INDUSTRIAL PRODUCTION OF POLYHYDROXYALKANOATES USING *ESCHERICHIA COLI*: AN ECONOMIC ANALYSIS

R. J. VAN WEGEN, Y. LING and A. P. J. MIDDELBERG (MEMBER)

Biodegradable Technologies Group, Department of Chemical Engineering, University of Adelaide, South Australia 5005

Disposal of plastic, in particular packaging, is a serious problem confronting many countries. Part of a solution lies in using biodegradable plastics such as polyhydroxyalkanoates (PHAs), which can be produced by bacterial fermentation. This paper presents an economic analysis of PHA production by fermentation using recombinant *Escherichia coli*, in order to identify areas where significant cost reductions are possible. Based on an annual production of 4300 tonnes of poly-hydroxybutyrate (PHB) from glucose supplemented with complex nitrogen sources, the break-even selling price per kilogram of plastic is US1995$6.08. A ten year plant life, 40% tax rate, 10% discount rate and a conservative process design based on existing technology are assumed. This cost is highly sensitive to PHB expression level and recovery strategy, and moderately sensitive to medium cost and cell growth yield on glucose. Maximum cell density achieved has little effect for the process design used in this analysis. At the same level of production, the cost can be easily reduced to between US1995$5.63 kg^{-1} and US1995$3.59 kg^{-1} by using dairy whey as a partial replacement for glucose as the carbon source. The price variation represents different constraints on whey availability and credits for whey disposal. Further price reduction will require significant changes to existing process technology. For example, a scenario incorporating 60% glucose substitution with concentrated dairy whey and a significantly altered processing strategy has the potential to reduce PHB production cost to US1995$2.67 kg^{-1}. Significant work remains to develop and optimize such cost-effective designs.

Keywords: polyhydroxyalkanoate; Escherichia coli, economic analysis; dairy whey; fermentation

INTRODUCTION

In 1990, 10^8 tons of plastic were manufactured worldwide[1]. Disposing of plastic products at the end of their useful life poses significant problems, in particular landfill volume. Of the non-biodegradable component of municipal waste sent to landfill in the United States, plastic typically makes up around 35–55% by volume. Furthermore, this proportion is expected to reach 45–65% by the year 2000[2]. There is increasing attention worldwide on ways to reduce landfill volume, e.g., composting, pyrolysis, recycling. The focus in many countries, most notably Germany, has been to remove plastics from the landfill waste stream using recycling programs. Unfortunately, this is not a cost effective solution for many types of plastic articles, especially packaging. Hence, biodegradable plastics have a significant role to play in solving the plastic disposal problems.

Several types of biodegradable plastics are available, for example starch-based, polylactate (PLA), polyvinylacetate (PVA) and polyhydroxyalkanoates (PHA). Each type has relative advantages and disadvantages, but PHA is one of the most promising: its strength and toughness are good and can be varied over a wide range by altering its composition,

it is completely resistant to moisture, and has very low oxygen permeability[3,4]. The simplest type of PHA is polyhydroxybutyrate (PHB). Pure PHB possesses several undesirable mechanical properties, so the copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is generally used. Commercially-produced PHBV, marketed under the name 'BIOPOL', is used to manufacture disposable items such as shampoo bottles, disposable razors and food trays. The market price of BIOPOL is around 1995US$16 per kilogram compared with less than $1 per kilogram for conventional commodity plastics[5]. The price difference on a typical $17 \mu m$ shopping bag (6.6 g each) is $0.09, and for a 1 mm injection moulded shampoo bottle (60 g) it is $0.84. Obviously, PHA cannot be a serious competitor to more traditional polymers such as polyethylene (PE) and polyethylene terepthalate (PET) or other biodegradable alternatives (e.g., PLA) until its price is substantially reduced. There are many research groups around the world focusing on this goal. However, without due attention to the production process as a whole, efforts to optimize each step individually will waste much effort and result in overall suboptimality. The best approach for reducing PHA cost can only be identified using economic

analysis. The purpose of this paper is therefore to estimate the production cost of PHA using *E. coli*, identify key areas where improvements will have a large impact, and indicate which tradeoffs (e.g., cell density vs fermentation time) will reduce production cost.

PROCESS DESCRIPTION
Fermentation

The most appropriate way to produce PHA at present is by bacterial synthesis. The non-recombinant bacterium *Alcaligenes eutrophus*, which produces polymer in response to nutrient limitation, has been used for PHBV production[6,7]. In 1988, the genes for PHA production were cloned from *A. eutrophus* into *Escherichia coli* on high copy number plasmids[8,9,10]. *E. coli* has a number of advantages over *A. eutrophus* for PHA production, including:

- easier PHA recovery due to cell fragility and larger granule size;
- higher growth rates and PHA production levels;
- more extensive research experience and a greater range of tools (e.g. plasmids) for genetic engineering;
- nutrient limitation is not required, hence process control is easier.

When grown on minimal media, current strains of recombinant *E. coli* generally do not produce high levels of PHA. Most literature examples of high yield PHA fermentations are with complex media (e.g. Luria-Bertani broth), economically marginal for large-scale application. However, glucose supplemented with corn steep liquor, casein hydrolysate and yeast extract yields excellent results with fed batch operation[11]. This medium achieved $113 \, g \, l^{-1}$ dry cell weight (DCW) with 72% PHB in 41 hours. Above $75 \, g \, l^{-1}$, sparging with pure oxygen was necessary to maintain sufficient oxygen tension, and this is prohibitively expensive at large scale. A conservative final cell density of $75 \, g \, l^{-1}$ is therefore assumed for this analysis, using a relatively high air flowrate of 2.0 v.v.m.

PHBV is generated in the same manner as PHB, except that propanoic acid is added to the medium during the fermentation. The toxicity of propanoic acid causes reduced cell yield and PHA percentage and consequently PHBV will be more costly than PHB. There has, however, been some progress in developing mutant strains which can produce PHBV without the need for propanoic acid addition, thus avoiding these problems[12,13,14]. Very little large-scale experimental data are available for PHBV production, hence this paper necessarily considers PHB production only. Apart from the fermentation step, all other process operations are identical for both PHB and PHBV.

Recovery

After fermentation, the contaminating cell components must be separated from the PHA granules. In particular, contaminating proteins and nucleic acids give PHA an offensive odour and cause decolouration during thermal processing[15]. A number of PHA recovery methods have been suggested. Most of them are restricted to laboratory use, and are technically and economically difficult to scale-up. PHA solubilization methods such as chloroform extraction can achieve high PHA purity[16]. However, they require large quantities of toxic and volatile solvent, which obviously has adverse environmental consequences and also raises total production cost. Hypochlorite or sodium hydroxide treatments for the digestion of non-PHA cell materials hold promise as cheap recovery methods compared to solvent extraction[17,18]. A chloroform and hypochlorite dispersion method has also been suggested[19]. However, extensive work is still required to optimize these processes before large-scale use, particularly with respect to minimizing solvent use.

