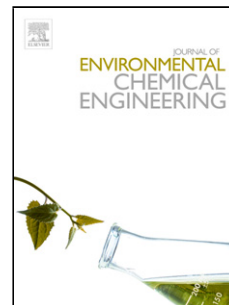


Accepted Manuscript

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Author: Ian Levett Greg Birkett Nick Davies Aidan Bell
Alexandra Langford Bronwyn Laycock Paul Lant Steven Pratt



PII: S2213-3437(16)30283-4
DOI: <http://dx.doi.org/doi:10.1016/j.jece.2016.07.033>
Reference: JECE 1205

To appear in:

Received date: 30-5-2016
Revised date: 18-7-2016
Accepted date: 28-7-2016

Please cite this article as: Ian Levett, Greg Birkett, Nick Davies, Aidan Bell, Alexandra Langford, Bronwyn Laycock, Paul Lant, Steven Pratt, Techno-economic assessment of poly-3-hydroxybutyrate (PHB) production from methane—The case for thermophilic bioprocessing, Journal of Environmental Chemical Engineering <http://dx.doi.org/10.1016/j.jece.2016.07.033>

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**Techno-economic assessment of poly-3-hydroxybutyrate (PHB)
production from methane – the case for thermophilic bioprocessing**

Ian Levett, Greg Birkett, Nick Davies, Aidan Bell, Alexandra Langford, Bronwyn Laycock, Paul Lant, Steven Pratt *

School of Chemical Engineering, The University of Queensland, St Lucia, Qld, Australia, 4072

* Corresponding author: Steven Pratt (s.pratt@uq.edu.au); +61 7 33654943

Highlights

- CH₄ is abundant and relatively cheap; its use does not adversely impact food supply
- A full process flowsheet for biopolymer from CH₄ is developed
- Techno economic assessment reveals that biopolymer production from CH₄ is competitive
- Heat removal to allow for mesophilic operation contributes nearly a third of the operating cost
- Thermophilic methanotrophs could significantly reduce cooling costs

Abstract

A major obstacle preventing the large scale production of polyhydroxyalkanoates (PHAs) has been the lack of a reliable, low cost, large volume feedstock. The abundance and relatively low price of methane therefore marks it as a substrate of interest. This paper presents a techno-economic assessment of the production of poly-3-hydroxybutyrate (PHB) from methane. ASPEN Plus was used for process design and simulation. The design and economic evaluation is presented for production of 100,000 t/a PHB through methanotrophic fermentation and acetone-water solvent extraction. Production costs were estimated at \$4.1-\$6.8/kg PHA, which compares against a median price of \$7.5/kg from other studies. Raw material costs are reduced from 30-50% of production for sugar feedstocks, to 22% of production for methane. A feature of the work is the revelation that heat removal from the two-stage bioreactor process contributes 28% of the operating cost. Thermophilic methanotrophs could allow the use of cooling water instead of refrigerant, reducing production costs to \$3.2-5.4/kg PHA; it is noted that PHB producing thermophilic methanotrophs are yet to be isolated. Energy consumption for air compression and biomass drying were also identified as significant capital and operating costs and therefore optimisation of bioreactor height and pressure and biomass moisture content should be considered in future research.

Keywords: poly-3-hydroxybutyrate, methane, methanotrophs, thermophilic, energy, techno-economic

1. Introduction

Polyhydroxyalkanoate (PHA) bioplastics are widely recognised as outstanding candidates to replace conventional plastics. Their mechanical properties are good, they are biodegradable, and unlike many alternatives, they don't rely on oil-based feedstocks. Further, they are the only commodity polymer that can be synthesised intracellularly, ensuring stereoregularity. However, despite offering enormous potential for many years, they are still not making a significant impact. This is broadly because commercial uptake has been limited by variable performance (inconsistent polymer properties) and high production costs of the raw polymer. The cost of the raw polymer is strongly impacted by the feedstock used (1). With the world currently experiencing a natural gas boom, there is growing interest in utilising methane for synthesis of higher value products.

This paper presents the first techno-economic assessment of large scale production of poly-3-hydroxybutyrate (PHB) from methane. The specific objectives of the work are to:

- Design a full process for utilising methane as a feedstock for large scale PHB production, incorporating biotechnology specific for utilising gas substrates as well as state-of-the-art non-chlorinated solvent extraction based downstream processing.
- Perform a techno-economic assessment on the design, with the view to evaluating process viability, and to identifying challenges regarding (i) utilising methane as a feedstock and (ii) biotechnology for PHB production more generally.

Industrial production of PHB, the simplest form of PHA, typically involves accumulation of polymer in pure cultures using plant-derived carbon sources as the feedstock. Table 1 summarises current large-volume commercial PHA production from pure cultures. DaniMer

Scientific and Meredian Inc. opened the doors to the world's largest PHA manufacturing plant in October 2012 (2).

Table 1: Current industrial production of polyhydroxyalkanoates

Pure culture production using plant-derived feedstocks can compete with food supply, and potentially have indirect adverse impact on natural environments; such processes require expensive refined substrates and need sterilization, limiting widespread commercialisation (11). Techno-economic studies have shown that a major cost of pure culture production is the carbon feedstock, estimated to be up to 40% of the product cost (1, 12, 13). Consequently many research groups are investigating the potential of using waste streams for PHA production, such as dairy whey waste, waste lipids, sugar industry waste streams, agricultural crop residues, petrochemical waste, syngas and glycerol (14).

The problems with waste streams, however, are their limited abundance and distributed nature. In contrast, methane is a cheap, abundant and widely available carbon source. Also, the robust, self-regulating nature of mixed methanotrophic cultures (15) offers the opportunity to operate under non-sterile conditions, thereby reducing operating costs on an industrial scale. Over 300 bacterial strains, including the methanotrophs: *Methylocystis paravus*, *Methylosinus trichosporium*, *Methylosinus sporium*, *Methylocystis* spp. GB25, MTS, and *Methylocella tundra* (1), have shown potential to synthesise and store PHB.

