Production of natural fiber reinforced thermoplastic composites through the use of polyhydroxybutyrate-rich biomass

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Received 14 February 2006; received in revised form 26 March 2007; accepted 26 March 2007
Available online 15 June 2007

Abstract

Previous research has demonstrated that production of natural fiber reinforced thermoplastic composites (NFRTCs) utilizing bacterially-derived pure polyhydroxybutyrate (PHB) does not yield a product that is cost competitive with synthetic plastic-based NFRTCs. Moreover, the commercial production of pure PHB is not without environmental impacts. To address these issues, we integrated unpurified PHB in NFRTC construction, thereby eliminating a significant energy and cost sink (ca. 30–40%) while concurrently yielding a fully biologically based commodity. PHB-rich biomass synthesized with the microorganism Azotobacter vinelandii UWD was utilized to manufacture NFRTCs with wood flour. Resulting composites exhibited statistically similar bending strength properties despite relatively different PHB contents. Moreover, the presence of microbial cell debris allowed for NFRTC processing at significantly reduced polymer content, relative to pure PHB-based NFRTCs. Results further indicate that current commercial PHB production yields are sufficiently high to produce composites comparable to those manufactured with purified PHB.

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Keywords: Polyhydroxybutyrate (PHB); Polyhydroxyvalerate (PHV); Natural fiber reinforced thermoplastic composite (NFRTC); Modulus of elasticity (MOE); Modulus of rupture (MOR)

1. Introduction

Synthetic-based composites are commercially appealing due to the ability to engineer products with known properties to meet a variety of diverse applications, however, these commodities represent an environmental liability both implicit in the raw materials production and upon disposal. Natural fiber reinforced thermoplastic composites (NFRTCs) represent an opportunity to partially ameliorate the environmental impacts by integrating biodegradable filler material, such as flax, hemp, and wood flour, in lieu of synthetic fillers, such as glass, carbon, or steel; perhaps more critically, the cost of some biodegradable filler materials is better than an order of magnitude less than synthetic reinforcing fibers (Mohanty et al., 2000). Moreover, NFRTCs can be engineered for a variety of applications wherein synthetic-based composites would be overdesigned (Mohanty et al., 2000). However, integration of biodegradable material, even if waste derived, does not completely address the environmental liabilities; these commodities remain bio-recalcitrant upon disposal (Scott, 2000) based on the use of synthetic thermoplastics as the principal binding material, and the manufacture of petroleum-based thermoplastics is not without environmental ramifications (Mecking, 2004).

More recently, the use of polyhydroxyalkanoates (PHAs) in NFRTC construction has been recognized as
an opportunity to further reduce the environmental impact of composite products (Dufresne et al., 2003; Fernandes et al., 2004; Mohanty et al., 2000; Reinsch and Kelley, 1997; Shanks et al., 2004). PHAs are natural thermoplastic polyesters – essentially carbon storage reserves – that over 300 different microbial species are known to synthesize and accumulate in the form of cytoplasmic granules (Lee, 1996). Poly-3-hydroxybutyrate (PHB or P3HB) was the first PHA discovered (over 75 years ago), and hence is the most extensively characterized type (Lemoigne, 1926); however, many forms of hydroxyalkanoic monomer units have since been identified. Common precursors to PHA synthesis include simple sugars such as glucose and fructose, and organic acids such as acetic and propionic acid. The carbon substrate form dictates the polymeric structure of the PHA (Madison and Huisman, 1999), with some of the most common forms including PHB, polyhydroxyvalerate (PHV), and poly-4-hydroxybutyrate (P4HB). Each form of PHA exhibits different polymeric properties. PHB exhibits similar characteristics to polypropylene, including melting temperature and crystallinity, but the polymer is brittle upon crystallization and thus exhibits little stress resistance (Madison and Huisman, 1999). Copolymerization with PHV (e.g., PHB-co-PHV) yields a less brittle, less crystalline thermoplastic with a lower processing temperature; NFRTCs produced with PHB-co-PHV yielded improved mechanical properties (Shanks et al., 2004).