The PHA granules themselves do not contain appreciable amounts of contaminant. Hence high-purity PHA can be produced by processes that wash the granules instead of dissolving them. An industrial method for PHB recovery described by Harrison[15] was based on detergent and enzyme digestion of non-PHB cell materials after PHA release by homogenization, coupled with extensive centrifugation. Hydrogen peroxide was also incorporated into the extraction process to further remove nucleic acids and protein. This caused a reduction of PHB molecular weight to some extent. The complexity and cost of this treatment regime raises total recovery cost. The PHA obtained is also of a relatively low quality.

Homogenization and centrifugation are common unit operations in bioprocessing. A PHA extraction process combining homogenization, disc-stack centrifugation, and sodium hypochlorite treatment has recently been developed[20,21]. It achieves a high PHA recovery and purity, and is well suited to large-scale operation. The main process steps are shown in Figure 1.

The recovery process begins with homogenization for the release of PHA granules. In developing the process a low cell concentration ($4.3 \, l^{-1}$ DCW) was employed in the homogenizer feed because only limited material was available[21]. However, a higher cell concentration (e.g. $25–50 \, g \, l^{-1}$ DCW) would be employed at production scale because disruption efficiency is relatively insensitive to feed concentration[22]. Three homogenizer passes are sufficient to substantively micronize cell debris, an important step that increases centrifuge separation efficiency. A single
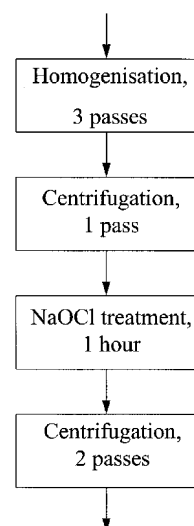

Figure 1. Basic design of the PHA recovery process developed by Ling *et al.*[21]

centrifugation pass is then employed to remove soluble cellular materials, especially nucleic acids. This step is essential since chemical treatment prior to nucleic acid removal led to low purity, possibly due to DNA adherence to the granule surface. The PHA sludge is then resuspended in buffer, and treated with NaOCl (0.085% w/v active chlorine) for 1 hour at ambient temperature. Finally, two centrifugation steps are employed to wash the PHA granules. In developing the process, a low centrifuge feed concentration was again employed because of limited material[21]. In practice, a higher feed concentration would be used, since centrifuge performance decreases only slightly with feed concentration. For example, Wong[23] concluded that 'diluting homogenate at a fixed feedrate can slightly improve (protein) inclusion body recovery, but not its purity'. We have conservatively chosen to limit the maximum centrifuge feed concentration for the first pass to $50 \, \mathrm{g \, l^{-1}}$ DCW. Subsequent passes can tolerate a higher solids loading (estimated at 70–$75 \, \mathrm{g \, l^{-1}}$) because most of the nucleic acids have been removed and hence broth viscosity is greatly reduced.

The experimentally determined PHA recovery and purification after each centrifuge pass are detailed in Table 1[21]. An analysis of the final PHA purity confirmed low contaminant levels following this recovery process (protein <0.01% w/w, DNA <0.03% w/w, >96% v/v cell debris removal).

PROCESS FLOWSHEET

Figure 2 shows the Process Flow Diagram for the PHB production plant used in this economic analysis. The five main sections are feed pretreatment, fermentation, homogenization, centrifugation, and drying. In order to reduce equipment cost, two fermenters operate in parallel and several storage tanks are used. The simplified batch schedule diagram is given as Figure 3.

Feed medium (glucose, complex supplements, and salts)

Table 1. Values of parameters used in the simulation. All prices are in 1995US$.

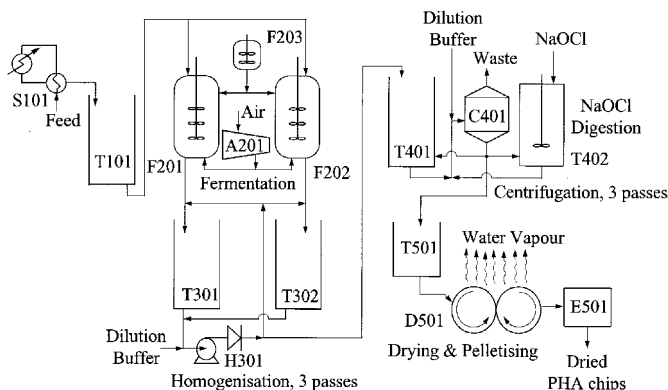|			Pass 1	Pass 2	Pass 3	
Centrifuge	$(Q/\Sigma)_{ref}$	$1 \times 10^{-8} \, \mathrm{m \, s^{-1}}$				see text
	Q_{Cref} (for power)	$1.6 \, \mathrm{m^3 \, hr^{-1}}$				[35]
	W_{Cref}	$27 \, \mathrm{kW}$				[35]
	maximum inlet solids ($\mathrm{g \, l^{-1}}$ dry weight)		50	70	75	see text
	exit solids loading ($\mathrm{g \, l^{-1}}$ dry weight)		150	150	150	see text
	PHA collection by weight		82%	99%	98%	see text
	debris collection by weight		38%	24%	35%	see text
Homogenizer	ΔP_H	$55 \, \mathrm{MPa}$				see text
	η_{he}	90%				[36]
	η_{Hm}	90%				[37]
Compressor	k	1.395				
	P_1	$101325 \, \mathrm{Pa}$				
	P_2	$303975 \, \mathrm{Pa}$				[38]
	$\hat{Q}_A$	$2.0 \, \mathrm{vvm}$				[39]
	η_{Ae}	92.5%				[36]
	f	60%				assumed
Fermenter	$\dot{W}_r$	$2.0 \, \mathrm{kW \, m^{-3}}$ average				[39]
	cell growth yield	45%				see text
	final cell density	$75 \, \mathrm{g \, (DCW) \, l^{-1}}$				see text
	final PHB level	72% of DCW				[11]
	working volume	75% of total volume				assumed
Inoculator	inoculum volume	5% of fermenter				assumed
Dryer	$\hat{m}_w$	$9 \times 10^{-3} \, \mathrm{kg \, m^{-2} \, s^{-1}}$				[40]
	ρ_s	$1200 \, \mathrm{kg \, m^{-3}}$				previous work
	η_{Dt}	55%				[40]
Sterilizer	ε_s	$0.1 \, \mathrm{kg^{-1}}$				[41]
Utility Costs	γ_s (lumped cost)	$\$20 \, \mathrm{t^{-1}}$				[39]
	γ_e	$\$55 \, \mathrm{(MWhr)^{-1}}$				Electricity Trust of South Australia
	γ_{vsol}	$\$0.22 \, \mathrm{kl^{-1}}$				[26]
	γ_{BOD}	$\$0.26 \, \mathrm{kg^{-1}}$				[26]
Maintenance	materials	Fixed Capital Investment $\times 3\%$				[42]
	labour	FCI $\times 3\%$				[42]
	general overheads	FCI $\times 3\% \times 60\%$				[42]
Labour	base salary	$\$16.77 \, \mathrm{hr^{-1}}$				assumed, 40 hr wk
	supervisory labour	$\times 15\%$				[42]
	labour burden	$\times 25\%$				[42]
	general overheads	$\times 60\%$				[42]
	(total):	230%				