The economic feasibility of PHB production from methane was first reported by Listewnik, et al. (16). They studied relatively small-scale production (500 t/a) and found biosynthesis of PHB from methane to cost \$8.5/kg. This was extrapolated to \$15.1-18.3/kg when accounting for downstream processing costs. It was estimated expansion to 5,000 t/a could enable a 30-35% price reduction, but considering PHB can be produced for \$2.00-6.50/kg and bio-alternatives are in the order of \$2-5/kg (Table 2), the cost would still be relatively high. Still, Newlight Technologies have commercialised proprietary greenhouse gas-to-plastic technology, ramping

up from pilot scale to 500+ t/a production in 2013 (Table 1) (10). This is the first technology to utilise methanotrophs for industrial scale PHA production, converting waste methane from wastewater treatment facilities, anaerobic digesters, landfills and energy facilities (17). Other companies, like Mango Materials, are now following, albeit at relatively small scale.

Table 2: Price comparison for biodegradable polymers

For large scale production (in the order of 100,000 t/a), a readily accessible and reliable feedstock is needed. Here the feasibility of using methane for the production of PHB is investigated. A process is proposed for production of 100,000 t/a of PHB with at least 98% purity. Capital and operating costs were estimated.

A feature of the work is analysing the energetics for large scale PHB production. At the scale investigated, very large bioreactors are required, which reduces the surface area to volume ratio, limiting the potential for heat removal. The benefit of operating the bioreactors at higher temperatures is considered, highlighting the potential for thermophilic methanotrophs to reduce production costs. For PHB production from methane, this is the first investigation into the energy requirements for maintaining bioreactor operating temperature and for downstream processing.

2. Methods

2.1 Process Development

A simplified process flow diagram (PFD) for PHB production from methane is shown in Figure 1 with detailed PFDs and mass balance tables available in the Supplementary Material. A list of the key model assumptions is given in Table 3.

Table 3: Summary of key model assumptions

The process can be broken down into the following steps: (i) bioreactors: for biomass growth and for accumulation of PHB, (ii) biomass treatment: to harvest and dry PHB rich biomass, (iii) solvent extraction: to release PHA from the PHB rich biomass, and (iv) PHB precipitation and purification: to recover and dry the PHB product.

Figure 1: Process flow diagram for the process design

2.1.1 Bioreactors

A two stage growth and accumulation strategy for PHB production by a mixed methanotrophic culture was selected. Mixed methanotrophic cultures have been found to self-regulate, giving stable populations under non-sterile conditions (15), thereby offering significant savings in capital and operating cost. The bioreactors for both growth and accumulation were assumed to operate under an elevated pressure of 5 bar in the head space to improve gas-liquid mass transfer efficiency (15). Operating temperature was set at 38 °C. Due to high volumetric gas requirements air-lift bioreactors with a concentric internal draft tube were selected to reduce mixing costs (26). The flue gas from both growth and accumulation was set at 2% (v/v) methane. The gas was assumed to be sent to a catalytic converter for heat recovery and low pressure steam generation. This step has not been explicitly considered in the design. It is expected to be approximately cost neutral compared with purchasing the heating that the catalytic converter could have offset.

Stage one of the two stage growth and accumulation strategy involves continuous biomass growth. At the large scale considered here, operating the bioreactors at the dilution rate of 0.17 /h reported by Wendlandt et al. (24, 25) may be unrealistic due to mass transfer limitations, so a conservative value of 0.085 /h was assumed. The bacteria will store low levels of PHB in the growth phase (assumed PHA content in biomass of 3 wt%).

Stage two refers to the accumulation phase. Biomass is semi-continuously harvested from the growth reactors for 24 hour batch accumulations. Methane and air are supplied to the

accumulation reactors but phosphorus is limited, as per the strategy outlined by Wendlandt et al. (25). In the absence of phosphorus (an essential element of DNA) cell division ceases and methane is converted into PHB and CO₂.

2.1.2 Biomass treatment

Biomass treatment refers to harvesting and drying the PHA rich biomass. Drying is necessary since water residues impact the efficiency of the selected extraction process. At the end of each batch accumulation the broth is acidified with sulphuric acid to stop metabolism and so prevent bacteria consuming the intracellular PHB product. The acidified broth is then neutralised with sodium hydroxide and dewatered to 65 wt% moisture with continuous decanter centrifuges (*biomass centrifuge 1*). The dewatered biomass is then conveyed to rotary biomass dryers to reduce the moisture content further to 10 wt% by direct co-current drying with steam heated air entering at 100 °C.

2.1.3 Solvent extraction and recovery

To obtain a marketable PHA powder, the intracellular granules need to be isolated and separated from the dewatered biomass and then washed and dried. Many downstream processing options have been proposed in the literature, including: bead milling, high-pressure homogenisation, flotation, supercritical fluid extraction, chemical and enzymatic digestion, and solvent extraction (27). Solvent extraction has been most widely adopted for PHA recovery due to high efficiency, endotoxin removal, negligible polymer degradation and potential for solvent recovery through distillation (28). The two-step process comprises the release and solubilisation of PHA into the solvent, followed by non-solvent precipitation. Frequently, halogenated solvents such as chloroform or 1-2-dichloroethane are used, but they have major environmental concerns. Acetone, coupled with water to precipitate the polymer, is a renewable and environmentally benign alternative (29). As such, acetone-water PHA recovery is selected for this study.

Dry biomass is loaded into the solvent extraction vessel with acetone in a 9:1 acetone to biomass ratio. The mixture should have less than 6 wt% water and the acetone fed to solvent extraction will contain 5 wt% water due to imperfect separation from water in the recovery section (29). The vessel is heated with steam to 90 °C and 3 bar and mixed for 2 h. PHB will solubilize under these conditions and can be separated from insoluble biomass by centrifugation (*biomass centrifuge II*) operated at the same pressure and temperature as the solvent extraction vessel to prevent acetone flashing or PHB precipitation. Since the biomass cake contains significant quantities of acetone, the cake is rinsed with water using 3 wash displacements. The acetone-water mixture is combined with the filtrate from the rotary filter. This mixture will contain low levels of soluble contaminants such as salts, ammonia and solubilized lipids. To prevent fouling in the distillation column, an ultrafiltration unit is used to remove lipids. The acetone-water mixture is then distilled to yield a top product of 95 wt% acetone. The low boiling point of acetone results in very low acetone concentration in the bottoms, however, it will still contain dissolved salts that could potentially accumulate if not purged. Nanofiltration of the bottoms product allows safe reuse of the water for precipitation.

