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CONTROL OF GLUCOSE FEEDING USING EXIT GAS DATA AND ITS APPLICATION TO THE PRODUCTION OF PHB FROM TAPIOCA HYDROLYSATE BY *ALCALIGENES EUTROPHUS*

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SUMMARY

A method to estimate the glucose concentration in the culture broth using CO_2 evolution rate (CER) data from a mass spectrometer was developed. *Alcaligenes eutrophus* was cultivated to produce poly(3-hydroxybutyric acid) (PHB) from tapioca hydrolysate using this method. The k value (g glucose/mol CO_2), defined as the glucose consumption per CO_2 evolution, decreased with culture time and was automatically changed using CER data. The glucose concentration in the culture broth could be controlled at 10 to 20 g/L. A final cell concentration of 106 g/L, PHB concentration of 61 g/L, and PHB content of 58 % of dry cell weight were obtained after 59 h of cultivation.

INTRODUCTION

Poly(3-hydroxybutyric acid) (PHB) is a biodegradable thermoplastic which can be produced by many microorganisms as an environmentally alternative to petrochemically derived plastics (Steinbuchel, 1991). The high production cost of PHB can be decreased by improving fermentation and separation processes and/or using a cheap carbon source.

Alcaligenes eutrophus is widely used for the production of PHB (Byrom, 1987; Doi, 1990). Recently, we found that maintaining glucose concentration between 10 and 20 g/L was important for efficient production of PHB by *A. eutrophus*, and obtained a high concentration of PHB (121 g/L) in a fed-batch culture with glucose concentration control using exit gas data from a mass spectrometer or using an on-line glucose analyser (Kim et al., 1994).

When using CO_2 evolution rate (CER) data for glucose concentration control, however, it was necessary to manually change the value of k (g glucose/mol CO_2) which represents the glucose consumption per CO_2 evolution. In this study, we developed a method to estimate the k value with time from simple stoichiometry and an empirical method to automatically change the k value with time from the variables that could be monitored. Using this method, we carried out PHB production by *A. eutrophus* from tapioca hydrolysate.

MATERIALS AND METHODS

Organism and media

Alcaligenes eutrophus NCIMB 11599 was grown on medium containing (per liter) glucose, 20 g; $(NH_4)_2SO_4$, 1 g; KH_2PO_4, 1.5 g; $Na_2HPO_4 \cdot 12H_2O$, 9 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; trace element solution, 1 mL. The composition of the trace element solution was (per liter) $FeSO_4 \cdot 7H_2O$, 10 g; $ZnSO_4 \cdot 7H_2O$, 2.25 g; $CuSO_4 \cdot 5H_2O$, 1 g; $MnSO_4 \cdot 4\text{-}5H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 2 g; $Na_2B_4O_7 \cdot 10H_2O$, 0.23 g; $(NH_4)_6Mo_7O_{24}$, 0.1 g; 35 % HCl, 10 mL.

For the fed-batch culture, the initial medium was (per liter) $(NH_4)_2SO_4$, 4 g; KH_2PO_4, 13.3 g; $MgSO_4 \cdot 7H_2O$, 1.2 g; citric acid, 1.7 g; trace element solution, 10 mL. Tapioca hydrolysate containing 20 g/L glucose was added to the initial medium as a carbon source. The pH of the medium was adjusted to 6.8 with NaOH.

Preparation of tapioca hydrolysate

Tapioca hydrolysate was prepared from tapioca starch through liquefaction and saccharification processes with α-amylase and glucoamylase. The concentration of the total sugar after the saccharification process was about 250 g/L, and the composition was glucose, 89.9 %; maltose, 7.5 %; maltotriose, 0.3 %; etc., 2.3 %. The solution containing 500 g/L glucose was used as a feeding medium for the fed-batch culture, which was obtained by concentrating the tapioca hydrolysate using a rotary evaporator.

Cultivation and analytical procedures

The details on cultivation and analytical methods are described in our previous paper (Kim et al., 1994).

Control of glucose feeding using CER data from mass spectrometer

The feeding strategy is based on the assumption that both cell growth and PHB production are associated with CO_2 evolution. The glucose consumption rate, r_S, was expressed as

$$r_S = k \cdot CER \tag{1}$$

where k is a proportionality parameter which varies with culture time. The details on the determination of feeding rate are described in our previous paper (Kim et al., 1994).

RESULTS AND DISCUSSION

We found that a high concentration of cells (124 g/L) and PHB (94 g/L) could be obtained when ammonia feeding was stopped at 55 g/L cell concentration from glucose as a carbon source by glucose concentration control using CER data (Kim et al., 1994). Since the value of k in equation (1) changed during the course of culture, we had to recalculate it manually at every sampling time. To solve this problem, we developed a method to change the k value automatically with culture time.

First, the theoretical k value was calculated by estimating the coefficients in the following simple stoichiometric equation.

$$a\,C_6H_{12}O_6 + b\,NH_3 + c\,O_2 \rightarrow CH_{1.666}N_{0.2}O_{0.27} + d\,CO_2 + e\,H_2O + f\,C_4H_6O_2 \tag{2}$$

It was assumed that glucose ($C_6H_{12}O_6$) was consumed to produce cell ($CH_{1.666}N_{0.2}O_{0.27}$), PHB ($C_4H_6O_2$), and CO_2. The chemical formula of the cell was taken from that of typical bacteria (Bailey and Ollis, 1986). Six coefficients (a, b, c, d, e, and f) were determined from balances on the four elements (C, H, O, and N) and two additional relationships provided by experimental determination of the yield of residual biomass from glucose ($Y_{R/S}$) and PHB yield from glucose ($Y_{P/S}$).

$$\text{C}: 6a = 1 + d + 4f \tag{3}$$

$$\text{H}: 12a + 3b = 1.666 + 2e + 6f \tag{4}$$

$$\text{O}: 6a + 2c = 0.27 + 2d + e + 2f \tag{5}$$

$$\text{N}: b = 0.2 \tag{6}$$

$$Y_{R/S} = \frac{20.7}{180a} \tag{7}$$

$$Y_{P/S} = \frac{86f}{180a} \tag{8}$$

The k value was calculated by the equation.

$$k = \frac{180a}{d} \tag{9}$$

Fig. 1 shows the time course of k value. Empty squares and filled squares represent values determined from equation (9) and values actually used during the experiment, respectively. Both values continuously decreased with culture time even though they did not exactly coincide with each other.

