 207−214.

Zabriskie, D. W. and Humphrey, A. E., 1978. Estimation of fermentation biomass concentration by measuring culture fluorescene. *Appl. Environ. Microbiol.*, 35: 337−343.

國 文 抄 錄

Palm oil 醱酵중의 微生物 菌體濃度의 測定方法

Palm oil 醱酵過程중에 微生物菌體를 신속하고 정확하게 測定하기 위한 여러 方法을 檢討하였다. 微生物培養液에 有機溶媒(1,4−dioxane : ethylacetate=3 : 2)를 添加하여 殘存油脂를 용해한 후 菌體현탁액을 조제하고 560 nm에서 吸光度를 測定하는 方法에 의해 좋은 結果를 얻었다. 또한 培養液중에 있는 微生物菌體의 蛋白質의 測定, jar培養중의 알칼리 消費量의 測定, 乾燥菌體量의 測定方法도 有效하였다.