2.1.4 PHB precipitation and purification

PHB is recovered by precipitation. Dissolved PHB in acetone from the solvent extraction vessel is cooled to 40 °C and mixed with water for 1 h in a well-mixed precipitation vessel at a 2:1 acetone to water ratio. PHB is insoluble under these conditions and will rapidly precipitate out of solution. Crystallized PHB is filtered, washed with acetone to remove lipids, and washed with water to displace acetone, in a single rotary filtration unit. Finally the filter cake, containing 75% solids, is dried to 0.5% moisture content with rotary drum dryers to produce 98% pure PHB powdered product.

2.2 Mass and energy balance

Key process specifications and unit operation design outcomes are summarised in Table 5, with further details available in the supplementary material. Balances were based on 100,000 t/a of PHB production assuming 8640 h of operation per year (360 days).

Bioreactors were balanced using standard chemostat kinetics assuming ash free biomass composition of $\text{CH}_2\text{O}_{0.5}\text{N}_{0.25}$ (1). Yields for the conversion of methane into biomass and PHB (Table 6) were based on literature values (1) and it was assumed that scaling up would not impact the process performance. The remaining yields were calculated using elemental balances. Methane conversion was fixed to achieve a safe flue gas composition of 2% (v/v), below the explosive limit of 5% (15). Methane was assumed to be available off the grid at 5 bar pressure.

To approximate the heats of reaction in the bioreactors, the heat of combustion of biomass and PHB were approximated to be 560 kJ/C-mol (30) and 472 kJ/C-mol (31), respectively.

2.3 Sizing

Growth vessels were sized based on growth kinetics, assuming a 40 minute batch filling time. Periodic filling of batch reactors results in fluctuating growth reactor volumes. Non steady-state mass balances were solved (see supplementary material) with the maximum required working volume used to size the growth vessels. Batch PHA accumulation vessels were sized based on a 24 h total cycle time. All bioreactors were sized based on 10% headspace and 10% gas holdup, with the volume of cooling coils accounted for. Mass transfer of methane into solution was estimated using a simplified plug flow model, which was used to estimate the bioreactor heights. Further details on bioreactor sizing are provided in the supplementary material.

Centrifuge type was selected based on sigma theory (32) and sized based on liquid and solid loading (33). Dryers were sized assuming a number of transfer units of 1.5, a maximum air flux of 10,000 kg/m².h and maximum diameter of 4 m (34, 35). Rotary drum filters were sized according to Perry, et al. (33) with a maximum loading of 336 kg/m².h. Compressors, heat exchangers and the distillation column were sized using ASPEN Plus software.

2.4 Capital and operating costs

As a result of recent developments in hydraulic fracturing, the United States currently has huge methane availability at low price. For this reason, and for ease of comparison with other studies, the plant location was assumed to be the U.S. with all costs indexed to US2014\$. Note pricings from previous studies were inflated and converted to US2014\$ using inflation of 2.3% per annum and current conversion rates.

Equipment was priced according to charts from Ulrich and Vasudevan (36) and Peters, et al. (37) using key sizing parameters and indexed to 2014 prices. Where possible prices were checked against Matches (38) and an average price was used where significant differences existed. Pricing equipment from historical data is considered accurate to within $\pm 25\%$ (32). Total installed capital investment was estimated by scaling up the unit-by-unit equipment pricing using a Lang factor of 4 to account for installation, contingency, piping, instrumentation, insulation, auxiliary buildings and other required paraphernalia. Typical Lang factors are between 3 and 5, with 4.1 suggested for solid-fluid processes (36).

Operating costs include raw materials, utilities, maintenance and labour. Raw material prices were estimated at \$235/t CH₄ (\$4.71/mmBtu) (39), \$144/t ammonia, \$1290/t acetone, and \$0.5/m³ main water (36). The economic impact of other material costs is insignificant. Operating labour was estimated from process equipment requirements at \$60/h base salary assuming 3 shifts per day (36). Calculations for other economic components are provided in Table 4.

Table 4: Additional operating costs

Utility costs were estimated using the following correlation

$$C_{S,u} = a \times CE \text{ Plant Cost Index} + b \times C_{S,f}$$

where $C_{S,u}$ is the price of the utility, a and b are utility cost coefficients, and $C_{S,f}$ is the price of fuel in \$/GJ (36). Parameter a accounts for the annualised costs of capital and labour that are subject to inflation and b is the estimated price of the gas/electricity. The results of these calculations are presented in Table 4.

Table 5: Utility prices estimated from Ulrich and Vasudevan (36)

Refrigeration costs were estimated based on a coefficient of performance of 3. The addition of annualised capital expense for a mechanical refrigeration unit resulted in an overall utility cost for refrigeration of approximately 1 ¢/MJ of heat removed.

Electricity consumption for unit operations such as stirred vessels, centrifugation, and fans for dryers were estimated as described in Perry, et al. (33). Similarly, steam consumption for heating and drying was calculated based on energy balances around individual units. Steam costs could be reduced through catalytic conversion of the flue gas, however, capital and running costs were considered to be similar to steam utility costs and potential savings were not considered significant.

Wastewater and waste biomass were considered cost neutral considering the waste biomass could be anaerobically digested to generate methane which could be used to supplement the feed to the bioreactor or to produce electricity; Zamalloa et al. (40) concluded that the production and anaerobic treatment of (algal) biomass for electricity generation would be cost neutral given the electricity prices assumed in this work.

PHA production costs were estimated assuming a 20 year plant lifetime and 30% tax rate with linear capital depreciation over the first 10 years of production. Capital was invested over the first two years and the first year of production was 50% of full capacity to account for plant commissioning. The break-even PHA price was calculated based on a net present value of zero. A nominal discount rate of 20% was used for the base case of the analysis due to the risk of the

project. However, breakeven PHA prices for a range of discount rates (10-30%) were calculated as part of the sensitivity analysis.

