Effect of pH on the production of PHB by *Acaligenes eutrophus* from whey

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Abstract

The production of polyhydroxybutrate(PHB) by *Alcaligenes eutrophus* NCIB 11599 was studied in a synthetic medium from whey as a sole carbon source. Especially pH-effect was treated and compared in this study.

At the end of fermentation (A) unadjusted to pH, the dry cell weight, PHB concentration, and PHB conversion rate were 10.3g/L, 3.1g/L, and 30%, respectively. At the end of fermentation (B) adjusted to pH(7.0~7.5), the dry cell weight, PHB concentration, and PHB conversion rate were 12.5g/L, 4.8g/L, and 41%, respectively. PHB conversion rate was about 10% higher on the fermentation (B) than on the fermentation (A).

Introduction

Growing concern about the environmental fate of polymeric materials has to have much interest in the development of biodegradable and biocompatible materials. Thus, various investigations have been focused on the biosyntheses of poly-hydroxybutrate(PHB), one of polyester. PHB is naturally occurring, optically active polyesters that are produced metabolically from the bioconversion of alkanes and alkanoic acids by a number of bacterial strains.[1)]

A. eutrophus has been studied extensively for the production of PHB due to its ability to accumulate large amount of PHB, ca. 80%(w/w) of dry cell mass, from simple carbon source(ICI, now ZENECA Bio Products, UK).[2)]

Initially *A. eutrophus* was grown in a glucose-salts medium containing only the calculated amount of phosphate to support a desired amount of cell growth.[4)]

Cells encounter phosphate limitation after about 60hrs, and accumulate PHB during the next 40 to 60hrs from glucose supplied.

This paper describes strategies for the high cell density cultivation of *A. eutrophus* for the production of PHB from whey based-medium, very cheap carbon source.

And this stduy stands for pH as a very important factor when *A. eutrophus* grows up and PHB is accumulated.

Materials and Methods

Bacterial strain, medium, and growth condition

A. eutrophus NCIMB 11599 was used in this experiments.

Fermentation (A) and (B) were carried out in a 4.5L jar fermenter(Korea Fermenter Company, Incheon, Korea). In fermentation (A) and (B), the intial amount of medium was 3L containing 80g/L whey(whey consists of minimum 11% protein and approximatively 65% lactose). Whey solution was pretreated to remove precipitates.

The initial medium consisted of(L^{-1}) $MgSO_4 \cdot 7H_2O$ 0.36g, KH_2PO_4 3.4g, $(NH_4)_2HPO_4 \cdot 2H_2O$ 2.67g, citric acid 0.43g, NaClO 10mL, and trace element solution 2.5mL(the constituents in the trace element are listed in Table 1.) in 1L distilled water.[3), 5)] Cells were cultured at 30℃, 250rpm, and 10kg/㎠g and initial pH was adjusted at 6.5~7.0 by the addition of 2N NaOH or HCl. Initial inoculation was carried out as 10%(v/v) of initial medium.

Table 1. Constituents in the trace elements solution

constituent	concentration(per liter)
HCl	5g
$FeSO_4 \cdot 7H_2O$	10g
$CaCl_2 \cdot 2H_2O$	2g
$ZnSO_4 \cdot 7H_2O$	2.2g
$MnSO_4 \cdot 4H_2O$	0.5g
$CuSO_4 \cdot 5H_2O$	1g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.1g
$Na_2B_4O_7 \cdot 10H_2O$	0.02g

Analytical procedures

Cell growth was monitored by measuring the absorbance at 660nm. Cell concentration was defined as cell dry weight per liter of culture broth. PHB concentration was determined by gas chromatography(Carbowax 20M, Hewlett-Packed, USA) using benzoic acid as an internal standard. The PHB content was defined as the ratio of PHB concentration to cell concentration given in as percent. The concentration of lactose was determined by liquid chromatography(μ Porasil, 3.9㎜×300㎜, Waters, USA) using acetnitrile as

mobile phase.

Results and discussions

Figure 1 and Figure 2 show the time profiles of cell concentration, PHB concentration, and pH during fermentation.

At the end of fermentation (A)(50hrs), 10.3g/L of the dry cell weight, 3.1g/L of PHB concentration, 30%(max. 40%) of PHB conversion rate, 9.84g/L of lactose concentration, and 8.34 of pH were shown.

At the end of fermentation (B)(50hrs), 12.5g/L of the dry cell weight, 5.1g/L of PHB concentration, 41%(max. 50%) of PHB conversion, 7.03g/L of lactose concentration and 7.0~7.5 of pH were shown.

This study demonstrated that PHB could be produced efficiently through the proper adjustment of pH(7.0~7.5). In case (B), pH was fallen at initial and as the time went up, pH was raised, which might result from protein compound.

In addition, if initial inoculation concentration is high, and precipitates is removed by pretretment efficiently, the production of PHB might be more increased from whey based-medium.

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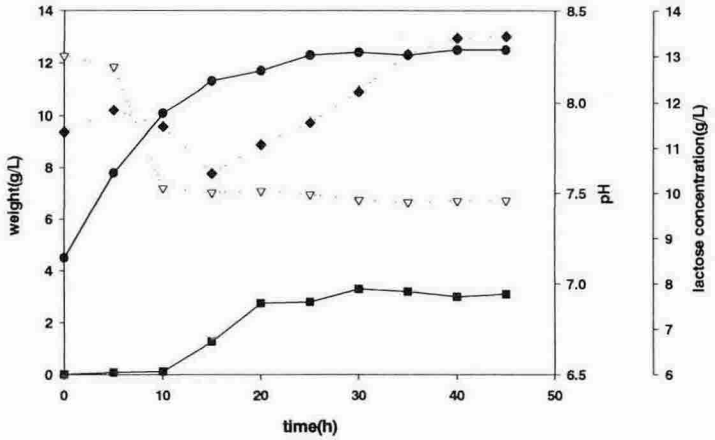

Fig1. Time profiles of cell concentration(●), PHB concentration(■), lactose concentration(▽) and pH(◇), in fermentation A

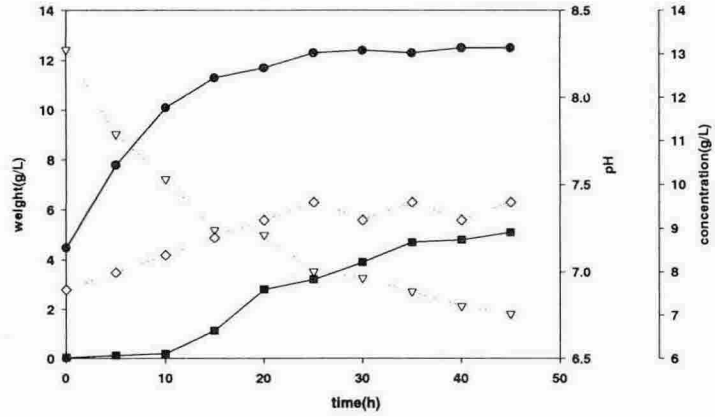

Fig2. Time profiles of cell concentration(●), PHB concentration (■), lactose concentration(▽), and pH(◇) in fermentation B.