Effects of Glucose concentration on the production of poly(3-hydroxybutyrate) by high cell density culture of *Ralstonia eutropha*

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Abstract

The effects of glucose concentration on the production of PHB by fed-batch culture of *Ralstonia eutropha* were investigated. In the range of glucose concentration of 2.5 ~ 40 *g/l*, it was found that the high glucose concentration was not favorable for the PHB formation after the phosphate limitation. It was further confirmed by the specific PHB synthesis rates and yields. The PHB concentration decreased much with the increase of glucose concentration. But if the glucose concentration was very low, e.g. 2.5 *g/l*, the cell growth and PHB synthesis also could be limited because of inadequate glucose supply. Itwould be better to maintain the glucose concentration at about 9.0 *g/l* to obtain high DCW, PHB concentration and productivity.

Introduction

Carbon source, as one of the major nutrients for cell growth, has a strong effect on the growth of microorganisms. In most cases of fermentation, the high nutrient concentration (e.g. Carbon) may inhibit the cell growth, and the low nutrient concentration limits the cell growth, as shown by the Monod equation. So the fed-batch culture coupling with a suitable nutrient feeding strategy has been most often used to overcome the inhibition of high nutrient concentration, and to obtain high cell density and high productivity1, 2).

In the fed-batch fermentation for producing poly-hydroxyalkanoate (PHA), numerous studies have been carried out on the feeding of glucose3-5), glucose and organic acid4. 6) to achieve a high cell density and productivity. Kim et al4) has pointed out that the maintenanceof glucose concentration in the range of 10 to 25 *g/l* is very important to get high cell density and productivity by testing several glucose feeding strategies, such as pH-stat, on-line glucose controller and

another one based on the CER (carbon dioxide evolution rate). But in all of them, the glucose concentrations controlled varied in a big range. There has been no report on the determination of glucose concentration effect on the process of PHA production by high cell density cultures.

Here, an on-line glucose analyzer was used to determine the glucose concentration and control it at a certain value, which just fluctuates less than 2 *g/l*. The cell growth and PHB formation were determined in the fed-batch culture of *R. eutropha*under different glucose concentrations. The effects of glucose concentration on the cell growth rate and PHB formation rate were analyzed too.

Materials and methods

Microorganism and medium: *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*) NCIMB 11599 was used in this study. The compositions of culture medium and the trace element solution were the same as previously reported7).

Culture conditions: Seed cultures were prepared in a 500 *ml* flask containing 150 *ml* medium by incubating at 30 C for about 30 hours. All fed-batch cultures were carried out in a 5 *l*jar fermentor with initial volume of 1.5 *l* at 30C. The pH was controlled at 6.7 with a 2 N HCl solution and a 28% NH4OH solution. Thedissolved oxygen concentration was maintained at 20% of air saturation by automatically increasing the agitation speed up to 950 rpm and supplying pure oxygen. The glucose solution of 700 *g/l* was used as feeding solution.

Analysis methods: The methods used to determine the optical density (OD), dry cell weight (DCW), residual cell weight (RCW), and PHB concentration were the same as the previous description7). The glucose concentration was monitored and automatically controlled by a glucose analyzer (Model 2730, Yellow Springs Instruments, USA) to maintain the glucose concentration in 2.5, 9.0, 16, and 40 *g/l*, respectively.

Results and discussion

A set of experiments was carried out to investigate the effects of glucose concentration on the cell growth and PHB formation in fed-batch cultures of *R. eutropha* with the phosphate limitation. The highest DCW (208.1 *g/l*) and PHB concentration (138.7 *g/l*) were obtained when the glucose concentration was controlled at 9.0 g/L, and the lowest DCW (158.6 *g/l*), PHB concentration (85.2 *g/l*) obtained at the glucose of 40 *g/l*. Except for the case of glucose of 2.5 *g/l*, all the DCW, PHB concentration and productivity decreased with the increase of glucose concentration controlled in culture broth (see Table 1).

Table 1. Summary of the fed-batch cultures in different controlled glucose concentrations

Glucose conc. (g/L)	Fermentation time (h)	The final DCW (g/L)	The final PHB conc. (g/L)	The final RCW (g/L)	The final PHB Content (wt%)	Productivity of PHB (g /h-L)
2.5	40	154.5	96.4	58.1	62.4	2.41
9.0	45	208.2	138.7	69.4	66.3	3.08
16	43	178.2	111.9	66.2	62.8.	2.60
40	45	158.6	85.4	73.2	53.8	1.90

The accumulation of RCW and PHB in the fermentations was shown in Fig. 1. It shows that RCW increased very fast after 10 hours, then kept a constant in a short time when RCW reached about 120 g (65 *g/l*), which is resulted from the phosphate limitation, and increased thereafter again except for the case of glucose concentration of 2.5 *g/l*, where the cell growth is muchlower than the others. It was attributed to the shortage of glucose. The PHB was continuously accumulated after the phosphate limitation occurred. In 40-hour fermentations, PHB of 216, 322, 262, and 200 g were produced at the glucose concentration of 2.5, 9.0, 16.0, and 40 *g/l*, respectively. The PHB obtained at glucose concentration of 9.0 *g/l* is 1.6 times of that obtained at the 40 *g/l*. These results suggest that glucose concentration has a strong effect on the PHB formation in the fed-batch culture of *R. eutropha*.

