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Monitoring of itaconic acid production by a 2-dimensional fluorescence sensor

[1]정상윤, [2]이종일,
전남대학교, [1]물질생물화공과, [2]화학공학부
전화 (062)530-0847, Fax (062)530-1847

Abstract

The fluorescence sensor is utilized to monitor the complex fluorescence patterns of intra- and extracellular components in cultivation processes. Especially biogenic fluorophores such as proteins and peptides (tryptophan, phenylalanine), coenzymes (FAD, NAD(P)H) and vitamins (riboflavin, pyridoxine) within cells are detected by a fluorescence sensor. In this work a 2-dimensional fluorescence sensor has been used to monitor a production process of itaconic acid by *Aspergillus terreus* and the on-line monitored spectra data can be correlated to off-line data measured by a few methods.

Introduction

Itaconic acid (methylene succinic acid) is an unsaturated dicarboxylic acid produced by *A. terreus*. It is an important intermediate for the manufacturing polyester resins or other polymeric materials as well as N-substituted pyrrolidones in many chemicals(1,2). For the high productivity of itaconic acid during fermentation process, it is necessary to monitor some factors such as phosphate concentration, oxygen supply, pH, temperature etc. On-line and in-situ monitoring of the bioprocess is important for the understanding metabolic pathway of the biological system in a bioreactor. It provides opportunity to detect some problems early and to solve those as soon(3). In a fermentation process of *A. terreus* it is not easy to take samples and measure some components such as cell mass because of mycelial growth on bioreactor's walls. Furthermore analysis of itaconic acid is time-consuming.

Optical sensor systems are very effective to monitor and control biological processes due to their stability in high temperature conditions. Among optical sensors fluorescence sensors have been often used to monitor some components

in biological processes. Fluorescence sensors had normally fixed excitation and emission wavelengths, so they could monitor only limited fluorophores such as NAD(P)H. Recently 2 dimensional fluorescence sensors has been developed and used for the monitoring of substrates or products in the bioprocesses(3,4).

In this study a 2-D fluorescence sensor has been employed for process monitoring of itaconic acid production and some relationship is investigated among fluorescence spectra, off-line data and on-line data monitored by a FIA (flow injection analysis) system as well as also by an optic fiber biosensor.

Materials and Methods

Microorganisms and medium composition. Strain used in this study was *Aspergillus terreus* DSMZ 5770. The spores of the strain were kept in glycerol stock solution. The growth culture medium consisted of (g/L): glucose, 20; $(NH_4)_2SO_4$, 2.5; $MgSO_4 \cdot 7H_2O$, 1; KH_2PO_4, 2.5; NaCl, 2; $CuSO_4 \cdot 5H_2O$, 0.005; $FeSO_4 \cdot 7H_2O$, 0.02; $ZnSO_4 \cdot 7H_2O$, 0.005. For the production medium more glucose (50~100 g/L) was added to the growth culture medium.

Cultivation. The spore stock solution (1 mL) of *A. terreus* was first inoculated into the growth culture medium of 100 mL in a 250 mL Erlenmeyer flask and cultivated for 2 day on a shaking incubator (170 rpm) at 37℃. Cultured cell broth of 40 mL was inoculated into production medium of 5.5 L in a jar fermenter (7 L, Kobiotech Co.). The fermentation process was monitored and controlled under the following operating conditions: 37℃, 300 rpm, pH 2.0, and aeration rate of 1 vvm.

Analytical methods Culture broth was filtered on filter paper (Whatman, No. 2). In order to analyze the cell mass, the mycelium on the filter paper was washed with distilled water before being dried in a oven at 45℃ for one day. Concentrations of reduced sugar (PHBAH method), ammonia (DIN 38406, Germany) and phosphate in the filtrate were analyzed by a few colorimetric methods. Concentration of itaconic acid was analyzed by HPLC (Alliance 2690, Waters Co.) with a 3.9 x 150 mm column (Symmertry C_{18}) at 30℃, with a 5 mM phosphate buffer as the mobile phase (1 mL/min). A photodiode detector (Waters detector) was used and overall procedures were controlled by a software, Millennium^{32} (ver. 3.2).

