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Utlization of Hydrolyzed Sago Starch as Main Substrate for the Production of Bioplastic Poly Hydroxy Butyrate (Phb) By *Rasltonia eutropha* on Fed Batch Cultivation System

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Abstracts

The objective of this research was to examine the utilization of hydrolyzed sago starch as the main substrate to produce poly hydroxy butyrate (PHB) by *Ralstonia eutropha* using fed batch cultivation method. The results showed that the hydrolyzed sago starch could be used as carbon source for the production of PHB by *Ralstonia eutropha.* A higher formation and accumulation of PHA could be achieved through feeding of hydrolyzed sago starch on a fed batch cultivation system. The best treatment could increase the specific product yield up to 76.54% and product concentration up to 3.72 g/L.

Keywords: polyhydroxyalkanoates, polyhydroxybutyrate, biopolymer, bioplastic, Ralstonia eutropha, hydrolyzed sago starch, fed-batch cultivation system

Introduction

The increasing concern on quality and sustainability of the environment has led to the investigation on alternative sources of plastics from renewable resources which are biologically degradable by existing natural microorganisms. Poly hydroxy alkanoates (PHAs) in general and poly-3-Hydroxybutyrate (PHB) in particular, are promising alternatives.

Compared to other biopolymers such as starch and protein, PHAs have many advantages. These polymers are hydrophobic, resistant to humidity and have low permeability against oxygen (van Wegen *et al.*, 1998). These polymers have obtained an increasing attention to be used as materials for coating, carrier, disposable products, surgical strings, and packaging materials (Lee *et al,* 1999; Brandl *et al.* in Babel and Steinbüchel, 2001).

Even though PHAs have many advantages, however, its commercialization is hindered by economic problems. Its production cost is relatively higher than that of petrochemical based plastics, leading to a higher selling price. Reduction of production cost is possible through the use of cheaper cultivation substrates and development of a more productive cultivation process.

One of cheaper candidates for cultivation substrate is hydrolyzed sago starch. Sago starch is a cheap and abundantly available source of hydrolyzed starch, especially in the tropical regions. The use of hydrolyzed sago starch as cultivation substrate for PHB production hopefully can reduce the production cost while giving an added value to sago starch.

PHB is accumulated by various bacteria as carbon and energy stock in cytoplasm at unbalanced conditions, such as limitation of essential nutrients and excess of carbon (Lee et al, 1999). Application of fed-batch strategy in which excess carbon is fed into the system with limited other essential nutrients may result in a higher formation and accumulation of PHB.

The purpose of this research was to investigate the possibility of using hydrolyzed sago starch as the carbon source for *Ralstonia eutropha* to produce PHB. The specific objective is to investigate the application of fed-batch strategy in which excess carbon source (hydrolyzed sago starch) is fed while limiting other essential nutrients to induce the formation and accumulation of PHB.

Materials and Methods

The *Ralstonia eutropha* IAM 12368 was obtained from IAM Culture Collection, Institute of Molecular and Cellular Biosciences, the University of Tokyo, Japan. Batch cultivations were carried out in 2L bioreactor (working volume of 1 L) for 96 hours, at pH 6.9, temperature of 34°C, and aeration rate of 0.2 vvm. Hydrolyzed sago starch with the total sugar of 25 g/L was used as the carbon source while $(NH_4)_2HPO_4$ was used as nitrogen source to make the C/N ratio of 10:1. Other media components in 1 L were 5.8 g K_2HPO_4, 3.7 g KH_2PO_4, 10 ml $MgSO_4$ solution 0.1 M and 1 ml microelement solution.

Fed-batch cultivations were carried out in a 2 L bioreactor with the initial volume of 1 L. Feedings of substrates at certain dilution rate were conducted at the beginning of stationary phase (Hahn et al., 1995). The treatments carried out were as follows:

(F1) Feeding of complete media with the same composition as batch media;
(F2) Feeding of hydrolysed sago starch only;

(F3) Feeding of hydrolysed sago starch + $MgSO_4.7H_2O$;

(F4) Feeding of hydrolysed sago starch + $MgSO_4.7H_2O$ + $(NH_4)_2HPO_4$.

