

# Production and Characterization of Poly-3-hydroxybutyrate From Biodiesel-Glycerol by *Burkholderia cepacia* ATCC 17759

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*Glycerol, a byproduct of the biodiesel industry, can be used by bacteria as an inexpensive carbon source for the production of value-added biodegradable polyhydroxyalkanoates (PHAs). Burkholderia cepacia ATCC 17759 synthesized poly-3-hydroxybutyrate (PHB) from glycerol concentrations ranging from 3% to 9% (v/v). Increasing the glycerol concentration results in a gradual reduction of biomass, PHA yield, and molecular mass ( $M_n$  and  $M_w$ ) of PHB. The molecular mass of PHB produced utilizing xylose as a carbon source is also decreased by the addition of glycerol as a secondary carbon source dependent on the time and concentration of the addition.  $^1H$ -NMR revealed that molecular masses decreased due to the esterification of glycerol with PHB resulting in chain termination (end-capping). However, melting temperature and glass transition temperature of the end-capped polymers showed no significant difference when compared to the xylose-based PHB. The fermentation was successfully scaled up to 200 L for PHB production and the yield of dry biomass and PHB were 23.6 g/L and 7.4 g/L, respectively. © 2009 American Institute of Chemical Engineers Biotechnol. Prog., 26: 424–430, 2010*

*Keywords: biodiesel-glycerol, polyhydroxyalkanoates, Burkholderia cepacia, polyhydroxybutyrate, end-capped PHB*

## Introduction

Polyhydroxyalkanoates (PHAs) are accumulated as microbial intracellular carbon and energy reserves. These polymers represent a class of compounds with physical-chemical characteristics similar to petroleum-derived plastics such as polypropylene, polyethylene and polystyrene, but are environmentally compatible and totally biodegradable to carbon dioxide and water.<sup>1–3</sup> A number of microorganisms, including *Ralstonia eutropha*, *Alcaligenes latus*, and several species of *Pseudomonas*,<sup>4–7</sup> have been shown to produce various polyesters with different subunits.<sup>8</sup> The homopolymer PHB and the copolymer poly-3-hydroxybutyrate-co-poly-3-hydroxyvalerate (PHB-co-PHV) are the most widely studied and have been produced commercially to manufacture some finished products, which are primarily used in medical applications such as tissue engineering.<sup>9</sup> *Burkholderia* (formerly *Pseudo-*

*monas*) *cepacia* has been shown to efficiently synthesize short-chain-length (scl) PHAs, such as PHB, PHV, and PHB-co-PHV. By incorporating PHV with PHB to form the copolymer, lower crystallinity and better elongation can be obtained, which have been shown to exhibit more desirable mechanical properties.<sup>10</sup>

Many carbon sources, including xylose, galactose, glucose, glycerol and levulinic acid, have been used to support growth and scl-PHA production by *B. cepacia*.<sup>10,11</sup> Although it is feasible for these carbon sources to be used to produce PHAs in the laboratory, high production costs hamper large-scale commercial production and the cost of fermentation feedstocks can account for up to 50% of the overall production cost.<sup>4,12</sup> Several process stream feedstocks, such as cheese whey permeate,<sup>13</sup> wood hydrolysate,<sup>14</sup> sugarcane molasses, and corn steep liquor,<sup>15</sup> have been used to produce PHAs in an attempt to reduce production costs.

Glycerol (approximately 10% of the final weight of biodiesel)<sup>16</sup> is the major byproduct of the biodiesel industry. As