3. Results and Discussion

Detailed process design for utilising methane as a feedstock for large scale PHB production, incorporating biotechnology specific for utilising gas substrates as well as state-of-the-art non-chlorinated solvent extraction based downstream processing, is summarised in Figure 1 and Table 6. The full design, including the mass flows, is presented in the Supplementary Material.

Table 6: Process specifications and design outcomes

The capital and operating costs of major unit operations are presented in Figures 2 and 3. The total installed capital cost for the PHB production plant was estimated at a total of \$383 million, and the annual operating cost was estimated at \$443 million. Break-even production cost at 20% discount rate was estimated at \$5.4/kg PHB. Considering $\pm 25\%$ uncertainty results in a price range of \$4.1-\$6.8/kg PHB. These estimates are significantly lower than those previously reported by Listewnik, et al. (16) who estimated pure methanotrophic PHB production costs at \$15.4-18.7/kg depending on the downstream process. This highlights the economy of scale since those results were based on small scale (500 t/a) production.

PHB prices estimated here are approximately double the lowest current PHB purchase price, however they are within the range of current prices (Table 2). Also, the estimates are similar to those reported in other techno-economic studies for PHA production, which highlights the relative viability of the new design developed in this work. PHA from glucose was estimated at \$8.6/kg at 4,300 t/a production scale, reducing to \$5.1-7.9/kg if dairy whey was used as a partial replacement for glucose as the carbon source (42). Choi and Lee (12) estimated production costs at \$3.7-11.9/kg from sucrose depending on the fermentation process chosen.

The lowest reported production cost was reported by Posada, et al. (43) at \$2.0-2.6/kg for PHB from waste glycerol as a by-product from biodiesel production.

Figure 2: Installed capital cost for unit operations, in order of process flow.

Both elevated operating pressure (5 bar-as discussed in Section 2.1) and tall bioreactors result in very high air compressor capital cost. This is also reflected in high electricity consumption for air compression, and highlights the need to optimise operating conditions to minimise capital and operating cost of air compression. Since growth is mass transfer limited due to the low solubility of methane (45), reducing operating pressure would reduce the growth and PHB accumulation rates, increasing the cost of the bioreactors. Experimental literature on the sensitivity of growth and accumulation rates to operating pressure is scarce and could be a point of interest for future studies.

A larger number of shorter bioreactors could be utilised to reduce the head required, provided sufficient mass transfer is achieved to avoid explosive limits. However, this would result in additional capital for bioreactors. Better knowledge of mass transfer of methane in this system would allow optimisation of compressor capital and operating expense. Yazdian, et al. (46) investigated the hydrodynamics and mass transfer of natural gas in a forced-liquid vertical loop bioreactor operated at a maximum cell density of 1.5 g/L. The highest mass transfer coefficient (k_{La}) obtained was 0.034 /s, which was significantly influenced by liquid circulation rate. Correlations for mixing time, gas hold up and k_{La} of methane and oxygen were reported. Mass transfer coefficients were correlated to superficial gas and liquid velocities and the height-to-diameter ratio. Further study of the influence of pressure, temperature and cell density on mass transfer would aid optimal bioreactor design of large scale methanotrophic PHB production.

Figure 3: Annual production cost comparison for operating bioreactors under different conditions.

Drying the biomass prior to solvent extraction is also expensive, with capital estimated at \$39 million and \$11.5 million annual costs for electricity to run fans and steam consumption. In addition to the drying cost itself, handling dry biomass adds further expense and operational risk. In the dryers, biomass was considered to be dried to 10 wt% moisture. There is considerable uncertainty with the drying behaviour of the biomass and as to whether this drying extent is feasible. The drying needs to be optimised together with the acetone/water separation in the acetone recovery section to deliver less than 5 wt% water to the solvent extraction section.

The single greatest operating cost, however, is the refrigeration cost to cool the bioreactors, accounting for 28% of the annual production costs (Figure 3). The energy balance highlights the intensity of heat generation in each stage of fermentation, with total heat removal of 540 MW from the bioreactors. In some cooler areas of the world, cooling water could potentially be used in conjunction with an external heat exchanger (at least in the cooler months). However, cooling water was assumed to be delivered from the cooling tower at 30 °C, a reasonable assumption in many areas of the world. Moderate operating conditions of 38 °C negates the feasibility of using cooling water at 30 °C for heat removal in these bioreactors due to unrealistic transfer areas required. Even with chilled water entering and leaving at 5 °C and 10 °C respectively, very large transfer areas are necessary, and preliminary design indicates that a cooling jacket plus multiple coils would be required.

3.1 Sensitivity analysis

Sensitivity of the production cost was assessed for the most influential factors, shown in the torpedo plot in Figure 4. A range of $\pm 25\%$ was considered for each parameter and production cost ranges are given for a discount rate of 20%.

Figure 4: Sensitivity analysis.

Energy consumption is the most significant cost for mesophilic PHB production resulting in costs being highly sensitive to electricity prices. Electricity price was estimated at 10 ¢/kWh, however industrial prices can be as low as 5 ¢/kWh in states such as Iowa and Kentucky, dropping production costs by 15% to \$3.5-5.8/kg PHB. Figure 4 indicates that production costs are the most sensitive to operating cost, particularly the price of energy but also sensitive to the initial capital investment and raw material prices.

Methane accounts for 22% of operating costs. This value is significantly lower than techno-economic assessments on other substrates, for example glucose (~47%) (42) or sucrose (~30%) (12). However, methane contributes significantly more than waste sources such as glycerol (~8%) (43). PHA from methane is therefore sensitive to gas prices, which are currently falling, particularly in the United States. Makeup acetone costs are 10%, and methods to minimise acetone loss, particularly that lost with waste biomass, should be further investigated to determine if savings could be made.

3.2 Benefits of thermophilic fermentation

One approach to reduce cooling costs is to increase the operating temperature of the bioreactors so that cooling water could be utilised. Similar transfer areas would be required using cooling water at 30 °C, leaving at 40 °C, if bioreactors were operated at 60 °C.

