Journal of Metals, Materials and Minerals, Vol.28 No.1 pp.52-61, 2018

Enhancement of mechanical, thermal and antibacterial properties of sisal/ polyhydroxybutyrate-co-valerate biodegradable composite

Thorsak KITTIKORN[1,*], Raminatun MALAKUL[2], Emma STROMBERG[3], Monica EK[3], and Sigbritt KARLSSON[3]

[1]*Department of Materials Science and Technology, Faculty of Science, Prince of Songkla University, Songkhla, 90112, Thailand*
[2]*Department of Microbiology, Faculty of Science, Prince of Songkla University, Songkhla, 90112, Thailand*
[3]*Department of Fibre and Polymer Technology, Royal Institute of Technology, Stockholm, 10044, Sweden*

***Corresponding author e-mail:** thorsak.k@psu.ac.th

Received date:
14 March 2018
Accepted date:
7 June 2018

Keywords:
Sisal
PHBV
Surface modification
Thermal properties
Antimicrobial

Abstract

Lignocellulosic biocomposite is a promising biodegradable materials, though improvement of the interfacial adhesion between cellulose fibre and polymer matrix is still challenged. Therefore, this work investigated the effect of propionylation of sisal reinforced fibre in the sisal/polyhydroxybutyrate-co-valerate (PHBV) biocomposite. Propionylation involved esterification substitution of propionic anhydride to hydroxyl group of sisal fibre, where ester group (COOR) of propionylated fibre was successfully observed by Fourier transform Infrared spectroscopy (FTIR). Then mechanical and thermal properties were evaluated and biodegradation characteristics were assessed. The tensile strength and modulus of propionylated sisal/PHBV biocomposite were greater than unmodified sisal/PHBV, which revealed better compatibility at the interface. In addition, propionate moieties of sisal fibre could induce crystalline formation of PHBV, as determined by an increase of crystalline phase. The higher decomposition temperature (Td) and activation energy (Ea) of 155 kJ·mol^{-1}, determined by thermal gravimetric analyser (TGA), were strong confirmation of good thermal resistance of the propionylated sisal biocomposite. The storage modulus, as characterized by dynamic mechanical thermal analyser (DMTA), also revealed the improvement of stiffness. Bacterial growth tests evaluated the inhibition of bacterial growth on the PHBV biocomposites. It was clear that propionylation of sisal fibre decreased colonization of *Staphylococcus aureus* (SA) and *Escherichia coli* (*E.coli*).

1. Introduction

Biodegradable polymers like polylactic acid (PLA) or polyhydroxyalkanoates (PHAs) are increasingly used in many applications. Derived from renewable resources, these polymers have many benefits for commodity plastics and their biodegradability offers sustainability for the environment, economy and society [1,2]. Of all PHAs, Polyhydroxybutyl-co-valerate (PHBV) receives most interest because its properties are closely comparable to polypropylene. Furthermore, because it is produced from microprobes of bacteria, it can biodegrade faster than polylactic acid [3]. These properties make it very attractive in various applications, particularly biomedical materials or packaging. However, since PHBV is still very expensive and degrades especially easily by enzymatic action [4,5] the incorporation of low cost antimicrobial lignocellulosic fibres in PHBV seems to be an appropriate method of enhancing its performance, especially, in long term usage. Although lignocellulosic fibres have been used in composites for many decades [6,7], due to their inherently hygroscopic nature, these cellulose fibres cause problems which require a further stage of processing before the final product is ready for use [8,9]. Therefore, the addition of hydrophobic lignocellulosic fibres, such as an esterified fibre, is able to prevent the deterioration of PHBV biocomposites because it can enhance the interfacial adhesion in these biocomposites [8,10,11]. In consequence, the mechanical and thermal properties of the biocomposites are also improved. Esterified fibres also reduce chemical hydrolysis because of the reduced water uptake [9,12] and can adequately prevent the growth of microbes such as fungi or bacteria on the biocomposite due to the increased hydrophobicity of the material's surface. As previously reported [13,14], the hydrophobicity of a polymer surface could prevent the formation of biofilm. Because the cell structure of biofilm

DOI: 10.14456/jmmm.2018.08

microbes mainly consists of protein, they adhere to and grow on a hydrophilic surface rather than a hydrophobic surface. Therefore, increasing the hydrophobicity of a PHBV biocomposite by reducing surface free energy is one possible method of preventing microbial growth and delaying enzymatic degradation during the service life of a product [15]. Thus, the aim of this work is to prepare and produce a sisal/PHBV biocomposite with improved mechanical, thermal and antibacterial properties to optimize its long term performance. Propionylation of fibre is a simple technique and is always used to improve fibre-polymer matrix interaction, especially in polyester biocomposites. However, it is quite rarely reported in research of lignocellulosic fibre/PHBV biocomposites. Hence, this work could elucidate the potential of propionylation to improve the mechanical, thermal and antimicrobial characteristics of a sisal/PHBV biocomposite. The results of this study provide information to be further evaluated for the development of PHBV biocomposite products such as packaging or household appliances [16].

2. Experimental

2.1 Materials

Polyhydroxybutyrate-co-velerate (PHBV), ENMAT Y1000P (grade of injection moulding) was purchased from TianAn Biopolymer (China). Sisal fibre was kindly provided by the Hubkapong royal project farm (Thailand). Propionic anhydride (Fluka, 96%) was used as a reagent for sisal modification. Acetone (96%) and NaOH were also used in modification process.

