*บทความวิจัย*

# การใช้ไคโตซานและอนุพันธ์ในการยับยั้งการเจริญของเชื้อรา *Aspergillus niger* บนไบโอคอมพอสิตพอลิแล็กติกแอสิด/ป่านศรนารายณ์

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## บทคัดย่อ

ปัจจุบันการใช้พลาสติกจำนวนมหาศาลประกอบกับการจัดการรีไซเคิลที่ไม่สมดุล ส่งผลให้พลาสติกกลายเป็นประเด็นปัญหาสิ่งแวดล้อมระดับโลก ไบโอคอมพอสิตหรือวัสดุเชิงประกอบฐานชีวภาพจึงเป็นวัสดุที่ได้รับการพัฒนาเพื่อมุ่งลดและแทนที่การใช้วัสดุเชิงประกอบฐานปิโตรเคมีและใยแก้ว อย่างไรก็ตาม ปัญหาในการพัฒนาไบโอคอมพอสิต คือ ความเข้ากันได้ระหว่างสารเติมแต่งที่มาจากชีวมวลกับเนื้อพื้นพอลิเมอร์ งานวิจัยครั้งนี้จึงมุ่งพัฒนาไบโอคอมพอสิตจากพอลิแล็กติกแอสิด (poly (lactic) acid, PLA) ซึ่งเป็นพลาสติกย่อยสลายได้ และเสริมแรงด้วยเส้นใยป่านศรนารายณ์ ซึ่งเป็นวัสดุชีวมวล และศึกษาการใช้ไคโตซานและอนุพันธ์ประเภทบิวไทเรตในการยับยั้งการเจริญของเชื้อรา โดยใช้เครื่องมือในการวิเคราะห์สมบัติทางความร้อน โครงสร้างและสัณฐานวิทยาของวัสดุไบโอคอมพอสิตที่เตรียมขึ้น ได้แก่ Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), differential scanning calorimeter (DSC) และ thermogravimetric analysis (TGA) ผลการศึกษาพบว่า การดัดแปรเส้นใยป่านศรนารายณ์และไคโตซานด้วยการทำปฏิกิริยาเอสเทอริฟิเคชั่น กับบิวไทริกแอนไฮไดรด์ ช่วยปรับปรุงความเข้ากันได้ระหว่างป่านศรนารายณ์ที่เติมเข้าไปกับเนื้อพื้น PLA ได้ดี เห็นได้จากภาพ SEM ทำให้อุณหภูมิการเกิดผลึก (Tc) เพิ่มขึ้น ในขณะที่ระดับความเป็นผลึก (%Xc) ลดลง หลังการทดสอบเพาะเชื้อ *Aspergillus niger* พบว่า ไบโอคอมพอสิตเกิดการเสื่อมสภาพเนื่องจากเอนไซม์ สังเกตได้จากการที่หมู่เอสเทอร์ของ PLA ลดลง แต่เกิดหมู่ไฮดรอกซิลขึ้นมาแทน ในขณะที่ไบโอคอมพอสิตที่เติมไคโตซานไม่มีหมู่ไฮดรอกซิลเกิดขึ้น จะเห็นได้ว่า ไคโตซานและอนุพันธ์บิวไทเรตไคโตซานมีผลในการยับยั้งการเจริญของเชื้อราบนวัสดุไบโอคอมพอสิต PLA/ป่านศรนารายณ์

**คำสำคัญ:** ไบโอคอมพอสิต วัสดุคอมพอสิตชีวภาพ พลาสติกย่อยสลายได้ทางชีวภาพ ไคโตซาน การยับยั้งเชื้อรา

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# Use of Chitosan and Its Derivative as Fungal *(Aspergillus niger)* Inhibitor on Poly(lactic) acid/ Sisal Biocomposite

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## ABSTRACT

Presently, massive plastic consumption and unbalanced recycle management lead us to an environmentally global issue. Biocomposite has been considered as beneficial material to reduce and replace typical glass fibre reinforced composite derived from petrochemicals. However, one major concern when developing biocomposite is compatibility between biomass-based reinforced filler and polymeric matrix. The objectives of this research were to develop a biocomposite composed of poly(lactic) acid (PLA), a biodegradable plastic, and biomass-based reinforced filler, sisal, and study the role of chitosan and its butyrate modified derivative as fungal inhibitors. Several techniques, such as Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), differential scanning calorimeter (DSC), and thermogravimetric analysis (TGA) were utilized to investigate the chemical structure, surface morphology, thermal and mechanical behaviour of PLA/sisal biocomposite. It is shown in SEM images that the modification of chitosan and sisal filler through butyric anhydride esterification, improves compatibility between sisal reinforced filler and PLA matrix, resulting in higher crystallization temperature (Tc), and lower %crystallinity (%Xc). After *Aspergillus niger* culture test, enzymatic degradation of biocomposite can be noticed from the reduction of ester group of PLA and the addition of hydroxyl group. However, the antifungal activities of all PLA/sisal biocomposites with chitosan added are improved where no identical hydroxyl group is observed after the tests. As a result, chitosan and its butyrate modified derivative are effective antifungals for PLA/sisal biocomposite.

