

Article

# Development and Characterization of Polymer Eco-Composites Based on Natural Rubber Reinforced with Natural Fibers

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**Abstract:** Natural rubber composites filled with short natural fibers (flax and sawdust) were prepared by blending procedure and the elastomer cross-linking was carried out using benzoyl peroxide. The microbial degradation of composites was carried out by incubating with *Aspergillus niger* recognized for the ability to grow and degrade a broad range of substrates. The extent of biodegradation was evaluated by weight loss and cross-linking degree study of composites after 2 months incubation in pure shake culture conditions. Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR) have proved to be precious and valuable instruments for morphological as well as structural characterization of the composites before and after incubation with *Aspergillus niger*.

**Keywords:** eco-composites; natural rubber; natural fibers; *Aspergillus niger*

## 1. Introduction

Biodegradable composites have recently become of great interest, particularly for applications in various industry sectors, as a replacement for conventional products with low sustainability. More than that, the depletion of petroleum resources coupled with the concern for global environmental problems makes it necessary to find alternatives in new green materials that are compatible with the environment. Most studies have aimed to obtain biodegradable plastic materials, but there are also many studies on obtaining biodegradable elastomeric materials based on natural rubber [1–3], nitrile [4,5] and butadiene [4,6] rubber. Plastics are mostly thermoplastic or thermosetting polymers of high molecular mass (polystyrene, polymethacrylate, polyamide, polyvinyl chloride, phenol-formaldehyde resins, etc.). Plastics are organic synthetic or processed materials, in the manufacture of which petroleum is used both as feedstock and as energy [7,8]. Elastomers are polymers with viscoelasticity and very weak inter-molecular forces. When compared to other materials, they have low Young's modulus and high failure strain. The main characteristics of elastomeric materials (natural rubber, polybutadiene, neoprene, silicone, etc.) are the high elongation and elasticity of these materials, against breaking or cracking. A polymer of isoprene (cis-1,4-polyisoprene), natural rubber (NR) is the most widely used natural polymer. In spite of the natural rubber production of only 40% of the total rubber demand, the superiority of natural rubber over synthetic rubber stems from its molecular structure and high-molecular mass (>1 MDa), leading to properties such as elasticity, resistance to abrasion and impact, efficient heat dispersal and resilience and malleability at low temperatures. Natural rubber has unique, and to a certain extent, undefined secondary compounds (proteins, lipids, carbohydrates,

minerals), which makes it impossible to replicate its properties in synthetic rubber [8–10]. On the other hand, it is known that natural rubber is the most used elastomer worldwide, due to its low price; it is renewable, non-toxic and shows excellent physical properties. The mechanical and thermal stability of rubber is conferred by curing (cross-linking), which may be carried out by sulfur vulcanization, peroxides, ultraviolet light, electron beam irradiation, microwave, etc. The natural rubber is not used alone, only in the form of mixtures which generally contain: active fillers, plasticizers, cross-linking agents and other ingredients that give different characteristics to the final product [9,10]. A new environmentally-friendly elastomeric material with an improved biodegradability rate can be obtained using natural fibers in rubber blends instead of hazardous active fillers such as carbon black or silica. Natural fibers (FN) are traditional renewable materials that have recently resurfaced [11,12] due to their beneficial properties and the fact that they serve as adequate substitutes for synthetic fibres in polymer composites. Their low density reveals their potential for an outstanding reinforcement in lightweight structures. Natural fibers are a renewable resource, and therefore easily biodegradable [12], comprised mainly of cellulose, the content of which depends on the type of fiber. Cellulose is a polysaccharide composed of molecules of  $\beta$ glucose linked by 1,4- $\beta$ -glycoside bonds which may be broken by microorganisms via enzymatic hydrolysis. The intensity of cellulose decomposition depends on the reduction in the degree of polymerization, breakage of the fiber structure and reduction in tearing strength, while total decomposition is possible only in extreme cases. In addition to cellulose, natural fibers also contain small amounts (up to 10–30%) of compounds such as hemicellulose and lignin, providing fibers with rigidity, and pectins, which act as a kind of glue. Hemicelluloses and pectins may be decomposed by enzymes such as xylanase, galactosidase, mannosidase, glucuronidase, pectinesterase, glycosidase and others, produced by various microorganisms [13–16]. The structure of lignin makes it the slowest plant component to decompose, as phenylpropane compounds are linked by ether and carbon bonds and are therefore highly resistant to enzymatic decomposition. There are, however, certain species of fungi and bacteria able to decompose lignin, such as *Chaetomium*, *Paecilomyces*, *Fusarium*, *Nocardia*, *Streptomyces*, *Pseudomonas*, *Arthrobacter* and others. [17,18]. In recent years, the inefficiency of conventional disposal methods for these pollutants and the environmental problems they cause [19] have led to the attempt of using microorganisms to biodegrade rubber or plastic waste. Biodegradation or “biotic degradation” is basically a chemical degradation of materials caused by the biological decomposition action of microorganisms such as bacteria, fungi and algae [20,21]. The use of microorganisms for degradation of waste rubber products, as mentioned in many previous studies, is clear evidence of the power of those microorganisms in attacking the structure of natural rubber and breaking down its chains [22–24].

This paper focuses on obtaining composites based on natural rubber and natural fibers (flax and sawdust), and cross-linking the elastomer with benzoyl peroxide, with the aim of investigating the degree of biodegradation. The biodegradation degree was determined by comparing the initial dry weight of composites before and after incubation with the microorganism (weight loss) against the final one, as well as the cross-linking degree before and after incubation with the microorganism. Scanning electron microscopic (SEM) analysis was performed in order to show morphological changes.

## 2. Experimental

### 2.1. Materials Used for Composites Preparation

The following materials were used in the study: natural rubber Thick Pale Crepe 1X (purchased from Malwatte Valley Plantations PLC, Colombo, Sri Lanka), antioxidant Irganox 1010 (purchased from BASF Schweiz AG, Basel, Switzerland), polyethylene glycol PEG 4000 (purchased from Advance Petrochemicals LTD, Ahmedabad, India), wood sawdust (dimension of max 1 mm), ground flax wastes (thread length of max 3 mm) and dibenzoyl peroxide Perkadox 14-(purchased by Akzo Nobel Chemicals, Barcelona, Spain) as vulcanizing agent.