Figure 2. Process Flow Diagram for the PHA production plant employed in this analysis.

is continuously sterilized by S101 and stored until needed in Tank T101. Over a two hour period, fermenter F201 is filled from T101 and then inoculated from the inoculum fermenter F203. The temperature is 37°C. Air is supplied by an axial compressor, A201. F201 is fed with pulses of sterilized, concentrated glucose solution ($700 \, \text{g} \, \text{l}^{-1}$) as necessary to maintain carbon source concentration above zero. After 40 hours the cell density is $75 \, \text{g} \, \text{l}^{-1}$ and the cells contain large PHB granules, amounting to 72% of total dry cell mass. F201 is drained into T301 over two hours. After a two hour cleaning period, the fermentation cycle begins again.

The broth in T301 is diluted to $50 \, \text{g} \, \text{l}^{-1}$ dry cell weight with buffer ($1.4 \, \text{g} \, \text{l}^{-1}$ KH_2PO_4, $6.5 \, \text{g} \, \text{l}^{-1}$ NaCl), then homogenised by H301 and stored in T302. During the second homogenizer pass the broth is passed back to T301, and finally on pass 3 the homogenate is transferred to T401. One hour is provided between each pass, and the 3 passes take a total of 23 hours.

As T401 fills, the 3rd pass homogenate is centrifuged by C401 and the solids ($150 \, \text{g} \, \text{l}^{-1}$) are passed to the reactor tank T402 for storage. Supernatant is sent to a waste treatment facility. The solids stream is diluted with buffer to $70 \, \text{g} \, \text{l}^{-1}$ DCW, and sodium hypochlorite is added to give 0.085% w/v active chlorine. After a minimum reaction time of one hour, the mixture is centrifuged twice more, using T401 for intermediary storage of the solids stream. Dilution buffer is added before each pass to ensure that the maximum inlet cell density of the centrifuge is not exceeded. Supernatant is sent to the waste treatment facility. An average of 1 hour is provided between each centrifuge pass, and a total of 23 hours is required to complete the three passes.

The third-pass centrifuge solids are stored in T501 for processing by the double drum dryer D501, which operates

continuously. After drying, the powder is fed to the continuous extruder E501 and then chipped.

ECONOMIC ANALYSIS

All costs in this paper are given in 1995US$. Where necessary, costs were converted using the Marshall and Swift Index (MSI) for equipment, the Producers index for chemicals, and the Consumer Price Index (CPI) for miscellaneous costs (as obtained from *Chemical Engineering*). The plant location is assumed to be the United States and thus has a location factor of 1.

Capital Costs

The purchase cost of each item of equipment was estimated using literature cost-capacity correlations of the form

$$\text{Cost} = 10^{\alpha} \times X^{\beta}$$

The values of α and β are shown in Table 2. Fermenters were assumed to be stirred tanks made of 304 stainless steel, with the cost of controllers and other associated fittings included in the Lang factor. The total fixed capital cost and required working capital were estimated from the total equipment purchase cost, using the Lang Factors for a fluid processing plant (see Table 3). Based on the breakdown of Lang Factor into instrumentation cost, piping cost, etc., it was estimated that about 67% of the direct capital cost consists of depreciable equipment. The capital and operating costs of pumps were negligible compared to the other equipment items.

Sizing Calculations

The basis is an annual PHB production level of 4300 tonnes, which sets the volume of medium to be processed in a single year. Applying a 1.1 integration factor to account for unscheduled downtime and occasional reduced throughput gives the number of batches per year and hence batch size.

Nontrivial equipment design equations are listed in Tables 4 and 5. Performance data and general design parameters are shown in Table 1.

Rate of glucose usage during PHB production has not been reported in the literature, hence an estimate must be made. Firstly, the cell yield on glucose for non-recombinant *E. coli*
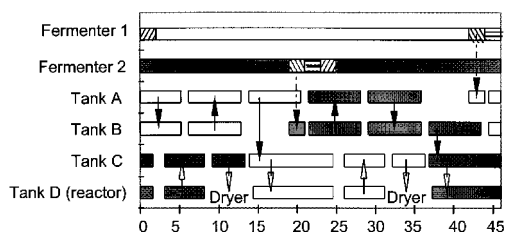

Figure 3. Batch schedule for equipment, times in hours. The order of events in the fermenters is fill, ferment, drain, clean. Solid arrows are homogenizer transfers, open arrows are centrifuge transfers, broken arrows are pump transfers.

Table 2. Cost correlation coefficients for major equipment items (1995US$).

Item	α	β	Scaling Variable	Capacity Limits	Source
Fermenter	3.96	0.54	volume (kl)	0.4-380	36
Homogenizer	4.85	0.43	inlet flowrate ($m^3 hr^{-1}$)	?	26
Centrifuge	3.69	0.33	effective area (m^2)	$-160k$	26
Unstirred tanks	3.65	0.56	volume (kl)	0.4–380	36
Double drum dryer	4.58	0.37	drum area (m^2)	4.8–46	43
Compressor	2.85	0.94	brake power (kW)	75–3200	36
Continuous sterilizer	4.97	0.6	flowrate ($m^3 s^{-1}$)	?	26
Continuous extruder and pelletizer	3.46	0.6	mass flowrate ($kg hr^{-1}$)	near 500 only	local supplier, corrected to USA

Table 3. Fixed capital estimation using Lang Factors[44].

equipment purchase cost:	100%
installation, ancillary equipment, site upgrade, etc:	+246%
total direct cost:	346%
indirect costs, contractors fee, contingency:	+137%
total fixed capital investment:	483%
working capital:	+86%
startup expenses:	+43%
total capital investment:	612%

is typically 0.4 to 0.45 grams DCW per gram glucose[24]. Secondly, the yield for PHB on glucose lies between 0.45 and 0.50 grams PHB per gram glucose[25]. Hence, 45% w/w growth yield on glucose is an appropriate value.