Although PHAs might appear to present an ideal opportunity to produce an entirely biologically based and biodegradable product, and thus yield an environmentally benign commodity, recent research demonstrated through an energy audit for a bacterial PHA production process, that over 30% of the energy and cost associated with the biological purification step. Polymer processing represents ca. 30–40% of the energy and cost associated with the biological production of PHA (Akiyama et al., 2003; Gerngross, 1999). In addition, the process of extracting bacterial PHA involves the use of various solvents, which generates waste streams that must be managed (Choi and Lee, 1997). Recognizing that the non-PHA material (e.g., cellular biomass) associated with microorganisms is organic, similar to the organic filler material in NFRTCs, the biopolymer arguably does not need to be purified in order to be utilized. The goal of this research was therefore to demonstrate that unpurified bacterial PHB (e.g., PHB-rich biomass) could be utilized in the manufacture of NFRTCs. The specific objectives were to: (i) design and operate a biological reactor for the purpose of mass producing PHB-rich biomass; (ii) harvest and process PHB-rich biomass for use in designing and testing composite products and (iii) produce and evaluate PHB-rich-biomass and pure PHB based NFRTCs following a factorial design to identify influencing factors and the potential significance of microbial biomass on composite material properties.

2. Experimental section

2.1. Materials

Azotobacter vinelandii UWD (ATCC 53799), derived from strain UW (ATCC 13705), was selected for this research because it has the demonstrated ability to hyper-produce PHB without any nutrient limitations (Manchak and Page, 1994; Page and Knosp, 1989). A. vinelandii UWD will synthesize the biopolymer under fully aerobic conditions due to a mutation associated with its respiratory NADH oxidase (Page and Knosp, 1989).

2.2. Reactor operations and culture media

A. vinelandii UWD was cultured in 10-L reactors aerated through a 9-inch diameter Sanitaire® Silver Series II membrane fine bubble disc diffuser (Brown Deer, Wisconsin, USA). Reactors were operated continuously in two 9-month periods for the sole purpose of producing large quantities of PHB-rich biomass. The reactors were operated as sequencing batch reactors, batch decanted and fed once daily at a dilution rate ranging from 0.5 d⁻¹ to 0.9 d⁻¹. The mixed liquor suspended solids (MLSS) concentration stabilized at ca. 5 g L⁻¹ in all reactors. The reactors were operated in environmentally controlled rooms with the temperature maintained at 30 °C. The growth medium consisted of the following, expressed in terms of g L⁻¹ (reagent grade): KH₂PO₄, 0.16; K₂HPO₄, 0.64; MgSO₄·7H₂O, 0.16; CaSO₄·2H₂O, 0.08; FeCl₃, 0.04; Na₂MoO₄·2H₂O, 0.008; glucose, 20; sucrose, 20. The medium was autoclaved prior to addition to the reactors.

2.3. PHA-rich biomass recovery and processing

Daily reactor decants were centrifuged at ca. 10,000g to recover the PHA-rich biomass. The biomass pellet was re-suspended in 6.25% sodium hypochlorite for 60 min to lyse the cells (Berger et al., 1989), recovered through centrifugation at ca. 10,000g, and dried at 60 °C. Chlorination was utilized to arrest the bacterial metabolic activity during the drying phase, thus preventing PHA biodegradation.