**Keyword:** biocomposite, biodegradable plastic, chitosan, antifungal

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## Introduction

Plastic material has been widely used in several applications since it was discovered in the world, from wrapped film in a kitchen, household appliances, and mobile cell phone to aerospace parts. Lightweight, easy processability and productivity, wide range of grade and quality are major advantages of plastic that lead to massive consumption every year. But presently, plastic material has been focused as environmentally global issues, though the problem of plastic utilizations is recycle management. Biodegradable plastic like poly(lactic) acid (PLA), poly(butylene) succinate (PBS), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) has become alternative material for ecological concern. Their specific properties are continually developed for appropriate applications, meanwhile, biomass-based composite has been proceeded to function as a biodegradable material. Under green society concept, several plants have been utilized as a reinforced filler in composite, such as cotton, hemp, sisal, jute, abaca, pineapple, banana, flax, oil palm empty fruit bunch, etc., [1-6] in order to reduce and replace typical glass fibre reinforced composite derived from petrochemicals. Cellulose, the major component of plants, not only enhances the strength of composite, but also promotes the biodegradable process [7-8]. Naturally, cellulose is a carbon source for microorganism, i.e. bacteria, and fungi. However, an applicable lifetime of cellulose reinforced composite cannot be neglected. Thus, the development of biodegradable composite should be comparable to their applications and enough strength at a certain period.

Due to a hot humid climate in Thailand, biocomposite may absorb moisture from surroundings, which leads to swelling and hydrolysis of the polymer matrix [5-6]. Then microorganisms grow up, resulting in the strength loss of composite itself and ultimately broken down [9-10]. Consequently, many researchers have emphasized to improve moisture resistance so as to reduce swelling of biocomposite and inhibit the growth of microorganism.

One of the most important factors in developing biocomposite is the compatibility between cellulose reinforced filler and polymer matrix, which determine the reinforcement efficacy. Various active compounds such as anhydride, i.e. acetic anhydride, benzoic anhydride; polyether, i.e. poly(glycidyl) ether, poly(propylene) oxide, have been used to modify a surface of cellulose as well as grafting cellulose with a silane coupling agent [4, 6, 8]. These modifications enhance the hydrophobicity of fibre, improve fibre-matrix compatibility, lessen void between filler and matrix, and ease fibre dispersion, resulting in better effective reinforcement. The less water is absorbed by fibre, the more hydro and heat resistances of composite are obtained accordingly [5]. Nevertheless, biodegradation of biocomposite is yet taken place, and antimicrobial treatment is another means to inhibit the growth of microorganisms. It was reported that chitosan, a naturally biological pesticide produced from an outer shell of crustaceans, effectively retarded fungal spread on biocomposite [11-13].

Since chitosan is hydrophilic and low heat resistance, there will be some troubles during melt mixing with a polymeric matrix, leading to phase separation at last. Esterification of the chitosan surface will reduce surface free energy, which enhances binding between chitosan and polymer, as well as dispersion of both components [14-15].

In this research, biocomposite of PLA and sisal was prepared where chitosan was applied as a fungal inhibitor. Sisal and chitosan were surface modification with butyric anhydride to improve their adhesion to PLA matrix [5]. Then, *Aspergillus.niger,* a fungus that caused black mold on certain fruits and vegetables, was exploited to observe the antifungal activity of the biocomposite in accordance with ISO-846: 1998 standard testing method [16]. Thermal and mechanical behaviour, chemical structure and surface morphology of the obtained biocomposite were properly analyzed. This research studied the role of chitosan as antifungal inhibitor on PLA/sisal biocomposite, in addition to chemical modification of sisal fibre and chitosan with butyric anhydride.

## Materials and Methods

### 1. Materials

PLA, a biodegradable polymer, injected grade 3052D was purchased from Nature Works, USA. Sisal fibre was obtained from Hub Krapong, the Royal Project, Thailand. Butyric anhydride, AR grade 97% from Sigma Aldrich, USA, and chitosan, commercial grade from Sea fresh company, Thailand were used as received. Aspergillus niger (TISTR 3153) was purchased from Thailand Institute of Scientific and Technological Research.

### 2. Esterification of sisal fibre and chitosan

The esterification procedure was modified from previous work [5]. Sisal fibre was immersed in 6.0 wt% sodium hydroxide aqueous solution for 48 hours to remove non-cellulosic components, and then thoroughly washed in water until neutral. Next, alkaline treated fibre was dried and ground to 1.0 to 2.0 mm length by a cutting mill. After that, it was reacted with butyric anhydride at 120°C for 2 hours in an oven, and washed with acetone to remove excess butyric anhydride. Chitosan was also modified by the same procedure.