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biodiesel production has increased dramatically from 500,000 gallons in 1999 to 450 million gallons in 2007 (National Biodiesel Board, 2008), crude glycerol generated from the transesterification of vegetable oil has also been produced in large quantities. Recent publications by Mothes et al.<sup>17</sup> and Cavalheiro et al.<sup>18</sup> described direct fermentation of biodiesel-glycerol to PHB by *Cupriavidus necator* with polymer production approaching 50% of dry microbial biomass. In addition, Papanikolaou et al.<sup>19</sup> demonstrated the production of 1,3-propanediol, citric acid, and cellular lipids (single-cell oil) from biodiesel-glycerol using three separate microbial fermentations. Despite the commercial use of glycerol in the food, pharmaceutical, cosmetics, and other industries,<sup>16</sup> it is expensive to refine crude glycerol to the purity needed for these applications. In order to examine alternative uses for low-value biodiesel waste glycerol, small scale fermentations were initially performed by *B. cepacia* in shake flasks and analyzed for PHB production using glycerol as a carbon source. Physical-chemical properties of PHB produced by *B. cepacia* grown on reagent-grade glycerol were determined, and it was established that the molecular mass decreased when glycerol was used as a carbon source. Subsequently, *B. cepacia* was used to convert biodiesel-glycerol to PHB via fermentation at a pilot plant (200-L) scale.

## Materials and Methods

### Microorganism and fermentation conditions

*B. cepacia* ATCC 17759 was used in both shake flask as well as pilot scale (200-L) fermentations. The nitrogen-limited mineral salts medium used in the fermentations was initially described by Bertrand et al.<sup>20</sup> For shake-flask experiments, all cultures were shaken at 30°C and 150 rpm and the concentration of nitrogen was further reduced to 1.5 g/L. Glycerol (99.5%, EMD, Gibbstown, NJ) and xylose (99% purity, Acros, Waltham, MA) were used to produce PHB for physical-chemical characterizations and were autoclaved separately. All shake flask experiments were performed in 500 mL flasks (baffled) containing 100 mL of medium with metal enclosures.

In experiments using both xylose and glycerol as carbon sources, xylose (2.2%) was initially added into the medium. At 24 h or 48 h, 2% or 5% glycerol was added to the medium, and cultures were harvested at 72 h. Only 2.2% xylose was used as a carbon source for a control. When using the 400-L fermentor (Model No: IF 400, New Brunswick Scientific Co., Edison, NJ), a fed-batch method was used and glycerol (85% purity) from a biodiesel-producing facility (Twin Rivers Technologies, Quincy, MA.) was added as the primary carbon source.

### Glycerol content

Glycerol concentration was measured using the Free Glycerol Reagent kit (Cat. F6428, Sigma, St. Louis, MO). The reactions were incubated for 5 min at 37°C and the absorbances were recorded spectrophotometrically at 540 nm (CARY 300 Spectrophotometer, Varian, Walnut Creek, CA) as described per the instructions of the manufacturer.

### Biomass and PHA isolation

Cultures were harvested from shake flasks by centrifugation at 7,000g for 10 min (Sorvall model SS-3, Thermo Fisher Scientific Inc., Waltham, MA). The biomass was subsequently washed with distilled H<sub>2</sub>O and centrifuged again

to remove the supernatant and stored at -20°C until lyophilization. For cultures grown in the 400-L fermentor, the broth was centrifuged at 16,000 rpm using a continuous flow centrifuge (CEPA High Speed Centrifuge Z81 G, New Brunswick Scientific, Edison, NJ).

The harvested biomass was then frozen and lyophilized at -80°C and 200 millitorr for 24 h. The dry biomass was subsequently ground to a powder, mixed with chloroform (10 mL chloroform/1 g dry biomass) and stirred at room temperature for 24 h.

For the extraction and purification of bench-scale quantities of PHA, the procedure of Keenan et al.<sup>10</sup> was used for all biomass samples. For the purification of polymers from cells produced at the pilot-plant scale, lyophilized cell mass (1 kg) was stirred in 9 L of chloroform at room temperature for 24 h. The polymer solution was concentrated by rotary evaporation at 70°C and a distillation system was used to recover chloroform. The polymer was precipitated from this solution via addition to cold methanol (1 vol PHA solution/5 vol methanol). Precipitated polymer samples exhibited a fibrous morphology and were washed with additional methanol.