2.4. Analytical procedures

Biomass PHA content was determined by gas chromatography/mass spectrometry (GC–MS), following the
method of Braunegg et al. (1978). Briefly, dried PHA-rich biomass samples were digested at 100 °C in 2 mL each of acidified methanol (3% v/v sulfuric acid) and chloroform. Benzoic acid was added to the chloroform as an internal standard. Following vigorous vortexing of the mixture with 1 mL deionized water, PHA-rich chloroform was recovered for analysis. The chloroform phase was dried with anhydrous sodium sulfate prior to analysis. GC–MS was performed on a Thermofinnigan PolarisQ iontrap GC–MS instrument (Thermo Electron Corporation, Waltham, MA, USA) in positive ei mode. The sample was introduced using split injection. Separation was achieved on a ZB1 (15 m, 0.25 mm ID) capillary column (Phenomenex, Torrance, California, USA) with helium as the carrier gas (1.2 mL min \(^{-1}\)) using a temperature program 40 °C (2 min) ramped to 200 °C at 5 °C min \(^{-1}\). Data was analyzed on the software program Xcalibur (Thermo Electron Corporation, Waltham, MA, USA). The identity of the compounds (e.g., form of PHA) was confirmed by retention time and mass spectral matching with known PHA standards (as methyl ester derivatives), and quantified based on the internal standard. Total cellular PHA content, and fractions thereof for the individual PHA forms, were determined and reported on a weight basis (e.g., mass PHA:mass of biomass, w/w).

2.5. Composite formulation and processing

Natural fiber reinforced thermoplastic composites (NFRTCs) were manufactured with the PHA-rich biomass, pure PHB obtained from PHB Industrial S/A (Biocycle 1000\(^{®}\)), and polypropylene obtained from Solvay S.A. (Brussels). The respective composite formulations are summarized in Table 1. Sixty-mesh wood (pine) flour was obtained from American Wood Fibers (Wisconsin, USA) and added to the composite formulation as the natural fiber reinforcement. Prior to injection molding (IM), the dry materials (below 2% moisture content) were melt-blended in a torque rheometer for 5 min at 170 °C. The mixed materials were then fed into a capillary rheometer with a barrel temperature of 170 °C. The capillary rheometer was used to inject the composite into a heated die creating two solid rectangular bars (5 × 12 × 94 mm). Once the bars were removed from the die, they were allowed to equilibrate for 1 week at 22 °C and 50% relative humidity.

Six specimens were produced and tested for each respective formulation. The conditioned specimens were tested for stiffness (modulus of elasticity, MOE), bending strength (modulus of rupture, MOR), and strain at break according to the ASTM D790 procedures (ASTM, 1997). NFRTC density was tested in accordance with ASTM D1037 (ASTM, 1999).

2.6. Statistical analyses

Analysis of variance (ANOVA) was performed for each of the respective NFRTC formulations to assess the effect of polymer content on both MOE and MOR. Main effects were assessed based on a 5% Type I error for the least squares means of the replicate specimens. Individual formulations were statistically contrasted utilizing a Tukey–Kramer pairwise comparison, with a 95% confidence limit. Statistical analyses were performed utilizing SAS software (SAS Institute, Inc., Cary, NC, USA).

<table>
<thead>
<tr>
<th>Thermoplastic type</th>
<th>Polymer/wood ratio</th>
<th>Density Average (kg/m³) COVe (%)</th>
<th>Modulus of elasticity Average (GPa) COVe (%)</th>
<th>Modulus of rupture Average (MPa) COVe (%)</th>
<th>Strain at break Average (mm/mm) COVe (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified PHB(^b)</td>
<td>70/30</td>
<td>1227</td>
<td>1.3</td>
<td>4.69</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>80/20</td>
<td>1211</td>
<td>0.6</td>
<td>4.50</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>90/10</td>
<td>1211</td>
<td>0.7</td>
<td>4.31</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>100/0</td>
<td>1186</td>
<td>0.8</td>
<td>3.93</td>
<td>2.1</td>
</tr>
<tr>
<td>Biomass PHB(^c)</td>
<td>70/30</td>
<td>1266</td>
<td>4.2</td>
<td>4.76</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>80/20</td>
<td>1305</td>
<td>1.1</td>
<td>5.00</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>90/10</td>
<td>1329</td>
<td>0.8</td>
<td>4.62</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>100/0</td>
<td>1358</td>
<td>1.8</td>
<td>4.51</td>
<td>7.8</td>
</tr>
<tr>
<td>Biomass PHB(^d)</td>
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<td>1280</td>
<td>3.0</td>
<td>4.24</td>
<td>21.0</td>
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<tr>
<td>Polypropylene(^b)</td>
<td>60/40</td>
<td>1003</td>
<td>2.4</td>
<td>3.1</td>
<td>18.7</td>
</tr>
</tbody>
</table>

