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Process analysis and economic evaluation for Poly(3-hydroxybutyrate) production by fermentation

Jong-il Choi, Sang Yup Lee

Abstract Several processes for the production and recovery of poly(3-hydroxybutyrate) (PHB) by *Alcaligenes eutrophus*, *Alcaligenes latus*, *Methylobacterium organophilum*, and recombinant *Escherichia coli* were designed based on the previously reported data and analyzed by computer-aided bioprocess design. PHB productivity, content, and yield significantly affected the final price of PHB. For the annual production of 2,850 tonnes of purified PHB, the process employing *A. eutrophus* with the recovery method of surfactant-hypochlorite digestion resulted in lowest price of PHB, $ 5.58/kg. As the production scale increased to one million tonnes per year, the price of PHB dropped to $ 4.75/kg. The cost of carbon substrate significantly affected the overall economics in large production scale. Therefore, the production cost can be considerably lowered when agricultural wastes, such as whey and molasses, are used.

1 Introduction

In response to the problems and harmful effects of plastic wastes on the environment, there has been considerable interest in the development of biodegradable plastic materials (Dawes, 1990; Hocking and Marchessault, 1994; Swift, 1993). Among the various biodegradable polymer materials, polyhydroxyalkanoates (PHAs) are attractive substitutes for conventional petrochemical plastics because of their similar material properties to various thermoplastics and elastomers, and complete degradability upon disposal under various environments (Brandl et al. 1990; Byrom, 1991; Doi, 1990; Steinbuchel, 1991). Even though there are more than 250 different microorganisms synthesizing PHAs, only several of these, such as *Alcaligenes eutrophus* (Kim et al. 1994), *Alcaligenes latus* (Yamane et al. 1996), *Azotobacter vinelandii* (Page and Knosp, 1989), methylotrophs (Kim et al. 1996), *Pseudomonas oleovorans* (Brandl et al. 1988) and recombinant *Escherichia coli* (Lee and Chang, 1994; Lee et al. 1994) are suitable for the production of PHAs to a high concentration with high productivity. More than 90 different monomer units have been identified as constituents of PHAs in various bacteria (Steinbuchel and Valentin, 1995), but only a few members of PHAs including poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/V), and poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate) (PHHx/O) have been produced to relatively large quantities and characterized (Lee, 1996a; 1996b; Lee and Chang, 1995). PHB is most widespread and best characterized polymer among these, and has material properties similar to polypropylene (Byrom, 1987).

A major drawback to the commercialization of PHAs is their much higher price compared with conventional petrochemical based plastic materials. Until recently, Zeneca Bio Products (Billingham, UK) produced approximately 1,000 tonnes per year of PHB/V copolymer and sold under the tradename BIOPOL^{TM} at ca. US $ 16/kg (personal communication from L.A. Naylor, Zeneca). If we consider the price of conventional petrochemical plastics, such as polyethylene and polypropylene, which is less than US $ 1/kg, PHAs may be considered too expensive to be used as bulk plastic materials. However, it is unfair to compare the price of PHAs with polyethylene or polypropylene since the latter is not biodegradable. Therefore, comparisons should be made with other biodegradable polymers such as polylactide, diol-diacid based aliphatic polyesters, and starch-based polymers, which are currently sold at US $ 5– $ 12 per kg. Recently, transgenic *Arabidopsis thaliana* harboring the *A. eutrophus* PHA biosynthesis genes (Poirier et al. 1992) was developed. PHB up to 10 mg per g fresh weight of cell, which is relatively low, could be produced (Nawrath et al. 1994). Therefore, until there is much improvement in the transgenic technology, PHA will be produced by bacterial fermentation.

For the commercialization of PHAs, much effort has been devoted to reduce the production cost by the development of better bacterial strains and more efficient fermentation/recovery processes (Lee, 1996a; 1996b; Lee and Chang, 1995). Two different cultivation methods can be employed for efficient production of PHAs by different bacteria (Lee, 1996b). Many bacteria including

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A. eutrophus, methylotrophs, and *P. oleovorans* synthesize PHAs under the limitation of a nutritional element such as N, P, Mg, K, O, or S in the presence of excess carbon source. For the cultivation of these bacteria, two-step cultivation method is employed. Cells are first grown without nutrient limitation and then nutrient limitation is applied for PHA synthesis. The choice of limiting nutrient as well as the time point of applying nutrient limitation can significantly affect polymer production. However, some bacteria such as *A. latus, A. vinelandii,* and recombinant *E. coli* do not require nutrient limitation for the synthesis of PHAs, and can accumulate PHAs during growth. For these bacteria, the nutrient feeding strategy is most important for the success of fermentation and should be optimized for each bacterium (Lee and Chang, 1995).

