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ACCUMULATION OF POLY-(HYDROXYBUTYRATE) BY A NON-SULFUR PHOTOSYNTHETIC BACTERIUM, *Rhodobacter sphaeroides* RV AT DIFFERENT pH

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SUMMARY

Effect of pH of culture media on intracellular accumulation of poly-(hydroxybutyrate) (PHB) by a non-sulfur photosynthetic bacterium, *Rhodobacter sphaeroides* strain RV was studied in pH-stat cultures. Sub-optimal pH for growth, 8.0 or 8.5 gave the higher content of PHB rather than optimal pH 7.5 for growth. These results show that growth and PHB accumulation of the bacteria can be controlled by pH of culture media.

INTRODUCTION

Poly-(hydroxybutyrate)(PHB) which has been known as a bacterial storage material has been already commercially produced as a raw material for biodegradable plastics (Brandl et al., 1990) .One of main PHB producers has been a facultative chemo-autotroph, *Alcaligenes eutrophas*, which has been cultivated heterotrophically for PHB production. Autotrophic, especially photo-autotrophic production of PHB would be an environmentally acceptable technology since sunlight energy and CO_2 or biomass are directly converted to a material for biodegradable plastics.

There have been some investigation of PHB accumulation by photosynthetic bacteria (Brandl *et al.*, 1989; Dierstein *et al.*, 1974; Hashimoto *et al.*, 1992; Liebergesell *et al.*, 1991) and cyanobacteria (Philippis *et al.*, 1992; Stal, 1992). Liebergesell *et al.* (Liebergesell *et al.*, 1991) have reported PHB accumulating activity of various photosynthetic bacteria. Similarly as in the cases of heterotrophic bacteria, nitrogen-limited conditions have

been tested for PHB accumulation. Brandl *et al.* (Brandl *et al.*, 1989) have reported that poly-(hydroxyalkanoate) (PHA) content of non-sulfur photosynthetic bacterium, *Rhodospirillum rubrum* is reversely associated with ammonium sulfate concentration as nitrogen source. Liebergesell *et al.* (Liebergesell *et al.*, 1991) have determined PHA content of nitrogen-limited cultures of various photosynthetic bacteria.

We have found that PHB accumulation by our isolate, *Rhodobacter sphaeroides* strain RV occurs in the presence of nitrogen source but is affected by pH of the culture media. The maximal PHB content in RV strain reaches more than 40% of cell dry weight. We report here the optimization of pH conditions for growth and PHB accumulation by the bacterium.

MATERIALS AND METHODS

Rb.sphaeroides strain RV is our isolate (Mao *et al.*, 1984) which has been used for studies on hydrogen production (Miyake *et al.*, 1986; Tsygankov et al., 1994). The bacterium was cultured on 50 mM acetate as carbon and electron source in basal medium (Mao *et al.*, 1984) where 1000 mg/*l* of yeast extract (Difco, USA) was supplemented. The initial pH was adjusted by sodium hydroxide or phosphoric acid. Ammonium sulfate as nitrogen source was added to 1.3 g/*l* in all experiments.

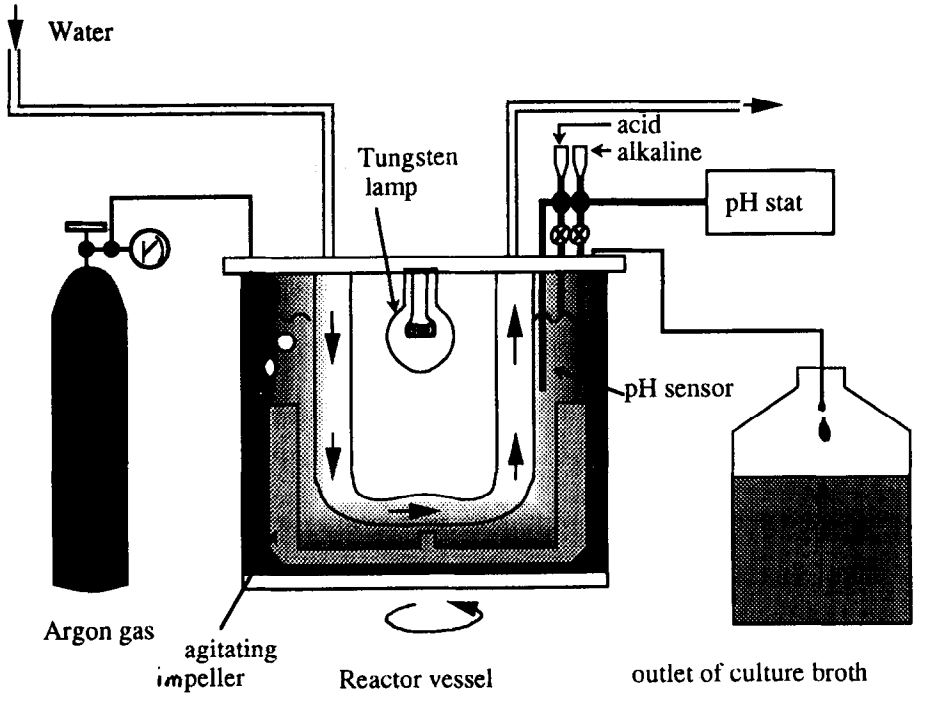

Figure 1. Photobioreactor system used.