## 2.2. Methods for Obtaining Elastomeric Composites

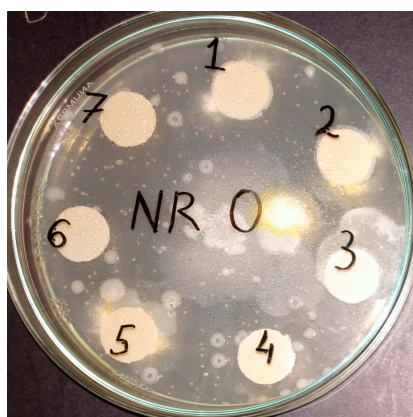
The composites were prepared using a laboratory sized two-roll mill. Therefore, the constituents were added in the following sequence and amounts: 100 phr of natural rubber (NR) roll binding (2 min), embedding 3 phr of PEG 4000 (parts to 100 parts rubber) and 1 phr of antioxidant Irganox 1010 (2 min), adding 10 and 20 phr wood sawdust or flax fibers respectively (2–4 min) and 8 phr of vulcanizing agent dibenzoyl peroxide Perkadox 14-4B (2 min). Finally, after the complete homogenization (4 min) the obtained mix was removed from the roll in the form of a sheet. The process variables were temperature  $40 \pm 10$  °C, friction 1:1.1 and total blending time from 8 to 14 min. The plates used for tests were obtained by pressing in a hydraulic press at 160 °C and 150 MPa and the curing time was 19 min. The full recipes of the composites are presented in Table 1.

**Table 1.** The composites samples synthesis details.

Samples Codes	Amount of Materials (phr)					
	Natural Rubber	PEG 4000	Irganox 1010	Perkadox 14-4B	Flax Fibers	Wood Sawdust
A	100	3	1	8	-	-
B-1	100	3	1	8	10	-
B-2	100	3	1	8	20	-
C-1	100	3	1	8	-	10
C-2	100	3	1	8	-	20

## 2.3. Sample Inoculation

The microorganism used for NR composites biodegradation was *Aspergillus niger* (from Microbiologics, MediMark Europe, Grenoble, France) and Sabouraud Dextrose Agar (Scharlab S.L., Barcelona, Spain) was used as a culture medium. Seven disks of each composite samples (1 cm diameter and 2 mm thick) were sterilised in 96% ethyl alcohol, washed with sterile distilled water and aseptically placed in Petri dishes with a diameter of 15 cm sterilized at 180 °C, containing Sabouraud Dextrose Agar medium (see Figure 1). The disks were placed in the form of a regular heptagon at a distance of 5 mm from the edge of the plate. Each disk was covered with *Aspergillus niger* suspension by spraying. Biodegradation was carried out at  $30 \pm 1$  °C and relative humidity of >85% for 60 days. Loss of water during incubation was supplemented with sterile distilled water.



**Figure 1.** Seven disks of control sample (free natural fibers) placed in Petri dish (15 cm diameter), containing Sabouraud Dextrose Agar medium.

#### 2.4. Laboratory Tests

**Mass Loss after Biodegradability Tests:** After having been exposed to fungal cultures for 60 days, composites were harvested, washed in ethanol to remove all fungal traces, and air-dried overnight at room temperature. Biodegradation of composite samples was determined by calculating the weight loss percentage before ( $m_i$ ) and after ( $m_f$ ) microbial treatment using an electronic balance, based on the following formula:

$$\text{Weight\_loss(\%)} = \frac{m_i - m_f}{m_i} \times 100 \quad (1)$$

All seven disks in each sample were analyzed and values were averaged.

The sol-gel analysis was performed on cross-linked natural rubber (with and without natural fibers) to determine the mass fraction of insoluble elastomer (the network material resulting from network-forming cross-linking process) samples (gel fraction). The gel fraction was calculated as:

$$\text{Gel\_fraction} = \frac{m_s}{m_i} \times 100 \quad (2)$$

where  $m_s$  and  $m_i$  are the weight of the dried sample after extraction and the weight of the sample before extraction, respectively [10,25].

The cross-link density ( $\nu$ ): was determined on the basis of equilibrium solvent-swelling measurements (in toluene at 23–25 °C) by application of the well-known modified Flory–Rehner equation for tetra functional networks, using the relationship:

$$\nu = -\frac{\text{Ln}(1 - \nu_{2m}) + \nu_{2m} + \chi_{12}\nu_{2m}^2}{V_1\left(\nu_{2m}^{1/3} - \frac{\nu_{2m}}{2}\right)} \quad (3)$$

$$\nu_{2m} = \frac{1}{1 + G} \quad (4)$$

$$G = \frac{m_g - m_s}{m_s} \times \frac{\rho_e}{\rho_s} \quad (5)$$

where  $V_1$  is the molar volume of solvent (106.5 cm<sup>3</sup>/mol for toluene),  $\nu_{2m}$  is the volume fraction of polymer in the sample at equilibrium swelling, and  $\chi_{12}$  is the Flory–Huggins polymer–solvent interaction term (the value of  $\chi_{12}$  is 0.393 for NR-toluene) [25,26].

**Rubber–filler interactions:** the extent of interaction between rubber and filler (wood sawdust or flax fibers) can be analyzed using the Kraus equation [27]. The Kraus equation is expressed as follows:

$$V_{ro}/V_{rf} = 1 - m \frac{f}{1 - f} \quad (6)$$

where

$$V_{rf} = \frac{[(D - FT)/\rho_r]}{[(D - FT)/\rho_r] + [A_0/\rho_s]} \quad (7)$$

where  $V_{ro}$  and  $V_{rf}$  are the volume fractions of rubber in the gum vulcanizate and in fiber filled swollen sample, respectively,  $f$  the volume fraction of filler,  $m$  the filler polymer interaction parameter,  $\rho_r$  and  $\rho_s$  are the densities of rubber samples and solvent (0.94–1.0 g/cm<sup>3</sup> for natural rubber and 0.866 g/cm<sup>3</sup> for toluene), respectively,  $D$  the deswollen weight of the test specimen (dry weight),  $F$  the weight fraction of the insoluble components,  $T$  the weight of the specimen and  $A_0$  the weight of the absorbed solvent at equilibrium swelling.