Several methods have been developed for the recovery of PHAs (mostly PHB) from the cells. Solvents such as chloroform, methylene chloride, propylene carbonate, and dichloroethane have been used for the extraction of PHB (Ramsay et al. 1994). However, the extracted polymer solution containing more than 5% (w/v) PHB was very viscous and the removal of cell residues was difficult. Digestion method using hypochlorite has been proposed as an alternative (Berger et al. 1989). However, during the digestion of non-PHA cellular materials (NPCM), severe degradation of PHB has been observed. Surfactant pretreatment and hypochlorite digestion under the optimized condition resulted in high purity of PHB with less degradation (Ramsay et al. 1990). An enzymatic digestion method was also developed by Zeneca (Holmes, P.A. and Lim, G.B. 1990. U.S. Patent 4,910,145). This process consisted of thermal treatment of biomass containing PHB, enzymatic digestion, and washing with an anionic surfactant to solubilize NPCM. Recently, PHB recovery using a dispersion of chloroform and sodium hypochlorite solution has been suggested (Hahn et al. 1994a). PHB could be recovered by this method with much less polymer degradation. For the recovery of PHB from *A. vinelandii*, a simple treatment with 1 N NH_4OH for 10 min resulted in the purity of 94% due to the fragility of the cells (Page and Cornish, 1993). Among these methods, surfactant-hypochlorite digestion, treatment with the dispersion of chloroform-hypochlorite, and enzymatic digestion allow PHB recovery with purity higher than 95%, and therefore can be used commercially.

To date, there has been no report on the process analysis and economic evaluation for PHB production by bacterial fermentation. Analysis of the entire process for the production and recovery of PHB by several bacteria will allow us to design the most efficient way of PHB production, and to evaluate the approximate price of PHB produced in a commercial scale. In this paper several processes for the production and recovery of PHB employing *A. eutrophus, A. latus, Methylobacterium organophilum*, and recombinant *E. coli* were analyzed. The processes were simulated based on the recently published information on the production and recovery of PHB (Hahn et al. 1994a; Kim et al. 1994; Kim et al. 1996; Lee and Chang, 1994; Ramsay et al. 1990; Yamane et al. 1996), and economics of each process were evaluated.

2 Materials and methods

2.1 Process description

Process analysis and economic evaluation for PHB production and recovery were carried out using the software tool BioPro Designer® from Intelligen Inc. (Scotch Plains, NJ). Two different process flowsheets for the production and recovery of PHB are shown in Fig. 1A and 1B. These two processes differ only in recovery steps: surfactant-hypochlorite digestion for Fig. 1A and the dispersion method for Fig. 1B. Several equipments and other auxiliary pieces such as piping and valves are omitted for simplicity in the flowsheets, but are taken into account in economic analysis.

2.2 Raw materials

The media for PHB production consist of a carbon source and various inorganic salts. Several different carbon sources have been used for different bacterial strains producing PHB (Lee and Chang, 1995). Process simulations were performed using the best carbon source for the production of PHB by each bacterium (Lee and Chang, 1995): glucose for *A. eutrophus* and recombinant *E. coli*, sucrose for *A. latus*, and methanol for *M. organophilum*. The prices of carbon sources and other major raw materials are ($ per kg): glucose, 0.5; sucrose, 0.3; methanol, 0.2; surfactant (Triton-X100), 1.5; chloroform, 0.7; hypochlorite solution, 0.1; water, 0.001. The costs of other medium components such as inorganic salts were not included because their contribution to the final cost of PHB was insignificant.