The photo-bioreactor used was made of three coaxial glass cylinders (Fig.1) which was similar as Tsygankov-type (Yakunin *et al.*, 1986). The total volume was 1.6 *l*, and the working volume, 1.2 *l*.

A seed culture (approximately 200 m*l*) was grown in a Roux bottle and inoculated into 1 *l* of fresh culture medium contained in the bioreactor. The light intensity of the outside surface of water-jacket was about 35 W/m^2. The culture was bubbled by argon gas with a rate of 20m*l*/min in order to keep anaerobic conditions. The pH value of the culture was controlled by pH-stat with automatic addition of phosphoric acid. The temperature was 30 ℃. The cell mass concentration was determined by measuring optical density of the culture broth or cell dry weight after overnight desiccation at 120℃.

Specific growth rate (μ)which was defined as $(1/X) \cdot dX/dt$ when X was bacterial cell mass concentration and t, time was calculated by approximations of $\{1/(X_1+X_2)/2\}$ $\{(X_2-X_1)/(t_2-t_1)\}$ using the data obtained.

PHB content was determined by the method of Braunegg et al. using gas chromatography (Braunegg et al., 1978).

RESULTS AND DISCUSSION

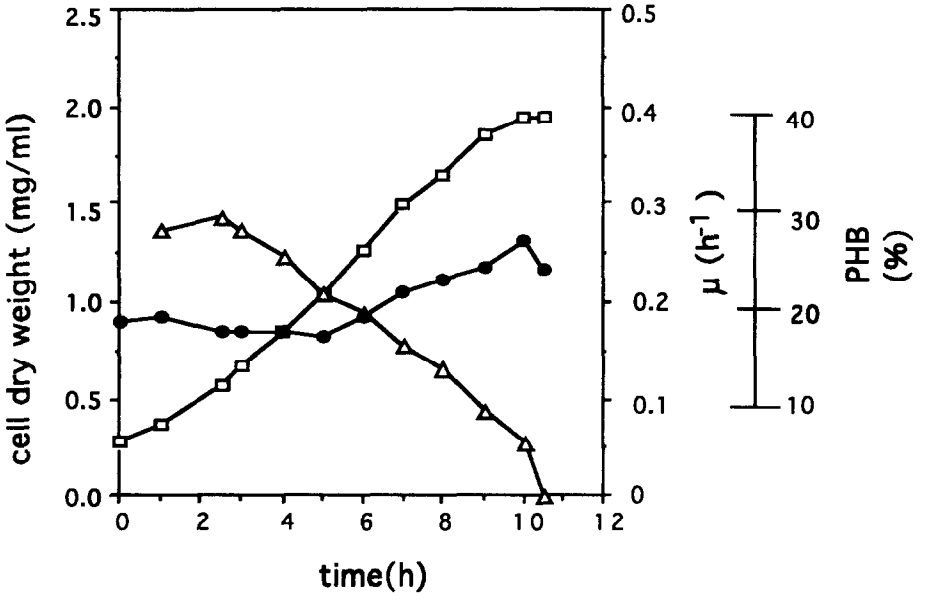

Figure 2. Growth of *Rb.sphaeroides* RV at pH 7.5.

□ ,cell concentration. △ ,specific growth rate(μ) ● ,PHB content(dry wt base).

We conducted pH-stat batch cultures of the bacteria with changing pH from 7.0 to 9.0. The time course of a pH-stat culture is shown in Fig.2. The strain, RV was not able to

grow in pH 9.0. From the results obtained in the various pH conditions, change of specific growth rate and PHB content are shown in Figs.3 and 4, respectively. Figure 3 shows that specific growth rate had the maximum in the early growth phase and declined thenafter. The optimal pH for the growth is estimated to be about 7.5. The PHB content was higher in high pH, 8.0 or 8.5.

The culture medium was buffered with sodium acetate that was electron and carbon source for the bacterium. The bacterial growth raises the pH of the culture medium due to the consumption of acetate. Therefore, we assumed that pH-uncontrolled culture may be useful since it would give optimal growth in the early growth phase and raised pH condition for PHB accumulation in the later phase. The results (Fig.5) show that PHB content increased according to the increase of pH of the culture medium and finally reached 42 % which was higher than pH-stat cultures.

