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POLIMERY

Influence of sisal fibre modification on the microbial stability of poly(hydroxybutyrate-*co*-valerate): thermal analysis

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Abstract: The effect of modification of sisal fibre with propionic anhydride and vinyltrimethoxy silane on the microbiological stability of poly(hydroxybutyrate-*co*-valerate) (PHBV) was investigated. The effect of the coupling agent – PHBV grafted with maleic anhydride (PHBV-*g*-MA) was also investigated. The best adhesion at the interface was observed for propionylation of sisal fibre, which improved the thermal properties of the composites. Composites with modified sisal fibre were characterized by higher activation energy (155 kJ/mol), which is related to stronger interactions at the matrix-fibre interface. In the microbial growth test, all biocomposites showed a decrease in molecular weight due to enzymatic degradation by *Aspergillus niger*. The most resistant to microorganisms was the composite containing propionylated sisal fibre. DMTA and TGA also confirmed the highest microbiological stability of the composite with the addition of propionylated sisal fibre, as evidenced by the smallest change in the properties after the microbiological growth test. In contrast, PHBV-*g*-MA caused significant enzymatic degradation due to the presence of large amorphous regions.

Keywords: poly(hydroxybutyrate-*co*-valerate), sisal fibre, surface modification, thermal analysis, microbial stability.

Wpływ modyfikowanego włókna sizalowego na stabilność mikrobiologiczną poli(hydroksymaślanu-*co*-walerianianu): analiza termiczna

Streszczenie: Zbadano wpływ modyfikacji włókna sizalowego bezwodnikiem propionowym i winylotrimetoksy silanem na stabilność mikrobiologiczną poli(hydroksymaślanu-*co*-walerianianu) (PHBV). Zbadano również wpływ środka sprzęgającego − PHBV szczepionego bezwodnikiem maleinowym (PHBV-*g*-MA). Najlepszą adhezję na granicy faz stwierdzono w przypadku zastosowania propionylowania włókna sizalowego, co poprawiło właściwości termiczne kompozytów. Kompozyty z modyfikowanym włóknem sizalowym charakteryzowały się wyższą energią aktywacji (155 kJ/mol), co wiąże się z silniejszymi oddziaływaniami na granicy faz osnowa-włókno. W teście wzrostu drobnoustrojów wszystkie biokompozyty wykazywały zmniejszenie masy cząsteczkowej na skutek enzymatycznej degradacji przez *Aspergillus niger*. Najbardziej odporny na działanie mikroorganizmów były kompozyt za-

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wierający propionylowane włókno sizalowe. DMTA i TGA potwierdziły również najwyższą stabilność mikrobiologiczną kompozytu z dodatkiem propionylowanego włókna sizalowego, o czym świadczy najmniejsza zmiana badanych właściwości po teście wzrostu mikrobiologicznego. Natomiast, PHBV-*g*-MA powodował znaczną degradację enzymatyczną ze względu na obecność dużych obszarów amorficznych.

Keywords: poli(hydroksymaślan-*co*-walerianianu), włókno sizalowe, modyfikacja powierzchni, analiza termiczna, stabilność mikrobiologiczna.

Currently, industrial policy emphasizes the importance of sustainable development for the environment, economy and society [1-6]. The use of bioplastics as raw materials for consumer products fully meets these requirements [6]. Polyhydroxybutyrate-covalerate (PHBV) is a biodegradable polymer of high interest. PHBV produced by bacteria has a faster biodegradation rate than polylactic acid [7, 8], which makes it an attractive alternative for all applications [9] where biodegradability is required. However, PHBV has some disadvantages, such as high production costs, moderate mechanical properties and a very high degree of biodegradation [10]. A solution may be the use of high-strength, cheap, surface-treated lignocellulosic fibres in order to improve their compatibility with the PHBV matrix. This approach leads to obtaining "green" PHBV composites, which are more practical than the conventional PHBV [11-15]. However, when developing green biocomposites, resistance to biodegradation should be taken into account, which is an important factor in assessing useful service life [16]. Therefore, to extend the service life of the lignocellulosic/PHBV composites for long-term use, an appropriate fibre modification method should be considered. It was reported that the rate of degradation of biocomposites was influenced by the molecular weight and crystallinity of the polymeric matrix [16]. The reduction of the molecular weight with the simultaneous increase of the amorphous phase plays an important role in the degradation of biocomposites by oxidation, hydrolytic degradation or biodegradation by microbial attack [17]. To enhance the performance and prolong the service life of lignocellulosic fibre/PHBV biocomposites without incorporating additives such as antioxidants or biocidal agents, the surface modification of the fibre is possibly a sustainable method which improves the interfacial adhesion between fibre and matrix and reduces moisture absorption [18,19]. Therefore, the appropriate modification of the fibre is of key importance to improve the mechanical and thermal properties as well as the durability of the fibre/PHBV composites.