The specific cell growth rate and specific PHB synthesis rate, two important parameters for determining the cell growth and product synthesis ability, were calculated based on the residual cell weight (Fig.2 and 3). The maximal specific cell growth rate (0.28g RCW/g RCW-h) appeared in about 14-hour, see figure 2. Before 20 hours, there is no obvious difference between the specific cell growth rates obtained at different glucose concentrations except the case of 2.5 *g/l*, at which the specific cell growth rate was low in most fermentation time. This may be resulted from the shortage of glucose. Carbon source is the main source for cell growth. If it is not supplied enough, the cell growth rate should be reduced. At the moment of phosphate limitation starting in about 25-hour, the cell growth almost ceased, and then grew again after the cells adapted the new environment.

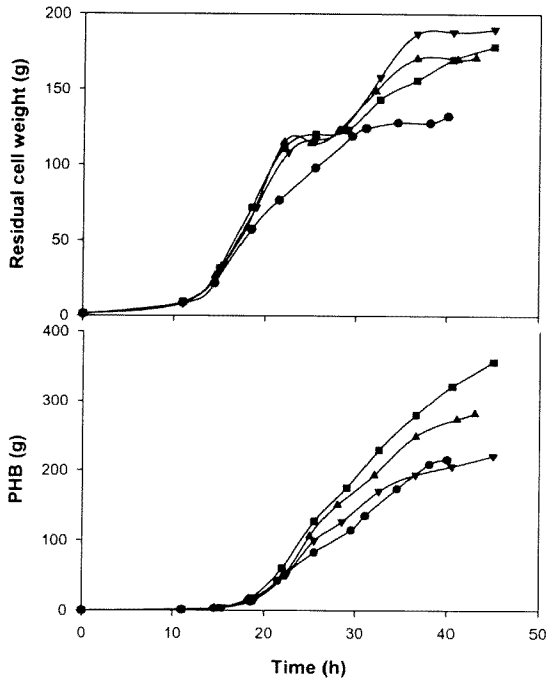

Fig. 1 Accumulation of RCW and PHB at different glucose concentrations: 2.5 g/L, 9.0 g/L, 16.0 g/L, 40 g/L

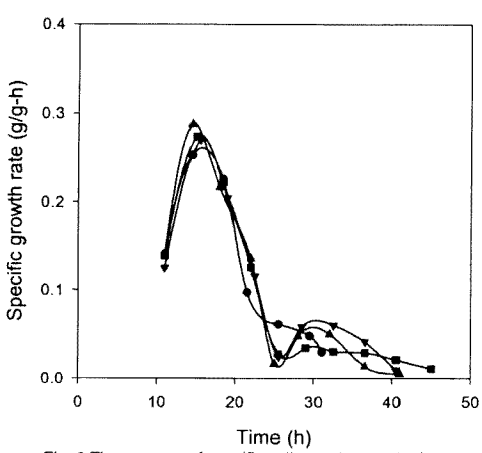

Fig. 2 Time course of specific cell growth rate obtained at different glucose concentration: 2.5 g/L, 9.0 g/L, 16 g/L, 40 g/L

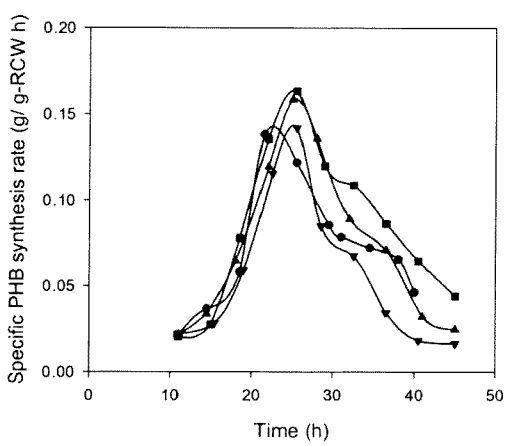

Fig.3 Time course of specific PHB synthesis rate obtained at different glucose concentrations: 2.5 g/L, 9.0 g/L, 16 g/L, 39 g/L

In summary, this set of experiments shows the highglucose concentration was not favorable for the PHB formation in the fed-batch culture of *R. eutropha*. With the increase of glucose concentration controlled in culture broth, the more cell mass and the less PHB was produced. It would be better to control the glucose concentration at about 9.0 *g/l* to increase the DCW, PHB concentration and productivity. This will be very important to overcome the high production cost of PHB, one of main problems met in its mass production.

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