Centrifuge size is governed by the necessary effective settling area, which is calculated from the volumetric flowrate by assuming a constant ratio of flowrate to area (Q/Σ). The recovery procedure outlined in the Process Description used a (Q/Σ) of 2.21×10^{-9} m s^{-1}. However this is a very conservative estimate for two main reasons. Firstly, the small solid-bowl centrifuge used for this work had highly non-ideal hydrodynamics, giving reduced performance[23]. Large scale centrifuges closely approach the ideal and operate at (Q/Σ) values as high as 7×10^{-9} m s^{-1} for relatively small protein inclusion bodies[26]. Secondly, the PHA granules used in developing the process were relatively small, approximately 0.65 micron mean Stokes diameter, whereas more typical values are 1.13 micron to 1.25 micron[27]. An estimate of 1 micron is therefore more realistic. By Stoke's law (Q/Σ) is proportional to the square of the particle diameter. Hence, applying these two corrections gives a conservative (Q/Σ) value of approximately 1×10^{-8} m s^{-1}. The sensitivity to this parameter is moderate, as established subsequently in the Discussion.

Operating Costs

These can be broken into several main categories, viz labour, maintenance, utility, and media plus chemicals costs.

The cost of labour, maintenance and associated general overheads are calculated as shown in Table 1. Assumed labour requirement is three shifts per day with two process workers per shift. Tables 5 and 1 detail the utility cost (steam and electricity) calculations.

Each kilogram of glucose also requires 0.0156 kg of yeast extract, 0.0900 kg of corn syrup liquor, and 0.0191 kg of casein hydrolysate[11]. Resuspension buffer and hypochlorite concentrations are detailed in the Process Description section. The prices of media and chemicals were obtained from the literature or from local distributors, and are shown in Table 6. The price of yeast extract was not available, hence brewers' yeast was used as a substitute.

General Economic Parameters

The tax rate is 40%. Straight-line depreciation with a ten year write-off period is used. Working capital is required in year 0 and is recovered at the end of the plant life. Startup expenses are incurred in year 1. Salvage value is assumed negligible. A plant life of 10 years is assumed, with a discount rate of 10%. This conservative discount rate is chosen because it reflects a 'no-risk' time value of money and thus represents the true cost of the process. To account for risk, a company would use a substantially higher discount rate for their analysis, 25% or more. This is examined further in the Discussion.

The net present cost to produce PHB is calculated on a year-by-year basis, where for each year:

$$\text{cost} = \text{fixed capital outlay} + \text{operating expenses}$$
$$+ \text{ startup expenses} + \text{working capital}$$
$$- \text{ tax credit}$$

and

$$\text{tax credit} = \text{tax rate} \times (\text{operating expenses}$$
$$+ \text{ startup expenses} + \text{depreciation})$$

The effective cost per kg of PHB is then obtained using the following equation:

$$\text{Cost per kg} = \frac{\sum_{i=0}^{10}\left[(\text{Cost for year } i) \times \left(\frac{1}{1 + \text{ discount rate}}\right)^i\right]}{(1 - \text{tax rate} \times (\text{total kg of PHB produced}) \times \sum_{i=0}^{10}\left(\frac{1}{1 + \text{ discount rate}}\right)^i}$$

Table 4. Equipment sizing formulae.

centrifuge	effective settling area	$\Sigma_c = \dfrac{Q_C}{(Q/\Sigma)_{ref}}$
compressor	inlet peak gas flow rate	$Q_A = \hat{Q}_A \times V_{Tw}$
	peak adiabatic power	$W_{Aa} = \dfrac{k}{k-1} \times P_1 \times Q_{A1} \times \left(\left(\dfrac{P_2}{P_1} \right)^{\frac{k-1}{k}} - 1 \right)$
	peak brake power	$W_{Ab} \approx \dfrac{W_{Aa}}{0.8}$
dryer	water evaporation rate	$\dot{m}_{Dw} = \rho_w \left(Q_D - \dfrac{\dot{m}_{Ds}}{\rho_s} \right)$
	drum area	$A_D = \dfrac{\dot{m}_{Dw}}{\dot{m}_w}$

This is the price for which the PHB must be sold in order to break even, taking the time value of money into account.

RESULTS AND DISCUSSION

The mass balance is shown in Figure 4. The size and cost of major equipment items is given in Table 7. The effective production cost of PHB is 1995US $6.08 per kg over the 10 year lifetime of the plant. Estimates of the type conducted in this paper generally have an accuracy within $\pm 25\%$.

Figure 5 shows that medium cost is by far the most significant contributor to overall PHB price, approximately two and a quarter times greater than the capital cost of equipment. Electricity is also a large cost, with the major consumers of electrical power being the homogenizer and the compressors.

As shown in Figure 6, much of the capital cost is due to the centrifuges. The required settling area greatly exceeds the maximum centrifuge size ($160,000\,\text{m}^2$), and hence eight centrifuges operating in parallel are used. Compressors are also a significant cost.

The cells used for experimental testing of the recovery process contained 52% PHB. However, the industrial fermentation is based on 72% PHB, hence attaining the same final purity during separation is simplified. Furthermore, low levels of contamination by cell mass (5%) are not important for some commodity applications provided that the product is essentially free of nucleic acids and protein. Hence, optimizing the downstream processing (e.g. sacrificing PHA purity to obtain higher PHA recovery) will reduce centrifuge requirements and consequently PHA cost.

The centrifuge size depends on the assumed value for (Q/Σ), as described in the Sizing Calculations section. This parameter has an assumed value of $1 \times 10^{-8}\,\text{m s}^{-1}$, however, the uncertainty in this estimate is high, and the true value may possibly lie up to a factor of two on either side. As the assumed (Q/Σ) value increases from $0.5 \times 10^{-8}\,\text{m s}^{-1}$ to $2 \times 10^{-8}\,\text{m s}^{-1}$, the PHB cost varies by US1995 $0.96\,\text{kg}^{-1}$. Hence the analysis is moderately sensitive to the assumed centrifuge performance and further experimental work is required to define it more accurately.

It is clear from Figure 5 that the overall PHA price would

Table 5. Formulae used to calculate operating costs.

water disposal		$C_{Wd} = \gamma_{vol} \times V_{wd} + \gamma_{BOD} \times m_w$
steam	dryer usage	$\dot{m}_{Ds} = \dfrac{\dot{M}_{Dw}}{\eta_{Dt}}$
	continuous sterilizer usage	$m_{Ss} = V_{Tw} \times \varepsilon_S$
	total cost	$C_s = (\dot{m}_{Ds} \times t_b + m_{Ss}) \times \gamma_s$
electricity	compressor usage	$W_A = \dfrac{W_{Ab} \times f_A}{\eta_{Ae}}$
	tank stirring	$W_T = \hat{W}_T \times \sum V_{TW}$
	centrifuge	scaled from literature, viz.
		$W_C = W_{Cref} \times \dfrac{Q_C}{Q_{Cref}}$
	homogenizer	$W_H = \dfrac{Q_H \times \Delta P_H}{\eta_{Hm} \times \eta_{he}}$
	total cost	$C_e = \gamma_e \times \displaystyle\sum_{I=A,C,H,T} \times t_1$
media	glucose-related cost	$C_M = \left(\displaystyle\sum_i \dot{m}_{Mi} \times C_{Mi} \right) \times \dot{m}_{glc} \times V_M$

Table 6. Cost of media and chemicals (1995US$)[45,39].