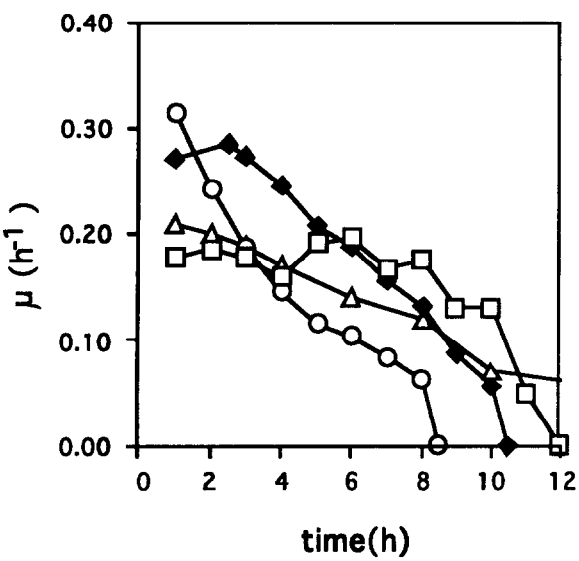

Figure 3. The effect of different pH on specific growth rate(μ).

□ ,pH7.0. ◆ ,pH7.5 . ○ ,pH8.0. △ ,pH8.5.

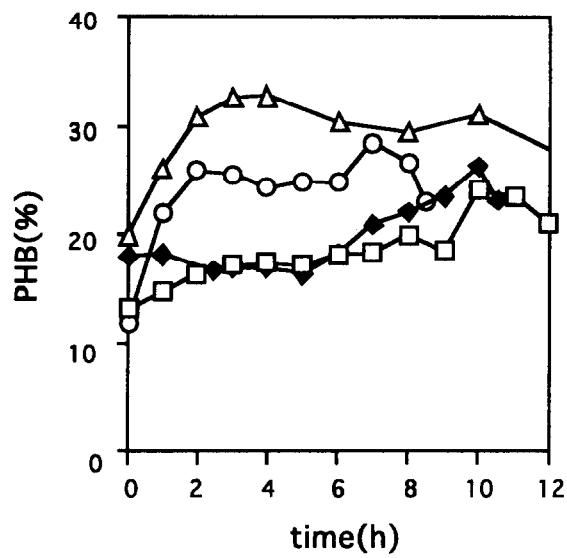

Figure 4. The effect of different pH on PHB accumulation

The symbols were the same as in Fig.3.

Furthermore, the productivity of PHB was defined as (specific growth rate, μ) · (PHB (content). By using the data from middle growth phase, the maximum productivity was obtained in the pH-uncontrolled culture (Table 1).

The pH-uncontrolled culture gave the best results of PHB production at this moment. Further studies are required to improve the PHB productivity by controlling pH of culture media, *i.e.*, temporal separation of the growth and PHB accumulation processes.

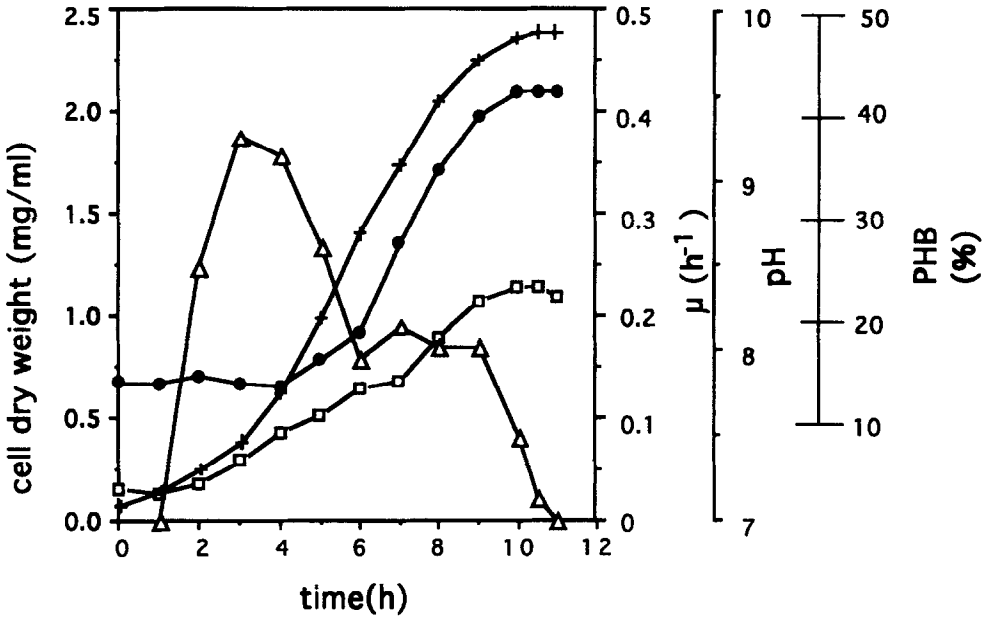

Figure 5. Growth of *Rb.sphaeroides* and PHB accumulation without pH control.

□,cell concentration. △ ,specific growth rate(μ) +,pH of the culture medium.

●,PHB content

Table 1. The productivity of PHB under different conditions.

pH	μ (h^{-1})[a]	PHB (%)[b]	μ ×PHB(%)
uncontrolled	0.189	27.1	0.0512
7.0	0.176	16.9	0.0298
7.5	0.253	16.8	0.0424
8.0	0.173	25.2	0.0437
8.5	0.155	31.3	0.0485

a. Average of specific growth rate in middle growth phase.

b. Average of PHB content in middle growth phase.

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