**Water uptake test:** the effect of water absorption on fiber reinforced natural rubber composites are investigated in accordance with SR EN ISO 20344/2004. The water uptake was calculated as:

$$\text{Water uptake (\%)} = \frac{W_5 - W_1}{W_1} \times 100 \quad (8)$$

where,  $W_5$  is the weight of the sample saturated with water, determined at periodic intervals and  $W_1$  is the initial weight of the oven-dried specimen.

Notes: For sol-gel analysis, cross-link density, rubber-filler interactions and water uptake test, both incubated and un-incubated disk samples were analyzed. Four samples of the total seven were used and values were averaged for sol-gel analysis, the cross-link density and rubber-filler interactions, and for water uptake test, three samples of the total seven were used and values were averaged. Also, work methods employed for all these tests were described in previous studies [9,10].

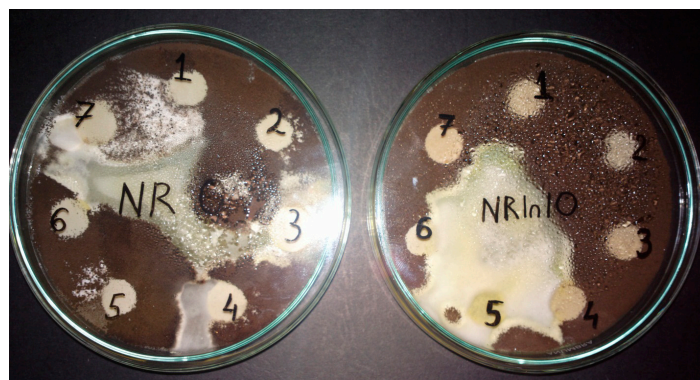
Fourier transform infrared spectroscopy (FT-IR): was used for investigation in changes in the chemical structure of the elastomeric composites. The device used was FT-IR spectrophotometer—TENSOR 27 (Bruker, Bremen, Germany) by ATR measurement method. Samples spectra are the average of 30 scans carried out in absorption in the range of 4000–600  $\text{cm}^{-1}$ , with a resolution of 4  $\text{cm}^{-1}$ .

Scanning Electron Microscopy (SEM): was used for examination of the surface texture of the elastomeric composites. The device used was scanning electron microscope (FEI Company, Hillsboro, OR, USA). All the surfaces were placed on an aluminum mount, sputtered with gold-palladium, and then scanned at an accelerating voltage up to 30 kV.

### 3. Results and Discussion

#### 3.1. Mass Loss after Biodegradability Tests

The *Aspergillus niger* strain is a widespread mould in the environment and may develop on almost anything: coffee, various foods, textiles, wood, paper and leather goods, which is why this mould was used to test resistance of NR/natural fibers (FN) to fungi. This mould is invasive, developing quicker than *Penicillium* or other types of fungi, with the tendency of extending to the detriment of other species [28]. *Aspergillus niger* was selected in this paper as a promising microbial agent in the degradation of NR/natural filler composite materials. It is known that natural fibers may be incorporated into polymeric matrices as a rapidly biodegradable component [29]. Figure 2 presents the appearance of NR and NR with 10 phr flax samples after 15 days. Furthermore, the weight loss is used as a quantitative measure for polymer biodegradation. The results of experiments are presented in Figure 3.



**Figure 2.** Appearance of NF (free natural fibers) and NR with 10 phr flax samples after 15 days.

Biodegradation depends on the nature of pretreatment and polymer characteristics such as mobility, tacticity, crystallinity, molecular weight, the type of functional groups and substituents present in its structure, and additives added to the polymer. In the presence of  $\text{O}_2$ , aerobic microorganisms decompose complex materials, resulting in microbial biomass,  $\text{CO}_2$ , and  $\text{H}_2\text{O}$  [30]. As we can see, the control sample, NR only (Figure 2) shows the slightest coverage with *Aspergillus niger*.

Both natural rubber biodegradation and the growth of bacteria with rubber as a carbon source are slow processes, and in the first step of rubber degradation, oxidative cleavage of the double bond in the poly(cis-1,4-isoprene) backbone takes place [31]. Degradation by microorganisms via different enzymatic activities and bond cleavage occurs in sequential steps, as follows: bio-deterioration (altering the chemical and physical properties of the polymer), bio-fragmentation (polymer breakdown in a simpler form via enzymatic cleavage) and assimilation (uptake of molecules by microorganisms) and mineralization (production of oxidized metabolites after degradation) [32]. Composites based on natural rubber and natural filler (flax and sawdust) are excellent substrates for fungal growth. It is known that, natural fibers may be incorporated into polymeric matrices as a rapidly biodegradable component [29]. The composition of the natural fibers used in the experiment is different. So, wood sawdust consists mainly of cellulose (40–50%), hemicellulose (25–30%), lignin (16–33%), extractives (3–8%) and ash (0.2–0.8%) [33] and the main constituents of a flax fiber consist of cellulose (70–75%), hemicellulose (17–18%), wax (1.7%), lignin (2–2.2%) and pectin (1.8–2.2%), in varying quantities [34,35]. It can be seen that flax fibers have a higher content of cellulose (70–75%) and lower content of lignin (2–2.2%) than that of wood sawdust (40–50% and 16–33%, respectively). In conclusion, the results are consistent with those presented by other researchers [13–16] who claim that cellulose degrades rapidly in the presence of fungi, whereas lignin degrades less. The results in Figure 3 show weight loss indicating both a biodegradation of natural rubber and NR/FN composites after incubation with microorganism. Weight loss is higher when increasing the amount of FN in mixtures and a higher weight loss is also seen in mixtures containing flax fibers (higher cellulose content) as compared to sawdust ones.

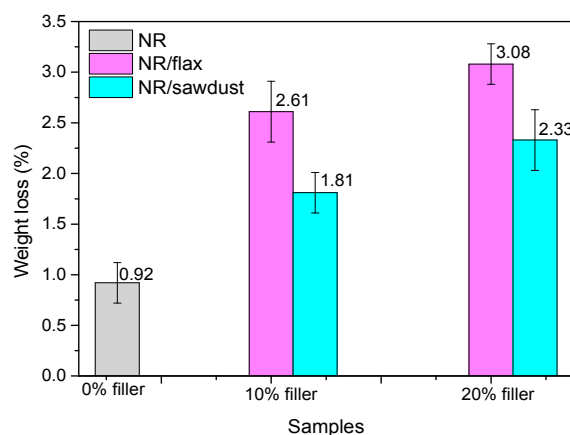


Figure 3. Weight loss of the composites.