2.3 Fermentation

Fermentation medium prepared in blending tank (V-101) was sterilized in a continuous heat sterilizer (ST-101). The axial compressor (G-101) and the air filter (AF-101) provided sterilized air (and pure oxygen if necessary) to the fermentor. The seed fermentors not shown in the flowsheet were used to inoculate the production fermentor (R-101). In all cases cells were cultivated in fed-batch mode, and fermentation data used in the simulations are summarized in Table 1. After the fermentation, culture broth was transferred to the holding tank (V-104 in Fig. 1A and V-102 in Fig. 1B).

2.4 Recovery of PHB by surfactant-hypochlorite digestion (Fig. 1A)

Cells were harvested by continuous centrifugation (CF-101) of fermentation broth collected in the holding tank (V-104). Surfactant solution (1%, w/v) was added to biomass and mixed at 25 °C for 1 hour of mean residence time (V-103). This treatment was followed by hypochlorite digestion in a flow-through manner (M-101). PHB was then separated from the aqueous solution containing dissolved NPCM by centrifugation (CF-104). PHB granules were rinsed with water (V-102, CF-102) and were finally spray-dried (SPD-101).

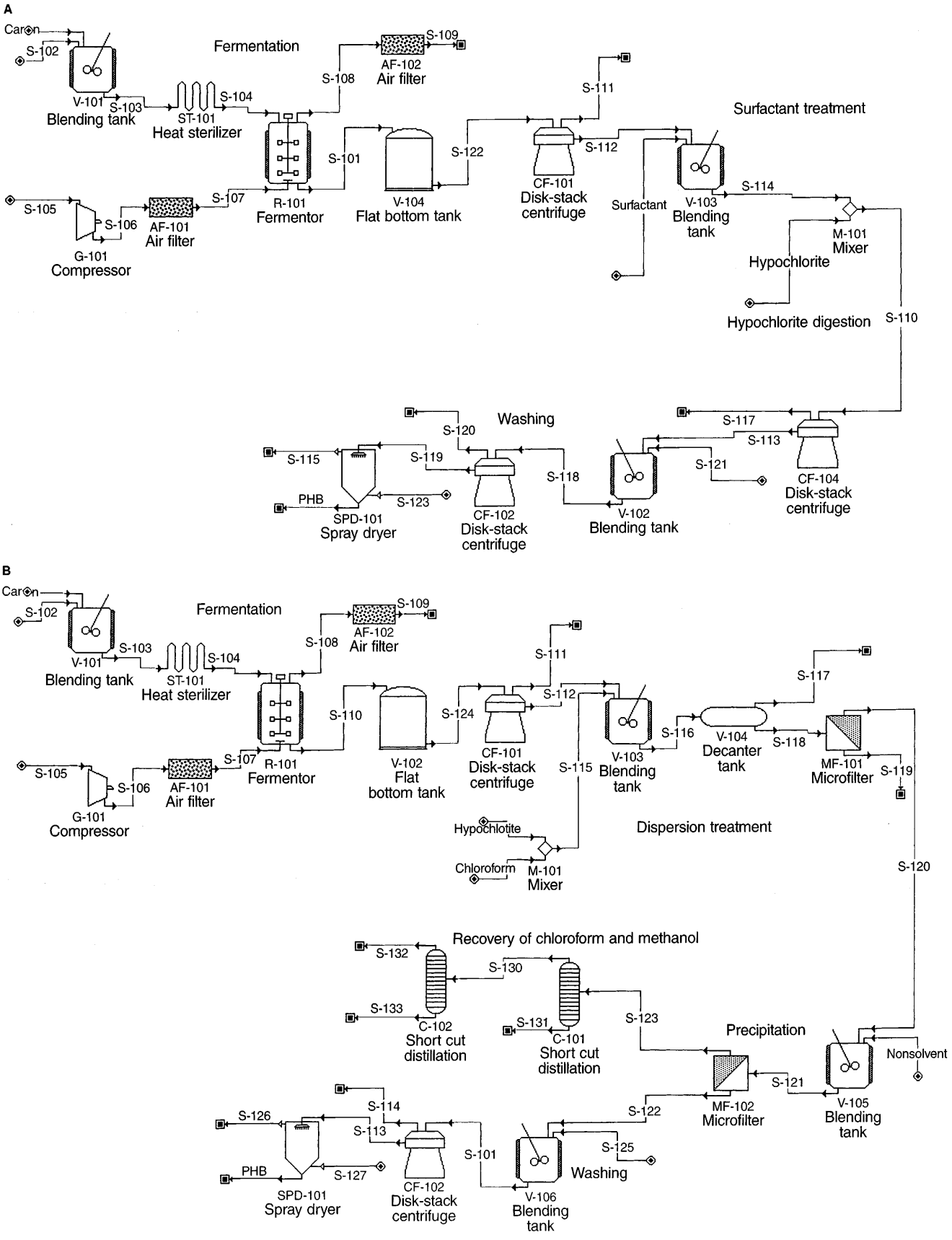

Fig. 1A,B. Process flowsheet for PHB production with two different methods: **A** surfactant-hypochlorite digestion and **B** treatment by dispersion of chloroform and hypochlorite

2.5
Recovery of PHB by treatment with the dispersion of chloroform and hypochlorite (Fig. 1B)

The harvested biomass containing PHB (CF-101) was treated with dispersion of 0.1 m^3 chloroform and 0.1 m^3 diluted sodium hypochlorite solution per 8 kg biomass (V-103). After treatment at 30 °C for 90 minutes of mean residence time, two phases were separated in a decanter tank (V-104). The light hypochlorite phase was removed and the heavy chloroform phase containing PHB was filtered (MF-101). The filtrate was precipitated by non-solvent consisting of methanol and water (7:3 by volume) (V-105), and precipitate was filtered (MF-102). Finally, PHB was washed with water (V-106, CF-102) and spray-dried (SPD-101). Two distillation columns were employed to recover chloroform (S-132) and methanol (S-133).

3
Results and discussion

Processes for the production of PHB by *A. eutrophus, A. latus, M. organophilum*, and recombinant *E. coli* with two different recovery methods were designed and analyzed. The process flowsheets for the production of PHB and recovery by two different methods are shown in Fig. 1. The targeted amount of purified PHB in the simulations was 2,850 tonnes per year. Since the recovery efficiency of 95% was assumed when