THE PRODUCTION OF POLY-β-HYDROXYBUTYRATE BY HYDROGEN BACTERIA, *ALCALIGENES EUTROPHUS* H16 FROM ALGINIC ACID

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Introduction

Poly-β-hydroxybutyrate (PHB), which is synthesized by many bacteria[1], has attracted industrial attention as a possible candidate for large-scale biotechnological production. This thermoplastic biopolymer has a high tensile strength, and is completely biodegradable to an innocuous compound, D(-)-3-hydroxybutyric acid. *Alcaligenes eutrophus*, presently used by Imperial Chemical Industries for PHB production[2], synthesizes this polymer from a variety of carbon sources under appropriate conditions such as limitation of nitrogen.

On the other hand, seaweeds contain a large amount of polysaccharides such as alginic acid and agar. Alginic acid, a linear polysaccharide composed of β-D-mannuronic acid and α-L-guluronic acid, is a main cell-boundary constituent of brown algae[3]. At present, alginate is chiefly used as a food additive such as a stabilizer or an emulsifier. Seaweed is abundant as a natural resource and is a promising carbon source for the fermentation industry, but most varieties are not utilized.

In the present study we used alginic acid and degradation products of alginic acid as a carbon source for the production of biodegradable plastic, PHB by Alcaligenes eutrophus H16, and the possibility of PHB production from seaweed will be discussed.

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1. Materials and Methods

All chemicals used in this study were reagent grade and were purchased from Wako Pure Chemical Industries. Degradation products of alginic acid were prepared in the following manner. A 0.25 dm^{-3} solution containing 20 g of sodium alginate was added to an equal volume of 0.6 M hydrochloric acid, and hydrolyzed at 100°C[4]. Alginic acid hydrolyzed for 0.5, 6, 12 and 24 hours were neutralized by the addition of sodium hydroxide solution and used as carbon sources for PHB production by *A. eutrophus*.

Alcaligenes eutrophus H16 was obtained from the Institute of Applied Microbiology, Tokyo University. *A. eutrophus* was first grown at 30°C in 1 dm^3 of nutrient-rich medium[5]. The cells were harvested after 24 hours and washed with distilled water. Under these culture conditions no accumulation of polyesters in the cell was observed. To promote polyester production, a certain amount of the washed cells was transferred into a nitrogen-free medium[6] containing sodium alginate or degraded alginic acid as the carbon source for PHB production. The cells were cultivated in these media (0.5 dm^3, pH7) at 30°C under aerobic conditions (aeration rate 8 $cm^3 \cdot sec^{-1}$), harvested by centrifugation, washed with acetone, and finally dried under reduced pressure at room temperature. PHB was extracted from the dried cells with hot chloroform in a Soxhlet apparatus for 5 hours, and was purified by reprecipitation with hexane. The PHB content in *A. eutrophus* was determined from the weight of dried cells and the reprecipitated PHB. Parallel determinations were carried out for several sam-

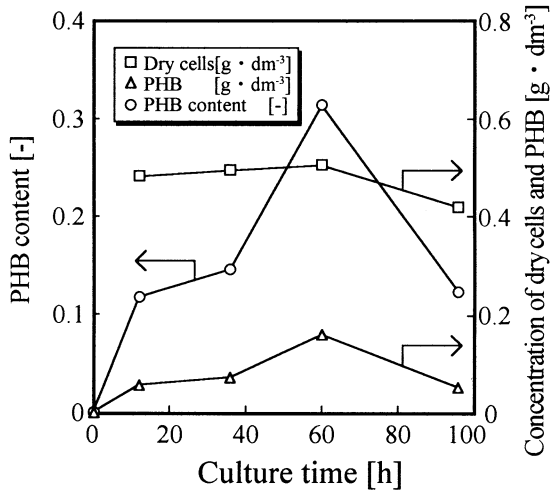


Fig. 1 Growth and PHB accumulation in *Alcaligenes eutrophus* H16 under aerobic conditions (8 $cm^3 \cdot sec^{-1}$) at pH 7.0, 30°C. (□) Concentration of cell [dry-g·dm^{-3}]; (△) Concentration of PHB [dry-g·dm^{-3}]; (○) PHB content in cell body [dry-g·dry-g^{-1}].