Next, we investigated an empirical method to evaluate the k value more accurately. We found that the k values are associated with the ratio of CER at time t to total CO_2 evolution from time zero to t. Fig. 2 shows the relationship between k and the ratio of CER to total CO_2 evolution. The following empirical equation was obtained.

$$k(t) = \frac{134.1U(t)}{U(t) + 0.02065} \tag{10}$$

where

$$U(t) = \frac{CER_t}{\int_0^t CERdt} \tag{11}$$

The solid line in Fig. 2 represents the value calculated by equation (10) and agrees well with the values used during the experiments (filled squares). Also in Fig. 1, the estimated k values (solid line) from equation (10) correlated very well to the values used every sampling time during the experiment (filled squares). Therefore, glucose concentration could be controlled by automatically changing the k value with time using CER data.

This method was applied to the production of PHB from tapioca hydrolysate. The set point for the glucose concentration was 15 g/L. Fig. 3 shows the time courses of the concentrations of cell, PHB, glucose, and PHB content. Nitrogen limitation was applied at the cell concentration of 67 g/L at 37.5 h. The final concentrations of cell and PHB obtained in 59 h were 106 and 61 g/L, respectively, resulting in PHB content of 58 %.

These values were lower than those of PHB (121 g/L) and PHB content (75 %) when using purified glucose as a carbon source (Kim et al., 1994). It seems that some products including cyanide compounds within the tapioca hydrolysate inhibited the cell metabolism. This phenomenon was also observed in the experiments on ethanol production by yeast from tapioca hydrolysate (Lee et al., 1994).

The glucose concentration in the culture broth was maintained at 10 to 20 g/L during the culture. This supports the adequacy of using CER data for the estimation of k value. This culture technique may be useful for the production of other metabolites when maintaining an optimum substrate concentration is important.

ACKNOWLEDGMENT

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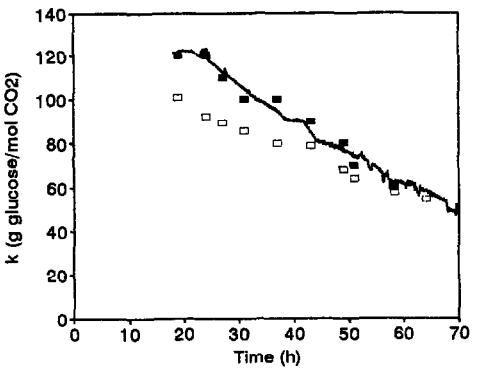


Fig. 1 Time course of the k value.
Solid line indicates the k value calculated from equation (10).
(■) k value used during experiment
(□) k value calculated from equation (9)
The k value (g glucose/CO_2 evolution) represents the glucose consumption per CO_2 evolution.

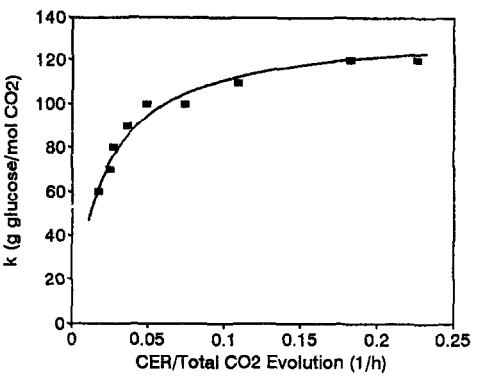
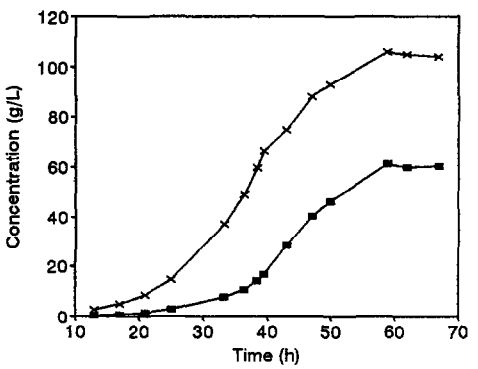


Fig. 2 Relationship between the k value and the ratio of CER (CO_2 evolution rate) to total CO_2 evolution at time t.
Symbols are the same as in Fig. 1.



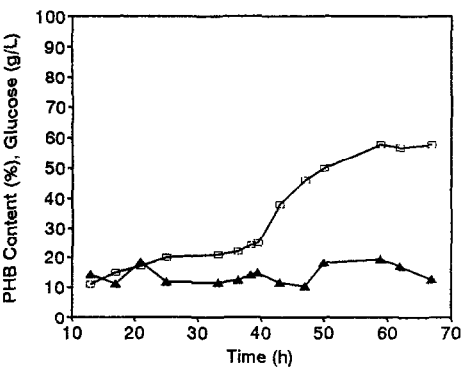


Fig. 3 Fed-batch culture of *A. eutrophus* from tapioca hydrolysate.
(x) dry cell concentration (g/L), (■) PHB concentration (g/L)
(□) PHB content (% of dry cell weight), (▲) glucose concentration (g/L)