### 3. Fabrication of PLA/sisal biocomposite

In order to get well dispersed and even mixed result, PLA/sisal biocomposite was dry-mixed in an internal mixer, called Brabender. Firstly, all components were oven dried at 80°C for 6 hours, then melted in Brabender at 180°C, 50 rpm for 5 minutes. The ratios of mixing are shown in Table 1. Each blend was crushed into small pieces by using a milling

machine, followed by hydraulic pressing to $10 \times 10 \times 0.5$ cm$^3$ sheet. The compression temperature was at 200$^{\circ}$C under 5 tons pressure for 2 minutes [6]. The obtained biocomposite sheets were then cut to small pieces of $3 \times 3$ cm$^2$ for fungal growth test.$^{3}\times^{\circ}{}_{1}^{2}$

**Table 1** Mixing ratio of PLA/sisal biocomposite.

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| bio-composite | mixing ratio of PLA/sisal biocomposite (wt%) | | | | |
|---|---|---|---|---|---|
| | **PLA** | **sisal** | **butyrate sisal** | **chitosan** | **butyrate chitosan** |
| S-PLA | 90 | 10 | 0 | 0 | 0 |
| S-C-PLA1 | 85 | 10 | 0 | 5 | 0 |
| S-C-PLA2 | 85 | 0 | 10 | 5 | 0 |
| S-C-PLA3 | 85 | 0 | 10 | 0 | 5 |

## 4. Characterization

### 4.1 Fourier transform infra-red spectroscopy (FTIR)

The chemical modifications of sisal fibre and chitosan were investigated by using FTIR with an attenuated total reflectance (ATR) device, as well as the changes of chemical structure of biocomposite surface before and after a fungal growth test. The FTIR was run in the range of wavenumber 4,000 to 600 cm$^{-1}$, average 32 scans, and 4 cm$^{-1}$ resolution.

### 4.2 Bio-degradation evaluation by fungal growth test

The bio-degradation of PLA/sisal biocomposite was conducted according to the standard testing method ISO 846: 1997, section B. The spore suspension containing $4.5 \times 10^6$ spore/ml of *Aspergillus niger* was prepared, then amount of 100 µl aliquot was inoculated onto each of $3 \times 3$ cm$^2$ of biocomposite specimen, and placed on a nutrient agar plates. All plates were constantly incubated at 31$^{\circ}$C, 85% relative humidity for 28 days, with 3 replicates.

### 4.3 Differential scanning calorimeter (DSC)

Thermal properties of biocomposite were studied such as glass transition temperature (Tg), crystallization temperature (Tc), melting temperature (Tm) with differential scanning calorimeter (Netszch, 200F3, Germany). The temperature range was 27 to 200$^{\circ}$C with 10$^{\circ}$C/minute under nitrogen atmosphere, and the specimen weight was 7 to 10 mg. The degree of crystallinity was calculated from the following equation:

$$\% \text{ crystallinity} = \frac{\Delta H_f}{\Delta H_{100\%}} \times 100 \quad (1)$$

where $\Delta H_f$ was enthalpy of melting crystal (the area under heated peak)
$\Delta H_{100\%}$ was enthalpy of reference PLA ($\Delta H_{100\%}$ of PLA = 93.1 J/g)

### 4.4 Thermogravimetric analysis (TGA)

Thermal degradation behaviour of biocomposite was evaluated by using thermogravimetric analysis (TGA, Jupiter model 449 F3, Netszch, Germany). The heating temperature was 25 to 500°C, at 10°C/minute under nitrogen atmosphere.

### 4.5 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM, JEOL, model JSM-5800, Japan) was used to investigated surface morphology of biocomposite including cracked area and cross section of specimens. The biocomposite was cut into $5 \times 5$ $mm^2$ and sputtered with gold vapor, then observed under 15 keV.

In order to investigate the deteriorated surface of PLA/sisal biocomposite after fungal growth test described in 4.2, all specimens were thoroughly soaked in 5 ml of 70% (v/v) of ethanol solution for 10 seconds 3 times. Therefore the fungal hyphae were gently removed from the surface, and the specimens were cut into $5 \times 5$ $mm^2$ samples and gold-sputtered for further observation by SEM.

## Results

### 1. FTIR analysis of sisal and chitosan

FTIR spectra of sisal fibre and butyrate modified sisal are shown in Fig. 1. There was no typical bands of ester group (C=OR) around 1740 $cm^{-1}$ representing hemicelluloses and aromatic components of lignin observed on alkaline treated sisal [17]. After esterification, prominent peaks were observed at 1741, 2972, and 1380 $cm^{-1}$, which corresponded to C=O of ester group, C-H stretching and C-H bending of methyl group ($CH_3$) from anhydride, respectively. This observation indicated a successful modification of sisal fibre.