In this paper the effect of sisal fibre surface modification in terms of biodegradation and microbiological stability of PHBV was investigated. Sisal fibre was modified by propionylation and vinyl silanization [23, 24] and PHBV grafted with maleic anhydride was used as a coupling agent (CA) [13, 17, 25]. The microbial stability of sisal/PHBV composites was assessed in a microbial growth test conducted in accordance with the ISO 846 method B protocol, using *Aspergillus niger*. A response of sisal/PHBV composites to microbial attack was determined by means of thermal and molecular weight analysis. Also, the activation energy was calculated to evaluate the thermal stability after biodegradation.

EXPERIMENTAL PART

Materials

Polyhydroxybutyrate-co-velerate (PHBV, ENMAT Y1000P), injection moulding grade, was purchased from TianAn biopolymer (China). Sisal fibre was supplied by the Thai Royal Project in Thailand. Propionic anhydride (96 wt% of purity) and maleic anhydride (MA, 98 wt% of purity) were received from Fluka (Switzerland). Vinyltrimethoxy silane (98 wt% of purity) and dicumyl peroxide (98 wt% of purity) were suppied by Sigma Aldrich (USA).

Surface modification of sisal fibre

Sisal fibre was chopped in length (1-3 mm) before modification. Propionylation of sisal fibre was carried out using propionic anhydride as a reagent according to a previous work [13, 23]. Similarly, silanization of sisal fibre was carried out using vinyltrimethoxy silane according to previous work *[*13,24*]*.

Manufacturing of sisal/PHBV composites

Polyhydroxybutyrate-co-velerate was compounded with 10 wt.% of unmodified, propionylated and vinyl silanized sisal fibre, respectively, in an internal mixer (Brabender, Germany). The process was carried out at the temperature of 185°C, and 50 rpm for 5 min. In case of sisal/CA/PHBV composite, the PHBV-g-MA (coupling agent, CA) was obtained via reactive mixing of 2.5 wt% of maleic anhydride (MA) and 0.3 wt% of dicumyl peroxide (DCP) with PHBV for 5 min and then 10 wt% of sisal fibre was added and the mixing was continued for 5 min. Before mould compressing the biocomposites were grinded and dried at 80°C in a vacuum oven for at least 3 hours (to avoid hydrolytic degradation). Sheets with dimensions of 100 × 100 × 0.5 mm were obtained using a vacuum-pressure forming machine (Fontijne Presses).

A pressure of 100 kN, a temperature of 190°C and a holding time of 2 minutes were used. The sheets were automatically cooled.

Analyses

The microbial growth test was conducted according to ISO 846-1997 using method B [24]. *Aspergillus niger* was selected as the active microorganism for assessment of the microbial stability. The microbial growth test was maintained for 28 days, then the samples were taken out for further characterization**.**

The molecular weight was analysed before and after microbial growth test using a size exclusion chromatography (SEC). GPC 50 Plus system model Verotech PL equipped with a PL-RI detector and two PLgel 5μm MIXED-D (300 × 7.5 mm) columns from Varian were used. M_n, M_w and MWD were recorded and evaluated by CirrusTM GPC software.

Thermal stability was determined using a Mettler Toledo TGA/SDTA 851 (Greifensee, Switzerland). The kinetics of thermal decomposition were studied using heating rates of 5, 10, 15 and 20°C/ min in the temperature range of 30–500°C under nitrogen. The activation energy of thermal decomposition (*Ea*) was calculated using the Flynn-Wall-Ozawa method (OWF) through a kinetic study of non-isothermal degradation, the following maximum rate method equation was used:

$$\ln\left(\frac{\beta}{T_m^2}\right) = \ln\left(\frac{A\cdot R}{Ea}\right) - \left(\frac{Ea}{R\cdot T_m}\right)$$

where: β and T_m were the heating rate and temperature at maximum rate of decomposition, respectively**.**

Thermal properties were determined using a Mettler Toledo DSC820 calorimeter (Schwerzenbach, Switzerland). The first and second heating of composite specimens were run under heating/cooling rate of 10°C/min, from -20 to 200°C. The crystallinity was calculated from equation:

$$\%Xc = \frac{\Delta H_m \cdot 100\%}{\Delta H_m^0 \cdot (1-w)}$$

where ΔH was the enthalpy change of fusion, whereas a weight fraction and ΔH_{fusion} of 100% crystallinity of PHBV was 0.9 and 146 J/g [26], respectively.

Dynamic mechanical thermal analysis (DMTA) was conducted on a Q800 dynamic analyser (TA instruments, USA) before and after biodegradation.

The biodegradation behavior was also observed by means of SEM images using a Hitachi S-4800 field emission scanning electron microscope (Japan).