Figure 3 and Table 7 compare the annual and normalised production costs respectively, with bioreactors operated under mesophilic and thermophilic conditions. Under thermophilic conditions the refrigeration cost is eliminated (Figure 3), while the cooling water cost increases. If thermophilic bacteria performed comparably to the mesophilic methanotrophs, then PHB production costs can be reduced to \$3.2-5.4/kg by operating at 60 °C. It is assumed that high temperature fermentation has no impact on the cost of downstream processing and bioreactor sizing is assumed to be the same. At higher temperature, the solubility of methane in solution is reduced from 1160 µmol/L at 38 °C to 937 µmol/L at 60 °C, reducing the mass transfer of

methane and oxygen into solution. However, diffusion rates are higher. There is some risk of additional capital and operating costs to achieve required mass transfer at thermophilic conditions that has not been accounted for here.

Table 7: Total annual PHB production costs normalised to production capacity.

Figure 5 shows how the PHB price varies with the nominal discount rate applied. The median PHB price increases from \$4.9 to \$6.1 when the discount rate is increased from 10 to 30% for mesophilic fermentation and \$3.8 to \$5.0 for thermophilic fermentation.

Figure 5: Median PHA price for various nominal discount rates.

Although few thermophilic bacteria have shown PHB accumulating capacity, operating at thermophilic conditions is not a novel idea for PHB production. Additional advantages have been realised, such as increased diffusion rates, higher solubility of non-gaseous substrates and reduced risk of contamination (47). Production of PHB from thermophilic methanotrophs has not yet been reported. However, a few methanotrophs with the required pathway(s) have been discovered (Table 8). Of most interest are those that use the serine pathway for primary carbon assimilation. *Methylocystis Parvus* strain Se48, isolated from a thermal spring in the Transbaikal region, is a type II methanotroph found living in waters at 48-55 °C (48). Identification and testing of a thermophilic PHA accumulating methanotrophs would reduce PHA production costs and potentially aid large scale commercialisation of PHA from methane.

Table 8: Known thermophilic methanotrophs.

4. Conclusions

A detailed techno-economic assessment of a 100,000 t/a PHB from methane production facility has revealed a normalised production cost of \$4.1-\$6.8/kg PHB (at an assumed 25%

uncertainty). In addition to the bioreactors, air compression and biomass drying are the major capital cost items. Refrigeration costs were the major operating cost for mesophilic production. Thermophilic methanotrophic PHB production holds potential to significantly reduce production costs and could be an area of interest for future studies. Optimisation of bioreactor operating conditions, particularly temperature and pressure, offer significant potential for cost reduction. Further understanding of the mass transfer of methane at high cell density and elevated pressure would support the design and economic optimisation of the fermentation process. Acetone-water solvent extraction offers economic PHA extraction and purification, however minimisation of acetone loss and optimisation of biomass drying is still required to reduce costs further.

Acknowledgements

The authors would like to acknowledge funding from the ARC Discovery Project DP150103060. We also would like to thank Dr Geoff Covey from Covey Consulting for his technical review of the work.

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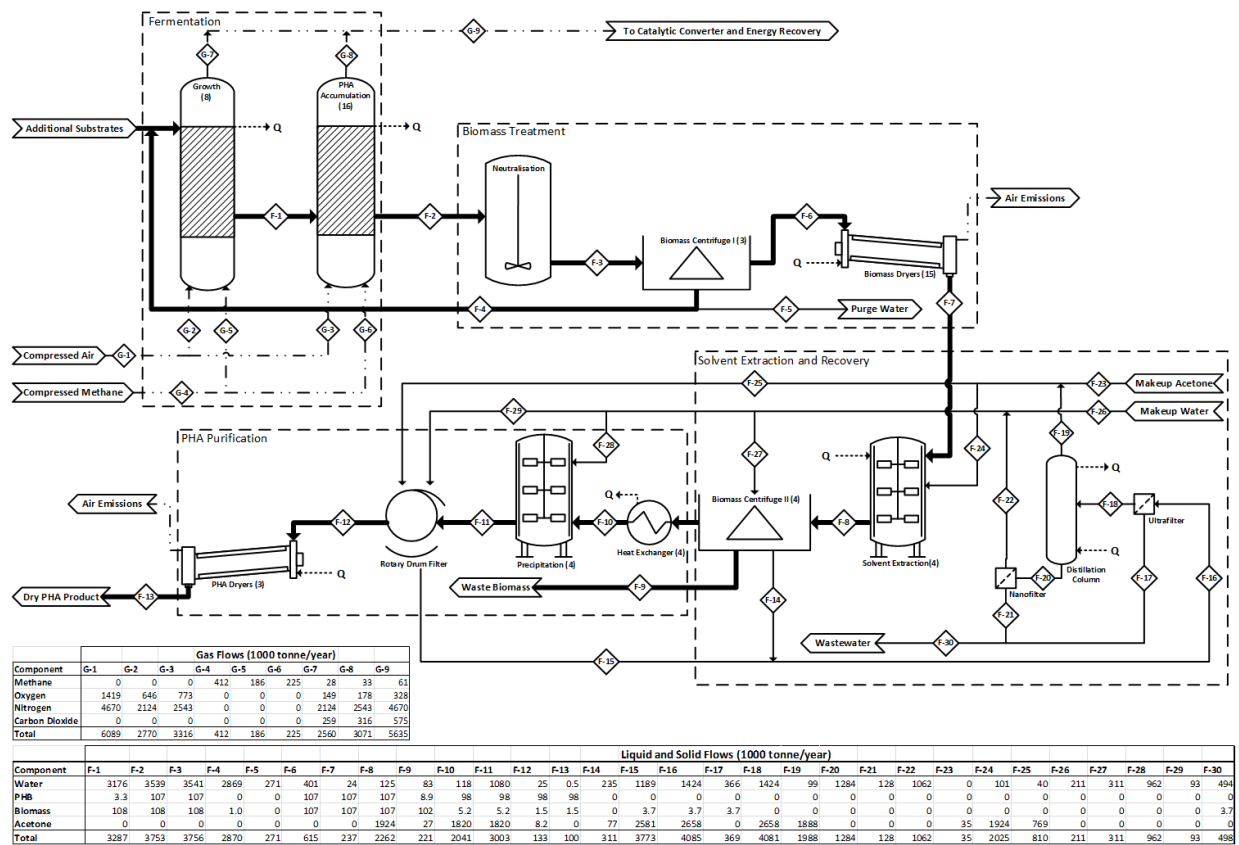