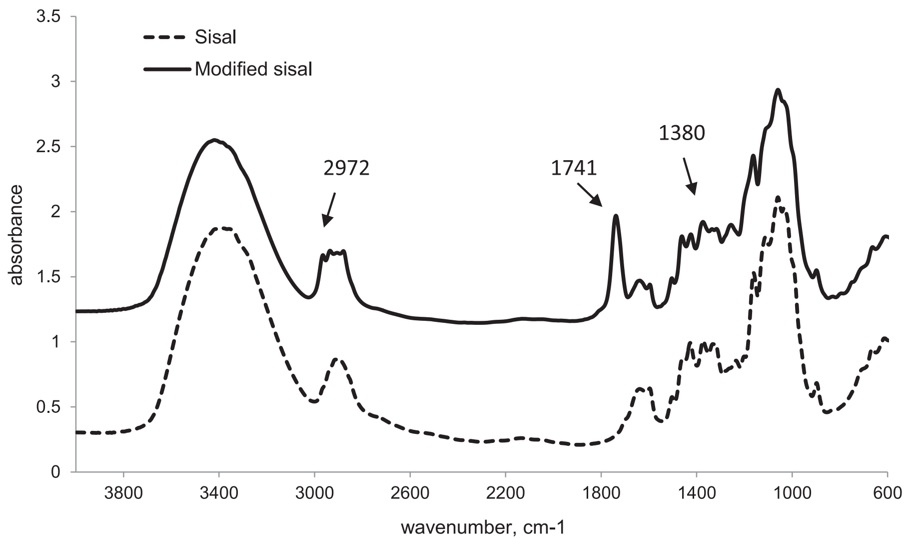


**Figure 1** FTIR spectra of sisal fibre and butyrate modified sisal.

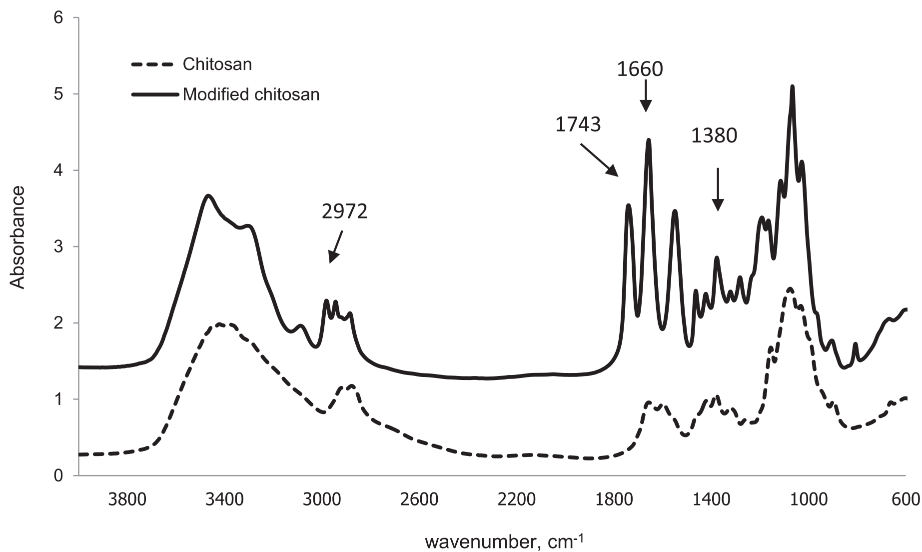


**Figure 2** FTIR spectra of chitosan and butyrate modified chitosan.

Evidently, the absorption spectrum of butyrate modified chitosan is different from virgin chitosan (Fig. 2). Characteristic bands of *N*-acetyl groups of chitosan around 1645 $cm^{-1}$ (C=O stretching of amide I), 1550 $cm^{-1}$ (N-H bending of amide II), and 1325 $cm^{-1}$ (C-N stretching of amide III) were not detected after esterification [18]. Whereas new peaks at 1735, 1660, and 1380 $cm^{-1}$, which corresponded to $COC_2H_5$ and $NHCOC_2H_5$ of butyric anhydride reacted at C6 and C2 of pyranose ring of chitosan, were observed, respectively.

**2. Bio-degradation evaluation by fungal growth test.**

A fungal growth test, in accordance with ISO 846: 1997 standard testing method, was adapted to evaluate bio-degradation PLA/sisal biocomposite by determining the action of fungi to the polymer. Aspergillus niger aliquot was cultured onto biocomposite specimen under specific condition for 28 days. It is found that fungi grew on all specimens, but mostly at the edge rather than center (Fig. 3). This occurrence is due to fungi readily spread through the cut line under the nutrient circumstance. Fungi also consume sisal fibre in biocomposite as a carbon food source combining with moisture in the atmosphere [19]. However, fungi growth on biocomposite S-PLA (A, neat PLA/sisal biocomposite) was obviously more than chitosan added sample S-C-PLA1 (B); whereas less of fungi expansion was observed onto S-C-PLA2 (C) and S-C-PLA3 (D), respectively.

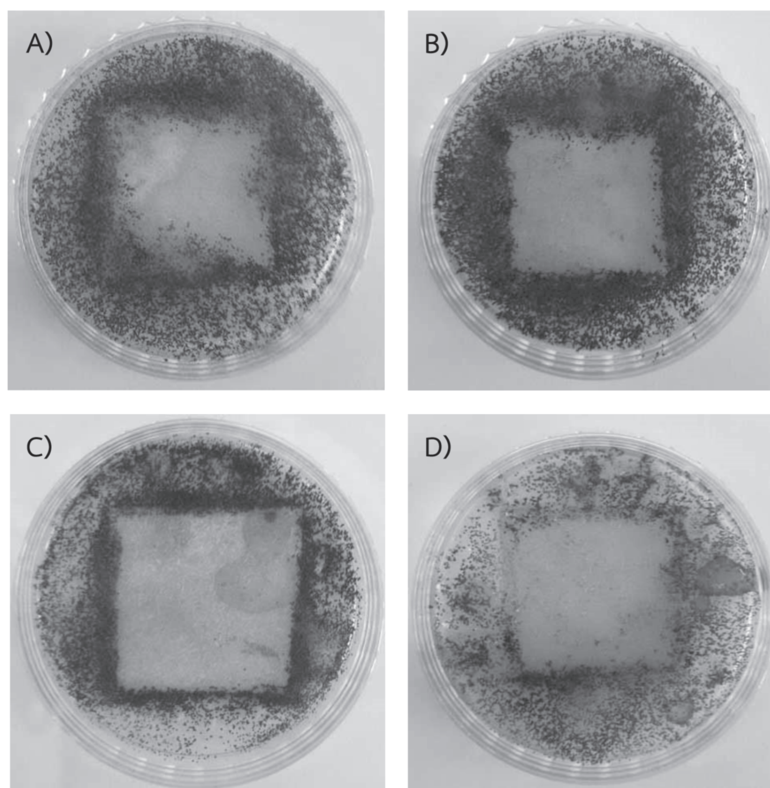


**Figure 3** Growth of *Aspergillus niger* on various surfaces of PLA/sisal biocomposite after 28 days of cultivation: A) S-PLA, B) S-C-PLA1, C) S-C-PLA2 and D) S-C-PLA3.