RESULTS AND DISCUSSION

The proliferation of *Aspergillus niger* was significantly better on the surface of the composite with unmodified sisal fibre (Fig. 1a) and the composite containing PHBV-g-MA as a coupling agent (Fig. 1b) than in the case of the composite with propionylated sisal fibre. (Fig. 1c). Due to the polysaccharide molecules, lignocellulosic fibres contained in biodegradable composites usually act as nutrients for microorganisms. Therefore, the incorporation of sisal fibre in the PHBV composite, besides improving its mechanical properties and reducing its costs, should also accelerate the rate of its biodegradation. Hence, the extent of the microbial growth on these composites indicated the response of *Aspergillus niger* to each type of PHBV composite. The lower rate of proliferation of the fungus on the surface of propionylated sisal/PHBV composite (Fig.1c), suggests that the surface modification improved microbial resistance and restrained microbial growth.

It was reported, that the reduced water absorption in fibres modified by esterification and silanization with vinyltrimethoxy silane was potentially due to enhanced hydrophobicity [13]. Moisture in biodegradable polymers always results in hydrolytic degradation, which promotes microbial growth. Furthermore, as previously reported [23], it was found that the modification of sisal with propionic anhydride had remarkable potential to delay bacterial growth. Therefore, increasing the hydrophobicity of the PHBV composites surface may inhibit the growth of microorganisms and reduce biodegradation.

Estimation of the PHBV composites molecular weight by SEC provided further evidence of the effects of the different sisal fibre surface modification on the biodegradation. The results are presented in Table 1. The molecular weight by number (M_n) of the sisal/CA/PHBV was the lowest of all the composites as considered before microbial growth. This was in agreement with the FTIR analysis. The decrease in M_n was due to oxidation and also hydrolytic degradation [27, 28]. On the other hand, the composite

Table 1. M_n and MWD of sisal/PHBV composites before and after microbial growth test

Samples	Before microbial growth test		After microbial growth test	
	M_n	MWD	M_n	MWD
Unmodified sisal/PHBV	89921	2.2	83572	2.2
CA/sisal/PHBV	66729	2.2	41380	2.4
Propionylated sisal/PHBV	99735	2.0	96052	2.0
Vinyl silanized sisal/PHBV	97333	2.1	88471	2.1

