POLYHYDROXYBUTYRATE ACCUMULATION AND HYDROGEN EVOLUTION BY Rhodobacter sphaeroides AS A FUNCTION OF NITROGEN AVAILABILITY

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Key Words
polyhydroxybutyrate accumulation, hydrogen evolution, purple nonsulfur bacteria, nitrogen availability

1. SUMMARY

Polyhydroxybutyrate (PHB) is a useful by-product of hydrogen production using photosynthetic bacteria. This current study was aimed at understanding the effect of different carbon substrates and nitrogen availability on the levels of PHB accumulation and H₂ evolution by Rhodobacter sphaeroides strain RV. Media containing acetate, lactate, and pyruvate as carbon sources and ammonium or glutamate as nitrogen sources were used, as well as nitrogen-free media. The highest levels of PHB were observed in the cells grown on ammonium and acetate (35–38% of dry cells), while those in the cells grown on other carbon substrates were low and/or stable. PHB accumulation under nitrogen-deprived conditions was observed on acetate (>38–41%), lactate (~12–25%), and to a lesser extent, on pyruvate (~15%). Hydrogen evolution by the culture was observed on lactate and, to a lesser extent, on pyruvate under nitrogen-deprived conditions and when cells were grown on glutamate. PHB levels, as well as H₂ evolution, were lower when cells were grown on carbon substrates with glutamate as the sole nitrogen source. The data presented indicate the possibility of controlling the bacterial cultivation process, whichever final product, PHB or H₂, is preferred.

BioHydrogen, edited by Zaborsky et al.
2. INTRODUCTION

Photosynthetic bacteria are promising organisms for biotechnological production of polyhydroxybutyrate (PHB), a source of biodegradable plastics. Production of PHB by phototrophic bacteria would provide a major advantage as an environmentally acceptable and friendly technology, where sunlight energy and CO₂ or organic acids of waste water could be converted directly to the polymer.

The purple nonsulfur bacterium, *Rhodobacter sphaeroides* strain RV, has been studied in our laboratory for its possibility for biotechnological application in H₂-gas-producing photobioreactors (Miyake et al., 1984; Mao et al., 1986; Tsygankov et al., 1994; Nakada et al., 1996; Nagamine et al., 1996). Recent work in our laboratory has shown that ammonium- and acetate-fed continuous pH-stat cultures of this strain accumulate higher amounts of polyhydroxybutyrate (PHB) at higher pHs (Suzuki et al., 1995). However, the PHB content in cells grown under pH-uncontrolled conditions was shown to be even higher. We are currently selecting cultivation conditions for *R. sphaeroides* RV that lead to both H₂ production and final accumulation of considerable amounts of PHB as a by-product. For this purpose, the relationship of the two processes has to be studied, as well as regulation of PHB accumulation.

The goal of the present investigation was to study the interrelationship of H₂ evolution and PHB accumulation processes during growth of *R. sphaeroides* RV on different carbon substrates under nitrogen-sufficient (with NH₄⁺ or glutamate as a source of nitrogen) and nitrogen-deprived growth conditions.

3. EXPERIMENTAL

*Rhodobacter sphaeroides* strain RV (Miyake et al., 1984) was cultivated anaerobically in 15-mL, screw-cap glass vials on medium with sodium succinate (40 mM, if not otherwise stated), ammonium sulfate (1.25 g/L), and yeast extract (1.0 g/L), at 30 °C, 2000 lux (~20 W/m²) and initial pH 6.8. Cultures for further use in experiments with nitrogen-sufficient growth conditions were pre-grown 12–15 h in 300 mL polystyrene flat (3 cm) culture flasks (Iwaki, Japan) at 5000 lux (~45 W/m²) and 30 °C on the same medium (6–8% inoculate). Cultures for experiments with nitrogen-limited conditions or with glutamate as a sole nitrogen source were pre-grown at the same time on the media with succinate substituted for acetate, lactate, or pyruvate (40 mM). Cells were harvested by centrifugation (6000 g, 10', 18 °C); resuspended in the corresponding fresh medium (OD₆₆₀ ~2.0); transferred from 45-mL to 60-mL polystyrene, see-through, double-seal test tubes (Iwaki, Japan); flushed with argon; and grown further under standard conditions.

Culture tubes were illuminated from two sides by two 100-W tubular tungsten lamps with multiple incandescence points that provided light intensity of 5000 lux and energy of ~45 W/m² on the front surface of the tubes from both sides. In the case of nitrogen-deprived conditions, or when glutamate was used as a sole nitrogen source, the polystyrene tubes were closed with a silicone stopper with a gas outlet. The hydrogen gas evolved during the culture growth was collected inside plastic graduated cylinders connected to each test tube. The initial cell concentration in the latter case was adjusted to OD₆₆₀ ~2.0. Cells in experiments were grown 14 h on media with ammonium, and 24 h on media with glutamate, or under nitrogen-deprived conditions. Test tubes were taken out sequentially at fixed time intervals to determine medium pH, cell dry weight, and PHB content of the cells. Determination of PHB was performed by gas chromatography (Braunegg et al.,