RECOVERY OF POLY-3-HYDROXYALKANOIC ACID GRANULES BY A SURFACTANT-HYPOCHLORITE TREATMENT

J. A. Ramsay, E. Berger, B. A. Ramsay* and C. Chavarie

Chemical Engineering, École Polytechnique de Montréal, C.P. 6079, succursale "A", Montréal, Canada, H3C 3A7

SUMMARY

When Alcaligenes eutrophus biomass was treated with a surfactant and then washed with hypochlorite, the recovered poly-3-hydroxyalkanoic acid (PHA) granules were 97 to 98% pure with a molecular weight (M_w) between 730,000 and 790,000, depending on the surfactant used. When treated with only surfactant, the M_w was slightly higher than that obtained with the surfactant-hypochlorite treatment but the purity was 10% lower. PHA of higher purity but lower M_w was obtained with just a hypochlorite treatment.

INTRODUCTION

Poly-3-hydroxyalkanoic acids (PHAs) are a family of microbial, biodegradable thermoplastics. The most common PHA is poly-3-hydroxybutyric acid (PHB), an intracellular storage product of many microorganisms. Other PHAs have been found in nature (Herron et al., 1978) and/or produced under laboratory conditions (Holmes et al., 1987; Doi et al., 1988; Haywood et al., 1989). Since PHAs are intracellular products, their separation from other biomass components can be complex and costly. A number of previously reported recovery processes involve extraction with organic solvents (Walker et al., 1982; Barham and Selwood, 1982) while others use sodium hypochlorite (Williams and Wilkinson, 1958), chloroform in combination with thioglycollic acid (Nuti et al., 1972) or enzymes and surfactants (Holmes and Lim, 1985).

Prior to Berger et al. (1989), the use of sodium hypochlorite to digest non-PHB biomass was believed to always result in severe degradation of PHB (Alper et al., 1963; Nuti et al., 1972) rendering it unsuitable as a thermoplastic. Berger et al. (1989) showed that by optimizing the conditions under which sodium hypochlorite digested the biomass and by balancing the ratio of hypochlorite to non-PHB biomass (which is the difference between the biomass dry weight and the PHB content), PHB of 95% purity with an average molecular weight
(Mₚ) of 600,000 was recovered. Although this Mₚ is sufficiently high for use as a thermoplastic, it was only 50% of the original Mₚ of 1,200,000. The present paper describes an improved process whereby the hypochlorite digestion is combined with a surfactant pretreatment to obtain granules of PHA which have a higher degree of purity than that obtained with just the surfactant pretreatment and Mₚ's higher than obtained with hypochlorite digestion alone.

**MATERIALS AND METHODS**

**Production and storage of PHB-containing biomass.** The production of PHB by *Alcaligenes eutrophus* DSM 545 was described by Berger et al. (1989). The biomass containing 50% PHB by dry weight was lyophilized and stored at -20°C until needed.

**Digestion of the biomass by hypochlorite.** Hypochlorite solutions were prepared according to the method of Williamson and Wilkinson (1958). After contacting the PHB-containing biomass with the hypochlorite solution at pH 10, PHB was separated from the aqueous portion (containing dissolved biomass) by centrifugation at 4000 x g for 15 min. The PHB granules were rinsed twice with water, recentrifuged, recovered by filtration and air-dried.

**Surfactant treatment of the biomass.** Unless otherwise stated, 1% (w/v) biomass was added to a 1% (w/v) surfactant solution at 25°C for 15 min with mixing. The aqueous portion was then removed by centrifugation at 4000 x g for 15 min and washed twice with distilled water. Sodium dodecyl sulfate (SDS) and Triton X-100 were obtained from Aldrich, Milwaukee, Wis. and were used at pH 10 and 13 respectively. These pHs were found to be optimal for PHA recovery.

**PHA analysis.** PHA samples were prepared according to the method of Braunegg et al. (1978). The methylesters of the PHA monomers were quantified by gas chromatography using a 25 m HP5 capillary column (Hewlett-Packard Co., Palo Alto, Ca.) under the conditions described by Berger et al. (1989).

**Molecular weight determination.** The Mₚ determination was done at 30°C by gel permeation chromatography (Berger et al., 1989) using the universal calibration method.

**Purity and recovery.** The purity of PHA was determined from a known mass of sample by gas chromatography using an internal standard, benzoic acid. From a known amount of PHA in the biomass, the percent PHA recovered was calculated based on the purity of the total mass of sample recovered from a given separation process.

**Impurities of PHA.** Protein concentration was determined by the Lowry method (Lowry et al., 1951) using bovine serum albumin as a standard. For diaminopimelic acid (DAPA), PHA samples were prepared as for