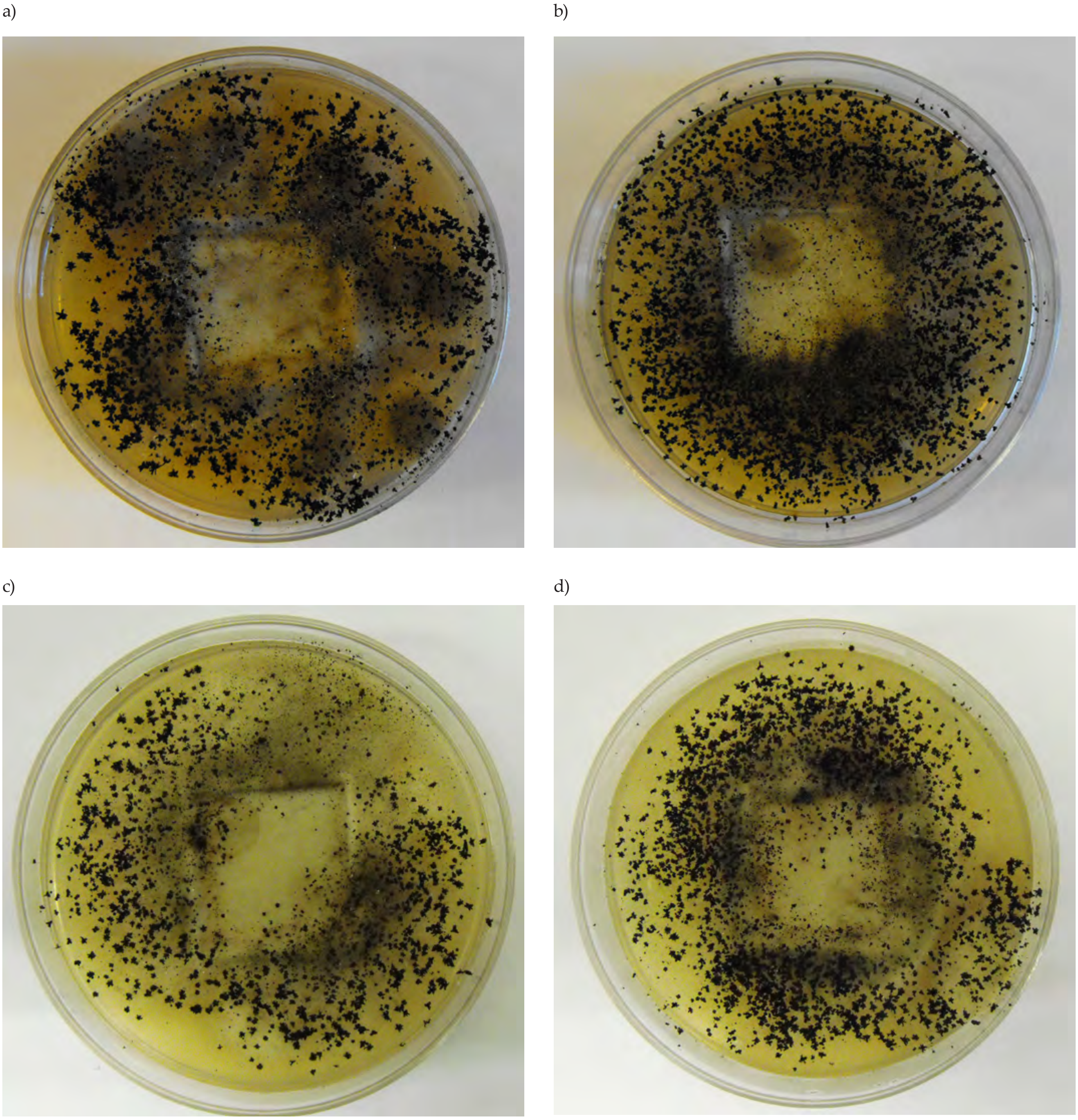

Fig. 1. Optical images of sisal/PHBV biocomposites on the 28 day of the microbial growth test: a) unmodified sisal fibre, b) CA, c) propionylated sisal fibre, d) silanized sisal fibre

with propionylated sisal fibre had the highest M_w, what is the evidence proving its ability to prevent degradation of PHBV. It is known that esterification of cellulosic fibres increases their hydrophobicity which prevents hydrolytic degradation. It also improves the thermal resistance of fibres, which can reduce thermal degradation.

After the microbial growth test, the M_n of the propionylated sisal/PHBV composite was slightly lower by 3.6% while the M_n of the composites with unmodified and vinyl silanized sisal fibre as well as with PHBV-*g*-MA used as coupling agent (CA) decreased by 7%, 9% and 38%, respectively. This could indicate that the interfacial adhesion between the fibres and the PHBV matrix contributed to slowing down of microbial growth and hence less surface erosion from enzymatic degradation. The improved interfacial adhesion not only increased the load transfer to the reinforcement fibre, but also reduced the risk of biodegradation. In consequence, the durability of the composites could be prolonged either storing on shelves or servicing. However, in the case of sisal/CA/PHBV composite, the substantial decrease in molecular weight following the microbial growth test

Table 2. Thermal properties of sisal/PHBV composites before and after microbial growth test

Sample	Microbial growth test	T_m, °C	ΔH_f, J/g	*Xc*, %
Unmodified sisal/PHBV	Before	171.5±0.8	85.4±1.9	58.5
	After	171.6±0.4	88.7±1.6	60.7
CA/sisal/PHBV	Before	166.6±1.2	85.6±4.4	58.6
	After	167.0±0.7	88.3±0.3	60.5
Propionylated sisal/PHBV	Before	171.7±0.1	88.3±0.8	60.5
	After	171.4±0.4	87.4±1.4	59.9
Vinyl silanized sisal/PHBV	Before	171.8±0.5	92.5±1.1	63.4
	After	171.5±0.2	84.6±2.0	58.0

Table 3. Activation energy (*Ea*) of sisal/PHBV composites before and after microbial growth test

Sample	Before microbial growth test		After microbial growth test	
	Ea	R^2	*Ea*	R^2
Unmodified sisal/PHBV	134	0.97	128	0.98
CA/sisal/PHBV	105	0.99	96	0.97
Propionylated sisal/PHBV	155	0.99	153	0.99
Vinyl silanized sisal/PHBV	155	0.99	146	0.99

was caused by the severe invasion of *Aspergillus niger* observed in Fig. 1b. Since the sisal/CA/PHBV composite had the lowest molecular weight, there were more areas of the amorphous phase in the PHBV matrix. These are the areas that *Aspergillus niger* prefers to attack [24].

According to the DSC data presented in Table 2, the crystallinity of the unmodified sisal/PHBV and CA/sisal/PHBV composites increased after the microbial growth test. The lower M_n of PHBV after biodegradation enabled the short chains of PHBV to recrystallize, increasing the crystallinity. Meanwhile, the crystallinity of PHBV in composites with propionylated sisal fibre practically did not change. This suggested that the structure of the composite with propionylated sisal fibre could resist microbial growth and delay biodegradation.

The kinetic study of sisal/PHBV composites were analysed and calculated using the Flynn-Wall-Ozawa method (OWF). The non-isothermal method of TGA was employed for this effort. The activation energy was calculated from the Arrhenius plot method as shown in Fig. 2.