### GC analysis for PHAs

Monomer composition and yield of PHAs were determined by gas chromatography on a GC2010 equipped with AOC-20i autosampler (Shimadzu, Columbia, MD) as described previously by Nomura et al.<sup>21</sup> Briefly, 15 mg of dry biomass were weighed, mixed with 2 mL of H<sub>2</sub>SO<sub>4</sub>/methanol (15:85) and 2 mL chloroform, and incubated at 100°C for 140 min to extract the polymer and subject it to a methanolysis reaction to form methyl ester monomers. The mixture was then cooled to room temperature and 1 mL of distilled water was added and the solution was vortexed for 1 min. The mixture was allowed to separate into an aqueous and an organic phase and the organic layer (0.5 mL) was removed, filtered (PTFE membrane, 0.22 μm), and mixed with 0.5 mL 0.1% caprylic acid in chloroform. This mixture was subjected to analysis by GC with a flame ionization detector (FID). Application of the sample was by split injection of 1 μL on to a 30-m Rtx<sup>®</sup>-5 (5% diphenyl-95% dimethyl polysiloxane) column with a 0.25 mm ID (Restek, Bellefonte, PA). The injection port was held at 280°C and the oven housing the column was held at 100°C for 3 min. The oven temperature was then raised 8°C min<sup>-1</sup> to 280°C where the column was held for 2 min. The oven temperature was raised to 310°C at a rate of 20°C min<sup>-1</sup> and held for 10 min to remove any residuals from the column before the next sample was applied. Products were detected by FID at a temperature of 310°C.

### Molecular mass determination

Number average molecular weight ( $M_n$ ) and weight average molecular weight ( $M_w$ ) were determined by gel permeation chromatography (GPC) using an LC-20AD Liquid Chromatograph equipped with a SIL-20A auto-sampler and RID-10A refractive index detector (Shimadzu, Columbia, MD). PHB polymers were dissolved in chloroform to a final concentration of 0.7 mg/mL and filtered (PTFE membrane, 0.22 μm) before analysis. A Styrene divinylbenzene (SDV) 8 × 300 mm column with 5 μm porosity was used as the stationary phase (Polymer Standards Service, Warwick, RI) with an oven temperature of 40°C. The mobile phase was chloroform at a flow rate of 1 mL/min.

Standard curves for molecular weight were created using polystyrene standards with a range from 682 to 1,670,000

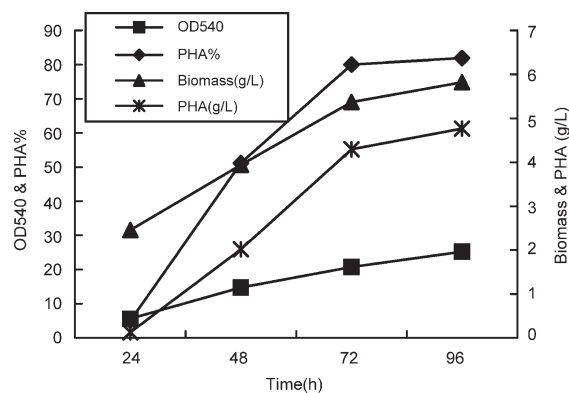


Figure 1. Growth and PHA production by *B. cepacia* using 3% biodiesel-glycerol in shake flasks.

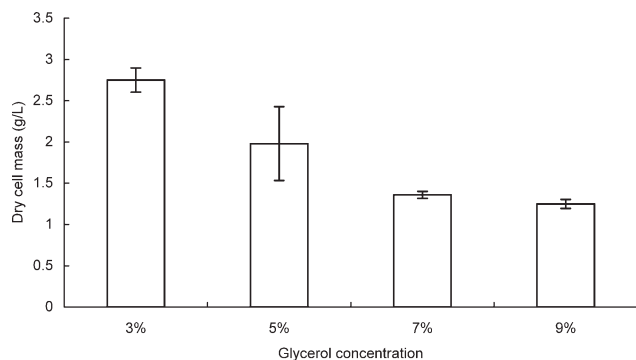


Figure 2. Dry biomass of *B. cepacia* grown in shake flasks on different concentrations of pure glycerol.