The results show that chitosan effectively retarded the growth of fungi onto biocomposite. In addition, by converting hydroxyl group of sisal fibre and hydroxyl and amine groups of chitosan to butyrate cellulose and butyrate chitosan, the hydrophobicity of sisal and chitosan was improved. Therefore, both fillers are able to disperse and bind PLA matrix, as well as reduced moisture absorption. The growths of fungi were restricted in sample C and D, which corresponded to the FTIR spectra of the specimen after growth test (Fig. 4).

Figure 4 shows the comparisons of FTIR spectral of all PLA/sisal biocomposites. The substantial decline of absorbance peak at 1745 $cm^{-1}$ as observed in S-PLA-post test specimen (B) indicates the depletion of COOR ester group of PLA. Whereas new peaks which are observed at wave number 3300 and 1661 $cm^{-1}$ corresponded to hydroxyl group (OH) and aldehyde group (HCOH), respectively. The reduction of ester group with addition of hydroxyl group reflects the violent bio-degradation of S-PLA-pre test specimen (A) at the surface through enzymatic degradation. The occurrence of aldehyde group is expected from oxidation reaction from fungi released enzymes, which accelerate the decomposition process. This leads to mineralization by assimilation inside fungal cells. The FTIR spectral (C), (D), and (E) representing the after growth test specimen of all chitosan added biocomposites, S-C-PLA1-post, S-C-PLA2-post, and S-C-PLA3-post, respectively, which are similar to the spectral of S-PLA pre-test (A). According to Babaee et al, chemical modification that turns hydrophilicity of the cellulose nanofibers reinforced starch biocomposites to hydrophobicity, resulted in a decrease in the decomposition rate and the micro-organisms activities [19]. Consequently, no reduction of absorbance at wave number 1645 $cm^{-1}$ is observed in all post-test specimens (C), (D), and (E), hence ester groups of PLA are not affected by the fungi. It is clearly revealed role of chitosan and its butyrate modified derivative, and butyrate modified sisal successively inhibit fungal growth on the PLA/sisal biocomposites.

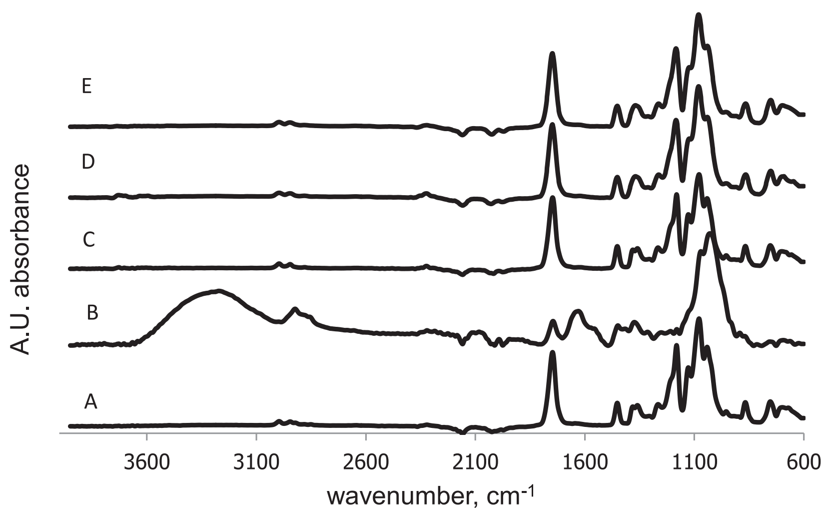


**Figure 4** FTIR spectra of pre-, and post- of fungal growth test: A) S-PLA-pre, B) S-PLA-post, C) S-C-PLA1-post, D) S-C-PLA2-post, and E) S-C-PLA3-post.

**3. Differential scanning calorimeter (DSC)**

Thermal behaviour of PLA/sisal biocomposite was analysed by DSC. It is observed that Tg, Tc, Tm and %Xc of S-PLA were quite close to those of S-C-PLA1 specimens (Table 2). These results reveal that the addition of chitosan does not directly affect the composite structure. However, Tc of S-C-PLA2 and S-C-PLA3 were increased to 123.9 and 129.8°C respectively, whereas the degree of crystallinity was clearly decreased. The crystallinity of S-C-PLA3 was 54.5% lower than S-PLA, indicating that new pendant configuration of butyrate modified sisal and chitosan obviously hinders their crystal formations.