PHB was recovered by surfactant-hypochlorite digestion, the amount of PHB to be produced by fermentation was 3,000 tonnes per year. When dispersion of chloroform and hypochlorite was used as a recovery method, 3,167 tonnes of PHB was produced by fermentation to give 2,850 tonnes of purified product (recovery efficiency of 90%). The total operating time was assumed to be 7,920 hours per year. The amount of PHB produced per run and the total number of runs per year varied among the processes employing different bacteria due to the different PHB productivity, concentration, and content obtainable, as summarized in Table 1. Since fed-batch fermentation takes 39 to 70 hours depending on the bacterium employed and the turnaround time of 12 hours is required for fermentor vessel cleaning and medium recharging, recovery can be done during this period. By incorporating a holding tank (V-104 in Fig. 1A and V-102 in Fig. 1B) prior to the recovery process, fermentation and recovery processes could be decoupled. The sizes of the equipments in the recovery process were determined so that the entire recovery operations could be carried out during the fermentation and turnaround time. In this way, the costs of equipments for recovery could be minimized. Seed cultures were also prepared during the fermentation period. Design of equipment specification, process analysis, and economic evaluation for the production of PHB by recombinant *E. coli* with the recovery method of surfactant-hypochlorite treatment are explained below as an example.

For the production of 3,000 tonnes of PHB per year (2,850 tonnes after purification) by recombinant *E. coli* with the fermentation time of 39 hours and the turnaround time of 12 hours per run, 155 fermentation runs can be carried out during the total annual operating time of 7,920 hours. Therefore, the two production fermentors of 185.37 m^3 were employed to produce 19,354.8 kg of PHB per run. The sizes of the recovery equipments were determined to perform entire recovery in 51 hours. The specifications and purchase costs of the major equipments are summarized in Table 2. Based on these results, the fixed capital cost and the annual operating cost were estimated and summarized in Table 3. Among the seven items contributing to the total annual operating cost, the cost of raw materials and direct fixed capital dependent cost were 39.5 and 28.4%, respectively, of the total cost (Table 3). The price of PHB (considering only the cost of PHB production, purification, and equipment depreciation, but not including sales cost and margin) is, $ 6.14/kg($ 17,488,000/2,850,000 kg) at this production scale.