	C_{Mi}
brewers' yeast	$0.539 \, \text{kg}^{-1}$
casein hydrolysate	$9.330 \, \text{kg}^{-1}$
corn steep liquor	$0.233 \, \text{kg}^{-1}$
glucose syrup	$0.530 \, \text{kg}^{-1}$
KH_2PO_4	$0.120 \, \text{kg}^{-1}$
NaCl	$0.133 \, \text{kg}^{-1}$
NaOCl (12.5% active Cl)	$0.280 \, \text{l}^{-1}$
$(NH_4)_2SO_4$	$0.120 \, \text{kg}^{-1}$
other salts, trace metals	(negligible)

be substantially reduced if a cheaper medium were used. If a 'free' medium were employed, then the production cost would be only 53% of the glucose-based case, or US1995$3.21 (from Figure 5). Diary whey is a problematic byproduct of cheese production, because it contains high levels of nutrient ($48 \, \text{g} \, \text{l}^{-1}$ lactose, $10 \, \text{g} \, \text{l}^{-1}$ protein) and thus requires treatment before disposal. Worldwide production is 130 million tonnes per year, less than half of which is put to useful purpose[28,29]. It thus has the potential to be such a 'free' medium, and may generate a positive cash-flow if credits can be obtained through reduced whey-disposal costs. Dairy whey gives good yields of PHA in shake flasks[30,31]. Whey permeate is readily utilized by recombinant *E. coli* if adequately supplemented with $(NH_4)_2SO_4$ (unpublished work, University of Adelaide). A simple strategy is to initially charge the fermenter with unconcentrated whey plus $(NH_4)_2SO_4$, then batch feed with glucose to achieve high cell density. Assuming that the Residual Cell Mass contains 13% w/w of nitrogen,[32] $0.069 \, \text{kg}$ of $(NH_4)_2SO_4$ per kg of lactose would be required.

Unfortunately, whey is available on a seasonal basis with little or no whey available for five months of each year. This restricted availability means that only 56 Ml of dairy whey can be used per year at the same PHB production level, giving a 16% reduction in total glucose requirement. The cost of PHB produced in this manner is 1995US$5.63 per kg, assuming no cost for the whey. The disposal cost of whey for the dairy industry is estimated as $13 per kl using
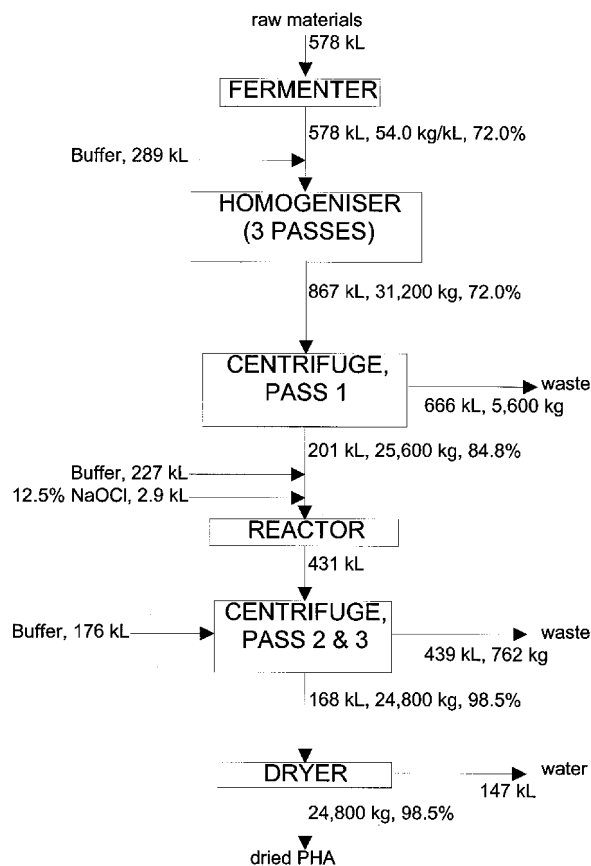
Figure 4. Simplified mass balance for glucose-based medium, basis is 1 full batch (46 hours). Volume in kl, mass of PHB in kg, PHB purity in %w/w.

the waste disposal equations in Table 5. If this is included in the analysis then the PHB cost is 1995US $5.46 per kg.

Fed-batch operation using concentrated whey during the fed-batch phase has not yet been proven, but it is likely to be readily achievable given the promising results achieved for batch fermentations. Based on capital and operating costs of dairy evaporators obtained from local suppliers, the PHB price using $200 \, \text{g} \, \text{l}^{-1}$ preconcentrated whey is

Table 7. Results of economic analysis using glucose-based medium (1995US$).

|	#	Total Size	Total Cost	Power Usage (MWhr/batch)	Steam Usage (tonnes/batch)
Inoculator F203	1	14.4 kl	$46k	1.33	–
Fermenters F201, F202	2	578 kl	$461k	48.5	–
Homogenizer H301	1	$65.0 \, \text{m}^3\text{hr}^{-1}$	$430k	49.0	–
Centrifuges C401	8	$1,170,000 \, \text{m}^2$	$1,983k	27.	–
Compressors A201	2	2,510 kW	$1,545k	81.5	–
Dryers D501	3	$46.0 \, \text{m}^2$	$385k	–	267
Extruder & Pelletizer E501	1	$540 \, \text{kg} \, \text{hr}^{-1}$	$126k	–	–
Sterilizer S101	1	$14.4 \, \text{m}^3\text{hr}^{-1}$	$463k	–	57.8
Tank T101	1	260 kl	$101k	–	–
Tanks T301, T302, T401	3	867 kl	$322k	–	–
Tank T402	1	215 kl	$91k	–	–
Tank T501	1	84.0 kl	$54k	–	–
		Medium Cost	$12,900k	per year	
		Total Fixed Capital Investment	$28,600k		
		Annual Operating Cost	$19,700k	per year (includes medium)	
		Effective Production Cost	$6.08	per kilogram of PHB	
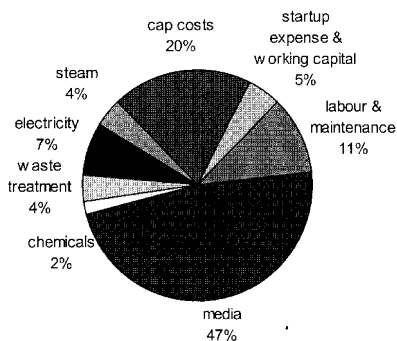
Figure 5. Breakdown of net present PHB price (4300 t(PHB) yr^{-1}, glucose medium).

