Biotechnology Techniques Vol 4 No 4 221-226 (1990)
Received as revised 9th May

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

(M_w) of 600,000 was recovered. Although this M_w is sufficiently high for use as a thermoplastic, it was only 50% of the original M_w 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 M_ws 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 M_w 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

amino acid analysis (Mulligan et al., 1989) and the hydrolysed material analysed according to the procedures of Spackman et al. (1958).

RESULTS AND DISCUSSION

At present, mostly Gram negative bacteria (e.g. A. eutrophus (Holmes et al., 1987), A. latus (Lafferty and Braunegg, 1988), Azotobacter vinelandii (Page, 1989) and Pseudomonas species (Haywood et al., 1989)) are of potential commercial interest. Gram positive bacteria such as Bacillus megaterium have been well studied (Macrae and Wilkinson, 1958) but do not accumulate enough PHA to be of commercial value. In this work, A. eutrophus was used as an example of a Gram negative bacterium to evaluate the method under investigation.

As the ratio (w/w) of Triton X-100 or hypochlorite to non-PHA biomass was increased, the purity of the PHA recovered increased, achieved a maximum and then levelled off (Fig. 1). At a ratio of 0.85 Triton X-100:non-PHA biomass, a maximum of 87% purity was attained while there was a maximum of 95% purity at a ratio of 11:1 (hypochlorite:non-PHA biomass). In the case of SDS, at a ratio of 0.3:1 (SDS:non-PHA biomass), a purity of 75% was achieved. Further improvements were attained as the SDS:non-PHA biomass ratio was increased but the improvements were small compared to the amount of SDS that was added. To achieve the same degree of purity as Triton X-100 (87%), a ratio of at least 2:1 (SDS:non-PHA biomass) or 2.4 times more SDS than Triton X-100 must be used.

The manner in which surfactants function in such a process is reasonably well understood (Helenius and Simons, 1975). At low concentrations, surfactant monomers insert themselves into the lipid bilayers of the cell membrane. As the surfactant concentration increased, more molecules are incorporated into the bilayers, increasing the volume of the cell envelope. Once saturated, further addition causes a disruption of the envelope structure and large micelles of surfactants and phospholipids are formed. This leaves the PHA granules enclosed in a peptidoglycan net surrounded by cellular debris. Surfactants also denature or solubilize proteins and hence make it easier to disrupt the cell membrane. Anionic

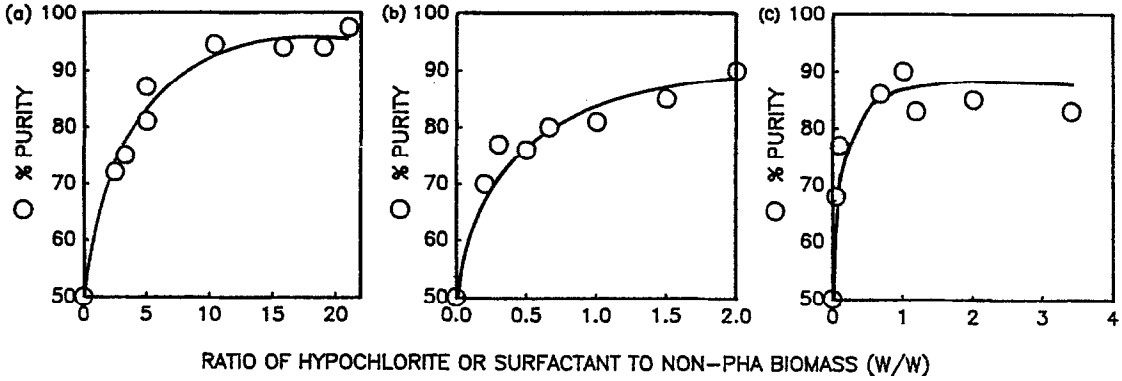

FIGURE 1 : THE EFFECT OF THE RATIO OF (a) HYPOCHLORITE, (b) SDS AND (c) TRITON X-100 TO NON-PHA BIOMASS ON THE PURITY OF THE PHA RECOVERED.

surfactants such as SDS denature proteins while non-ionic ones such as Triton X-100 solubilize them.

As the ratio of hypochlorite:non-PHA biomass was increased, there was an increasing loss in M_w. Berger et al. (1989) controlled this loss by adjusting the hypochlorite:non-PHA biomass ratio or the time of exposure to hypochlorite to obtain reasonably high M_w. Increasing concentrations of SDS or Triton X-100 did not affect the M_w of PHB or P(HB-HV).

When the PHA-containing biomass was treated at a ratio of 1:1 with SDS or Triton X-100, the purity of the PHA recovered was 87% (Table 1). However, when SDS or Triton X-100 treatment was followed by a one-minute hypochlorite wash, a higher purity of 97 to 98% was achieved than when treated with surfactant only. The M_w of the final product from the combined treatment was between 730,000 and 790,000 compared to 680,000 with the hypochlorite control. A change in the molecular weight distribution, as represented by the polydispersity index, was also observed. It was between 3 and 6 when only a surfactant was used but had a slightly higher range between 2 and 7 when the surfactant treatment was followed by hypochlorite. The loss in M_w and the slight increase in the polydispersity index from the surfactant-hypochlorite treatment were most likely due to the hypochlorite and pH conditions, as the surfactants themselves were

not found to degrade the polymer.