As shown in Table 3, the use of propionylated and silanized sisal fibre increased the Ea of PHBV from 134 to 155 kJ/mol, while in the case of sisal/CA/PHBV composite the Ea decreased significantly to 105 kJ/mol. The high Ea of propionylated sisal/PHBV and vinyl silanized sisal/PHBV composites indicated a strong internal structure due to the excellent interfacial interaction between the fibre and the PHBV matrix and high molecular weight. On the other hand, the low Ea of the sisal/CA/PHBV composite was due to the low molecular weight. Moreover, the low thermal stability of maleic anhydride in PHBV may also reduce the thermal resistance of the sisal/CA/PHBV composite. However, it was expected that the Ea of the composites would be reduced after exposure to *Aspergillus niger* because of the bond cleavage caused by enzymatic degradation of PHBV.

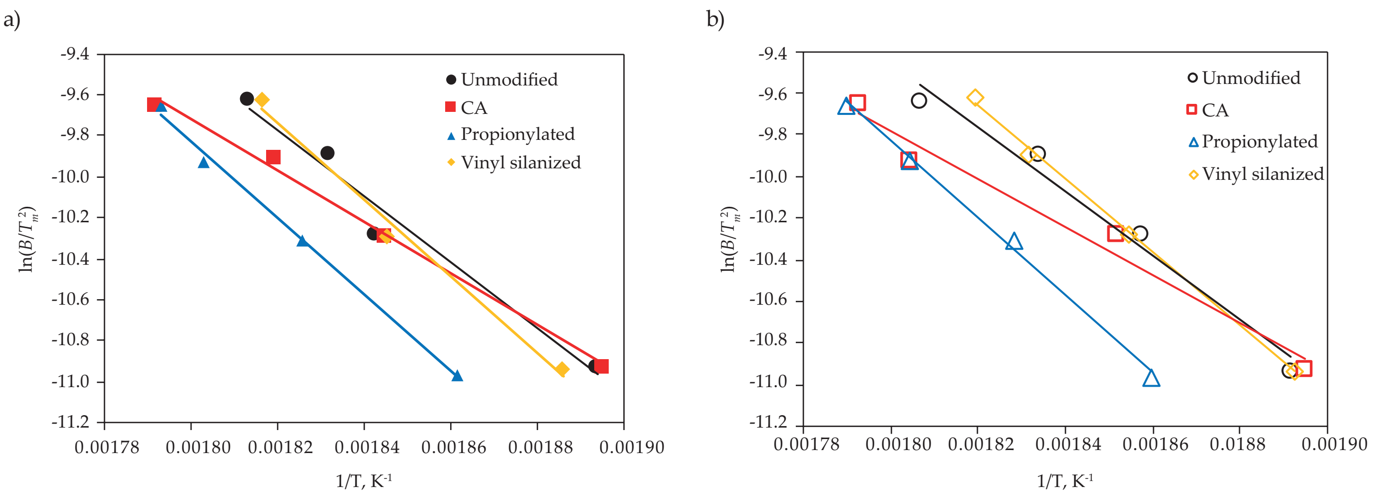

Fig. 2. The Arrhenius plot for activation energy of sisal/PHBV composites before: a) and after microbial growth test: b)

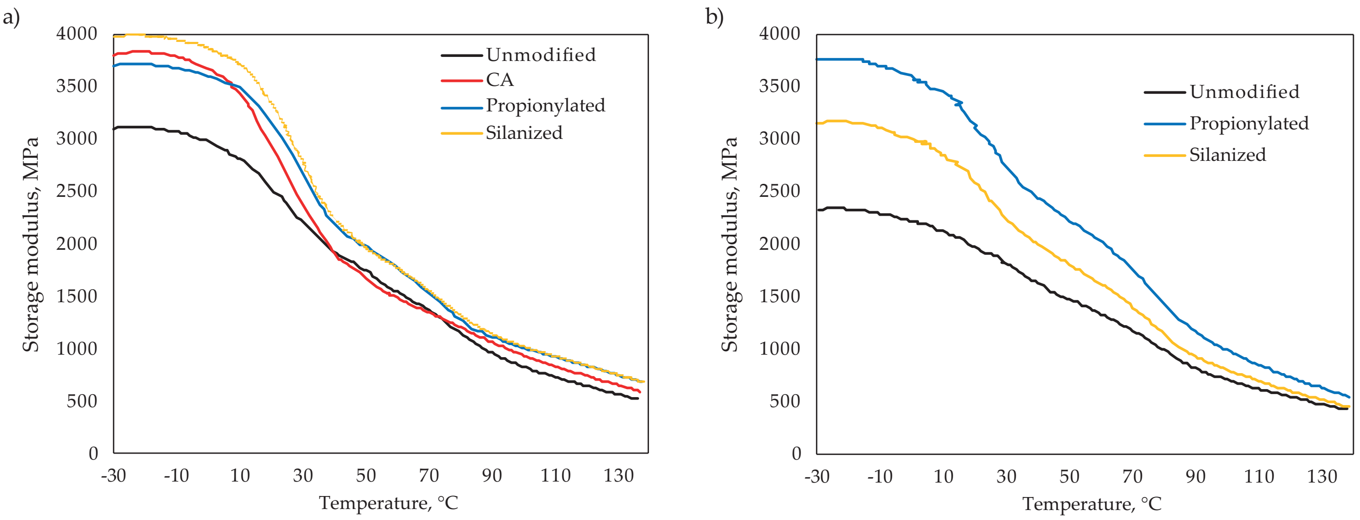

Fig. 3. Storage modulus of sisal/PHBV composites as a function of temperature before: a) and after microbial growth test: b)