### 3.2. Gel Fraction and Cross-Link Density of Blends

Natural rubber is a highly unsaturated polymer, very susceptible to oxidative degradation. The component hydrocarbon polymers of natural rubber have molecular weights ranging from  $10^5$  to  $10^6$ , and the network structure of the vulcanizate is insoluble in any organic solvent. Low molecular-weight fragments produced by a non-biological degradation are therefore considered to be metabolized by the microorganism in the microbial deterioration of rubber vulcanizates [36,37].

Figure 4 shows that gel fraction value is over 95% for all blends and varies irregularly depending on the amount of FN (wood sawdust or flax fibers) in the blend, but shows lower values for inoculated samples with *Aspergillus niger*. Starting from these results (shown in Figure 5), however, the content of the soluble fraction (soluble fraction =  $100 - \text{gel fraction}$ ) of the un-inoculated control sample (NR) was only 2.85% and after a 60-day incubation period over 96% of rubber remained an insoluble gel. Thus we can say that after 60 days, the soluble fraction increased from 2.85 to 3.64%. The same goes for samples with fillers (flax or sawdust): the gel fraction for the inoculated samples is lower than for the control samples (without *Aspergillus niger*). This means that after incubation with fungi for 60 days, the

percentage of soluble fraction, which can be both rubber and filler, is higher than the control samples, indicating a biodegradation process.

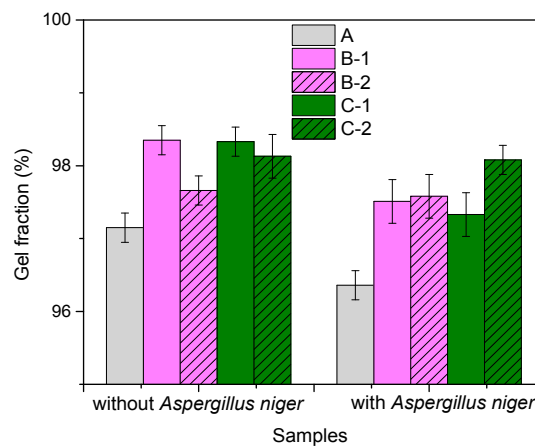


Figure 4. Gel fraction of the composites before and after incubation with *Aspergillus niger*.

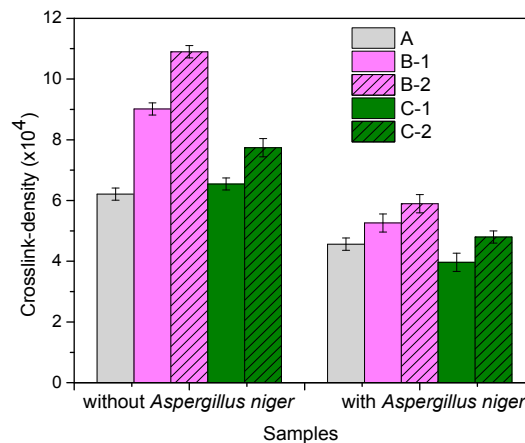


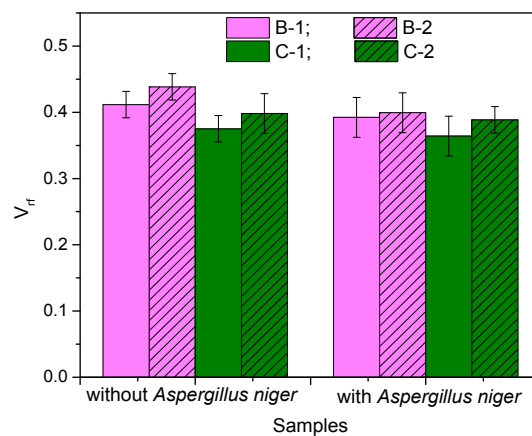
Figure 5. Cross-link density of the composites before and after incubation with *Aspergillus niger*.

Figure 5 shows the cross-link density of blends with and without *Aspergillus niger*. Cross-link density ( $\nu$ ) is found to increase with the increased amount of wood sawdust/flax fibers, due to FN acting as filler in natural rubber blends, reinforcing them. However, it is observed that for incubated samples, the cross-linking density is lower than the non-incubated samples. After 60 days, the cross-linking density decreased with 25.54% for NR, 41.64% for NR/10 phr flax, 45.92% for NR/20 phr flax, 39.38% for NR/10 phr sawdust and 38.01% for NR/20 phr sawdust, indicating a biodegradation process. These results indicate that the vulcanizate network was subject to a direct biological attack. There is an accumulation of soluble polyisoprene oligomers during the incubation, very likely caused by the enzymatic cleavage of polymeric chains in the network structure, with molecular weights ranging from  $10^4$  to  $10^3$ . The largest molecular weight of the extractable oligomers is found to correspond to the average-chain-segment molecular weight ( $M_c$  of about  $10^4$ ) between adjacent cross-links. Cross-links from fragments with molecular weight larger than  $M_c$  may not be easily extracted due to chain entanglements. Conversely, there are reports in the literature that oligomers of polyisoprene or polybutadiene with a molecular weight of up to approximately  $10^3$  can be rapidly utilized by microorganisms, while oligomers with a molecular weight exceeding  $2 \times 10^3$  are very slowly attacked [37–39]. The scission of longer polymeric chains of natural rubber produces short oligomers with molecular weights below  $10^3$ , which are rapidly consumed by the microorganism.