TABLE 1 :MOLECULAR WEIGHT (M_w), PURITY, PROTEIN AND DIAMINOPIMELIC ACID[1] (DAPA) CONTENT OF PHA RECOVERED BY SURFACTANT TREATMENT ALONE OR FOLLOWED BY A HYPOCHLORITE WASH.

TREATMENT	M_w	PURITY (%)	PROTEIN (%)	DAPA[1] (%)
UNTREATED BIOMASS	1,200,000	50	30.0	0.13
SURFACTANT ONLY				
SDS (1%)	810,000	87	3.1	0.13
TRITON X-100 (1%)	800,000	87	3.8	0.06
SURFACTANT FOLLOWED BY HYPOCHLORITE				
SDS (1%)	730,000	97	0.7	0.03
TRITON X-100 (1%)	790,000	98	0.1	0
HYPOCHLORITE ONLY[2]	680,000	87	1.2	0.01

[1] component of peptidoglycan
[2] treatment for 1 minute

Although treatment with surfactant followed by hypochlorite was highly effective, the reverse procedure did not work. Pretreatment with surfactants removed about 85% of the total proteins (Table 1) while the subsequent hypochlorite wash removed a further 10% proteins and nearly all the peptidoglycan (as represented by the diaminopimelic acid). Although the hypochlorite, itself, gave a PHA of 87% purity, its M_w was less than that obtained with surfactant only or surfactant followed by hypochlorite.

Another advantage of the surfactant-hypochlorite method is that PHA granules of reasonably high M_w can be obtained more quickly than with other techniques such as surfactant-enzyme treatment or solvent extraction. PHA granules possess a unique native morphology (Ellar et al., 1968) which can be useful in the production of certain materials from PHA. This morphology is disrupted by solvent extraction. The surfactant-hypochlorite treatment has the advantage

of retaining the native granule form allowing more diverse applications than solvent-extracted PHA.

ACKNOWLEDGEMENT

The authors acknowledge the financial support of Centre Québécois de Valorisation de la Biomasse and the Natural Sciences and Engineering Research Council of Canada. The technical assistance of B. Gibbs at the Biotechnology Research Institute was also appreciated.

REFERENCES

Alper, R., Lundgren, D.G., Marchessault, R.H., and Cote, W.A. (1963). Biopolymers. 1, 545-556.
Barham, P.J., and Selwood, A. (1982). Eur. Pat. Appl. 58,480.
Berger, E., Ramsay, B.A., Ramsay, J.A., Chavarie, C. and Braunegg, G. (1989). Biotechnol. Techniques. 3, 227-232.
Braunegg, G., Sonnleitner, B., and Lafferty, R.M. (1978). Eur. J. Appl. Microbiol. Biotechnol. 6, 29-37.
Doi, Y., Tamaki, A., Kunioka, M., and Soga, K. (1988). Appl. Microbiol. Biotechnol. 28, 330-334.
Ellar, D., Lundgren, D.G., Okamura, K., and Marchessault, R.H. (1968). J. Mol. Biol. 35, 489-502.
Haywood, G.W., Anderson, A.J., and Dawes, E.A. (1989). Biotechnol. Lett. 11, 471-476.
Helenius, A., and Simons, K. (1975). Biochimica Biophysica Acta. 415, 29-79.
Herron, J.S., King, J.D., and White, D.C. (1978). Appl. Environ. Microbiol. 35, 251-257.
Holmes, P.A., and Lim, G.B. (1985). Eur. Pat Appl. 145,233.
Holmes, P.A., Wright, L.F., and Collins, S.H. (1987). Eur. Pat. 69,497.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). J. Biol. Chem. 193, 265-275.
Lafferty, R.M., and Braunegg, G. (1988). Can. Pat. 1,236,416.
Macrae, R.M., and Wilkinson, J.F. (1958). J. Gen. Microbiol. 19, 210-222.
Mulligan, C.N., Chow, T.Y.K., and Gibbs, B.F. (1989). Appl. Microbiol. Biotechnol. 31, 486-489.
Nuti, M.P., de Bertoldi, M., and Lepidi, A.A. (1972). Can. J. Microbiol. 18, 1257-1261.
Page, W.J. (1989). Appl. Microbiol. Biotechnol. 31, 329-333.
Spackman, D.H., Stein, W.H., and Moore, S. (1958). Anal. Chem. 30, 1190-1206.
Walker, J., Whitton, J.R., and Alderson, B. (1982). Eur. Pat. Appl. 46,017.
Williamson, D.H., and Wilkinson, J.F. (1958). J. Gen. Microbiol. 19, 198-203.