DMTA was also used to evaluate the effect of fibre surface modification and coupling agent on the dynamic mechanical-thermal properties of sisal/PHBV composites. Fig. 3 presented storage modulus of the composites as a function of temperature before and after biodegradation. As expected, propionylated and vinyl silanized fibre as well as CA improved the PHBV storage modulus suggesting higher stiffness. The lowest storage modulus was observed when using unmodified fibre. However, after microbial growth, the storage modulus of all composites decreased, in particular of the sisal/CA/PHBV, which was not measurable due to extreme degradation made by *Aspergillus Niger*. Besides, a slight decrease in the storage modulus of the composite with propinylated fibre indicated its potentially high resistance to microorganisms. This also means that two constituent phases in the composite were less degraded and still maintain the good interfacial adhesion. This shows that the fungi of *Aspergillus Niger* rarely influence the propionylated sisal/PHBV composite.

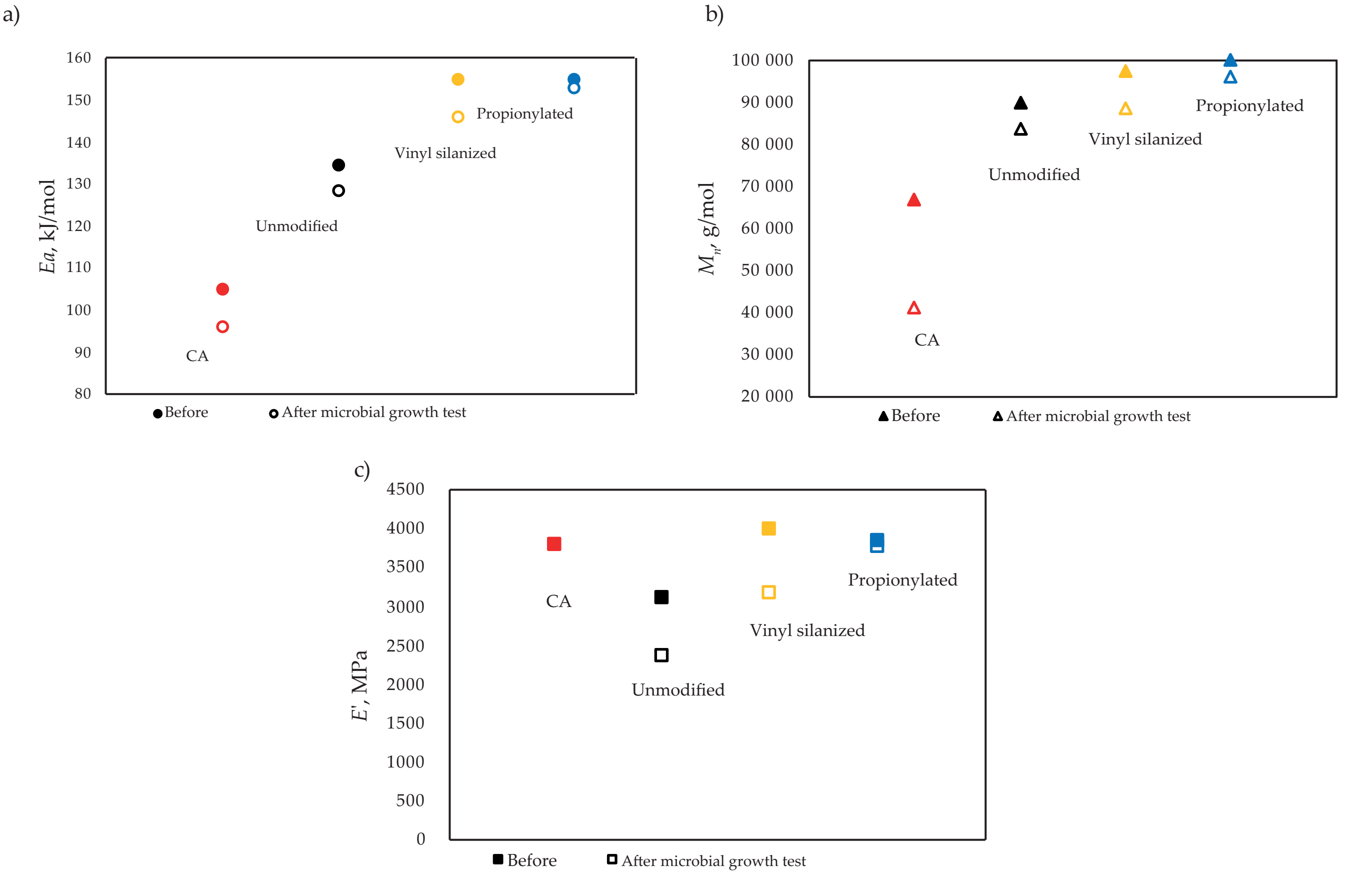

Fig. 4. Microbial stability of sisal/PHBV composites: a) activation energy (*Ea*), b) molecular weight by number (M_n) and c) initial storage modulus

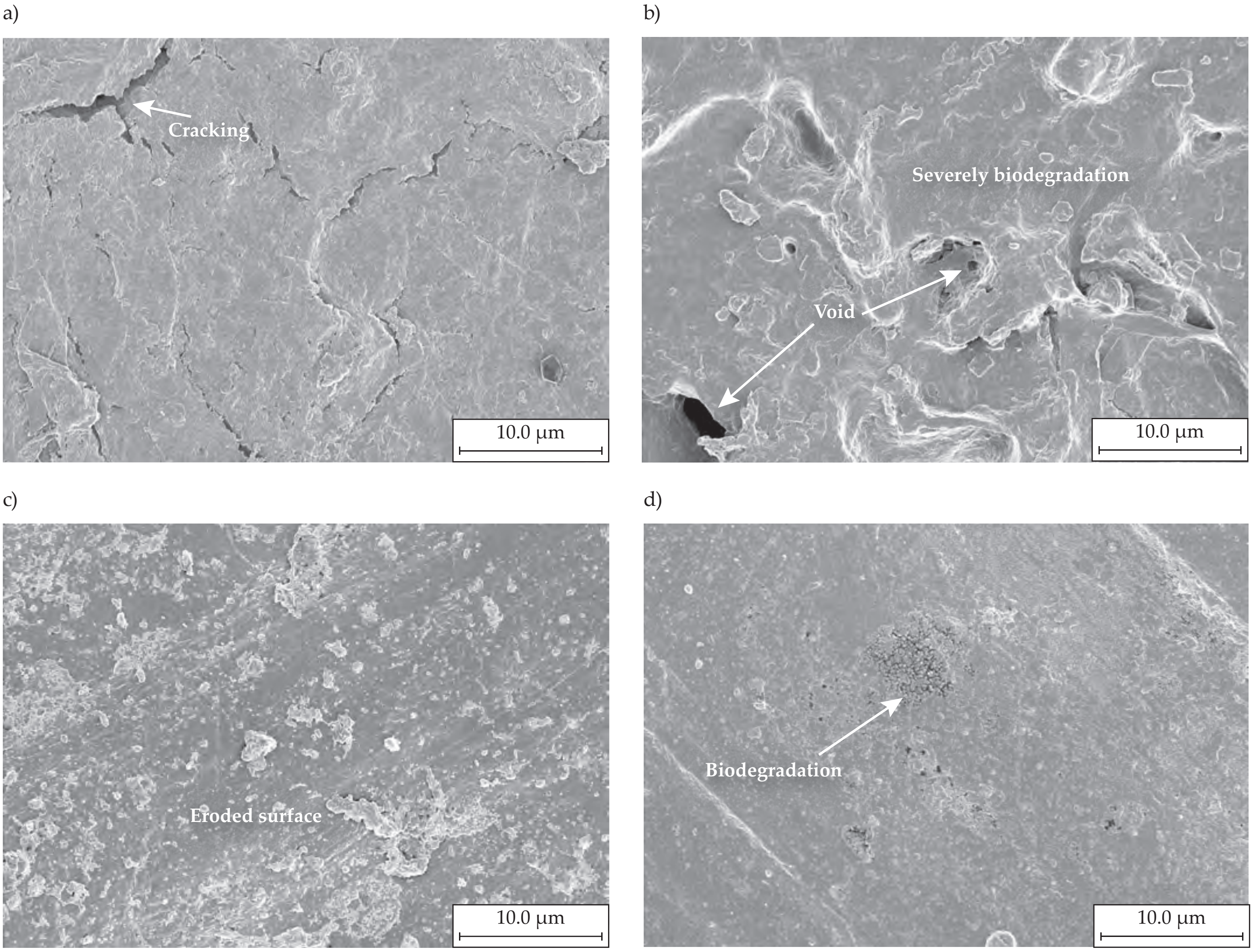

Fig. 5. SEM images of sisal/PHBV composites after microbial growth test: a) unmodified sisal fibre, b) CA, c) propionylated, d) vinyl silanized sisal fibre

Microbial stability of PHBV composites

The activation energy (*Ea*), molecular weight by number (M_n) and initial storage modulus (*E′*) were used to evaluate the effect of sisal fibre modification and maleic anhydride grafted PHBV as the coupling agent on the microbial stability of PHBV composites.