Though substitution of hydroxyl group at C6 of pyranose ring of cellulose, and substitution of hydroxyl and amine group at C6, and C2 of pyranose ring of chitosan with butyric anhydride cause polarity reduction of both groups, but larger molecules occurred obstruct crystallization of PLA matrix. As a result, crystallization temperatures (Tc) of S-C-PLA2 and S-C-PLA3 biocomposite are increased, whereas %crystallinity are decreased.

**Table 2** Thermal behaviour of PLA/sisal biocomposites of pre-and post-fungal growth test.

| Bio-composite | Microbial growth test | Tg | Tc | Tm | $\Delta H_{melt}$, J/g | %Crystallinity |
|---|---|---|---|---|---|---|
| S-PLA | pre | 60.3±0.3 | 117.2±0.2 | 155.8±0.4 | 27.8±0.5 | 29.9±0.6 |
| | post | 62.0±1.4 | 114.2±0.4 | 156.0±0.2 | 33.9±2.4 | 36.4±2.7 |
| S-C-PLA1 | pre | 59.3±0.3 | 116.4±0.8 | 156.6±0.4 | 27.6±0.4 | 29.6±0.4 |
| | post | 60.5±2.1 | 113.7±0.8 | 156.1±0.2 | 34.3±1.0 | 36.9±1.1 |
| S-C-PLA2 | pre | 59.5±0.7 | 123.9±0.6 | 154.7±2.3 | 26.1±0.7 | 28.0±0.7 |
| | post | 60.0±0.0 | 112.9±0.1 | 156.5±0.1 | 37.2±0.5 | 39.9±0.6 |
| S-C-PLA3 | pre | 60.0±0.0 | 129.8±0.6 | 154.4±0.1 | 12.6±0.0 | 13.6±0.1 |
| | post | 60.5±0.7 | 126.4±1.4 | 155.3±0.1 | 22.8±0.8 | 24.5±0.9 |

**4. Thermogravimatric analysis (TGA)**

Thermal degradation of biocomposite can be determined from %weight loss of specimen by using thermogravimetric analysis (TCA). Figure 5 reveals that decomposition temperature (Td) of S-C-PLA1 was lower than Td of S-PLA, because of low heat resistance characteristic of added chitosan. However, Td of S-C-PLA2 and S-C-PLA3 were ascended respectively. These increments demonstrate that chemical modification of sisal and chitosan with butyric anhydride are successfully improved compatibility between fillers and PLA matrix, leading to better thermal stability

In addition, glass transition temperature (Tg), melting temperature (Tm), and crystallinity (%Xc) of pre- and post- microbial growth test biocomposite specimen were drastically changed. Tg, Tm and %Xc of all post-test specimens were higher than pre-test specimens, whereas Tc was lower. According to previous observation, hydrolysis of PLA was caused by lipase and protease enzymes produced by *Aspergillus niger.* PLA matrix became food source for microbial growth through this enzymatic hydrolysis, resulted in weight loss of post-test specimen [20].

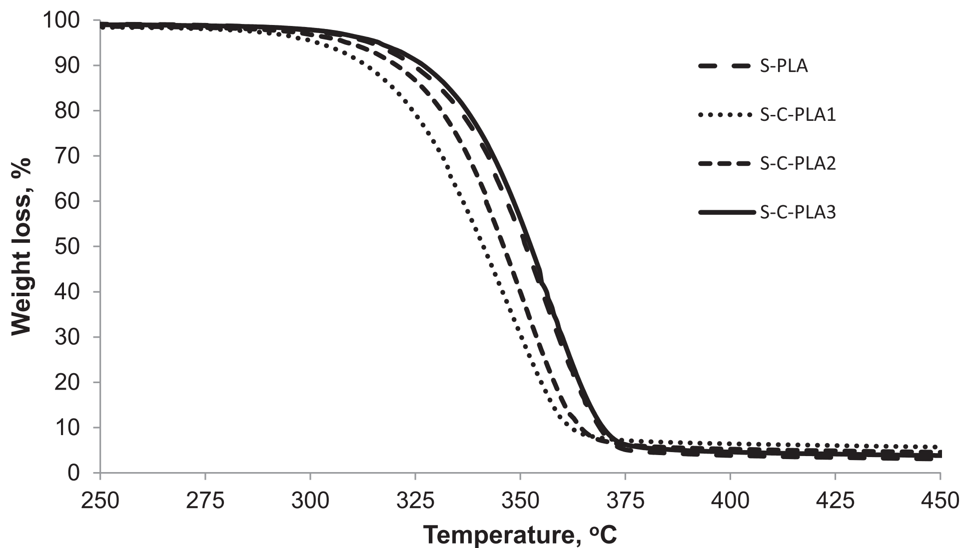


**Figure 5** %weight loss of PLA/sisal biocomposites at degradation temperature.

**5. Scanning electron microscope (SEM)**

Surface deterioration of PLA/ sisal biocomposites were investigated after 28 days of fungal growth test. All specimens were thoroughly soaked in ethanol to remove fungal hyphae from the surface. Some mycelia of *Aspergillus niger* were markedly observed on PLA/sisal biocomposite specimens's surfaces, which signified deteriorations of polymer matrix (Fig. 6). Though chitosan was added to PLA/sisal biocomposite S-C-PLA1, but *Aspergillus niger* was able to attack on biocomposite matrix because of inadequate fungi inhibition of virgin chitosan. Therefore, migration of hyphae largely occupied on S-PLA (6A) and S-C-PLA1 (6B), whereas S-C-PLA2 (6C) and S-C-PLA3 (6D) were barely visible. The inhibition efficacy is greater observed on butyrate modified chitosan and butyrate modified sisal, S-C-PLA2 and S-C-PLA3, because the modification enhances the binding between sisal reinforce filler and PLA matrix, reduces water absorption capability, and then retards biodegradation.