Da and low polydispersities (Polystyrene High MW Standards Kit, Polymer Standards Service, Warwick, RI).

### Thermal analysis

Thermogravimetric analysis (Hi-Res TGA 2950, TA Instruments, New Castle, DE) was used to determine the decomposition temperature ( $T_{\text{decomp}}$ ) of PHB. Ten milligrams of PHB film were folded into a platinum tray and subjected to a heating rate of 20°C/min from ambient to a final temperature of 500°C.

Differential scanning calorimetry (DSC, model 2920, TA Instruments, New Castle, DE) was used to characterize the melting temperature ( $T_m$ ) and glass transition temperature ( $T_g$ ) for all polymer samples. The temperature range for DSC varied from -50°C to 250°C at a heating rate of 10°C/min and a cooling rate of 5.0°C/min. Universal Analysis 2000 software was used for data analysis.

### Nuclear magnetic resonance

End-capped PHB was analyzed by  $^1\text{H}$  NMR spectroscopy using a Bruker BioSpin AVANCE 600 (Bruker BioSpin Corp., Billerica MA) operating at 600 MHz. Samples were prepared for analysis by dissolving solvent-cast film segments in deuterated chloroform [1% (w/v)] via mixing with mild heating. The analyses for glycerol and PHB were accomplished using XWIN-NMR version 3.1 software.

## Results

### Growth and PHB yield of *B. cepacia*

In shake flask experiments, yields of dry biomass approached 5.8 g/L, which was comprised of up to 81.9%

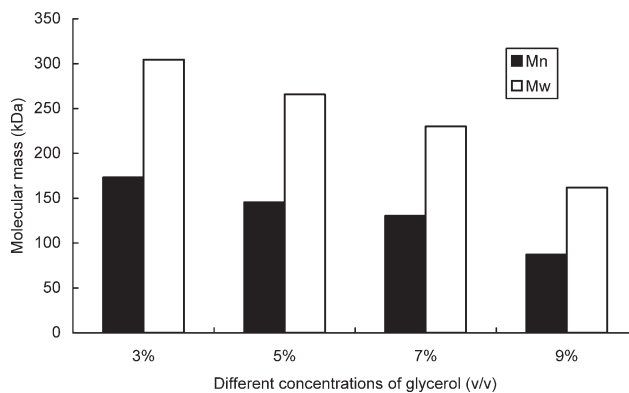


Figure 3. Number-average molecular weight ( $M_n$ ) and weight-average molecular weight ( $M_w$ ) of PHB produced by *B. cepacia* grown with different concentrations of glycerol.

Table 1. The Effect of Concentrations and Exposure Times of Glycerol on the Number-Average Molecular Weight ( $M_n$ ) of PHB

Glycerol Concentration (v/v)*	Exposure Time in Glycerol (h) <sup>†</sup>		
	0	24	48
No glycerol (control)	468.3 <sup>‡</sup>	—	—
2%	—	174.6	133.6
5%	—	138.4	114.0

\* All treatments contained 2.2% (w/v) xylose as the primary carbon source. <sup>†</sup> Glycerol additions were made at the time point of 24 and 48 h with all treatments harvested at 72 h. <sup>‡</sup> All data reported in kiloDalton (kDa).