\(^a\) NFRTCs manufactured with 40% pine fiber, with the remaining 60% composed of pure polypropylene, pure PHB, or biologically derived PHB plus microbial cell debris.

\(^b\) Pure polypropylene obtained from Solvay; pure PHB obtained from PHB Industrial S/A.

\(^c\) PHB produced biologically in Azotobacter vinelandii UWD (ATCC 53799). PHB content on a dry microbial cell basis (not wood fiber basis) was ca. 24%. The 70/30, 80/20, 90/10, and 100/0 formulations thus contained ca. 17%, 19%, 22%, and 24% PHB, respectively.

\(^d\) PHB produced biologically in Azotobacter vinelandii UWD (ATCC 53799). PHB content on a dry microbial cell basis (not wood fiber basis) was ca. 43%. The 60/40 formulation thus contained ca. 26% PHB.

\(^e\) COVe: coefficient of variation.
3. Results and discussion

3.1. Polyhydroxyalkanoate-rich biomass processing

Chlorination of the PHA-rich biomass, a process also referred to as alkaline hydrolysis, was utilized principally as a means to arrest bacterial metabolic activity and prevent depolymerization of cellular PHA, and not for polymer purification. However, the treatment is also a common first step in commercial purification of PHA (Middelberg, 1995). Moreover, saponification results in the hydrolysis of fatty acid ester bonds, thus subsequent centrifugation of the biomass material would be expected to remove some of the solubolized biomass constituents. Therefore, considering the research goal to generate an unpurified, biomass-based polymer, analyses on select samples of the PHA-rich biomass were performed to confirm that minimal polymer purification occurred. The samples analyzed were obtained from the respective chlorinated and unchlorinated PHB-rich biomass accumulated over both periods of bioreactor operations.

For unchlorinated biomass (Fig. 1a), PHB methyl ester was eluted at 2.71 min, while the esterified internal standard, benzoic acid, eluted at 8.06 min; the remaining peaks (24, 27.48, 28.01, and 31.3 min) consist principally of esterified cellular lipids. Chlorinated biomass yielded similar results for PHB and benzoic acid, however, the lipid constituents were significantly reduced (Fig. 1b). In both cases PHB was the only form of PHA detected. Average PHB content for the chlorinated biomass was determined to be ca. 24% (w/w), while non-chlorinated PHB content was estimated at ca. 17%, yielding ca. 29% loss of cellular material through alkaline lysis and centrifugation. Therefore, although the applied cell disruption technique did indirectly result in some biomass removal, the residual polymer-based matrix remained in an unpurified form.

As noted and indicated on the GC chromatograms (Figs. 1a and 1b), PHB in the biomass samples was readily detected and validated. Mass spectrometry analysis of the relative PHB peaks revealed a fragmentation pattern consistent with that of pure PHB samples (data not shown).

While the unique metabolic capabilities of this microorganism satisfied the research objective of producing a sufficient quantity of PHB-rich biomass to manufacture NFRTCs, the PHB yield (on a w/w basis) was significantly less than published for this and other PHB-producing microorganisms on glucose/sucrose. For example, research has shown that PHB yields in excess of 65% (w/w) on A. vinelandii UWD would be reasonable (Page and Knosp, 1989). One potential explanation for the reduced yield lies in the reactor operations. Analysis of two typical reactor operational cycles suggests that PHB yield peaked prior to biomass recovery for processing (Fig. 2). Moreover, the peak at 7 h was similar in magnitude to that determined by others (Page and Knosp, 1989). Hence, a shorter operating cycle, and thus a shorter hydraulic residence time, may have yielded higher PHB quantities. Nevertheless, regardless of the reduced polymer yields, the material served effectively in the production of NFRTCs.