Figure 1: Process flow diagram for the production of PHB from methane and simplified mass balance

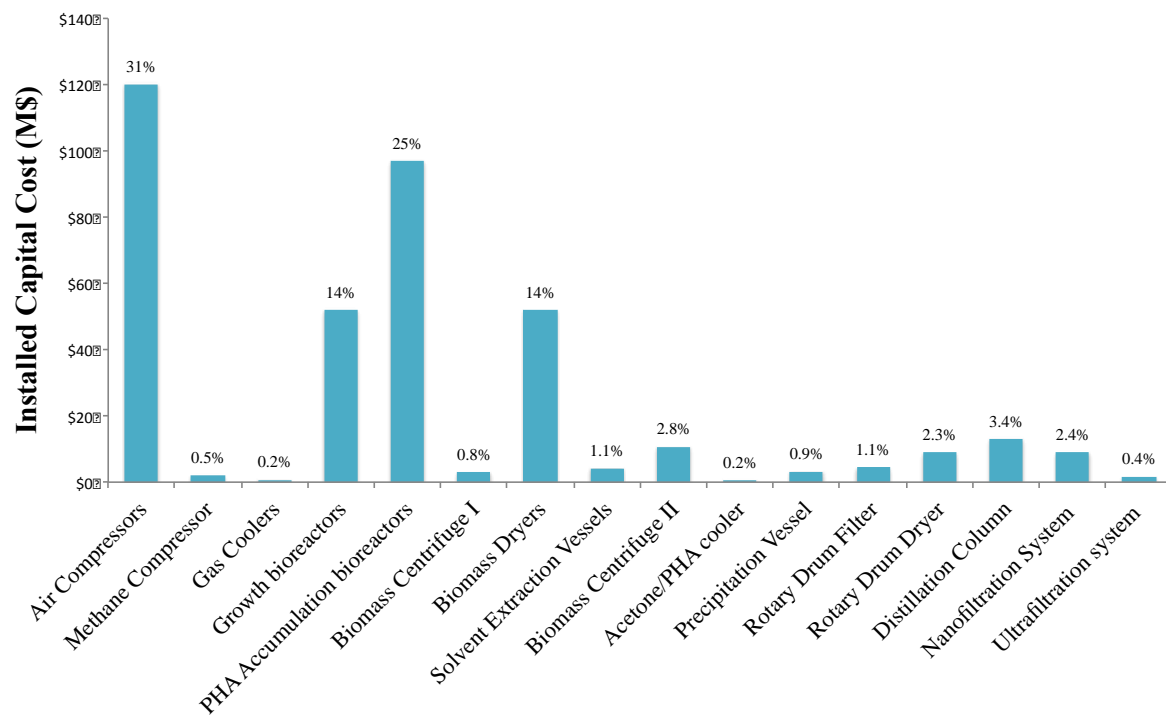


Figure 2: Installed capital cost for unit operations, in order of process flow

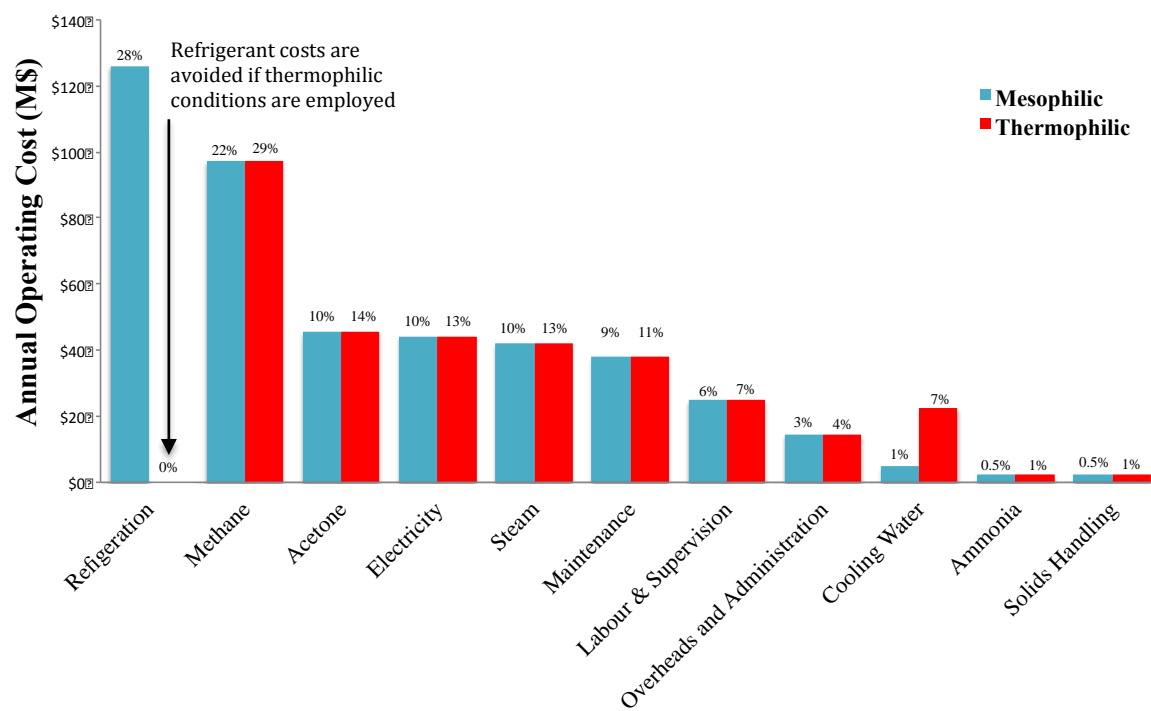


Figure 3: Annual production cost comparison for operating bioreactors under different conditions