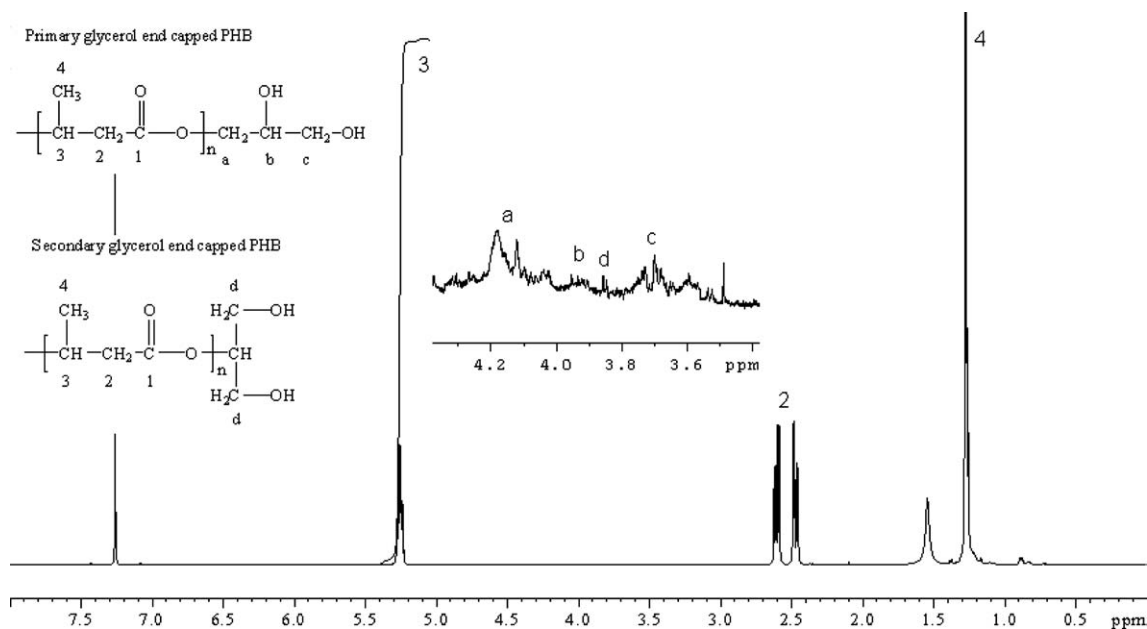
PHA when using 3% (v/v) biodiesel-glycerol as the carbon source. This resulted in 4.8 g/L PHB production after 96 h shaking at 150 rpm and 30°C (Figure 1).

When different concentrations of pure glycerol were added to the medium (Figure 2), the glycerol content influenced the amount of final dry biomass. Specifically, higher concentrations of glycerol resulted in lower amounts of dry biomass. For example, dry biomass reached 2.8 g/L at 3% (v/v) glycerol added in shake flask experiments, whereas when 9% (v/v) glycerol was added, the amount of dry biomass decreased to 1.3 g/L.

### Variation of molecular weights of PHB produced from glycerol in *B. cepacia*

Number average molecular weights ( $M_n$ ) and weight average molecular weights ( $M_w$ ) of thin PHB solvent-cast films were determined by GPC. When concentrations of glycerol were increased from 3% to 9%, both  $M_n$  and  $M_w$  decreased gradually from 173 kDa and 304 kDa to 87 kDa and 162 kDa, respectively (Figure 3).

Mixtures of xylose and glycerol were also examined as carbon sources. Initially, 2.2% (w/v) xylose was added to the cultures at the time of inoculation and glycerol was added at time points of 24 h and 48 h at concentrations of either 2% (v/v) or 5% (v/v). Subsequently, the cultures were harvested at 72h.  $M_n$  of PHB from 5% glycerol was lower than that from 2% glycerol added to the medium at the same time point. Higher glycerol concentrations resulted in the production of PHB with overall lower molecular mass (Table 1). Interestingly, when the cells were grown on the same concentration of glycerol (2% or 5%) with 2.2% xylose, PHB had lower  $M_n$  (133.6 or 114.0 kDa) as cells were exposed to



**Figure 4.**  $^1\text{H-NMR}$  of PHB produced by *B. cepacia* grown on 7% (v/v) glycerol as a carbon source. The expanded region indicates glycerol as the terminal end-group.