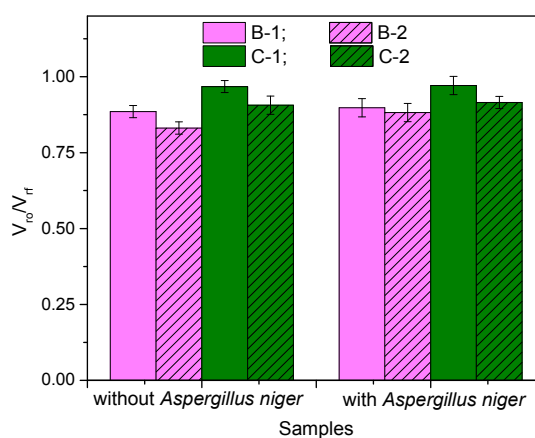
### 3.3. Rubber–Fiber Interactions

The extent of interaction between the rubber and the fibers was analyzed using the Kraus equation and the results are presented in Figures 6 and 7.

From Figure 6, it is observed that increased fiber content has led to a decreased equilibrium solvent uptake of the samples, which in turn, has caused an increase in  $V_{rf}$  for both types of composites. Also, since  $V_{r0}$  is a constant, the  $V_{r0}/V_{rf}$  ratio decreases, as shown in Figure 7, due to the increased hindrance of fibers at higher loadings. There is a strong connection between the diffusion mechanism in the composite and the ability of rubber to provide pathways for the solvent to progress in the form of randomly generated voids. Solvent uptake decreases proportionally with void formation. The  $V_{r0}/V_{rf}$  ratio designates the degree of swelling restriction of the rubber matrix due to the presence of fibers [27]. The decreased values of  $V_{r0}/V_{rf}$  values at higher loadings indicate the reinforcement effect of the fibers. If  $V_{r0}/V_{rf}$  values increase, we can say that the reinforcement effect decreases, and this happens with all samples incubated with *Aspergillus niger*. The most spectacular increase of  $V_{r0}/V_{rf}$  values was obtained for the NR/flax composites (with 1.44% and 6.18% for 10 phr and 20 phr, respectively), while for sawdust-based composites the growth was much lower, 0.39% and 0.93% for 10 phr and 20 phr, respectively. As opposed to un-bonded systems, a highly bonded system (small values for  $V_{r0}/V_{rf}$ ) is very resistant to swelling.



**Figure 6.**  $V_{rf}$  (volume fractions of rubber in the fiber-filled swollen composite) of the composites before and after incubation with *Aspergillus niger*.



**Figure 7.**  $V_{r0}/V_{rf}$  ratio of the composites before and after incubation with *Aspergillus niger*.

As we said above, the rubber–fiber interaction relies on the chemical nature of the polymer matrix and the fiber, matrix–fiber compatibility and interfacial adhesion. In this paper, the composites were



obtained by “classical” cross-linking using benzoyl peroxide, which confers to the composite a higher resistance to aging, as compared to those obtained using the classical cross-linking method with sulfur and vulcanization accelerators [9,40]. In addition to the natural rubber curing, benzoyl peroxide can produce a chemical modification of the fibres’ surface. Figure 8 illustrates the mechanism of natural rubber cross-linking using peroxide.

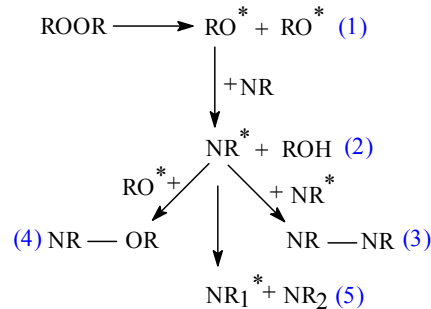


Figure 8. Mechanism of natural rubber cross-linking by peroxide.

Peroxide (ROOR) thermally decomposes to alkoxy pre-radicals (RO\*) at the vulcanization temperature due their low thermal stability (reaction 1). The formed alkoxy pre-radicals extract the hydrogen atom from the NR structure producing a site for cross-linking in NR (NR\*) (reaction 2). NR molecules combine through cross-linking sites to form vulcanized rubber, NR–NR (reaction 3). During this process some unwanted reactions may also occur (reactions 4 and 5). It is possible that fibers (flax or sawdust) will undergo chemical modification during natural rubber cross-linking, as illustrated in Figure 9. Therefore, free radicals may react with the hydrogen group of cellulose or lignin fibers of flax or sawdust [41–43].

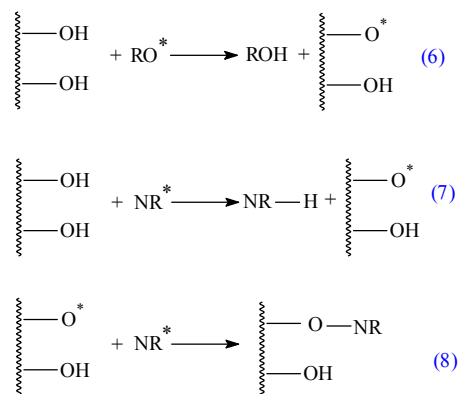


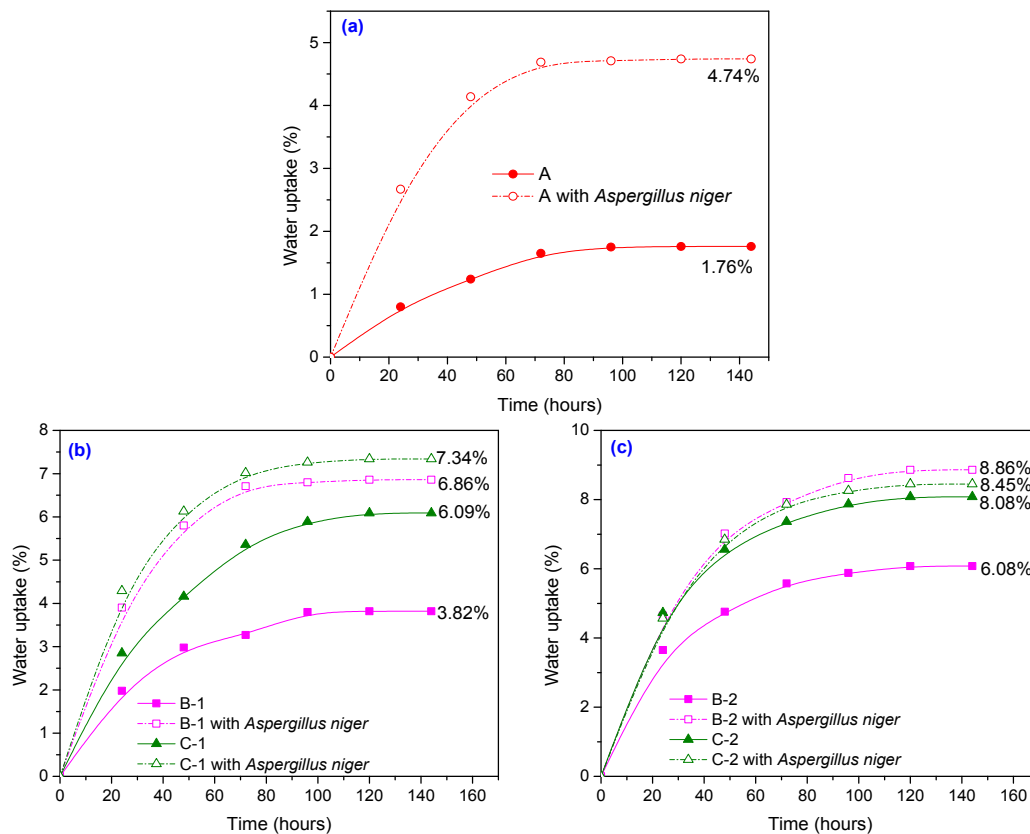
Figure 9. The chemical modification of flax or sawdust fibers by peroxide reaction.