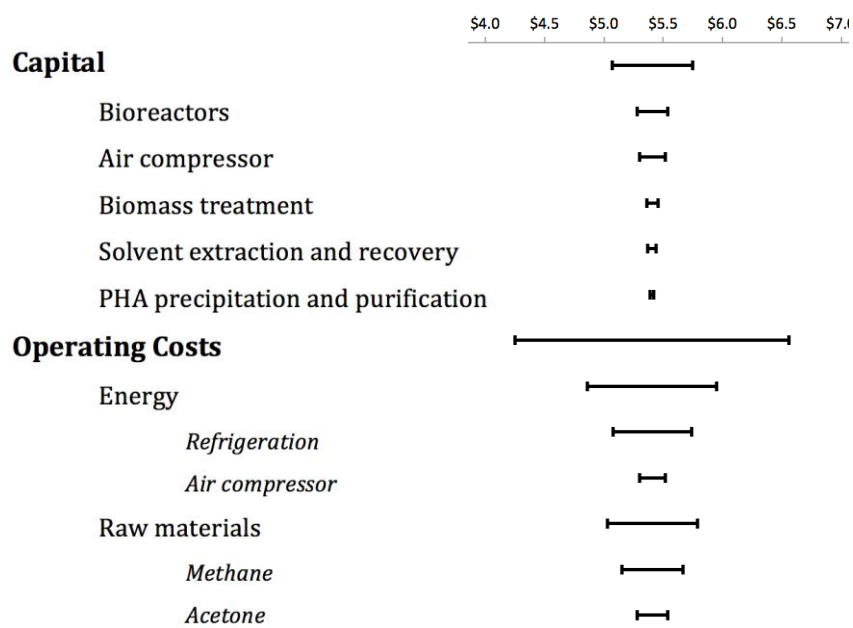


Figure 4: Sensitivity analysis

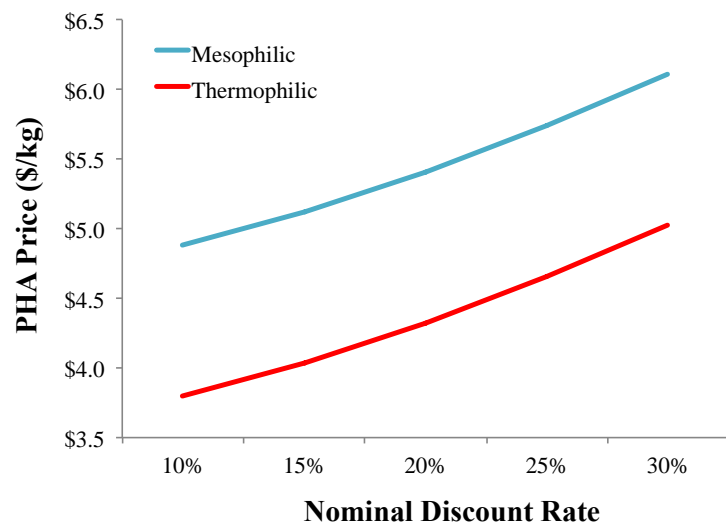


Figure 5: Median PHA price for various nominal discount rates

Table 1: Current industrial production of polyhydroxyalkanoates

Company name	Carbon Substrate	Product name	Production (t/a)	Ref.
DaniMer Scientific and Meredian Inc.	Canola oil	Seluma™	15,000	(2)
Metabolix/Antibióticos	Switchgrass, camelina, sugar cane	Mirel, Mvera™	10,000	(3)
TianAn Biologic Material Co	Corn/cassava starch	ENMAT	10,000	(4)
Tianjin GreenBio	Corn starch	SoGreen™	10,000	(5)
Bio-on	Beet or sugar cane	Bio-on™	10,000	(6)
Shenzhen Ecomann Biotech. Co	Corn starch		5,000	(7)
PHB Industrial	Sugar cane	Biocycle™	2,000	(8)
Kaneka	Vegetable oil	AONILEX™	1000	(6)
Biomer	Sugar (sucrose)	Biomer P™	1,000	(9)
Newlight Technologies	Waste methane	AirCarbon™	>500	(10)

Table 2: Price comparison for biodegradable polymers

Plastic	Price Range (US2014\$/kg)	Reference
Starch-derived bioplastics	2.60 - 5.80	<i>(18)</i>
Poly(lactic acid) (PLA)	2.00 - 3.45	<i>(19, 20)</i>
Poly(3-hydroxybutyrate) (PHB)	2.00 - 6.50	<i>(19, 21, 22, 23)</i>

Table 3: Summary of key model assumptions

Step	Assumptions
Bioreactors	<p>Bioreactors are operated for 8640 h (360 days) per year</p> <p>Biomass has 7% ash content with ash free content of $\text{CH}_2\text{O}_{0.5}\text{N}_{0.25}$</p> <p>Methane is available off the grid at 5 bar pressure</p> <p>Flue gas contains 2% (v/v) methane to avoid explosive limits of 5%</p> <p>Airflow is sufficient to achieve well mixed conditions</p> <p>Dissolved gases entering and leaving the reactors are negligible</p> <p>Bioreactors are operated with a minimum of 10% headspace and gas holdup contributes 10% of solution volume</p> <p>Biomass accumulates in growth reactor to 3 wt% of biomass</p> <p>PHA accumulation reactors fill in 40 minutes and the total batch cycle time (filling, accumulation, emptying, cleaning) takes 24 hours</p>
Biomass treatment	<p>Sulfuric acid treatment completely halts metabolism preventing any loss of PHB</p> <p>Dryers have 1.5 transfer units, maximum air flux of $10,000 \text{ kg/m}^2\cdot\text{h}$ and maximum diameter of 4 m</p>
Solvent extraction and recovery	<p>Negligible polymer degradation</p> <p>Complete endotoxin removal</p> <p>3 wash displacements are sufficient to removal enough acetone for safe handling of waste biomass cake</p> <p>Ultrafiltration removes all biomass-derived lipids and nanofiltration removes all salts from respective liquid inputs</p>
PHB precipitation and purification	<p>All dissolved PHB crystallises during precipitation</p> <p>All crystallised PHB is recovered through filtration and drying</p> <p>Rotary drum filters have a maximum loading of $336 \text{ kg/m}^2\cdot\text{h}$</p>

Table 4: Additional operating costs

Component	Cost
Maintenance	10% of fixed capital
Solids handling ¹	\$10/t
Supervision	20% of operating labour
Laboratory	20% of operating labour
Overheads and administration	50% of operating labour

¹ This includes additional cost of labour, maintenance and equipment associated with solids handling. Calculated based on wet biomass flow from biomass centrifuge II.