Annual operating costs for the processes employing four different bacteria as well as the prices of PHB obtainable with these processes are compared in Table 4. It can be seen that the operating costs for the processes with dispersion treatment were much higher than that with surfactant-hypochlorite digestion. The process with the former recovery method requires two distillation towers

Table 1. Fermentation data used in process analysis and economic evaluation

Bacterium	*A. eutrophus*	*A. latus*[a]	*M. organophilum*	Recombinant *E. coli*
Carbon source	Glucose	Sucrose	Methanol	Glucose
Limiting nutrient	Nitrogen	None	Potassium	None
Fermentation method	Glucose concentration control	pH-stat	methanol concentration control	pH-stat
Culture time (h)	50	28.45	70	39
Cell concentration (g/l)	164	143	250	110
PHB concentration (g/l)	121	71.4	130	85
PHB content (%)	76	50	52	77.3
PHB productivity (g/lh)	2.42	2.5	1.86	2.18
PHB yield (g PHB/g substrate)	0.3	0.17	0.19	0.29
kg substrate /kg PHB	3.33 kg glucose/kg PHB	5.88 kg sucrose/kg PHB	5.26 kg methanol/kg PHB	3.5 kg glucose/kg PHB
Reference	Kim et al. 1994	Yamane et al. 1996	Kim et al. 1996	Unpublished results[b]

[a] Data were converted from the reported values so that the inoculum size was similar to the other bacteria
[b] Results obtained by the pH-stat fed-batch culture of XL1-Blue(pSYL105) in a semi-defined medium containing small amount of yeast extract and corn steep liquor as previously reported (Lee and Chang, 1994)

Table 2. Major equipment specification and purchase cost (based on 1996 year) to obtain production of 2,850 tonnes of purified PHB per year by recombinant *E. coli* using the recovery method of surfactant-hypochlorite digestion

Equipment (Quantity)	Description	Cost ($)
Fermentor (2)	Volume = 185.37 m^3 Power = 312.80 kW	974,000
Seed Fermentor (1)	Volume = 35.04 m^3 Power = 78.85 kW	535,000
Seed Fermentor (1)	Volume = 3.50 m^3 Power = 7.88 kW	186,000
Seed Fermentor (1)	Volume = 0.35 m^3 Power = 0.79 kW	77,000
Blending Tank (1 EA)	Volume = 2.94 m^3 Power = 0.635 kW	10,000
	Volume = 46.73 m^3 Power = 8.34 kW	53,000
	Volume = 52.95 m^3 Power = 11.25 kW	55,000
Heat sterilizer (1)	Diameter = 0.10 m Length = 7.42 m	319,000
Compressor (1)	Pressure change = 5.00 bar Power = 478.95 kW	350,000
Air filter (1)	Throughput = 0.70 m^3/s	8,000
Disk-Stack centrifuge (1 EA)	Sigma factor = 2954.69 m^2 Power = 5.35 kW	66,000
	Sigma factor = 4956.20 m^2 Power = 6.58 kw	66,000
	Sigma factor = 27611.37 m^2 Power = 13.08 kW	108,000
Flat Bottom Tank (2)	Volume = 176.98 m^3	74,000
Spray dryer (1)	Dryer diameter = 0.80 m Dryer Height = 2.39 m	74,000

for the recovery of chloroform and methanol, more utilities for steam generation, and other consumables including filter cloth. The raw material costs are also higher due to the use of large amount of chloroform and methanol used as nonsolvent. The recovery efficiency of the former (90%) was lower than the latter (95%). The lowest price of $ 5.58/kg PHB was obtained with the process employing *A. eutrophus* as a host and surfactant-hypochlorite digestion for recovery, even though the cost of carbon source was highest ($ 0.5/kg glucose) among the tested. This was due to the combined effects of high PHB productivity, high PHB content, and high yield of PHB on the carbon source (Table 1). When the PHB productivity is high, more fermentation runs can be carried out for the given total annual operating time, resulting in the reduction of fermentor size and fixed cost. High PHB content allows processing of less amount of NPCM to obtain the same amount of PHB, resulting in the reduction of recovery cost. Finally, high PHB yield reduces the cost of carbon source, which is the major contributor to the total raw material costs. The price of PHB produced by *M. organophilum* was relatively high even though the cost of the carbon source ($ 0.2/kg methanol) was less than half that of glucose. This is due to the combination of low PHB content, low productivity, and low yield of PHB on methanol (Table 1). The price of PHB obtained by the process employing *A. latus* was highest even though the PHB productivity was highest with this bacterium. This was because the PHB yield and PHB content were the lowest among the examined (Table 1).