The effect of different composition of substrate feedings were investigated by measuring biomass concentration (g dry cell/L) and product concentration (g dry PHB/L). Specific yields of PHB (percent of g dry PHB/g dry cell) were then calculated. Experiments were repeated two times. Duplicated samples were taken and subjected to both biomass and PHB concentrations. PHB was obtained by the NaOH digestion method. (Lee *et al.*, 1999).

Results and Discussion

The results showed that under batch experimentations, *R. eutropha* grown on hydrolyzed sago starch as main substrate (total sugar of 25 g/L and C/N ratio of 10:1) entered a stationary phase after 48 hours of cultivation. At the end of batch cultivation, it yielded 4.41 g dry cell/L and 1.44 g dry PHB/L (32.6% of biomass).

Based on the previous research results in batch cultivations, feedings of substrates were started from 48th hour. The dilution rate was set at 0.1/h. The results (Table 1.) show that the fed-batch cultivations can induce the formation and accumulation of PHB as indicated by a higher specific yield of PHB and PHB concentration compared to that of batch cultivations. Comparison between all treatments for biomass, product and specific product yield is summarized in Table 1.

Table 1. Biomass, Product (PHB) and Specific Product Yield of PHB produced by R. *eutropha* in *batch* and *fed batch* cultivations

Treatments	Biomass (g dry cell/L)	Product (g dry PHB/L)	Specific Product Yield (% w/w)
Batch (control)	4.41 ± 0.32	1.44 ± 0.27	32.65±6.56
Fed batch			
F1	3.34 ± 0.11	2.15 ± 0.03	64.37±2.30
F2	4.86 ± 0.14	3.72 ± 0.24	76.54±5.41
F3	3.67 ± 0.28	2.12 ± 0.05	57.77±4.61
F4	4.58 ± 0.24	1.85 ± 0.06	40.39±2.49

Notes:

(F1) Feeding of complete media with the same composition as batch media;

(F2) Feeding of hydroised sago starch only;

(F3) Feeding of hydrolysed sago starch + $MgSO_4.7H_2O$;

(F4) Feeding of hydrolysed sago starch + $MgSO_4.7H_2O$+$(NH_4)_2HPO_4$.

Compared to control (*batch cultivations*), feeding of hydrolysed sago starch without additional nutrients (F2) gave the highest product formation. It produced PHB with a specific product yield of 76.54%, which is more than two folds that was produced under batch cultivations. The highest specific product yield in this current research (76.54%) is higher than that of Tanaka *et al* (1993) who used the two stage cultivation method to produce PHB using xylose as main substrate and produced PHB at the specific yield of 55%. Kim *et al* (1994) who cultivated *Alcaligenes eutrophus* using automatic fed batch technique of glucose with amonium limitation, also obtained the specific product yield of 76%. However, it is still lower than that of Ryu *et al* (1997) who obtained specific product yield of 80% in a 60-L fermentation of *Alcaligenes eutrophus* with phosphate limitation in a high cell density fed-batch culture.

The best treatment in the current research (F2) also increased the product accumulation as indicated by the increasing of product concentration at the end of fed-batch from 1.44 g/L (under batch cultivations) to 3.72 g/L (increasing of more than two folds). In addition, biomass was only slightly increased from 4.41 g/L (batch cultivations) to 4.86 g/L.

Conclusions

These results have proven that hydrolyzed sago starch can be utilized as carbon source for the production of PHB by *Ralstonia eutropha*. A higher formation and accumulation of PHB can be achieved through feeding of an excess of hydrolyzed sago starch as carbon source while limited other essential nutrients are maintained. Fed-batch cultivation strategy can increase both product concentration and specific product yield.

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