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Automation of Glutamic Acid Fermentation

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글루탐산 발효공정의 자동화

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Abstract

A strategy for the automation of glutamic acid fermentation has been developed by the use of CO_2 analyzer together with a controller. It was found that a linear relationship existed between growth and CO_2 level in the exit gas. Therefore penicillin addition at an appropriate biomass concentration to excrete glutamate could be achieved automatically. In addition, an automatic batch feeding method (fed-batch culture) provided a means of overcoming substrate inhibition effects on growth and glutamic acid production in batch culture, thereby increasing productivity and product yield.

Introduction

Glutamic acid is an amino acid currently produced on a large scale by fermentation using industrial substrates such as cane molasss[1]. Two common characteristics of glutamate overproducers represent the basic biochemical keys to their glutamate overproduction: a deficiency of α-ketoglutarate dehydrogenase[2] and a nutritional requirement for biotin. However, a molasses medium contains excess biotin which controls membrane permeability by its role in fatty acid synthesis[3]. Therefore the permeability should be altered to facilitate glutamate excretion by addition of agents such as penicillin or fatty acid derivatives[4].

In the present study, automatic addition of penicillin during the exponential growth phase was achieved at a suitable CO_2 level in the exit gas. Following the addition of penicillin, automatic batch feeding of molasses was carried out by measuring the decrease in CO_2 level which was an indication of the shortage of fermentable sugars.

Materials and Methods

The strain and medium composition have been described previously[4]. A schematic diagram of the overall system is shown in Fig. 1. The fermentor was

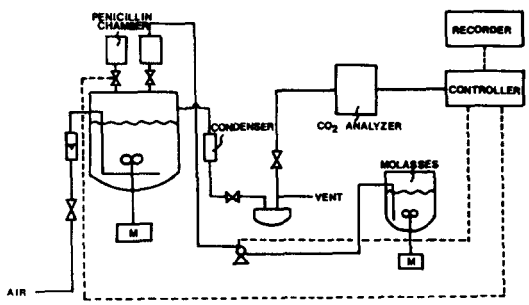

Fig. 1. Schematic diagram of the overall system for glutamic acid fermentation.

operated at a fixed aeration rate of 0.9 *vvm* of sterile air, and all experiments were carried out at pH 7.6 and temperature 31 ℃ before penicillin addition. After penicillin addition pH and temperature were increased to pH 7.8 and temperature 35 ℃.

Growth was measured using a spectrophotometer at 562 nm, and the total reducing sugar was estimated using the Bertrand method[5]. In order to determine glucose, fructose, and sucrose the HPLC system of Waters model 244 has been used. The procedure was similar to that used by Brandao *et al.*[6] Glutamic acid was analyzed using a Technicon Autoanalyzer and a procedure developed by Seidman and Blish[7].

Results and Discussion

A linear relationship between growth and CO_2 level in the exit gas is shown in Fig. 2. From the results of the three experiments, it was possible to introduce penicillin into a fermentor at an appropriate biomass concentration by triggering a solenoid valve shown in Fig. 1. For example, 0.8 units of penicillin G was fed within 0.5 min at an absorbance of 0.2 which corresponded to 2.7% of CO_2 for the given aeration rate.

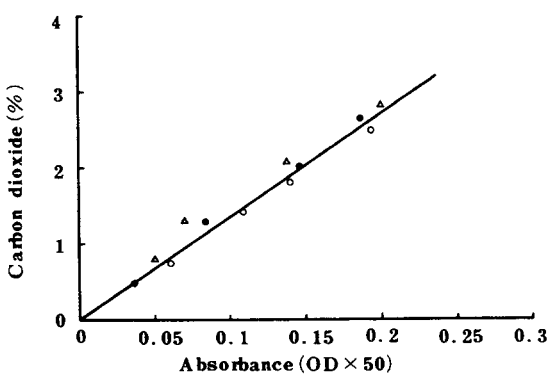

Fig. 2. Relationship between growth and CO_2 level in the exit gas

Following penicillin addition glutamate production was initiated as shown in Fig. 3. During the glutamic acid fermentation sucrose hydrolysis occurred to yield glucose and fructose. The concentration of glucose was found to be always lower than that of fructose, indicating a faster uptake of glucose. It was evident that the formation of significant amounts of CO_2 occurred during the uptake of fermentable sugars such as glucose, fructose, and sucrose. However at the end of fermentation when the fermentable sugars were fully metabolized, the cessation of CO_2 evolution was apparent. The value of CO_2 level, at which the molasses feed was desired, had been set on the controller. As a consequence, the set point triggered a timer (approximately 3 *min*) which, in turn, actuated a peristaltic pump (100 *ml/hr*).

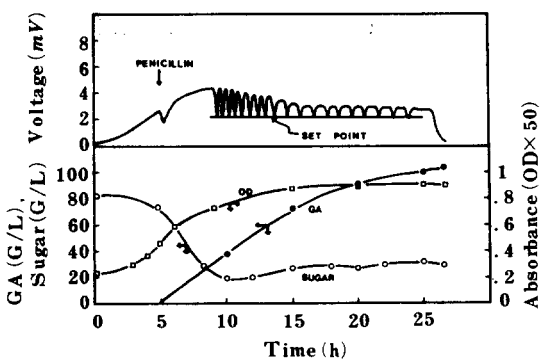

Fig. 3. Fed-batch culture kinetics for glutamic acid fermentation

Fig. 3 shows the results of fed-batch culture kinetics using 425 *g/l* molasses feed. As can be seen from Fig. 3, the level of total reducing sugar was maintained in the range 15-35 *g/l* throughout the fermentation. As a result, substrate inhibition effect on glutamic acid production could be minimized using the automatic batch feeding method. In fact, the molasses medium contained nonfermentable sugars such as hydroxymethylfurfural, which were accumulated up to 25 *g/l* as the fermentation progressed. At a culture time of 26.5 hr the final glutamic acid concentration was 104 *g/l* and the yield of glutamic acid $Y_{p/s}$ was 0.5 *g/g* based on the total amounts of reducing sugar.

요 약

이산화탄소 측정기 및 제어기를 사용하여 글루탐산 발효공정의 자동화 방법을 개발하였다. 이는 발효배기 가스중의 이산화탄소와 균체의 성장간에 직선관계가 있고 따라서 적절한 균체농도에서 페니실린 투여를 자동화할 수 있었다. 페니실린 투여후 균체성장 및 글루탐산 생성의 고농도당에 대한 저해작용을 감소시켜 주기 위한 방법으로서 회분식 추가당 첨가공정을 자동화할 수 있었으며, 그 결과로서 회분식 발효에 비하여 생산성과 수율이 향상되었다.

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