approximately $5.35 kg^{-1}. This is based on an annual limit of 112 Ml of whey, which is typical for a large cheese factory, and takes into account seasonal variability in cheese production as in the above scenarios. The same annual PHB production target of 4300 tonnes has also been maintained. Under these constraints, a total glucose replacement of 32% is attained. Removal of the whey availability constraint enables 61% glucose replacement and a decrease in PHB price to $4.66 kg^{-1} ($4.02 kg^{-1} if treatment credits are included). Further removal of the seasonality constraint enables 100% glucose replacement with a production cost of $3.59 kg^{-1}, neglecting any treatment credits. This last scenario could, for example, be based on the use of whey powder available on commodity markets. This approach will have its own costs (e.g., purchase and processing costs such as filtration) that have not been included. It is clear that the use of preconcentrated whey has the potential to further reduce PHA cost, but at the expense of increased process complexity.

Using dairy whey would have the significant advantage of reducing the dairy industry's whey disposal problem, and would compensate for the biomass and wastewater generated by the process. It is important that process waste be minimized and properly treated before discharge to the environment, hence the waste treatment cost is appreciable for this process. Ideally, waste digestion facilities would be designed as an integral part of the plant. Excepting the high electricity requirement, the process would then be very 'environmentally-friendly', a key marketing point for PHA.
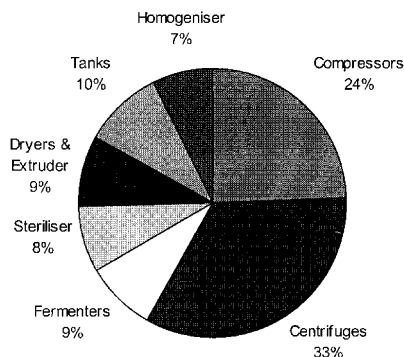
The 10% discount rate is conservative, and is appropriate for examining the effects of process parameters on the true, 'no risk' cost of PHA. A higher discount rate of 25% is applicable when risk must be accounted for, as in determining a minimum level of attractiveness for company investment. If the analysis is repeated at a discount rate of 25%, the minimum PHB price is 1995US $7.45 kg(PHB)$^{-1}$ for glucose and 1995US $5.12 kg(PHB)$^{-1}$ for the best-case dairy whey scenario.

Sensitivity Analysis

The sensitivity of product price to ten key process parameters is summarized in Figure 7. Other parameters such as the cost of electricity, labour cost, and waste treatment cost had dimensionless sensitivity less than 10%. A number of key points can be drawn from this graph:

The PHB content of the cells is of vital importance. Moderate variations in PHB% of ± 10 correspond to about 1995US $1.60 difference per kilogram of PHB. In contrast, duration of the fermentation is less important. Hence it may be advantageous to use strains which accumulate higher levels of PHB at the expense of growth rate.

The efficiency of downstream processing is also critical, as expected because this parameter is closely related to cell PHB content. The sensitivity is slightly higher for a reduction in PHB content because the increase in residual cell mass is greater. This affects centrifuge size and incurs higher waste treatment costs. A modest increase of first pass recovery to 90% would reduce the glucose-based PHB price by 1995US $0.51 per kg. Such an improvement in recovery is entirely feasible.

As expected from Figure 5, the total growth yield on carbon and the medium cost significantly influence overall PHB cost. Obviously, medium cost has less effect on the whey-based fermentation because it uses less glucose. Conversely, growth yield has a greater effect because the ratio of glucose to whey must change to maintain the same level of PHB production. A large proportion (30%) of the medium cost is the complex nitrogen supplements.

PHB granule size exerts only a small influence on the price, by virtue of the concomitant change in centrifuge size as described in the Sizing Calculations. However, granule size is more significant than Figure 7 suggests, because it also affects the differential sedimentation rate of PHB
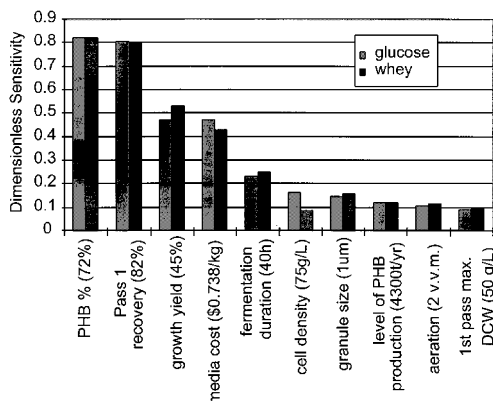

Figure 6. Equipment cost breakdown for the production of 4300 t(PHB) yr^{-1} from glucose.

Figure 7. Dimensionless sensitivity $(\partial \ln y / \partial \ln x)$ of PHB price to various parameters at a constant annual PHB production level.

granules and cell debris, influencing separation and hence recovery. Recent work has shown that phasins (granule bound proteins) have a strong influence on granule size in *Alcaligenes eutrophus*, and may give insights into increasing the granule size in *E. coli*[33,34].

Final cell density affects primarily the size of the fermenters and continuous sterilizer, because once the DCW is increased above $50 \, g \, l^{-1}$ the broth is diluted back to this level before downstream processing. The generally low significance of cell density is important, because much work in the literature has been directed at obtaining very high cell density. Such fermentations have extremely high aeration, stirring, and cooling requirements. It may be more cost-effective to use strains which accumulate PHB to high levels more rapidly, shortening the fermentation at the expense of cell density. Interestingly, there is even less effect of cell density on price for the whey-based fermentation. This can be explained by considering that raising the cell density has two main opposing effects: firstly the size of some equipment decreases, reducing capital cost. Secondly however, the glucose to whey ratio increases and hence the medium becomes more expensive.

Scaleup effects are quite insignificant at this level of production. Figure 5 shows that most of the cost is due to factors which scale linearly with production level. However, it is important to note that the slope of capital cost against size is discontinuous when an equipment item reaches its maximum size limit (e.g. centrifuges). In the vicinity of a discontinuity, this effect can grossly distort the relative parametric sensitivities.

Non-media costs are a higher proportion of the total cost for the whey-based medium than for the non-whey medium. Sensitivity parameters such as aeration and fermentation duration affect only the non-media related costs, hence their effect on price is greater for the whey medium.