Fig. 1a. Total ion current gas chromatograph plot of non-chlorinated PHB-rich biomass sample. HB methyl ester eluted at 2.71 min. Other peaks include the benzoic acid methyl esters (8.06 min) and esterified cell-wall associated lipids (24, 27.48, 28.01, and 31.3 min).
3.2. PHB-rich biomass composites

Molding of PHB-rich biomass composites was hindered with increased wood flour content incorporated into the formulation, principally due to the relatively low polymer content in the biomass and concomitant higher viscosity of the PHB-rich biomass relative to a pure polymer. Above the 30% wood content level, the increased viscosity associated with the biomass-based polymer resulted in the inability for the material to flow efficiently to the outer extension of the mold. Therefore, the experimental design matrix was limited to composite formulations (%PHB-rich biomass:%wood) of 100:0, 90:10, 80:20, and 70:30 which yielded actual PHB contents (total composite weight basis) of ca. 24%, 21%, 19%, and 17%, respectively (Table 1).

The respective NFRTC formulations were statistically evaluated to determine the effects of the formulation elements on the composite material properties. A one-way analysis of variance (ANOVA), with the PHB:wood ratio as the model parameter, revealed that both PHB content and composite density significantly affected the MOE (e.g., stiffness). Therefore, in order to independently consider the effects of polymer content on composite stiffness, the statistical analyses were modified to include density as a covariate. A Tukey–Kramer pairwise comparison was then applied to the data set on MOE. The PHB:wood ratio was found to have an insignificant effect on the stiffness properties between the 100% and 90% formulations; similar results were determined between the 80% and 70% formulations (Table 2). However, the two lower wood content formulations (e.g., higher PHB content) yielded statistically different, and lower, MOEs (Tables 1 and 2). These results suggest that the increased wood content and commensurate decreased polymer content yields an increased overall stiffness.

In contrast to the MOE results, a one-way ANOVA for MOR (e.g., bending strength), both with and without density as a covariate, revealed no statistically significant effect (5% Type I error) for either the polymer content or density. Moreover, a Tukey–Kramer pairwise comparison indicated no statistically significant difference (95% confidence limit) in bending strength between any of the biomass-based formulations (data not shown); the strain at break

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Fig. 1b. Total ion current gas chromatograph plot of chlorinated PHB-rich biomass sample. HB methyl ester eluted at 2.73 min. Other peaks include the benzoic acid methyl esters (8.33 min) and esterified cell-wall associated lipids (28.07 min).

Fig. 2. Summary of typical A. vinelandii UWD bioreactor operating cycles, highlighting PHB synthesis. Results are from two parallel, identical bioreactors operated concurrently.

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Table 2
Tukey–Kramer pairwise comparison summary for biomass-based natural fiber reinforced thermoplastic composite (NFRTC) formulations, on modulus of elasticity (MOE) (%PHB-rich biomass to %wood flour content)

<table>
<thead>
<tr>
<th>%PHB-rich biomass to %wood flour content</th>
<th>100:0</th>
<th>90:10</th>
<th>80:20</th>
<th>70:30</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>1.530</td>
<td>0.006</td>
<td>0.006</td>
<td>0.0013</td>
</tr>
<tr>
<td>90:10</td>
<td>1.530</td>
<td>0.0126</td>
<td>0.0063</td>
<td></td>
</tr>
<tr>
<td>80:20</td>
<td>0.0066</td>
<td>0.0126</td>
<td>0.7724</td>
<td></td>
</tr>
<tr>
<td>70:30</td>
<td>0.0013</td>
<td>0.0083</td>
<td>0.7724</td>
<td></td>
</tr>
</tbody>
</table>

Results shown are the F > F for the least squares means, based on a 95% confidence interval. Ratios represent %PHB-rich biomass:%wood flour content.