Also, the chemical composition of natural fibers used in the production of composite materials is very important when their biodegradation is desired. Each fiber, containing mostly cellulose, hemicellulose and lignin, is basically a composite in itself, consisting of a soft lignin and hemicellulose matrix in which rigid cellulose microfibrils are immersed. Accordingly, the overall properties of the fiber is the sum of the properties of each constituent. Thus, hemicellulose is the least resistant, facilitating biodegradation, moisture absorption and thermal degradation of the fiber, while lignin is thermally stable but susceptible to UV degradation [44]. The flax fibers have a higher content of cellulose (70–75%) and lower content of lignin (2–2.2%) than that of wood sawdust (40–50% and 16–33%, respectively). In conclusion, the results are consistent with those presented by other researchers [13–16] who claim that cellulose degrades rapidly in the presence of fungi, whereas lignin degrades less. Also, a highly bonded system (small values for  $V_{r0}/V_{rf}$ ) will exhibit high resistance to swelling compared to

the unbonded systems. When the  $V_{r0}/V_{rf}$  values increase, we can say that the reinforcement effect decreases, and this happens with all samples incubated with *Aspergillus niger*. Decreasing of the reinforcement effect can be explained by some broken bonds, both from the vulcanized network of natural rubber and from the natural rubber-fiber network, due to the biodegradation effect.

### 3.4. Water Uptake

The water uptake results of all samples (before and after incubation with *Aspergillus niger*) are presented in Figure 10a–c, showing that the percentage of water absorption in the NR/natural fiber composites is dependent on fiber amount and fiber compositions.



**Figure 10.** Water uptake depending on the amount of filler in composites at  $23 \pm 2$  °C: (a) 0 phr; (b) 10 phr; (c) 20 phr.

Increased fiber content resulted in a higher water uptake, due to the hydrophobic nature of the rubber, the hydrophilic nature of the fiber as well as the greater interfacial area between the fiber and the natural rubber matrix. The number of free –OH groups from the cellulose and hemicellulose inside the fiber increases proportionally to the amount of natural fibers. Upon contact with water, these free –OH or hydroxyl groups form hydrogen bonding, leading to composites gaining weight [45,46]. From Figure 10a–c it is observed that, before incubation with *Aspergillus niger*, NR has an absorption of only 1.76%, while for the NR/flax composites the water uptake it is of 3.82% and 6.8% (for 10 phr and 20 phr, respectively), and for NR/sawdust 6.09% and 8.08% (for 10 phr and 20 phr). Moisture penetrates composite materials by three different mechanisms. The main one consists of diffusion of water molecules into the microgaps between polymer chains. Another mechanism is capillary transport into the gaps and flaws at the interfaces between fibers and polymer, due to incomplete wettability and impregnation, and the third mechanism consists of transport by microcracks in the matrix, formed during the compounding process [47].

Rubber degradation by microorganisms is known to initiate with an oxidative attack at the double bonds of the polymeric chains [48]. The reduction of double bonds and the presence of aldehydes indicate oxidative cleavage as the mechanism of degradation of natural rubber (Figure 11). The network of the natural rubber is attacked directly by the biological action during microbial degradation, with the oligomers produced by the scission of polymeric chains being used by the organism as growth substrate [37–39].