2.2 Chemical modifications of sisal fibre

Short sisal fibres (length of 3-5 mm) were treated in 6% NaOH solution (w/v) for 48 h under ambient temperature. Afterward, the fibres were neutralized using water and then dried in an oven at 80^{o}C for 12 h and kept in a desiccator for further process. The propionylation of fibres was carried out accordance with the procedure of a previous work [9]. After modification, the propionylated fibres were washed in hot acetone and water, respectively for removal the residual reagent and followed by drying at 80^{o}C for 24 h in an oven. After that, the fibres were kept in a plastic zip bag and stored in a dessicator.

2.3 Fabrication of biocomposites

2.3.1 Compounding process

PHBV pellets and the sisal fibres (both unmodified and propionylated) were dried in a vacuum oven at 60^{o}C for 12 h. After that, compounded biocomposites were prepared by mixing together between PHBV pellets and 10% by weight of unmodified or propionylated sisal fibres in an internal mixer (Brabender, Germany). The process parameters of screw speed, duration time of mixing and temperature were set at 50 rpm, 5 min and 185^{o}C, respectively.

2.3.2 Compression moulding

The compounded PHBV biocomposites were ground to small granules using a grinding machine. The granules were dried at 80^{o}C in a vacuum oven for at least 12 h before manufacturing in order for preventing a hydrolytic degradation due to residual moisture during the thermal process. The biocomposite sheets were fabricated using a compression moulding process. The granules were put in a mould with dimensions of 100 x 100 mm^2 and then placed in a press machine. The press was operated at a force of 100 kN, process temperature of 190^{o}C and holding time for 2 min, and then cooled by automatic water cooling.

2.4 Fourier-transform infrared spectroscopy (FTIR)

FTIR spectrometer of Bruker (model Tensor 27) was exploited for characterization of fibre structure and also evaluation of inhibition of biodegradation of PHBV biocomposites after bacterial growth test. Each sample was triply measured based on 16 scans with a resolution of 4 cm^{-1} in mode of attenuated total reflection (ATR)

2.5 Tensile test

The tensile properties of all biocomposites were determined by means of an Instron 5566 universal electromechanical testing machine (Instron Corporation, High Wycombe, UK). The tensile tests with a load cell of 1 kN were performed accordance with the ASTM D638 standard. The tensile strength, tensile modulus at 0.5% of tensile strain, and elongation at break were measured under

condition at 23°C and 55% relative humidity and a crosshead speed of 5 mm/min.

2.6 Thermal gravimetric analysis (TGA)

The thermal degradation of the biocomposites was determined using a Mettler Toledo TGA/SDTA 851 (Greifensee, Switzerland). Approximately 4-6 mg of each sample were heated from 35°C to 500°C in an alumina crucible (70 μL), at a heating rate of 10°C·min^{-1} in N_2 atmosphere. In order to study the kinetics of thermal decomposition, the non-isothermal method was exploited by varying the heating rates of 5, 10, 15 and 20°C·min^{-1}. The activation energy, Ea was determined accordance with the Flynn-Wall-Ozawa (FWO) method.

2.7 Differential scanning calorimetry (DSC)

The information of melting temperature (T_m) and polymer crystallinity were determined using a Mettler Toledo DSC820 calorimeter (Schwerzenbach, Switzerland) calibrated with indium standard. Approximately 5 mg of each sample were placed in a standard 40 μL aluminium crucible in an N_2 atmosphere with flow rate of 50 mL·min^{-1}. Both the first and the second heating runs were recorded and the heating/cooling rate used was ± 10°C·min^{-1}, from -20 to 200°C. The results were obtained from the first and the second heating run as an average value of three samples.

2.8 Dynamical mechanical thermal analysis (DMTA)

Specimens with dimension of 20 x 5 x 0.1 mm^3 were analysed using TA-instrument Q800 in tensile mode under multiple frequencies. Experiments were carried out at temperatures of -30°C to 140°C, with a heating rate of 3°C·min^{-1} and a soaking time of 5 min. The storage modulus, loss modulus as well as Tan δ vs. temperature were recorded and analysed.

2.9 Field emission scanning electron microscopy (SEM)

The cross section of PHBV biocomposites and degraded surfaces after bacterial growth test were monitored by means of a Field Emission Scanning Electron Microscope, JEOL model JSM 5800 (Tokyo, Japan). The samples were cut into small pieces and dried in a desiccator for 48 h prior to the analysis. The specimens were sputter-coated with a gold-palladium layer using a high resolution sputter coater, equipped with a thickness monitor controller.

2.10 Bacterial growth test

Staphylococcus aureus (SA) and *Escherichia coli* (*E. coli*) were chosen as the target bacteria to study the effect of fibre modification on bacterial growth. To determine CFUs of both bacteria, a diluting concentration technique was used. The experiment was carried out as follows. First, each PHBV biocomposite was cut into samples 2 x 3 cm^2 and put into glass tubes containing potato dextrose broth. The experiment was separated into two sets, one to test SA and one for *E. coli* and each set was composed of a control (blank), an unmodified sisal/PHBV sample and a propionylated sisal/PHBV sample. Each sample was tested in triplicate. Equivalent quantities of SA and *E. coli* were inoculated into the glass sample tubes. Then, the glass tubes were incubated for 18 h, 35°C in the incubator. After 18 h, the biocomposite samples were taken out of the glass tubes and cleaned using ethanol solution 70% (v/v). The samples were then kept in a desiccator at least 24 h before taking to characterization of SEM and FTIR, respectively. Meanwhile, the bacterial growth of each replicate was determined by calculating CFUs remaining in the glass tubes.