The simulations performed in this study were based on the actual fermentation data reported. Therefore, the price of PHB estimated can be further lowered by developing more efficient process. We can see from the simulation results that it is important to develop a fermentation strategy that not only allows high PHB productivity but also high PHB content with high yield on the carbon source in order to lower the final price of PHB. If PHB productivity of 4 g/l h and the PHB content of 80% were obtained by fed-batch culture of *A. eutrophus* using glucose as a carbon source at the same production scale of 3,000 tonnes per year, the price of PHB obtained would be $ 5.1/kg, which is $ 0.48 lower per kg PHB than that obtained with the productivity of 2.42 g/l h. Development of more efficient recovery method will also lower the price of PHB. For example, PHB can be recovered with 96% purity from recombinant *E. coli* by surfactant treatment only (Hahn et al. 1994b). If this recovery method is used, $ 0.3 can be reduced for every kg of PHB.

The cost of the carbon source also contributes significantly. For the process with recombinant *E. coli*, it was as high as 30.7% of the total operating cost (see Table 3). Use of cheaper carbon sources can lower the final price of PHB considerably, but should be thoroughly investigated if reasonably high PHB productivity, content, and yield can be obtained with these substrates. If hydrolyzed corn starch ($ 0.2/kg) can be used instead of glucose for recombinant *E. coli* without changing the fermentation performance shown in Table 1, the price of PHB is $ 5/kg, which is $ 1.14 lower per kg PHB than that obtained with glucose. Therefore, other inexpensive carbon sources, especially agricultural wastes such as cheese whey, cane and beet molasses, and hemicellulose hydrolysate should be considered as potential carbon sources for PHB pro-

Table 3. Economic analysis of PHB production (2,850 tonnes/a) by recombinant *E. coli* with the recovery method of surfactant-hypochlorite digestion

FIXED CAPITAL ESTIMATE SUMMARY (1996 prices)		
A. TOTAL PLANT DIRECT COST (TPDC) (physical cost)		cost ($)
a. Equipment Purchase Cost	(PC)	4,039,000
b Installation (summed over all units)		2,993,000
c. Process Piping	(0.35 × PC)	1,414,000
d. Instrumentation	(0.40 × PC)	1,616,000
e. Insulation	(0.03 × PC)	121,000
f. Electrical	(0.10 × PC)	404,000
g. Buildings	(0.45 × PC)	1,818,000
h. Yard Improvement	(0.15 × PC)	606,000
i. Auxiliary Facilities	(0.40 × PC)	1,616,000
		14,629,000
B. TOTAL PLANT INDIRECT COST (TPIC)		
a. Engineering	(0.25 × TPDC)	3,657,000
b. Construction	(0.35 × TPDC)	5,120,000
		8,777,000
C. OTHER COSTS (OTC)		
a. Contractor's fee	(0.05 × (TPDC + TPIC))	1,170,000
b. Contingency	(0.10 × (TPDC + TPIC))	2,341,000
		3,511,000
D. DIRECT FIXED CAPITAL (DFC)		
TPDC + TPIC + OTC		26,917,000
ANNUAL OPERATING COST (1996 prices)		
A. DFC-DEPENDENT ITEMS (DFC = $ 26,917,000)		
Depreciation		2,557,000
Maintenance Material	(summed over all units)	258,000
Insurance	(0.01 × DFC)	269,000
Local Taxes	(0.02 × DFC)	538,000
Factory Expense	(0.05 × DFC)	1,346,000
		4,968,000
B. LABOR-DEPENDENT ITEMS		
a. Operating labor	(31,335 h × 18.0 $/h)	727,000
b. Maintenance labor	(summed over all units)	226,000
c. Fringe benefits	(0.40 × (a + b))	381,000
d. Supervision	(0.20 × (a + b))	191,000
e. Operating supplies	(0.10 × a)	73,000
f. Laboratory	(0.15 × a)	109,000
		1,707,000
C. ADMINISTRATION AND OVERHEAD EXPENSE (0.6 × (a+b+c))		800,000
D. RAW MATERIALS		
a. Carbon source		5,369,000
b. Water		39,000
c. Surfactant (Triton-X100)		655,000
d. Hypochlorite solution		844,000
		6,907,000
E. OTHER CONSUMABLES		
Membrane or filter cloth		0
		0
F. UTILITIES		1,505,000
G. WASTE TREATMENT/DISPOSAL		1,601,000
Total annual operating cost		17,488,000