SEM images in Figure 7 also illustrate that gap between chitosan filler and PLA matrix of S-C-PLA3 (7B) is less than occurrence of S-C-PLA1 (7A). Hence, the esterification of chitosan and sisal with butyric anhydride directly improves filler-matrix compatibility, and mechanical property.

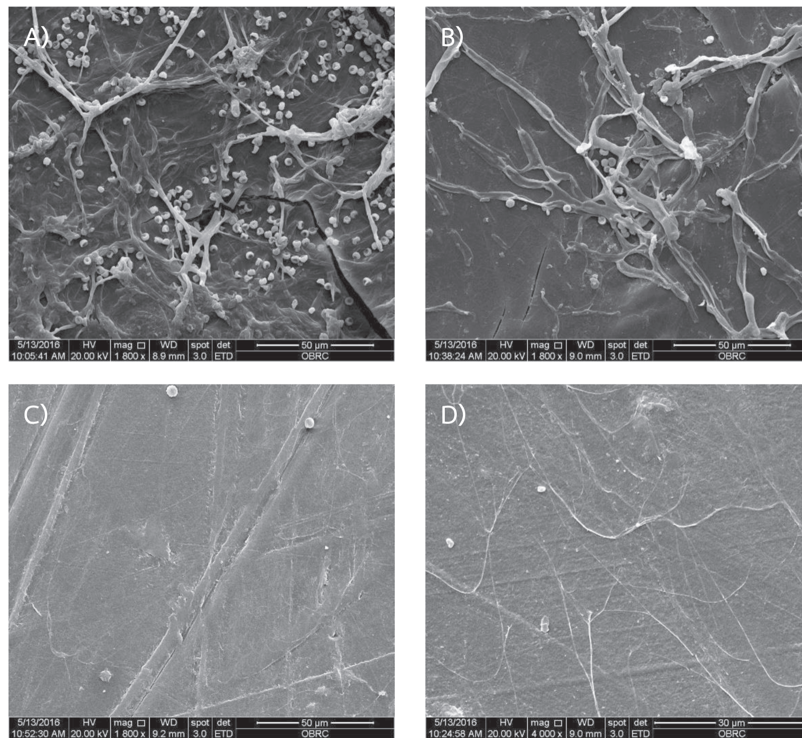


**Figure 6** SEM images of PLA/sisal biocomposite surface after 28 days of fungi growth test. A) S-PLA, B) S-C-PLA1, C) S-C-PLA2 and D) S-C-PLA3

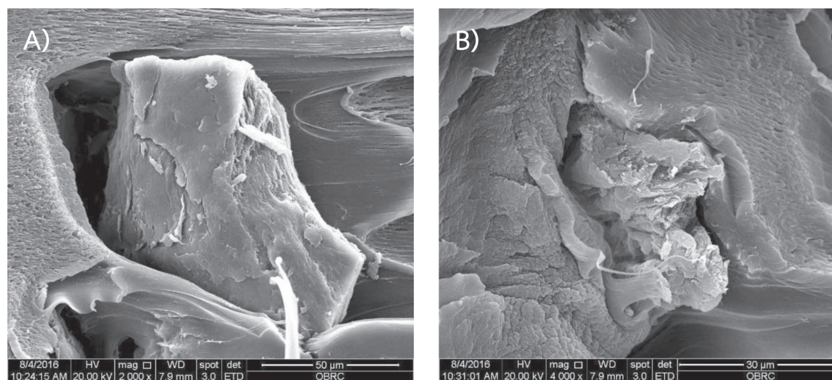


**Figure 7** Chitosan particle embedded in PLA matrix. A) S-C-PLA1, and B) S-C-PLA3

## Conclusions and Discussion

This research showed that butyrate derivative of chitosan and sisal successfully improved the compatibility between sisal reinforced filler and PLA matrix, which directly altered their thermal behaviour. Crystallization temperature (Tc) was higher, whereas %crystallinity was lower, because of pendant characteristic of the butyrate modified group hindering a crystal formation of PLA matrix [20].

Modification of chitosan and sisal filler with butyric anhydride enhanced fungi retardation of PLA/sisal biocomposite, because of better adhesion between both components [21].

FTIR spectrum and SEM images revealed that *Aspergillus niger* was able to grow on surface of PLA/sisal biocomposite. The incursion of fungi caused enzymatic degradation of biocomposite, which could be noticed from the reduction of ester group of PLA, together with addition of hydroxyl group [19]. However, chitosan and its butyrate modified derivative added, effectively retarded fungi growth.