Results further supports this finding. This outcome is of particular interest, considering the relatively significant difference in PHB content between the formulations (e.g., from 24% at 100:0 to 17% at 70:30). Finally, as another interesting comparison with the MOE results, wood content appears to not affect overall NFRTC strength within the respective formulations.

In the course of synthesizing the requisite large quantities of PHB-rich biomass for the purpose of producing the NFRTCs, initial bioreactor operations during the first 9-month bioreactor operating period yielded a small amount of higher PHB content biomass (43% PHB content, microbial w/w). With this higher PHB content biomass, the effects of increased microbial PHB content and decreased cell debris content on composite material properties, relative to constant %PHB content, could be assessed. Utilizing this material, a 60:40 PHB-rich biomass composite formulation was produced; this NFRTC formulation was contrasted with the 100:0 PHB-rich biomass formulation (which was produced with the 24% PHB-rich biomass). The formulations (%PHB:biomass:wood) were 26:34:40 and 24:76:0, respectively. Interestingly, the 60:40 formulation yielded the highest MOR of all biomass-based composites (ca. 29% higher than the 100:0 formulation – Table 1). Furthermore, the bending stiffness properties were significantly improved, with the 60:40 formulation yielding a 100% increase in the strain at break relative to the 100:0 formulation (Table 1). Considering that the PHB content between the 100:0 and 60:40 formulations was essentially the same, these results, coupled with the other PHB-rich biomass formulations, strongly indicate that (1) increased PHB content within the microbial cell, and thus decreased cellular debris content in the NFRTC, would measurably improve composite material properties and (2) the non-PHB cellular material does not offer comparable structural properties to wood flour.

3.3. Pure PHB-based composites

To better understand the potential effects microbial cell debris and wood content might have on composite material properties, pure PHB-based NFRTCs were produced. The experimental design matrix included composite formula-
facture of NFRTCs. For the PHB-rich biomass composites, wood flour appears to compensate for decreased polymer content (and an associated increase in microbial cell debris content) to maintain composite strength, although concurrently yielding a slightly stiffer composite. Improved composite material properties can be obtained through the use of PHB-rich biomass containing higher cellular polymer content. Recognizing that typical commercial PHB production yields significantly higher polymer content (Lee et al., 1999; Madison and Huisman, 1999), clearly there are opportunities to address this issue. Moreover, utilizing polymer-rich biomass containing PHA co-monomers (e.g., PHB-co-PHV) would be expected to yield significantly improved material properties. Finally, the microbial cell debris appears to convey material processing advantages in that significantly lower polymer content composites could be produced utilizing the PHB-rich biomass relative to the pure PHB NFRTCs. This is important because arguably there are applications even for the lower PHB-rich biomass formulations produced in this study.

While minimizing or mitigating environmental impacts is often cited as a driving factor in improving commodity manufacturing, ultimately cost competitiveness controls practice. Only through addressing the two factors together will we make significant strides toward minimizing anthropogenic impacts of manufacturing processes on the natural environment. By integrating unpurified PHB in NFRTC manufacturing to yield a product that is entirely biologically derived and biodegradable, we have concurrently addressed a significant energy and cost sink (Akiyama et al., 2003; Gerngross, 1999) in the polymer production process.

Further investigations are necessary to better understand the affects of cellular biomass versus conventional filler or fibers on the NFRTC material properties. For example, natural fibers have been shown to increase PHB crystallinity (Dufresne et al., 2003; Reinsch and Kelley, 1997), although it is not clear that the composite material properties are thus improved. Biomass may also potentially serve as a plasticizing agent in the composite formulations, and certain plasticizers have been shown to improve NFRTC material properties (Fernandes et al., 2004).

Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant Number DMI-0400337. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the funding agency.

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