duction. Recombinant *E. coli* is a good candidate for PHB producer since it can utilize all of these inexpensive carbon substrates. Preliminary simulations showed that the price of $ 4.47, $ 4.79, and $ 4.61 per kg PHB can be obtained when whey, cane molasses, and hemicellulose hydrolysate, respectively, are used to obtain 2,850 tonnes of purified PHB annually.

It is estimated that the market for biodegradable plastic material will reach 3–6 million tonnes per year by the year 2000 (Technical Insights, Inc. 1994. Biopolymers/Natural polymers, p. 10). The price of PHB will decrease with increasing production scale. The effect of production scale on the price of PHB is shown in Fig. 2. When one million tonnes of PHB is produced by *A. eutrophus* and recombinant *E. coli* from glucose, the price will be $ 4.75 and $ 5.01 per kg PHB, respectively. As the process scale changes, the contribution to the overall operating cost by raw materials, fixed capital dependent items, and others also changes. As

Table 4. Annual operating cost for PHB production (2,850 tonnes/a) by *A. eutrophus*, *A. latus*, *M. organophilum*, and recombinant *E. coli* recovered by (A) surfactant-hypochlorite digestion and by (B) dispersion treatment of chloroform and hypochlorite

In 1,000 US dollar	*A. eutrophus*		*A. latus*		*M. organophilum*		Recombinant *E. coli*	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
Direct fixed capital dependent items	4,299	6,056	5,320	6,966	5,821	8,195	4,968	6,330
Labor dependent items	1,386	1,746	1,737	2,131	1,747	2,184	1,707	1,984
Administration and overhead expense	652	825	817	1,004	823	1,035	800	934
Raw materials	6,628	10,214	10,278	13,249	7,626	10,521	6,907	10,128
Other consumables	0	1,844	0	2,874	0	2,762	0	1,736
Utilities	1,334	4,722	1,843	6,823	1,774	5,519	1,505	5,239
Waste treatment/disposal	1,599	699	3,290	1,043	3,153	999	1,601	641
Total	15,898	26,106	23,285	34,090	20,944	31,215	17,488	26,992
Production cost (US$ /kg PHB)	**5.58**	**9.16**	**8.16**	**11.96**	**7.34**	**10.95**	**6.14**	**9.45**

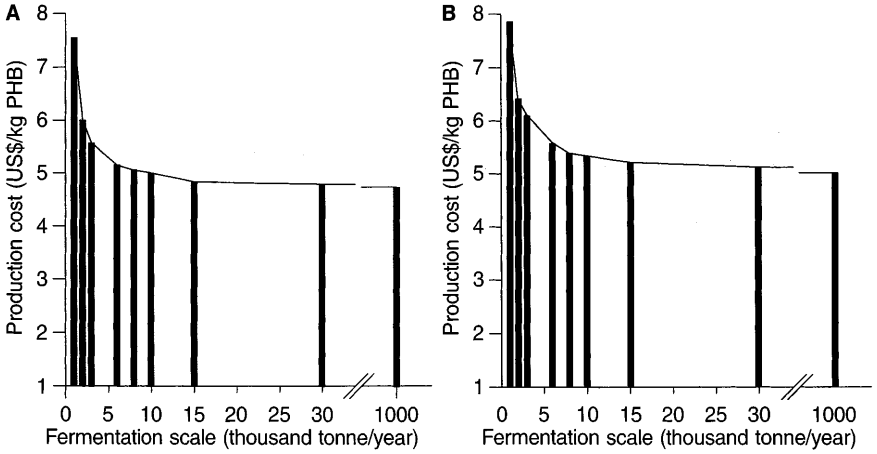

Fig. 2. The effect of production scale on the final price of PHB using (A) *A. eutrophus* and (B) recombinant *E. coli* with the recovery method of surfactant-hypochlorite digestion