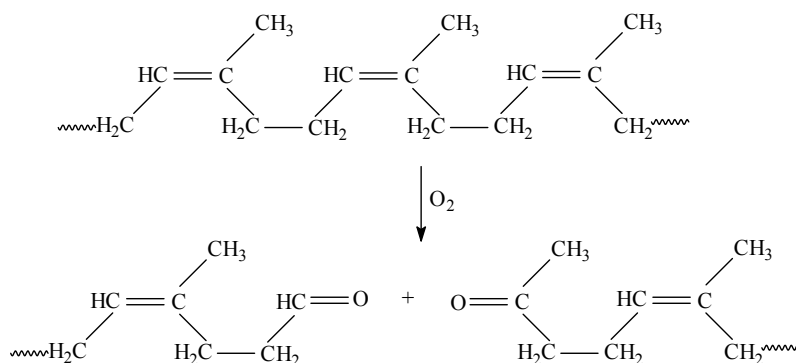


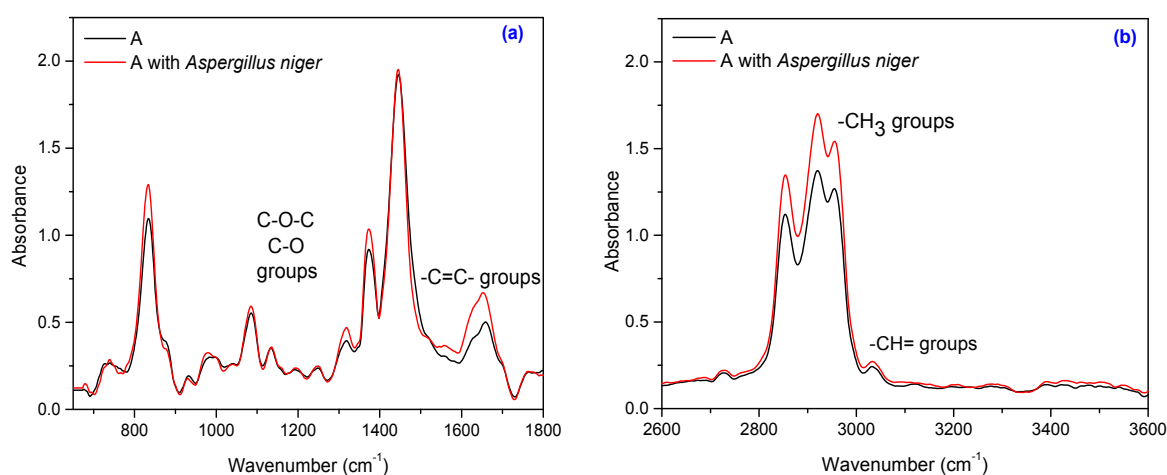
Figure 11. Biodegradation reaction mechanism of natural rubber.

On the other hand, the three polymers that make up lignocellulose, the major component of biomass, namely cellulose, hemicellulose, and lignin are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross-linkages. These macromolecules may be fragmented by a wide range of fungi and bacteria via hydrolytic or oxidative enzymes [49]. Cellulose, mostly found in plants (approximately 45% of the dry weight of wood), is a linear polymer consisting of D-glucose subunits linked by  $\beta$ -1,4 glycosidic bonds that form cellobiose molecules. These in turn form long chains (elemental fibrils) linked together by hydrogen bonds and van der Waals forces. Cellulose can also appear in crystalline form (crystalline cellulose), as well as in non-organized cellulose chains to a smaller extent, forming amorphous cellulose, which is more susceptible to enzymatic degradation [49,50]. Cellulose biodegradation may be affected by its association with other plant substances. Hemicellulose is a polysaccharide with a lower molecular weight than cellulose and consists of D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acids. Sugars are linked together by  $\beta$ -1,4- and occasionally  $\beta$ -1,3-glycosidic bonds. As opposed to cellulose, hemicellulose exhibits branches with short lateral chains consisting of different sugars, making them easily hydrolyzable polymers that do not form aggregates. Lignin is an amorphous water-insoluble and optically inactive heteropolymer consisting of phenylpropane units joined together by various bonds [49]. There has been a keen interest in degradation of cellulose, hemicellulose, and lignin by means of fungi, which are known for their ability to degrade these three polymers. Due to the insolubility of substrates, degradation by fungi and bacteria occurs exocellularly (in association with the outer cell envelope layer or extracellularly). Cellulose and hemicellulose are degraded via the hydrolytic enzymatic system of microorganisms that produces hydrolases, while lignin is depolymerized by the unique oxidative ligninolytic system [49]. As we said before the wood sawdust consists mainly of cellulose (40–50%), hemicellulose (25–30%) and lignin (16–33%), extractives (3–8%) and ash (0.2–0.8%) [33] and the main constituents of a flax fiber consist of cellulose (70–75%), hemicellulose (17–18%), wax (1.7%), lignin (2–2.2%) and pectin (1.8–2.2%), in varying quantities [34,35]. The flax fibers have a higher content of cellulose (70–75%) and lower content of lignin (2–2.2%) than that of wood sawdust (40–50% and 16–33%, respectively). And other authors suggest that the interactions between fungi and eubacteria cause total degradation of cellulose, with the release of carbon dioxide and water under aerobic conditions [49–51], whereas lignin degrades less. Even if a part of cellulose, which is hydrophilic, was biodegraded, the results obtained in terms of water absorption shows that the highest biodegradation was that of natural

rubber. Thus, after incubation with *Aspergillus niger*, an increased value of water uptake is seen, as compared to unincubated samples, the highest value being that of NR sample (169.3%), followed by NR/10 phr flax (79.6%), NR/20 phr flax (44.74%), NR/10 phr sawdust (20.53%), and NR/20 phr sawdust (4.58%) respectively. This may be due to biodegradation of the composites which might lead to reduction of molecular mass (with the effect of decreasing hydrophobicity) and to the formation of several functional polar groups:  $-\text{OH}$ ,  $-\text{O}^-$ ,  $-\text{COOH}$ ,  $-\text{COO}^-$  etc., (that enhance the hydrophilicity of materials) [52].