glycerol for a longer time period (48 h), and higher  $M_n$  (174.6 or 138.4 kDa) when exposed to glycerol for a shorter time period (24 h), respectively. The same trend was observed for  $M_w$  of PHB produced from 2% or 5% glycerol with 2.2% xylose (data not shown). When 2.2% xylose was provided as the sole carbon source, the  $M_n$  reached 468.3 kDa (Table 1). The xylose-based PHB polymer was found to be of greater molecular mass than PHA produced from or exposed to glycerol. High concentrations of glycerol increased the opportunity for incorporation of glycerol into the polymer. Also, exposing the cells to glycerol for a longer time period increased the possibility to terminate the elongation of PHB. Therefore, higher concentrations of glycerol or longer exposure to glycerol resulted in PHB with lower molecular masses.

#### Characterization of PHB end-capped with glycerol by $^1\text{H NMR}$

$^1\text{H NMR}$  shows the results from PHB synthesized in the presence of 7% glycerol (Figure 4). The full spectrum showed the expected resonances for PHB as demonstrated by the methyl group at 1.25 ppm, the methylene group between 2.45 and 2.65 ppm, and the methine group at 5.25 ppm. However, expansion of the spectral region between 3.0 and 4.5 ppm revealed the presence of additional resonances corresponding to terminal glycerol groups. The expanded region with three resonances at 3.7 ppm, 3.95 ppm, and 4.18 ppm showed the terminal esterification of glycerol to PHB through the primary hydroxyls ( $\text{C}_1$  or  $\text{C}_3$  positions of glycerol). Resonance at 3.86 ppm showed glycerol end-capping of PHB through the secondary hydroxyl group. Because glycerol is composed of 2 primary and 1 secondary hydroxyl groups, the possibility exists that the glycerol termination of PHB polymers could also be the result of covalent bonding at the secondary hydroxyl group of glycerol. These results were identical to those reported by Ashby et al.<sup>22</sup>

**Table 2.** Physical-Chemical Properties of PHB Produced From Xylose and Glycerol

PHB	$T_m$ ( $^{\circ}\text{C}$ )	$T_g$ ( $^{\circ}\text{C}$ )	$T_{\text{decomp.}}$ ( $^{\circ}\text{C}$ )
Xylose-based PHB*	178.6	-6.6	268.6
Glycerol-based PHB <sup>†</sup>	181.9	1.6	281.5

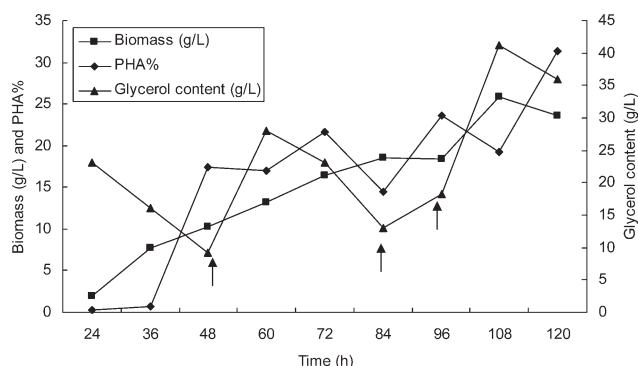
\* PHB produced from 2.2% (w/v) xylose as sole carbon source. <sup>†</sup> PHB produced from 3% (v/v) glycerol as sole carbon source.

#### Mechanical properties of PHB

Thermal characterization by DSC and TGA of PHB films are shown in Table 2. Although the molecular mass of glycerol-based PHB was lower than that of xylose-based PHB, there was no significant change for both  $T_g$  and  $T_m$ ; however,  $T_{\text{decomp.}}$  increased from 268.6 $^{\circ}\text{C}$  to 281.5 $^{\circ}\text{C}$ . Higher decomposition temperature provides a broader separation between the required melting temperature for injection molding and thermal degradation of the polymers.