It is clear from Fig. 4 that PHBV composites show significantly different potential to inhibit biodegradation by *Aspergillus niger* depending on the modification of the sisal fibre and the use of a coupling agent. Their potential could be written in sequence as the following: propionylated sisal fibre > vinyl silanized sisal fibre > unmodified sisal fibre> CA. The difference in microbiological stability observed in each of the biocomposites can be attributed to factors such as the sisal-PHBV interaction, hydrophobic properties, and also the molecular weight of the PHBV. The purpose of the sisal fibre surface modification was to improve its compatibility with the polymer matrix. Each type of chemical modification can change the surface properties of the sisal fibre, resulting in improved properties of the composite.

The biodegradation behavior of sisal/PHBV biocomposites was also investigated by scanning electron microscopy (Fig. 5). Clear cracks are visible on the surface of the unmodified sisal/PHBV composite (Fig. 5a) after the microbial growth test. This could be caused by a depolymerase enzyme excreted by *Aspergillus niger* including moisture uptake during testing.The very rough surface with erosion and voids as appeared on sisal/CA/PHBV (Fig. 5b) was caused by fungus as seen in Fig.1b. As discussed earlier, the lowest M_n of its PHBV matrix, promoted the growth of *Aspergillus niger*, which attacked the biocomposite through enzymatic degradation. Furthermore, the presence of pores on the surface was strong evidence of an extremely enzymatic degradation in these areas.

When considering the propionylated sisal/PHBV composite after microbial growth test (Fig. 5c), practically no voids were found on its surface, indicating that it was less biodegradable than the other composites. The obtained results confirmed the high resistance of the propionylated sisal/PBHV composite to the fungus.

CONCLUSION

The propionylation of sisal fibre performed the best potential of surface modification for manufacture of sisal/PHBV biocomposite. The propionylated sisal fibre/PHBV composite showed the best mechanical and thermal properties and also exhibited the best potential to resist microbial growth, as evidenced by the lower levels of biodegradation. The M_n of the sisal/PHBV composites had a significant effect on biodegradation. The use of maleic anhydride grafted PHBV as coupling agent caused the side effect of chain scission during reactive blending and induced more favourable conditions of low M_n for *Aspergillus niger* to grow quickly. In accordance with activation energy, molecular weight, and storage modulus, the effect of surface modification to microbial stability of biocomposites could be written in sequenced as the following: propionylated sisal fibre > vinyl silanized sisal fibre > unmodified sisal fibre > CA. The least change with the highest of Ea, M_n and E′ after fungal exposure indicated the strong internal structure of the biocomposite. By itself, the propionylated sisal fibre could act as a good barrier and perform the best microbial stability.

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farb, lakierów, surowców, klejów, materiałów budowlanych, technologii

i koncepcji z nimi związanych.

Najważniejsze Fakty dotyczące Kongresu:

- Program ETCC2022 zawiera 138 referatów oraz 68 prezentacji posterowych przedstawianych przez 200 autorów z wielu krajów całego świata. Szczegóły w programie kongresu: *www.etcc2022.org/en/congress/congress-program*
- Możliwość publikowania wystąpień kongresowych w renomowanych czasopismach, szczegóły: *www.etcc2022.org/en/congress/publication-in-journals*
- Przygotowano wystawę towarzyszącą kongresowi, szczegóły dostępne na *www.etcc2022.org/en/congress/registration-form-for-exhibitors*
- Ustalono program sesji “Summer School” dedykowanej młodym naukowcom. *www.etcc2022.org/en/congress/summer-school*

ETCC2022. Innowacje i Wymierne Korzyści:
W ciągu trzech dni kongresu odbędą się **prezentacje plenarne, 6 sesji równoległych, prezentacje posterów oraz wystawa.**
Prelegenci reprezentują największe firmy, instytuty i uniwersytety całego świata

Wszystkie dotychczasowe warunki i ustalenia, w tym:

- Rejestracja do udziału w kongresie
- Dokonane opłaty kongresowe
- Przesłane wystąpienia: referaty oraz postery
- Rejestracje do udziału w wystawie
- Zgłoszenia do sesji „Summer School” dla młodych naukowców

Pozostają ważne w nowym terminie kongresu.

Organizatorzy kongresu ETCC2022:
Europejska Federacja FATIPEC - Federation of Associations of Technicians for Industry of Paints in European Countries (*www.fatipec.com*), oraz
Stowarzyszenie Inżynierów i Techników Przemysłu Chemicznego SITPChem (*www.sitpchem.org.pl*)
W przypadku pytań prosimy o kontakt, e-mail: etcc2022@sitpchem.org.pl

www.etcc2022.org