The original CFUs of SA and *E. coli* were calculated at 2.1 x 10^7 and 1.9 x 10^7, respectively.

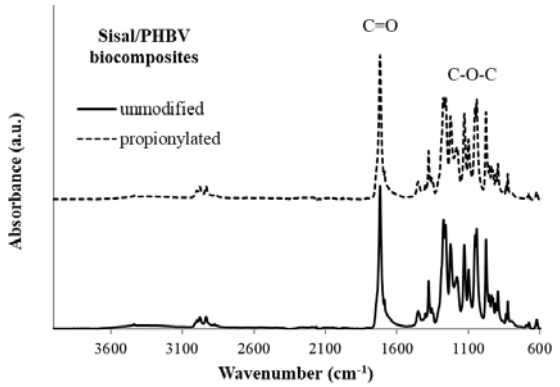

Figure 1. FTIR spectra of sisal fibre/PHBV biocomposites.

The FTIR spectra of both sisal/PHBV biocomposites before bacterial growth test, was presented in Figure 1.

3. Results and Discussion

3.1 Analysis of functional groups by FTIR

The new peaks of ester groups at 1745 cm^{-1} (Figure 2) were strong evidence of successful propionylation. Furthermore, the reduction of peak intensity of the hydroxyl group at 3400 cm^{-1} and 1660 cm^{-1} also confirmed that the hydroxyl group had been substituted by a propionate group. Actually, the loss of hydroxyl groups on the fibre surface was a necessary outcome for reducing water uptake and better interfacial interaction in the PHBV biocomposite.

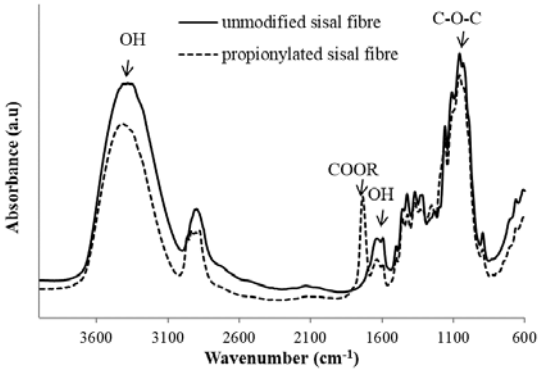

Figure 2. FTIR spectra of unmodified and propionylated sisal fibre.

3.2 Tensile properties

The stress-strain relationship as presented in Figure 3 and Table 1 showed the mechanical behaviour of PHBV biocomposites in terms of tensile properties. Propionylation of the sisal increased tensile strength, tensile modulus and also elongation at break. This was due to better interfacial adhesion after surface treatment of the sisal fibre. The increased tensile strength and modulus could indicate that the load transfer from the PHBV matrix into the propionylated fibre was more effective in reinforcing the composite than it was in the unmodified fibre composite. Additionally, the higher tensile strain (elongation at break) of the propionylated sisal/PHBV biocomposite also confirmed a good fibre dispersion and fewer voids in the PHBV matrix phase. These results confirm that propionylation of the sisal fibre reduced surface energy which led to enhanced interfacial adhesion between the two phases of the composite.

The higher tensile strength of the propionylated fibre/PHBV biocomposite was attributed to a better reinforcement produced by the propionylated fibre in the PHBV matrix.

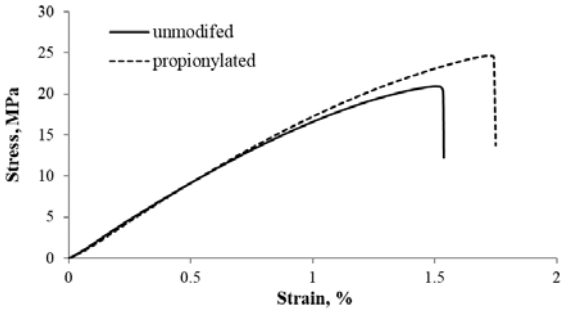

Figure 3. Stress-strain curve relationship of unmodified and propionylated sisal/PHBV biocomposites.

3.3 Analysis of thermal properties

The melting temperature of the composite of unmodified sisal fibre was similar to that of the propionylated sisal/PHBV biocomposite (Table 2). The slight increase of the crystalline domain (as calculated ($\Delta H_{f\,100\%}$ = 114 $J \cdot g^{-1}$) [17]) observed in the propionylated biocomposite could indicate that the propionate moiety of the modified cellulose influenced the induced crystalline arrangement. However, the TGA analysis revealed that the propionylation of cellulose improved thermal resistance compared to the unmodified cellulose biocomposite. The increase in decomposition temperature from 265ºC to 274ºC after modification, was attributed to the greater interfacial adhesion between the surface of the propionylated fibre and the PHBV matrix.

The SEM micrographs of cross sections of fractured biocomposites (Figure 4), show a large number of PHBV fragments covering the propionylated fibre surface. This morphology was very distinct from the appearance of the unmodified fibre which did not present those fragments of matrix on the fibre surface. These SEM results, could explain how the unmodified fibre loosely adhered to the PHBV matrix and had evidence of fibre pullout after failure. Therefore, propionylated sisal more effectively reinforced the PHBV biocomposite.