Setup of system Process variables such as pH (pH electrode, METTLER Co.), DO (O_2 sensor, METTLER Co.), exhaust gas (O_2/CO_2 gas analyzer, LOKAS Co.), were monitored in real time and controlled by a software, Notebook (Lebtech Co.). The fluorescence spectra during fermentation process were measured by a fluorescence sensor. The fluorescence sensor consisted of a fluorescence spectrophotometer (Model F-4500, Hitachi Co.) and 2 m-bifurcated liquid light conductor connected to a bioreactor(Fig. 1).

Result and Discussion

The spectra were collected every 10 min during the fermentation of itaconic acid in step of 10 nm (excitation range: 250-700 nm, emission range: 280-700 nm). Each fluorescent amino acids, coenzymes and vitamins were detected in their unique 2-D fluorescence regions(e.g. NAD(P)H; excitation: 340-360 nm, emission: 450-460 nm).

Fig. 2 showed important biogenic fluorophores (e.g. tryptophan, tyrosine, phenylalanine, NAD(P)H, FAD, vitamins) which were present in the mycelium or in the medium and detected by a 2-D fluorescence spectroscopy. The regions of tryptophan and tyrosine in the Fig. 2 highly increased with cultivation time (from 50 to 96 hour). In order to correlate the separated fluorescent components to off-line data the fluorescence plotting at time '**t**' should be extracted from on-line monitored 2-D fluorescence spectra.

Conclusion and outlook

During a fermentation process of itaconic acid on-line and off-line data have been obtained by a 2-D fluorescence sensor and other monitoring systems (e.g. FIA, optic fiber sensor). The on-line monitored fluorescence spectra will be correlated to other on-line data and off-line data such as phosphate, ammonia concentrations etc. The 2-D fluorescence sensor will be used to monitor few biological processes such as fed-batch and continuous fermentation system.

Acknowledgment

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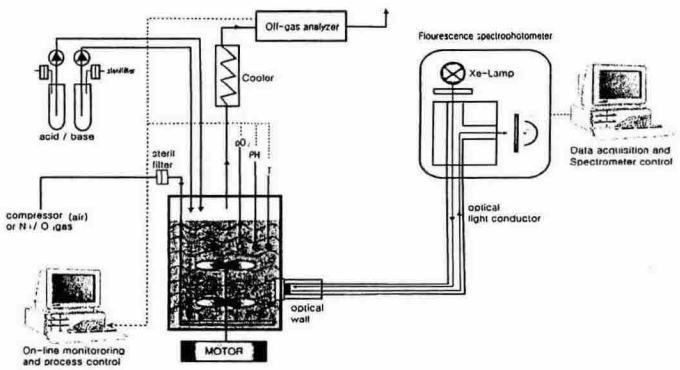

Fig. 1 Schematic drawing of the experimental setup with the bioreactor and the 2-D fluorescence sensor and on-line monitoring equipments.

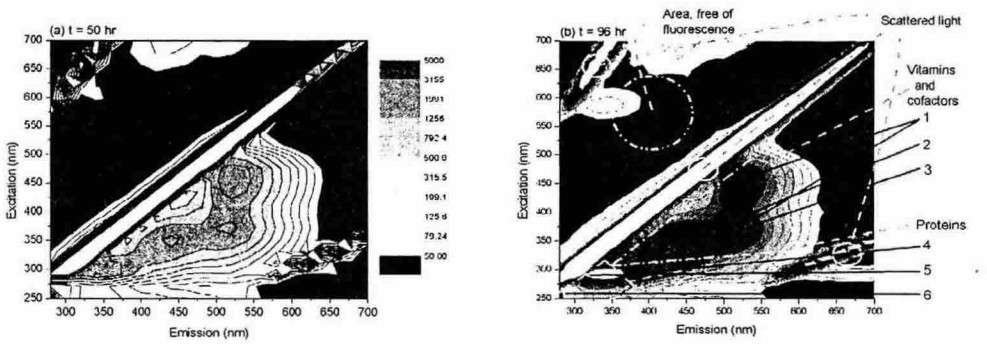

Fig. 2 Fluorescence spectra of *Aspergillus terreus* (at 50 hr (a), 96 hr (b) - 1: riboflavin, FAD, FMN; 2: NAD(P)H; 3: pyridoxine, pyridoxamine, pyridoxal-5'- phosphate; 4: tryptophan; 5: tyrosine; 6: phenylalanine)