## References

1. Fowler, P. A., Hughes, J. M., & Elias, R. M. (2006). Biocomposites: technology, environmental credentials and market forces. *Journal of the Science of Food and Agriculture, 86*(12), 1781-1789.
2. Bledzki, A. K., & Jaszkiewicz, A. (2010). Mechanical performance of biocomposites based on PLA and PHBV reinforced with natural fibres-a comparative study to PP. *Composites Science and Technology, 70*(12), 1687-1696.
3. La Mantia, F. P., & Morreale, M. (2011). Green composites: a brief review. *Composites: Part A: Applied Science and Manufacturing, 42*(6), 579-588.
4. Georgiopoulos P., Kontou, E., & Georgousis, G. (2018). Effect of silane treatment loading on the flexural properties of PLA/flax unidirectional composites. *Composites Communications, 10*, 6-10.
5. Kittikorn, T., StrÖmberg, E., Ek, M., & Karlsson, S. (2013). Comparison of water uptake as function of surface modification of empty fruit bunch oil palm fibres in PP biocomposites. *Bioresources, 8*(2), 2998-3016.
6. Gil-Castell, O., Badia, J. D., Kittikorn, T., Strömberg, E., Ek, M., Karlsson, S., & Ribes-Greus, A. (2016). Impact of hydrothermal ageing on the thermal stability, morphology and viscoelastic performance of PLA/sisal biocomposites. *Polymer Degradation and Stability, 132*, 87-96.

7. Batista, K. C., Silva, D. A. K., Coelho, L. A. F., Pezzin, S. H., & Pezzin, A. P. T. (2010). Soil biodegradation of PHBV/peach palm particles biocomposites. *Journal of Polymers and Environment, 18*(3), 346-354.
8. Kyutoku, H., Maeda, N., Sakamoto, H., Nishimura, H., & Yamada, K. (2019). Effect of surface treatment of cellulose fiber (CF) on durability of PLA/CF bio-composites. *Carbohydrate Polymers, 203*, 95-102.
9. Shimpi, N. G., Borane, M., & Mishra, S. (2014). Preparation, characterization, and biodegradation of PS: PLA and PS: PLA: OMMT nanocomposites using *Aspergillus niger. Polymer Composites, 35*(2), 263-272.
10. Nakajima-Kambe, T., Edwinoliver, N. G., Maeda, H., Thirunavukarasu, K., Gowthaman, M. K., Masaki, K., Mahalingam, S., & Kamini, N. R. (2012). Purification, cloning and expression of an *Aspergillus niger* lipase for degradation of Poly(lactic acid) and Poly ($\varepsilon$-caprolactone). *Polymer Degradation and Stability, 97*(2), 139-144.
11. Fernandez-Saiz, P., Lagaron, J. M., & Ocio, M. J. (2009). Optimization of the biocide properties of chitosan for its application in the design of active films of interest in the food area. *Food Hydrocolloids, 23*(3), 913-921.
12. Goy, R. C., de Britto, D., & Odilio, B. G. (2009). A review of the antimicrobial activity of chitosan. *Polimeros: Ciencia e Tecnologia, 19*(3), 241-247.
13. Martínez-Camacho, A. P., Cortez-Rocha, M. O., Ezquerra-Brauer, J. M., Graciano-Verdugo, A. Z., Rodriguez-Félix, F., Castillo-Ortega, M. M., Yépiz-Gómez, M. S., & Plascencia-Jatomea, M. (2010). Chitosan composite films: thermal, structural, mechanical and antifungal properties. *Carbohydrate Polymers, 82*(2), 305–315.
14. Jayakumara, J., Prabaharana, M., Reisa, R. L., & Mano, J. F. (2005). Graft copolymerized chitosan-present status and applications. *Carbohydrate Polymers, 62*(2), 145-158.
15. Choi, C. Y., Kim, S. B., Pak, P. K., Yoo, D. Il, & Chung, Y. S. (2007). Effect of *N*-acylation on structure and properties of chitosan fibers. *Carbohydrate Polymers, 68*, 122-127.
16. Atarijabarzadeh, S., Strömberg, E. & Karlsson, S. (2010). Biofilm formation on silicone materials containing various antimicrobial agents. *International Biodeterioration & Biodegradation, 65*(8), 1111-1118.
17. Orue, A., Jauregi, A., Pena-Rodriguez, C., Labidi, J., Eceiza, A., & Arbelaiz, A. (2015). The effect of surface modifications on sisal fiber properties and sisal/Poly (lactic acid) interface adhesion. *Composites: Part B, 73*, 132-138.

18. Fernandez Queiroz, M., TeodosioMelo, K. R., Sabry, D. A., Sassaki, G. M., & Oliveira Rocha, H. A. (2015). Does the use of chitosan contribute to oxalate kidney stone formation? *Marine Drugs, 13*, 141-158.
19. Babaee, M., Jonoobi, M., Hamzeh, Y., & Ashori, A. (2015). Biodegradability and mechanical properties of reinforced starch nanocomposites using cellulose nanofibers. *Carbohydrate Polymers, 132*, 1-8.
20. Lee, S. H., & Song, W. S. (2011). Enzymatic hydrolysis of polylactic acid fibre. *Applied Biochemistry and Biotechnology, 164*(1), 89-102.
21. Kittikorn, T., Kongsuwan, S., & Malakul, R. (2017). Investigation of the durability of sisal fiber/PLA biocomposite through evaluation of biodegradability by means of microbial growth. *Journal of Metals, Materials and Minerals, 27*(2), 23-34.