Table 1. Comparison of tensile properties of sisal/PHBV biocomposites.

Biocomposite	Tensile strength (MPa)	Tensile modulus (MPa)	Elongation at break (%)
unmodified	21 ± 2	1690 ± 90	1.5 ± 0.2
propionylated	26 ± 1	1920 ± 40	1.8 ± 0.1

Table 2. Information of thermal properties of sisal/PHBV biocomposites.

Biocomposite	Tm (°C)	ΔH_f (J·g^{-1})	% crystallinity	Td (°C)	Ea (kJ·mol^{-1})
unmodified	172	77	76	265	134
propionylated	172	80	78	274	155

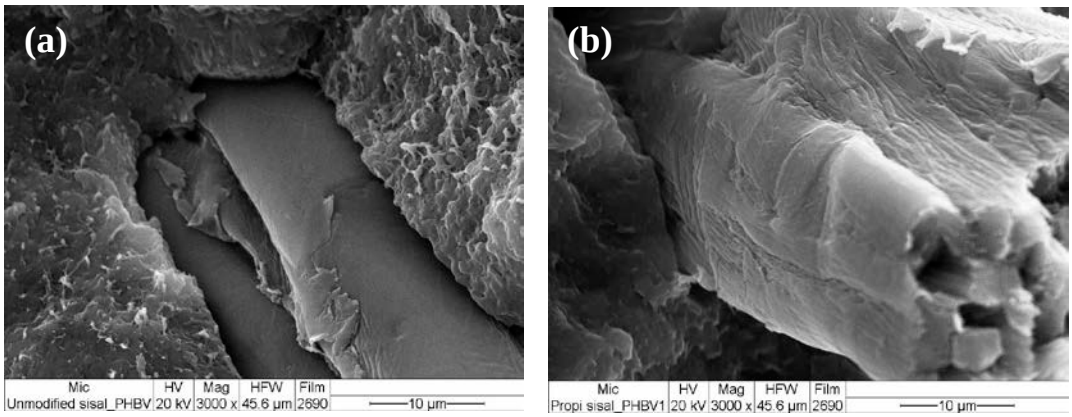

Figure 4. SEM micrographs of cross-section of (a) unmodified sisal/PHBV and (b) propionylated sisal/PHBV.

3.4 Kinetic study of thermal degradation

One of the methods used to evaluate the thermal performance of the biocomposites was the estimation of the energy required for the decomposition of the biocomposite by calculation of activation energy (E_a) in thermal decomposition. Basically, the thermal resistance of polymers is one of the crucial properties that affect many aspects of polymeric performance such as mechanical and thermal properties, transport phenomena or even degradation. Hence, determination of thermal resistance in terms of activation energy can determine some of the properties of polymers, particularly, resistance to polymeric degradations and also long-term durability. This experiment used the Flynn-Wall-Ozawa (FWO) method to calculate the value of E_a in the PHBV biocomposites, as illustrated in Figure 5.

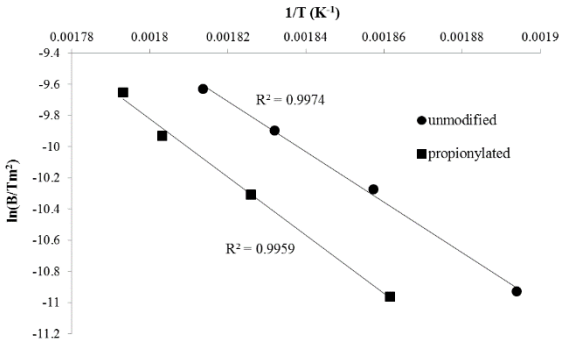

Figure 5. Relationship between $\ln\left(\frac{\beta}{Tm^2}\right)$ and $\left(\frac{1}{Tm}\right)$ using FWO method.

The activation energy of decomposition (E_a) was calculated via a kinetic study of non-isothermal

degradation. A maximum rate method was used and calculations were made from the equation $\ln\left(\frac{\beta}{Tm^2}\right) = \ln\left(\frac{A.R}{Ea}\right) - \left(\frac{Ea}{R.Tm}\right)$, where β and Tm were heating rate and temperature at maximum rate of decomposition, respectively. The E_a was then calculated from the slope of the linear plot.

The value of E_a of PHBV was presented previously by Kulkarni et al. [18] and estimated at 129 kJ·mol^{-1}. Meanwhile, Moliner et al (2018) studied and evaluated the kinetic decomposition of sisal/PHBV biocomposite through the non-isothermal technique of thermal gravimetric analysis, it was appeared that the E_a of unmodified sisal/PHBV biocomposite was 137 kJ·mol^{-1} [19]. In this study, the E_a of the unmodified and propionylated sisal/PHBV biocomposites were equal to 134 and 155 kJ·mol^{-1}, respectively. The increased E_a in the propionylated sisal biocomposite could be attributed to the higher thermal energy of decomposition (Td range ~ 300-320^{o}C) of the incorporated propionylated sisal fibre. However, considering the effect of surface modification, the increase in E_a to 155 kJ·mol^{-1} could result from the better interfacial adhesion described earlier. This means that the propionylation of cellulosic fibre not only improves mechanical and thermal properties but it also increases the thermal barrier of the biocomposite, thus extending the range of conditions tolerated in applications.