Table 5: Utility prices estimated from Ulrich and Vasudevan (36)

Utility	Price	Unit
Electricity	0.10	\$/kWh
Steam	0.14	\$/kg
Cooling water	0.057	\$/m ³

Table 6: Process specifications and design outcomes

Unit Operation	Operating Conditions/Design Basis	Outcomes/Process Design
Growth Bioreactor	Continuous airlift bioreactor Residence time = 13 h T = 38 °C, P = 5 bar in head space ^a Dilution rate = 0.085 /h ^a Cell density = 30 g/l ^a PHB concentration = 3 wt% $Y_{Xs} = 0.7 \text{ g X / g CH}_4$ ^b $Y_{ps} = 0.54 \text{ g PHB / CH}_4$ ^b	Total working volume = 4793 m ³ Total heat removal = 220 MW Total transfer area = 16,656 m ² Jacket and coils Number of reactors = 8 Height = 32.5 m Diameter = 5.6 m
PHA Accumulation Bioreactor	Batch airlift bioreactor Residence time = 24 h (batch cycle time) ^a T = 38 °C, P = 5 bar in head space ^a Cell density = 30-60 g/l ^a PHB concentration = 50 wt% ^a Limited nutrient = Phosphorus ^a $Y_{ps} = 0.54 \text{ g PHB / CH}_4$	Total working volume = 9900 m ³ Heat removal = 320 MW Transfer area = 24,112 m ² Jacket and coils Number of reactors = 16 Height = 31.9 m Diameter = 5.6 m
Air Compressor	Continuous centrifugal compressor Inlet pressure = 1 bar Discharge pressure = 8 bar Compression ratio = 2.1 Number of Stages = 3 Isentropic efficiency = 80%	Power = 52.8 MW Inter-stage Cooling = 32.6 MW Discharge temperature = 125 °C Air cooling duty = 17.2 MW Cooling transfer area = 3072 m ²
Methane Compressor	Continuous centrifugal compressor Inlet pressure = 5 bar Discharge pressure = 8 bar Compression ratio = 1.6 Number of Stages = 1 Isentropic efficiency = 80%	Power = 1.2 MW Discharge temperature = 58.8 °C Methane cooling duty = 1.2 MW Cooling transfer area = 280 m ²

Neutralisation Vessel	Continuous stirred tank reactor Residence time = 1 h pH = 7	Volume = 495 m ³ Stirrer power = 106 kW
Biomass Centrifuge I	Continuous decanter centrifuge Solids at outlet = 35 wt% ^c	Power = 0.9 MW Number of units = 3 Diameter = 1.1 m
Biomass Dryers	Continuous rotary drum dryer Air temperature (in/out) = 100/50 °C Moisture at outlet = 10 wt%	Heat duty = 2.7 MW Number of units = 15 Length = 36.3 m Diameter = 4 m
Solvent Extraction Vessels	Batch stirred vessel Residence time = 2 h ^d Temperature = 90 °C ^d Pressure = 3 bar ^d Acetone: solids = 9 ^d	Peak heat duty = 20 MW Number of units = 4 Volume = 183 m ³ Stirrer power = 82 kW
Biomass Centrifuge II	Continuous disk centrifuge Solids at outlet = 50 wt% ^c T = 90 °C, P = 3 bar ^d Water wash displacements = 3	Power = 0.8 MW Number of units = 8 Diameter = 0.8 m
Acetone/PHA Cooler	Continuous shell and tube heat exchanger Discharge temperature = 40 °C	Heat duty = 32 MW Number of units = 4 Transfer area = 566.4 m ²
Precipitation Vessels	Batch mechanically stirred vessel Residence Time = 1 h ^d T = 40 °C, P = 1 bar ^d Water: acetone = 0.33 ^d	Volume = 936 m ³ Number of units = 4 Stirrer power = 95 kW
PHA Filter	Continuous rotary drum filter P = 0.5 bar Solids Output = 75 wt%	Filter area = 35 m ²

	Water wash displacements = 3	
PHA Dryers	Continuous rotary dryer Air temperature (in/out) = 100/50 °C Solids output = 99.8 wt%	Heat duty = 5.7 MW Number of units = 3 Diameter = 2.59 m Length = 32 m
Distillation Column	Continuous operation Top product purity = 95% acetone	Reboiler = 124 MW/4542 m ² Condenser = 103 MW/ 4148 m ² Stages/height = 31/21.9 m Diameter = 8.84 m Reflux ratio = 0.32

Specifications adopted from:

^aWendlandt et al. (25)

^bKhosravi-Darani et al. (1)

^cTodaro and Vogel (40)

^dNarasimhan et al. (29)

Table 7: Total annual PHB production costs normalised to production capacity

Item	Normalised Production Costs (\$/kg)	
	Mesophilic conditions	Thermophilic conditions
Capital amortization	\$0.98	\$0.98
Utilities	\$2.49	\$1.40
Raw materials	\$1.45	\$1.45
Maintenance	\$0.38	\$0.44
Labour and supervision	\$0.31	\$0.31
Overheads and administration	\$0.10	\$0.10
Total	\$5.41	\$4.32

Table 8: Known thermophilic methanotrophs

Phylum Major	Genus	Species	Major Pathway	T. range/ opt. (°C)	Reference
y-proteobacteria	Methylothermus	Subterraneus	RuMP	37-65/55-60	(49)
y-proteobacteria	Methylothermus	Thermalis	RuMP	37-67/57-59	(49, 50)
y-proteobacteria	Methylococcus	Thermophilus	RuMP	37-55/*	(49)
y-proteobacteria	Methylocaldum	Szegediense	RuMP, Serine	30-62/55	(49, 51)
y-proteobacteria	<i>Methylocystis</i>	Parvus	Serine	15-53/ 37	(48)
Verrucomicrobia	Methylacidphilum	'Isolate V4'	RuBP	*/60	(52)
Verrucomicrobia	Methylacidphilum	Fumarolicum	RuBP	40-66/55	(53, 54)
Verrucomicrobia	Methyloacida	Kamchatkensis	RuBP	37-60/55	(55)

* no data available