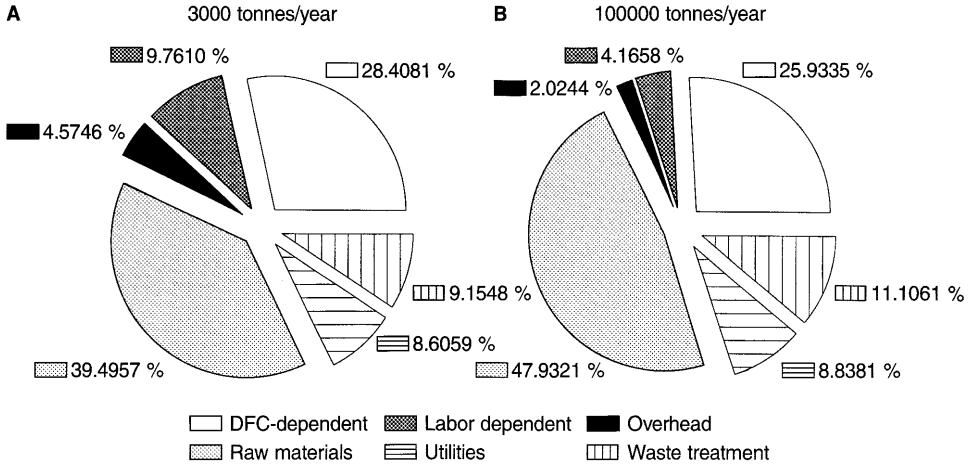

Fig. 3. The breakdown of operating cost for the production of PHB from glucose using recombinant *E. coli* with the recovery method of surfactant-hypochlorite digestion: (A) 3,000 tonnes fermentation per year and (B) 100 ,000 tonnes per year

shown in Fig. 3, the fraction of raw material cost increases most significantly with process scale-up. The cost of raw materials can account for as much as 50% of the total operating cost as the production scale increases. Therefore, the cost of raw materials becomes very important to the overall economics of PHB production in a large scale. Since the cost of carbon source accounts for 70 to 80% of total raw material cost, the price of PHB can be significantly lowered if cheap carbon substrate can be used. For example, the price of PHB will be as low as $ 3.84/kg when one million tonnes of PHB per year is produced by recombinant *E. coli* from hydrolyzed corn starch. The increase of waste treatment cost by using cheap carbon substrate was only 0.075% of the total production cost, which resulted in the increase of only $ 0.003 per kg PHB.

In this paper, we have designed and analyzed several processes for the production of PHB by four different bacteria. Process analysis and economic evaluation have clearly shown that PHB productivity, PHB content, PHB yield, and the cost of carbon substrate considerably affect the final price of PHB. Since fermentation strategies for the production of PHB have been relatively well developed,

isolation of a new strain or development of a recombinant strain that accumulates PHB with high productivity, high content, and yield from inexpensive carbon substrate seems to be more important. Development of an efficient recovery method is also important to lower the price of PHB. With these advances, the price of PHB will be likely to become $ 3−4 per kg when produced in a large scale. The price of other biodegradable polymer such as polylactide and diol-diacid based aliphatic polyesters will be similar to this value. Even though this price is still four times higher than petrochemical-based plastic materials, complete biodegradability of PHB can justify this higher price. If we consider manufacturing a shampoo bottle that requires 50 g of polymer per bottle, the cost difference of the bottles made of PHB and polypropylene will be only 15 cents. Consumers may be willing to pay extra 15 cents to participate in the protection of our environment. Since PHB, and more generally PHAs, can be used in a wide range of applications including packaging materials, disposable items, starting material for the synthesis of chiral compound, surgical sutures and wound dressing (Lee, 1996a), there is little doubt that PHAs will become a major biodegradable plastic material in the near future.

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