3.5 Dynamic mechanical thermal analysis (DMTA)

The behavior of the mechanical – thermal relationships in the PHBV biocomposites was explained through DMTA analysis. Propionylation of the sisal fibre improved the reinforcement of the PHBV biocomposite by increasing the storage modulus by roughly 25% (Figure 6). Additionally, it was also observed that the Tan δ (indicating glass transition temperature (Tg)) rose to 38^{o}C following the incorporation of propionylated sisal in PHBV, while Tan δ of the unmodified biocomposite was 29^{o}C. The substantial increase in Tg observed in the propionylated biocomposite confirmed the interaction of the fibre and matrix phases at the molecular level. This was possibly due to dipole – dipole interaction between the propionate group of the fibre and the ester group of PHBV in backbone chains. Moreover, a lower value of Tan δ in the propionylated biocomposite would indicate that its mechanical behavior tended to be governed by elasticity. In accordance with the expression of Tan δ (E''/E', loss modulus/storage modulus), a greater value of Tan δ would simply indicate that the unmodified biocomposite was performing like a viscous material because applied force energy was dissipated in a softer phase. Conversely, a low value of Tan δ could reflect that the modified biocomposite still exhibited the behavior of elasticity due to good interfacial adhesion with the propionylated sisal. However, above 51^{o}C, the value of Tan δ of the propionylated biocomposite was higher than that of the unmodified biocomposite. This might be caused by a disruption of dipole-dipole interaction as temperature increased. Interestingly, at around 61^{o}C a small bulb hill appears in the spectrum representing Tan δ of the propionylated biocomposite. This bulb hill was crucial evidence of a second Tg for the PHBV matrix. Although this behavior occurred at 41^{o}C in the unmodified biocomposite, the occurrence of the same behavior in the propionylated biocomposite at the substantially higher temperature of 61^{o}C confirmed the existence of considerable interfacial interaction. In consequence, the crystallinity of the PHBV matrix was induced and restricted the mobility of molecular chains. This was a reason why the stiffness of the biocomposite increased after incorporating propionyled sisal.

3.6 Evaluation of biodegradation of PHBV biocomposites through a bacterial growth test

In order to evaluate the possible application of PHBV biocomposites in food packaging, a bacterial growth test of *Staphylococcus aureus* (*SA*) and *Escherichia coli* (*E.coli*) was carried out to assess the bacterial resistance and biodegradation of the PHBV biocomposites.

The ability of the propionylated fibre in the biocomposite to inhibit *SA* and *E.coli* growth was evaluated via a bacterial growth test. The numbers of CFUs of bacteria after culturing for 24 h in broth with different types of PHBV biocomposites are presented in Figure 7.

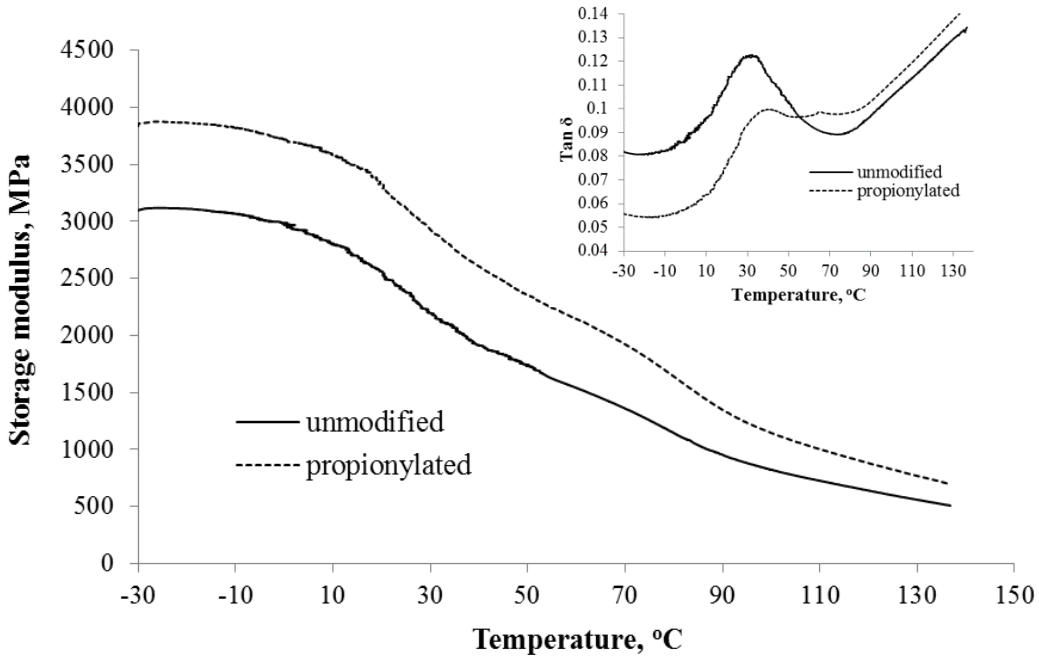

Figure 6. DMTA spectra (a) unmodified sisal/PHBV and (b) propionylated sisal/PHBV.