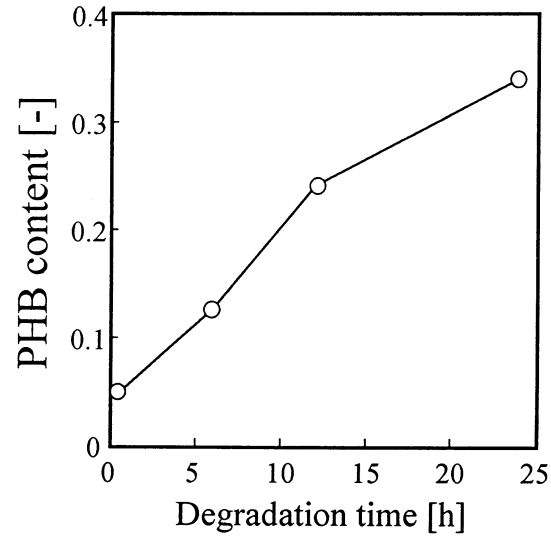


Fig. 2 Effect of the degradation time of alginic acid on PHB content in *Alcaligenes eutrophus* H16. The culture conditions were identical to those described in Fig. 1.

ples of PHB by a spectrophotometric method[7]. PHB was treated with 10 cm^3 of concentrated sulfuric acid in a water bath at 100°C for 10 minutes to resolve into crotonic acid, and the absorbance was measured at 235 nm.

2. Results and Discussion

2.1 Production of PHB from alginate

A. eutrophus was cultivated in 0.5 dm^3 of nitrogen-free medium containing 20 g·dm^{-3} of sodium alginate as the carbon source for 42, 68 and 82 hours. The initial pH of the medium was adjusted to 7.0 by adding 1 M sodium hydroxide solution.

PHB was not produced from alginic acid by *A. eutrophus*. Under the same culture condition, *A. eutrophus* produce PHB from sodium acetate (20 g·dm^{-3}) with a content of 0.41 g-PHB/g-dry cell at 72 hours of culture time, and 0.55 g-PHB/g-dry cell was obtained at 161 hours.

Alginic acid is a (1-4)-linked copolymer of β-D-mannuronic acid and α-L-guluronic acid, and the degree of polymerization of the copolymer is a few hundred[4]. This result suggests that *A. eutrophus* could not utilize alginic acid for PHB production because of this high degree of polymerization.

2.2 Production of PHB from degraded alginic acid

The degraded alginic acid 24h hydrolyzation was used as a carbon source for PHB production. *A. eutrophus* was cultivated in 0.5 dm^3 of nitrogen-free medium containing 20 g·dm^{-3} of degraded alginic acid. The culture conditions were identical to those described above. **Figure 1** shows the time course of PHB production by *A. eutrophus*. The maximum PHB content of 0.33 g-PHB/g-dry cell was observed at 60 hours of culture time, after which the PHB content decreased abruptly. This result is probably due to the heterogeneity of the degradation product of alginic acid.

The hydrolysis of alginic acid in 0.3 M hydrochloric acid is a heterogeneous reaction, and the limit of hydrolysis is approximately 30 %[4]. Therefore, it can be considered that the alginic acid degraded to available size for PHB production was consumed at 60 hours of culture time and that PHB in the cell body was reutilized for the energy source.

2.3 Effect of degradation time on the production of PHB

A. eutrophus was cultivated in 0.5 dm^3 of nitrogen-free medium containing 20 g·dm^{-3} of degraded alginic acid hydrolyzed for 0.5, 6, 12, 24 hours. The culture conditions were identical to those described in Fig. 1. **Figure 2** shows the effect of degradation time of alginic acid on the PHB contents in *A. eutrophus* at 72 hours of culture time. The result shows that the longer period of degradation resulted in higher PHB content. According to Haug *et al.*, the present degradation method increases the soluble fraction of alginic acid at pH 2.8 from 7.4 to 32 % and decreases the degree of polymerization of insoluble fraction from 52 to 22 % during a hydrolysis period from 40 minutes to 20 hours[4]. From the results in Fig. 2, it is apparent that PHB production from alginic acid by *A. eutrophus* depends strongly on the degree of degradation of alginic acid. PHB production from alginic acid degraded for 24 hours was 0.33 g-PHB/g-dry cell, which is 60 % of that produced from sodium acetate. This lower PHB content is probably due to the heterogeneous hydrolysis of alginic acid.

The experimental results in the present study suggest that the further degradation of alginic acid may yield a higher PHB content in *A. eutrophus*.

Conclusions

1) PHB was not produced from alginic acid by *A. eutrophus*.

2) PHB production strongly depends on the degree of degradation of alginic acid.
3) PHB production of 0.33 g-PHB/g-dry cell was attained from alginic acid hydrolyzed at 100°C in 0.3 M hydrochloric acid for 24 hours.

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