#### Pilot scale (200-L) fermentation using biodiesel-glycerol

The initial evaluation of physical properties of glycerol-based PHB demonstrated the potential application of glycerol as a carbon source to support growth and PHB production by *B. cepacia*. Subsequently, biodiesel-glycerol was used as the carbon source for a 200-L pilot scale fermentation. Dissolved oxygen (DO) and pH were controlled at 35–40% and 7.0, respectively. As shown in Figure 5, glycerol concentration was kept between 10 g/L and 40 g/L with periodic additions of glycerol. Biomass gradually increased and reached maximum density of 25.8 g/L at 108 h. PHA accumulated quickly from 0.7% at 36 h to 17.4% at 48 h, and PHA% fluctuated near 20% before increasing to the highest point of 31.4% at 120 h. In total, 26.5 L glycerol and 2.5 kg ammonium sulfate were added into the 400-L fermentor in a fed-batch mode and the cells were harvested at 120 h. The final yield of dry biomass and PHB were 23.6 g/L and 7.4 g/L, respectively.



**Figure 5.** Changes in biomass, PHA%, and glycerol concentration during a fed-batch fermentation, in a 400-L fermentor, with periodic additions ( $\uparrow$ ) of biodiesel-glycerol.

### Discussion

Due to environmental pollution caused by relatively recalcitrant plastics and the consumption of fossil fuels for their production, biodegradable PHAs have attracted attention for their unique properties as thermoplastics or elastomers. However, the high cost of PHA production, primarily due to the cost of the feedstock and PHA recovery, has hindered large scale industrial production.

When biodiesel-glycerol was used as the sole carbon source in shake flasks, *B. cepacia* produced 5.8 g/L dry biomass and contained up to 81.9% PHB of total dry biomass when using 3% (v/v) biodiesel-glycerol as a feedstock over 96 h of growth. *B. cepacia* showed higher dry cell weights and PHA production when grown on glycerol when compared to *Escherichia coli* and various *Pseudomonads* grown on glycerol (Table 3). However, increasing glycerol concentration from 3% to 9% (Figure 1) resulted in a decrease of microbial biomass of *B. cepacia* of almost 50%. In a recent study by Cavalheiro et al.,<sup>18</sup> similar effects of increasing concentrations of glycerol (on microbial growth) were observed. Specifically, at glycerol concentrations exceeding 30 g·L<sup>-1</sup>, the specific growth rate of *C. necator* decreased when using either pure glycerol or biodiesel-glycerol as sole sources of carbon.

In a recent publication by Cavalheiro et al.,<sup>18</sup> experiments were performed to establish the effects of gas composition and rate of addition to fed batch cultures of *C. necator*. The authors demonstrated significant increases in dry cellular biomass (g L<sup>-1</sup>) by increasing the flow rate from 1.5 to 3.0 L of air min<sup>-1</sup>. Additionally, exponential phase growth and final cell densities were achieved sooner by aeration with 2.0 L air min<sup>-1</sup> supplemented with 1.0 L pure oxygen min<sup>-1</sup>. Preliminary experiments for this work established that initially 1 VVM (volume of air per volume of medium per minute) was sufficient to maintain maximum growth. As the fermentation progressed, whenever the dissolved oxygen fell below 35%, the impeller speed and aeration (data not shown) were increased to maintain dissolved oxygen between 35 and 50%. The latter was found to be optimal for cell growth and PHA production by *B. cepacia* and this approach was used in all fermentor experiments.

The molecular mass of PHB varies from 50 to 3,000 kDa,<sup>2,3</sup> depending on the microorganism and growth conditions. Although the pathways of PHA synthesis using different carbon sources have been previously described,<sup>3,25</sup> the

**Table 3.** Comparison of Dry Cell Mass and PHA Content From Different Bacterial Strains Grown on Various Carbon Sources in Shake Flasks