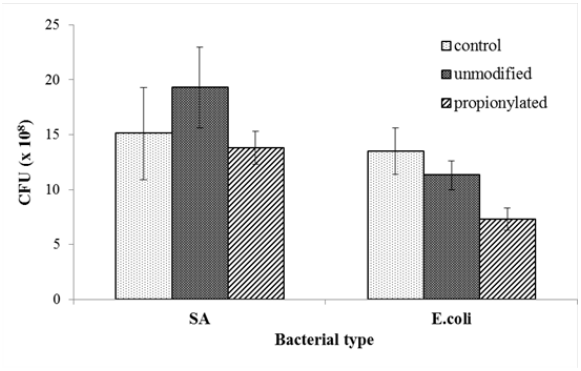

Figure 7. Numbers of CFUs of *Staphylococcus aureus* (*SA*) and *Escherichia coli* (*E.coli*) after culturing for 18 h with different PHBV biocomposites.

After culturing with the propionylated sisal/PHBV biocomposite, the detected numbers of CFUs of *E.coli* were significantly lower than they were after culturing with the control. Meanwhile, in case of *SA*, there was no significant difference between the control and the propionylated sisal/PHBV biocomposite. Additionally, compared to the unmodified sisal/PHBV biocomposite culture, the propionylated sisal/PHBV was more able to suppress the bacterial growth of *SA* and *E. coli*. Since the fewest CFUs of *SA* and *E. coli* were observed on the propionylated sisal/PHBV, this could be evidence that the growth of both bacteria was inhibited on this type of biocomposite. Conversely, the substantial increase of CFUs on the unmodified sisal/PHBV relative to the control, particularly obvious in the *SA* test, was strong confirmation that *SA* preferred to colonize the unmodified biocomposite.

The pattern of bacterial growth of *SA* and *E.coli* on the PHBV biocomposites was also assessed from SEM images. The SEM images (Figures 8 and 9) reveal significant differences in the patterns of bacterial growth of *SA* and *E.coli* on the unmodified sisal/PHBV and propionylated sisal/PHBV.

Clearly, SA and *E. coli* could grow on and biodegrade both these PHBV biocomposites. Equally clearly, comparison of the SEM images in Figures 8(a1-2) and 9(a1-2) and Figures 8(b1-2) and 9(b1-2) shows that the invasion of bacterial microprobes of both *SA* and *E. coli* was more severe on the unmodified sisal/PHBV biocomposite. The appearance of a large amount of both bacteria on the unmodified biocomposite confirmed that *SA* and *E. coli* preferred to colonize the unmodified biocomposite. In addition, the voids surrounding *SA* spores on the unmodified biocomposite (Figure 8(a2)) are evidence of the extreme biodegradation of the unmodified biocomposite by *SA*. These bacteria biodegrade polymer biocomposites by excreting extracellular enzymes such as esterase or cellulase to convert polymers into digestible oligomers for mineralization and the visible erosion of the surface of the unmodified biocomposite (Figures 8(a1) and 9(a1)) is strong evidence that this process had taken place. Contrastingly, the lower

numbers of *SA* and *E. coli* on the propionylated sisal/PHBV biocomposite (Figures 8(b2) and 9(b2)) indicated that the bacterial growth on the propionylated biocomposite had occurred at a lower rate. Actually, both PHBV and sisal fibre are natural nutrients for microprobes like bacteria or fungi and so, under the conditions of the bacterial growth test, both the PHBV biocomposite could be degraded by enzymatic action.

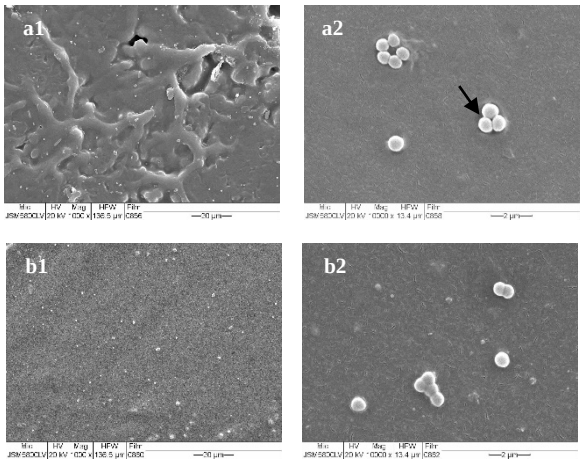

Figure 8. SEM images of bacterial growth (*Staphylococcus aureus* (*SA*)) cultured in broth, (a1-2) unmodified sisal/PHBV and (b1-2) propionylated sisal/ PHBV.

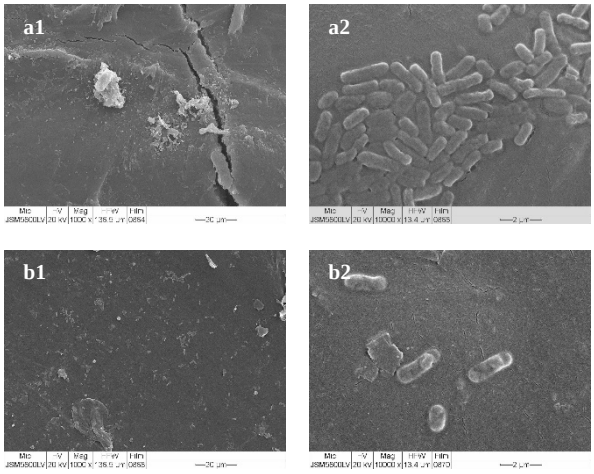

Figure 9. SEM images of bacterial growth (*Escherichia coli* (*E. coli*)) cultured in broth, (a1-2) unmodified sisal/PHBV and (b1-2) propionylated sisal/PHBV.