A Target Process

A benefit of this economic approach is the ability to guide research activity. A process has been examined which is based on largely-existing and demonstrated technology. Given the high level of research activity in this field, it is reasonable to ask what the cost for PHB production via a highly-simplified process might be. The following scenario was therefore considered:

- an increased fermentation yield of $75 \, g \, l^{-1}$ dry cell weight, with 75% w/w PHB content;
- reduced fermentation aeration requirements of 1.0 v.v.m. air;
- an optimized chemical-treatment regime eliminating the need for mechanical cell disruption;
- a streamlined centrifugation regime (2 passes) with no dilution of the fermentation broth before the first pass, an equivalent solids concentration on the second pass, and an overall PHB recovery of 90%;
- 60% glucose substitution by evaporator-concentrated whey (i.e., the seasonal constraint on whey availability is maintained, but not the availability limit);
- $13 \, kl^{-1}$ credit for whey disposal.

Under this scenario PHB production cost falls to $2.67 per kg, at the annual production target of 4300 tonnes. The above scenario is feasible, and is something that existing research should perhaps be trying to achieve.

CONCLUSIONS

This economic analysis shows that by using *E. coli* it may be possible to drastically reduce the production cost of PHA, to a point reasonably competitive with commodity plastics. It also indicates several key areas of sensitivity where future research would likely be most beneficial, and defines a target process designed to achieve drastic reduction in PHB cost.

NOMENCLATURE

A_D	drum area of dryer, m^2
C	cost per batch, US95$
d	particle effective settling diameter
f_A	fractional compressor loading over the batch
k	heat capacity ratio
m	mass per batch, kg
$\dot{m}$	mass flowrate, $kg \, s^{-1}$
$\hat{m}_{glc}$	mass of glucose per unit volume of medium, $kg \, kl^{-1}$
$\hat{m}_{Mi}$	mass of medium component per unit mass of glucose, $kg \, kg(glc)^{-1}$
$\hat{m}_W$	specific water evaporation rate, $kg \, m^{-2} s^{-1}$
m_{Ws}	mass of solids in wastewater, kg
P	absolute pressure, Pa
Q	volumetric flowrate, $m^3 s^{-1}$
Q_A	peak air flowrate (STP) per unit broth volume, $m^3 m^{-3}$, min^{-1}
t	time per batch, hr
t_D	duration of dryer operation per batch, s
V	volume, kl
W	power requirement, kW
W_{Aa}	compressor adiabatic power, W
W_{Ab}	compressor brake power, W
$\hat{W}_r$	stirrer power input per unit broth volume, $W \, kl^{-1}$
X	characteristic capacity for cost scaling

Greek Symbols

α	cost-capacity constant
β	scaling exponent
ε_S	steam usage for sterilization, $kg \, l^{-1}$
γ	unit utility cost, $US95\$ kWhr^{-1}$ or $US95\$ kg^{-1}$
γ_{BOD}	BOD dependent waste disposal cost, $US95\$ kg^{-1}$
γ_{vol}	volume dependent waste disposal cost, $US95\$ kl^{-1}$
η_{Ae}	compressor motor (prime mover) efficiency
η_{Dt}	dryer thermal efficiency
η_{He}	homogenizer motor (prime mover) efficiency
η_{Hm}	homogenizer mechanical efficiency
ρ	density, $kg \, m^{-3}$
Σ_C	effective settling area, m^2

Subscripts

1	inlet conditions
2	outlet conditions
A	compressor
C	centrifuge
D	dryer
H	homogenizer
M	fermentation medium
T	tank stirrers
e	electricity
s	solids
st	steam
w	water
wd	wastewater disposal
ref	literature value for scaling from

REFERENCES

1. Doi, Y., 1990, *Microbial Polyesters* (VCH publishers, New York) 128.

2. US Environmental Protection Agency, 1991, *Plastic Wastes—Management, Control, Recycling and Disposal* (Noyes data corp, United States).

3. Holmes, P. A., 1988, Biologically produced (R)-3-hydroxyalkanoate polymers and copolymers, *in* Bassett, D. C. (ed), *Developments in Crystalline Polymers-2* (Elsevier Applied Science, London) 1–65.

4. Brandl, H., Bachofen, R., Mayer, J. and Wintermantel, E., 1995, Degradation and applications of polyhydroxyalkanoates, *Can J Microbiol*, 41(Supp 1): 143–153.

5. Poirier, Y., Newrath, C. and Somerville, C., 1995, Production of polyhydroxyalkanoates, a family of biodegradable plastics and elastomers, in bacteria and plants, *Bioltechnology*, 13(Feb): 142–150.

6. Byrom, D., 1992, Production of poly-B-hydroxybutyrate:poly-B-hydroxyvalerate copolymers, *FEMS Microbiol Rev*, 103: 247–250.

7. Byrom, D., 1990, Industrial production of copolymer from *Alcaligenes eutrophus in* Dawes, E. A. (ed), *Novel Biodegradable Microbial Polymers* (Kluwer Academic Publishers, Dordrecht) 113–117.

8. Schubert, P., Steinbuchel, A. and Schlegel, H.G., 1988, Cloning of the *Alcaligenes eutrophus* genes for synthesis of poly-B-hydroxybutyric acid (PHB) and synthesis of PHB in *Escherichia coli*, *J Bacteriol*, 170: 5837–5847.

9. Slater, S. C., Voige, W. H. and Dennis, D. E., 1988, Cloning and expression in *Escherichia coli* of the *Alcaligenes eutrophus* H16 poly-B-hydroxybutyrate biosynthetic pathway, *J Bacteriol*, 170: 4431–4436.

10. Peoples, O. P. and Sinskey, A. J., 1989, Poly-beta-hydroxybutyrate biosynthesis in *Alcaligenes eutrophus* H16: Characterization of the genes encoding beta-ketothiolase and acetoacetyl-CoA reductase, *J Biol Chem*, 264: 15293–15297.

11. Lee, S. Y. and Chang, H. N., 1995, Production of poly(3-hydroxybutyric acid) by recombinant *Escherichia coli* strains: genetic and fermentation studies, *Can J Microbiol*, 41(1): 207–215.

12. Steinbuchel, A. and Peiper, U., 1992, Production of copolyesters of 3-hydroxybutyric acid and 3-hydroxyvaleric acid by a mutant of *Alcaligenes eutrophus* from single unrelated carbon sources, *Appl Biochem Biotechnol*, 37: 1–6.

13. Anderson, A. J., Williams, D. R., Dawes, E. A. and Ewing, D. F., 1995, Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in *Rhodococcus ruber*, *Can J Microbiol*, 41(Supp 1): 4–13.

14. Valentin, H. E. and Dennis, D., 1996, Metabolic pathway for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) formation in *Nocardia corallina*: inactivation of mutB by chromosomal integration of a kanamycin resistance gene, *Appl Environ Microbiol*, 62(2): 372.

15. Harrison, S. T., 1990, The extraction and purification of poly-β-hydroxybutyrate from *Alcaligenes eutrophus*, *PhD Dissertation* (University of Cambridge, UK).