Bacteria	Substrates	Polymer	Cell Weight (g/L)	PHA Content (%)	Ref.
<i>B. cepacia</i>	Glycerol	PHB	5.8	81.9	Current study
<i>B. cepacia</i>	Xylose + levulinic acid	PHB-co-PHV	4.4–5.3	42–56	10
<i>E. coli</i>	Glucose	PHB	4.9	27.6	23
<i>E. coli</i>	Glycerol	PHB	5.6	9.8	23
<i>E. coli</i>	Glycerol	PHB	3.6	34	24
<i>Pseudomonas corrugata</i> (2%)	Glycerol	PHB	3.4	19.7	19
<i>Pseudomonas oleovorans</i> (1%)	Glycerol	mcl-PHA	1.9	26.8	19

mechanism for regulating molecular mass in PHA production is still unclear. The activity of PHA synthase (moles of substrate converted per unit time) may influence the molecular mass and the polydispersity of the polymer. It has been shown previously that PHA synthases with higher activity produced lower molecular weight polyesters.<sup>26–28</sup> On the other hand, enhancing the specific activity of PHA synthase in a *phaC* mutant increased not only PHA accumulation but also weight-average molecular weight by 6% to 74%.<sup>29</sup> Thus, PHA synthase is not the only factor to control and regulate the molecular weight of PHAs, and other enzymes or compounds may also play an important role in chain termination of the polymer.

In addition to providing a carbon source, glycerol has a unique function as a terminal end group for PHB synthesis. Both *R. eutropha* (formerly *Alcaligenes eutrophus*) and *Pseudomonas oleovorans* are known to produce short-chain-length PHAs; however, when using glycerol as the sole carbon source, the molecular mass of PHB was substantially lower than that produced from glucose and whey sugars (glucose and galactose from lactose hydrolysis).<sup>30–32</sup> The molecular weight gradually decreased as glycerol concentration increased.<sup>22</sup> In this study, when glycerol content was increased from 3% (v/v) to 9% (v/v), both the molecular weight of PHB and dry cell weight decreased. One possible explanation for this phenomenon is that the organism was inhibited by osmotic stress as the glycerol content increased. The cells began to decrease their enzymatic efficiency and undergo chain termination earlier. PHB was capped by glycerol through covalent esterification in a chain termination position, resulting in lower molecular weights of the polymers produced. As the cells were in contact with glycerol for longer time periods, there was a higher possibility for chain termination to form the lower molecular mass polymers. The osmotic stress may also have caused a slower growth rate and explains the decreasing cell dry weight. Interestingly, there has been no evidence to show that medium-chain-length PHA (produced from *Pseudomonas corrugata*) polymers can be esterified with glycerol to form an end-capped polymer.<sup>22</sup> Perhaps, the PHA synthase of strains producing medium-chain-length PHAs (monomers greater than 6 carbons) cannot incorporate small molecules such as glycerol. However, the PHA synthase of *B. cepacia* may more readily incorporate glycerol as a terminal group because the enzyme may exhibit a specificity for shorter chain length substrates.

The glycerol-based PHB exhibited similar  $T_g$  and  $T_m$  compared with PHB polymers produced in cells grown on xylose or compared with commercially available polypropylene.<sup>10</sup>  $T_{decomp.}$  however, was 13°C higher in glycerol-capped PHB compared to xylose-based PHB. This advantage could provide a broader range for temperature exposure in certain industrial applications.

Although the thermal properties of the glycerol end-capped PHB in this study were not markedly different from PHB without end-capping, the molecular weights of the polymers were significantly lower. The lower molecular weights of the end-capped polymers may affect the tensile strength of the polymers.<sup>33</sup> On the other hand, the diol of these end caps provides an interesting pair of functional groups (—OH) through which these polymers have the potential to be further chemically modified to produce novel PHA derivatives.

In conclusion, when using glycerol as the carbon source, the concentration of glycerol needs to be strictly controlled. By regulating the glycerol content, different lengths of PHB can be produced to meet the diverse criteria of various industrial and medical applications. These studies provide another degree of regulation in the production of PHB by *B. cepacia*. Previous studies by Keenan et al.<sup>10</sup> have demonstrated the regulation of compositional content (PHB-co-PHV) using xylose and levulinic acid, and this investigation describes the regulation of molecular mass by end capping with glycerol.

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