The FTIR spectra of the composites after the SA bacterial growth test (Figure 10a) also confirmed that the unmodified sisal/PHBV biocomposite was more severely biodegraded than the propionylated sisal/PHBV. The obvious decline of the ester band at 1745 cm^{-1} with an increase of the hydroxyl band at 3400 cm^{-1} in the unmodified sisal/PHBV biocomposite (Figure 10a) indicates an extreme enzymatic degradation that hydrolyzed the PHBV backbone chain at the ester group. Meanwhile the slight change in the FITR spectrum of the propionylated sisal/PHBV indicates a greater resistance to biodegradation. However, in the case of the *E. coli* test (Figure 10b), only a slight difference can be observed between the peaks of the FTIR spectra of unmodified and propionylated sisal/PHBV.

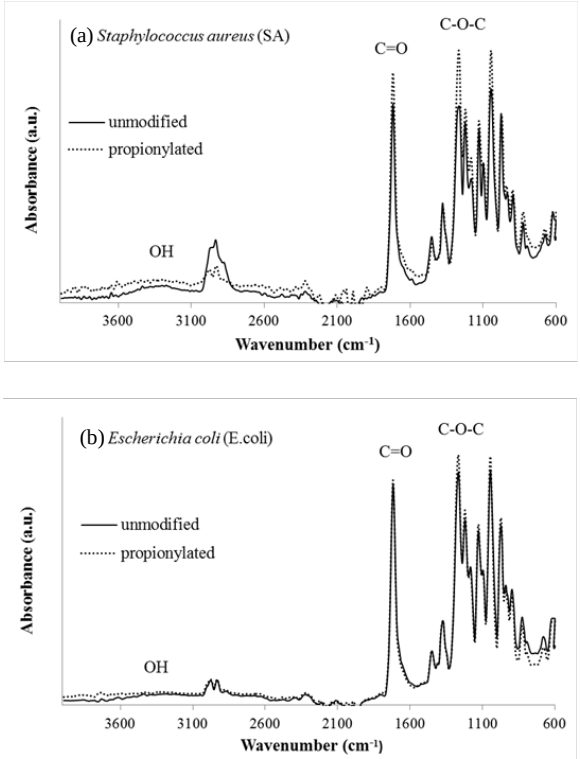

Figure 10. FTIR spectra of PHBV biocomposites after bacterial growth test, (a) *Staphylococcus aureus* (SA) and (b) *Escherichia coli* (E.coli).

The surface properties of the PHBV biocomposites could be one explanation of how the propionylation of fibres could inhibit bacterial growth and then resist biodegradation. The wide extent of bacterial spores on the surface of the unmodified sisal/PHBV indicated that the surface property of the unmodified biocomposite facilitated biodegradation while the surface property of the propionylated sisal/PHBV did not. As discussed previously, fewer bacterial spores on a less eroded surface, as observed on the propinylated biocomposite, could indicate that the surface property is unsuitable for bacterial colonization.

One possible reason for this phenomenon is the hydrophobic-hydrophilic property of the biocomposite, which is usually explained in terms of surface energy.

The relationship between surface energy and bacterial growth on the biocomposites was determined by the surface free energy of the PHBV biocomposites, evaluated through the contact angle test. The values of the surface energy of each biocomposite indicate that the propionylation of sisal fibres could reduce the hydrophilicity of the PHBV biocomposite. The increase of surface energy in the dispersion part and the decreased surface energy in the polar part (Table 3) indicate that the propionylated sisal was more compatible with the PHBV matrix and also reduced the hygroscopic property of the biocomposite. Samuelsson and Kirchman [15] reported that the hydrophobicity of plastics delayed bacterial growth more than the surface of hydrophilic polymers. Because the structure of bacterial cells is mainly composed of proteins, microbes commonly prefer to attach and grow colonies on the hydrophilic surface of polymers. On the other hand, this behavior is rarely seen on hydrophobic polymers because their surfaces are unsuitable for microbe growth. This means that we can extend the service life time of biocomposites by increasing the hydrophobicity of the surface to disrupt the mechanism of bacterial growth.

Table 3. Surface free energy of PHBV biocomposites.

PHBV biocomposite	Dispersion ($J \cdot m^{-1}$)	Polar ($J \cdot m^{-1}$)	Total ($J \cdot m^{-1}$)
Unmodified	20	14	34
Propionylated	22	11	33

4. Conclusions

4.1 The propionylation of sisal fibre produced an improvement of the mechanical and thermal properties of PHBV biocomposite due to an improved interfacial adhesion between the modified fibre and the PHBV matrix.

4.2 The improvement of the interfacial adhesion of the fibre-matrix phase by propionylation could increase the glass transition temperature as well as the activation energy of thermal decomposition. These improvements indicated good long-term stability of the biocomposite.

4.3 The improved hydrophobicity of the propionylated sisal/PHBV biocomposite, determined by surface energy, delayed degradation of the biocomposite due to reduced water uptake and opportunity for microorganisms.

4.4 The PHBV biocomposites were subjected to bacterial growth tests with *Staphylococcus aureus* (*SA*) and *Escherichia coli* (*E.coli*) to compare their ability to restrict bacterial growth. The propionylated sisal/PHBV biocomposite showed a better inhibition of bacterial colonization.

4.5 Surface erosion of the unmodified sisal/PHBV after bacterial growth was clearly observed over a wide area. This meant that substantial enzymatic degradation occurred on the unmodified sisal/PHBV. However, this pattern was less obvious on the surface of the propionylated PHBV, indicating that the propionylated PHBV could adequately prevent enzymatic degradation.