16. Doi, Y., 1990, *Microbial Polyesters* (VCH publishers, New York).

17. Berger, E., Ramsey, B. A., Ramsay, J. A., Chavarie, C. and Braunegg, G., 1989, PHB recovery by hypochlorite digestion of non-PHB biomass, *Biotechnol Tech*, 3: 227–232.

18. Lee, I. Y., Chang, H. N. and Park, Y. H., 1995, A simple method for recovery of microbial poly-β-hydroxybutyrate by alkaline solution treatment, *J Microbiol Biotechnol*, 5(4): 238–240.

19. Hahn, S. K., Chang, Y. K., Kim, B. S., Lee, K. M. and Chang, H. N., 1994, Optimisation of microbial poly(3-hydroxybutyrate) recovery using dispersions of sodium hypochlorite solution and chloroform, *Biotechnol Bioeng*, 44: 256–261.

20. Ling, Y., Williams, D. R. G., Thomas, C. J. and Middelberg, A. P. J., 1997, Recovery of poly-3-hydroxybutyrate from recombinant *E. coli* by homogenization and centrifugation, *Biotechnol Tech*, 11(6): 409–412.

21. Ling, Y., Wong, H. H., Thomas, C. J., Williams, D. R. G. and Middelberg, A. P. J., 1997, Pilot-scale extraction of PHB from recombinant *E. coli* by homogenization and centrifugation, *Bioseparation*, accepted.

22. Kleinig, A. R., Mansell, C. J., Nguyen, Q. D., Badalyan, A. and Middelberg, A .P. J., 1995, Influence of broth dilution on the disruption of *Escherichia coli*, *Biotechnol Tech*, 9: 759–762.

23. Wong, H. H., 1996, Modelling studies of the interaction between homogenisation, centrifugation, and inclusion body dissolution, *PhD Dissertation* (The University of Adelaide, Australia).

24. Varma, A. and Palsson, B. O., 1993, Metabolic capabilities of *Escherichia coli*: II. Optimal growth patterns, *J Theor Biol*, 165: 503–522.

25. Yamane, T., 1993, Yield of poly-D(-)-3-hydroxybutyrate from various carbon sources: a theoretical study, *Biotechnol Bioeng*, 41(1): 165–170.

26. Middelberg, A. P. J, 1996, The influence of protein refolding strategy on cost for competing reactions, *Biochem Eng J*, 61: 41–52.

27. Middelberg, A. P. J., Lee, S. Y., Martin, J., Williams, D. R. G. and Chang, H. N., 1995, Size analysis of poly(3-hydroxybutyric acid) granules produced in recombinant, *Escherichia coli*, *Biotechnol Lett*, 17(2): 205–210.

28. Zall, R. R., 1992, Sources and composition of whey and permeate *in* Zadow, J. G. (ed), *Whey and Lactose Processing*, 1st Edition (Elsevier Applied Science, London and New York) 1–72.

29. Hsu, S. T. and Yang, S. T., 1991, Propionic acid fermentation of lactose by *Propionibacterium acidipropionici*. Effects of pH, *Biotechnol Bioeng*, 38(6): 571–578.

30. Dennis, D. E., 1991, Improved production of poly-beta-hydroxybutyrate in transformed *Escherichia coli*, *Patent, WO91/18993*.

31. Janes, B., Hollar, J. and Dennis, D., 1990, Molecular characterisation of the poly-beta-hydroxybutyrate biosynthetic pathway of *Alcaligenes eutrophus* H16 *in* Dawes, E.A. (ed), *Novel Biodegradable Microbial Polymers* (Kluwer Academic Publishers, Dordrecht) 175–190.

32. Nielsen, J. and Villadsen, J., 1994, *Bioreaction Engineering Principles*, 1st Edition (Plenum Press, New York and London) Ch2.

33. Wieczorek, R., Pries, A., Steinbuchel, A. and Mayer, F., 1995, Analysis of a 24-kilodalton protein associated with the Polyhydroxyalkanoic acid granules in *Alcaligenes eutrophus*, *J Bacteriol*, 177(9): 2425–2435.

34. Wieczorek, R., Steinbuchel, A. and Schmidt, B., 1996, Occurrence of polyhydroxyalkanoic acid granule-associated proteins related to the *Alcaligenes eutrophus* h16 ga24 protein in other bacteria, *FEMS Microbiol Lett*, 135(1): 23–30.

35. Petrides, D., Cooney, C. L, Evans, L. B., Field, R. P and Snoswell, M., 1989, Bioprocess simulation: an integrated approach to process development, *Comput Chem Eng*, 13(4/5): 553–561.

36. Peters, M.S. and Timmerhaus, K.D., 1991, *Plant Design and Economics for Chemical Engineers*, 4th Edition (McGraw-Hill, New York) Ch14.

37. Genereaux, R. P., Mitchell, C. J. B., Hempstead, C. A., Curran, B. F., 1984, Transport and storage of fluids *in* Perry, R.H., Green, D.W. and Maloney, J.O. (eds), *Perry's Chemical Engineers' Handbook*, 6th Edition (McGraw-Hill, New York) Ch6.

38. Bartholomew, W. H. and Reisman, H. B., 1978, Economics of fermentation processes *in* Peppler, H. J. and Perlman, D. (eds), *Microbial Technology*, 2nd Edition (Academic Press, New York) 54.

39. Kalk, J. P. and Langlykke, A. F., 1986, Cost estimation for biotechnology projects *in* Demain, A. L. and Solomon, N. A., *Manual of Industrial Microbiology and Biotechnology* (American Society for Microbiology, Washington DC) 363–385.

40. (no listed author), 1978, *Introduction to Industrial Drying Operations*, 1st Edition (Pergamon Press, Sydney) 226.

41. Kalk, J. P. and Langlykke, A. F., 1986, Cost Estimation for Biotechnology Projects *in* Demain, A. L. and Solomon, N. A. (eds), *Manual of Industrial Microbiology and Biotechnology* (American Society for Microbiology, Washington DC) 226.

42. Peters, M. S. and Timmerhaus, K. D., 1991, *Plant Design and Economics for Chemical Engineers*, 4th Edition (McGraw-Hill, New York) Ch6.

43. Peters, M. S. and Timmerhaus, K. D., 1991, *Plant Design and Economics for Chemical Engineers*, 4th Edition (McGraw-Hill, New York) Ch16.

44. Peters, M. S. and Timmerhaus, K. D., 1991, *Plant Design and Economics for Chemical Engineers*, 4th Edition (McGraw-Hill, New York).

45. Atkinson, B. and Mavituna, F., 1987, *Biochemical Engineering and Biotechnology Handbook*, 2nd Edition (Macmillan, England) 1073.

ADDRESS

Correspondence concerning this paper should be addressed to Dr A. J. P. Middelberg, Department of Chemical Engineering, University of Cambridge, Pembroke Street, Cambridge CB2 3RA, UK. Fax: +44 1233 331 761.