5. Acknowledgements

The authors gratefully acknowledge Prince of Songkla University, Thailand for their financial support of this research. The authors also would like to thank Department of Fibre and Polymer Technology, Royal Institute of Technology (KTH), Sweden for the kind provision of thermally analytical instruments.

References

[1] F. P. La Mantia and M. Morreale, "Green composites: A brief review," *Composites Part A: Applied Science and Manufacturing*, vol. 42, pp. 579-588, 2011.

[2] A. K. Mohanty, M. Misra, and L. T. Drzal, "Sustainable bio-composites from renewable resources: opportunities and challenges in the green materials world," *Journal of Polymers and the Environment*, vol. 10, pp. 19-26, 2002.

[3] R. A. J. Verlinden, D. J. Hill, M. A. Kenward, C. D. Williams, and I. Radecka, "Bacterial synthesis of biodegradable polyalkanoates,"

Journal of Applied Microbiology, vol. 102, pp. 1437-1449, 2007.

[4] Y. X. Weng, Y. Wang, X. L. Wang, and Y. Z. Wang, "*Biodegradation behavior of PHBV films in a pilot scale composting condition," Polymer Testing*, vol. 29, pp. 579-587, 2010.

[5] K. C. Batista, D. A. K. Silva, D. A. K. Silva, L. A. F. Coelho, and S. H. Pezzin, " Soil biodegradation of PHBV/peach palm particles biocomposites," *Journal of Polymers and the Environment*, vol.18, pp. 346-354, 2010.

[6] A. K. Bledzki and A. Jaszkiewicz, "Mechanical performance of biocomposites based on PLA and PHBV reinforced with natural fibres-a comparative study to PP,"*Composites Science and Technology*, vol.70, pp. 1687-1696, 2010.

[7] O. Faruk, A. K. Bledzki, H. P. Fink, and M. Sain, "Biocomposites reinforced with natural fibres: 2000–2010," *Progress in Polymer Science*, vol. 37, p p. 1552-1596, 2012.

[8] T. Kittikorn, E. Strömberg, and S. Karlson, "The effect of surface modifications on the mechanical and thermal properties of empty fruit bunch oil palm fibre PP biocomposites," *Polymer from Renewable Resources*, vol. 3, pp. 79-100, 2012.

[9] T. Kittikorn, E. Strömberg, M. Ek, and S. Karlsson, "Comparison of water uptake as function of surface modification of empty fruit bunch oil palm fibres in PP Biocomposites," *BioResources*, vol. 8, pp. 2998-3016, 2012.

[10] H. P. S. Abdul Khalil, N. L. Suraya, N. Atiqah, M. Jawaid, and A. Hassan, "Mechanical and thermal properties of chemical treated kenaf fibres reinforced polyester composites," *Journal of Composite Materials*, vol. 47, pp. 3343-3350, 2012.

[11] L. Petersson, K. Oksman, and A.P. Mathew, "Using maleic anhydride grafted poly(lactic acid) as a compatibilizer in poly (lactic acid)/layered-silicate nanocomposites," *Journal of applied polymer science*, vol. 102, pp. 1852-1862, 2006

[12] M. S. Sreekala and S. Thomas, "Effect of fibre surface modification on water-sorption characteristics of oil palm fibres," *Composites Science and Technology*, vol. 63, pp. 861-869, 2003.

[13] S. H. Lee and S. Wang, "Biodegradable polymers/bamboo fibre biocomposite with bio-based coupling agent," *Composites Part A: Applied Science and Manufacturing*, vol. 37, pp. 80-91, 2006.

[14] S. Wallström, E. Strömberg, and S. Karlsson, "Microbiological growth testing of polymeric materials: an evaluation of new methods," *Polymer Testing*,vol. 24, pp. 557-563,2005.

[15] M. O. Samuelsson and D. Kirchman, "Degradation of adsorbed protein by attached bacteria in relationship to surface hydrophobicity," *Applied and Environmental Microbiology*, vol. 56, pp. 3643-3648, 1990.

[16] J. W. Rhim, H. M. Park, and C. S. Ha, "Bio-nanocomposites for food packaging applications," *Progress in Polymer Science*, vol. 38, pp. 1629-1652, 2013.

[17] N. M. Barkoula, S. K. Garkhail, and T. Peijs, "Biodegradable composites based on flax/polyhydroxybutyrate and its copolymer with hydroxyvalerate," *Industrial Crops and Products*, vol. 3, pp. 34-42, 2010.

[18] S. O. Kulkarni, P. P. Kanekar, S. S. Nilegaongar, S. S. Sarnaik, and J. P. Jog, "Production and characterization of a biodegradable poly(hydroxybutyrate-co-hydroxyvalerate) (PHB-co-PHV) copolymer by moderately haloalkalitolerant Halomonas campisalis MCM B-*1027* isolated from Lonar Lake, India," *Bioresource Technology*, vol. 101, pp. 9765-9761, 2010.

[19] C. Moliner, J. D. Badia, B. Bosio, E. Aratoa, T. Kittikorn, E. Strömberg, R. Teruel-Juanes, M. Ek, S. Karlsson, and A. Ribes-Greus, "Thermal and thermo-oxidative stability and kinetics of decomposition of PHBV/sisal composites," *Chemical Engineering Communications*, vol. 205, pp.226-237, 2018.