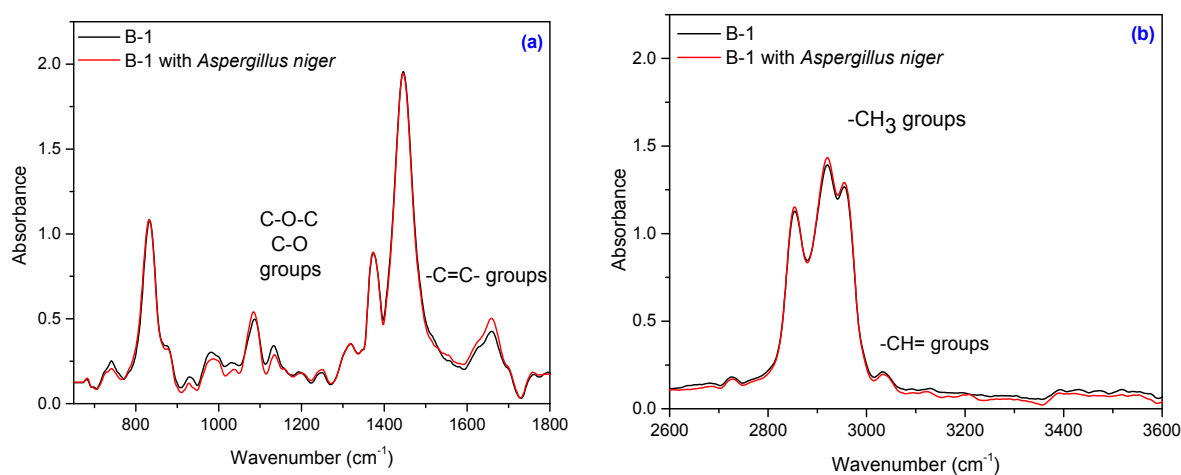
### 3.5. Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectrum of NR (Figure 12) contains some peaks, known to be characteristic of the NR structure. The characteristic bands of the saturated aliphatic  $\text{sp}^3$  C–H bonds are seen at  $2970\text{--}2830\text{ cm}^{-1}$ , assigned to  $\nu_{\text{as}}(\text{CH}_3)$ ,  $\nu_{\text{as}}(\text{CH}_2)$ , and  $\nu_{\text{s}}(\text{CH}_2)$ , respectively (as three corresponding bands) [53]. Absorption bands in the spectral region at  $1670\text{--}1640\text{ cm}^{-1}$  are due to the valence vibration of homogeneous double bonds ( $\nu_{\text{C}=\text{C}}$ ) in the NR structure. The absorption bands peaking at  $3050\text{--}3010\text{ cm}^{-1}$  corresponding to CH stretching in the  $-\text{CH}=\text{CH}_2$  group and the specific absorption bands of single bonds corresponding to  $\text{R}_2\text{C}=\text{CH}-\text{R}$  group are seen at  $850\text{--}830\text{ cm}^{-1}$ . The absorption bands of  $\text{CH}_2$  deformation and  $\text{CH}_3$  asymmetric stretching occur at  $1440\text{--}1460\text{ cm}^{-1}$  and  $1350\text{--}1380\text{ cm}^{-1}$ , respectively. Oxidation inevitably occurs during degradation of natural rubber (polyisoprene) backbone chain [31]. There are three metabolic pathways under aerobic condition: (i) oxidation of terminal methyl group with formation of carboxylic acid derivatives group; (ii) hydration of double bond to tertiary alcohol (aldehyde and ketones) and (iii) oxygenase-catalyzed cleavage of the internal bond resulting in polymeric molecular chain breakage [31,54]. The FT-IR spectrum of NR confirms the above by the fact that after inoculation, the following are seen: (i) an increase of absorption peaks due to formation of polar groups such as carbonyl and hydroxyl compound group frequencies ( $1260\text{--}1400\text{ cm}^{-1}$ ), and carboxylate or conjugated ketone ( $1550\text{--}1690\text{ cm}^{-1}$ ) respectively [55], that may be due to the first two metabolic pathways; (ii) cleavage of macromolecules with the decrease of molecular masses or the cross-linking degree highlighted by the increase of absorption peaks at  $1370\text{--}1380\text{ cm}^{-1}$ ,  $2850\text{--}2880\text{ cm}^{-1}$ ,  $2950\text{--}2980\text{ cm}^{-1}$ , corresponding to  $-\text{CH}_3$  groups, which may be due to the third metabolic pathway. At the same time, biodegradation leads to an increased number of double bonds indicated by the increased absorption peak at  $1640\text{--}1660\text{ cm}^{-1}$  correlated with the increase of the one at  $800\text{--}900\text{ cm}^{-1}$ , as well as changes in the degree of substitution of carbon atoms of the double bond indicated by the modification of absorption peaks at  $800\text{--}900\text{ cm}^{-1}$ ,  $1650\text{--}1680\text{ cm}^{-1}$ ,  $3010\text{--}3040\text{ cm}^{-1}$  [55].

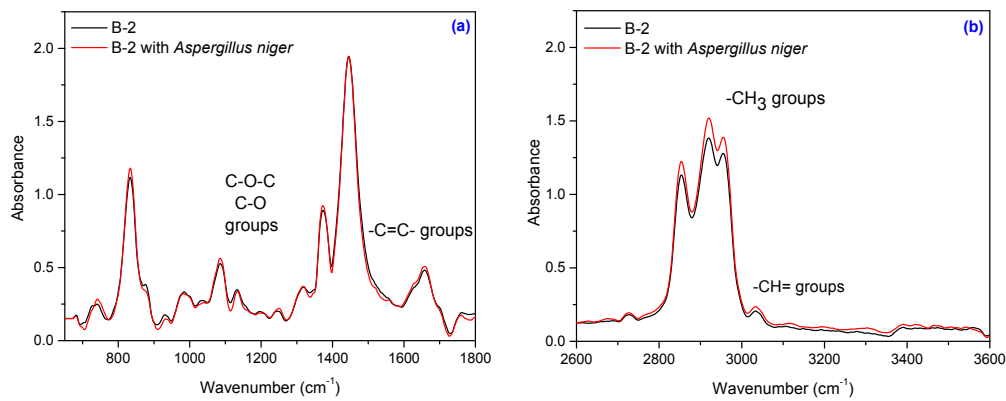


**Figure 12.** Infrared spectrum of the A sample: (a) in the range of  $650\text{--}1800\text{ cm}^{-1}$  and (b) in the range of  $2600\text{--}3600\text{ cm}^{-1}$ .

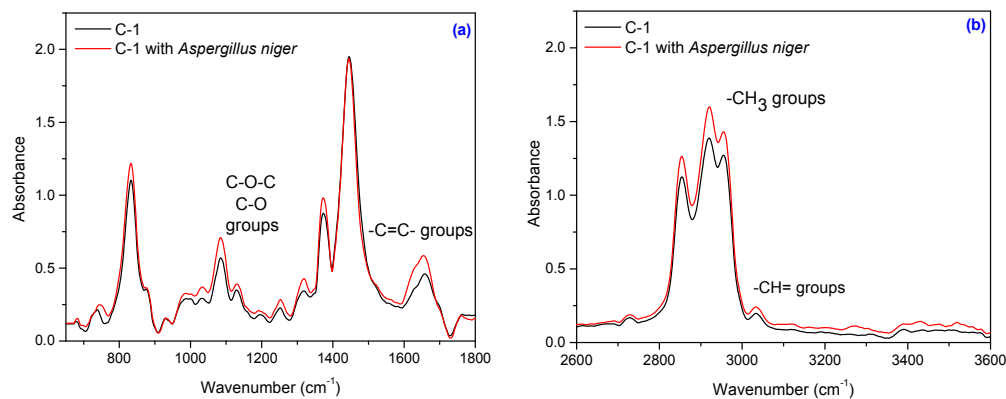
Composites subject to this study also contain natural fibers, namely flax and sawdust. The composition of natural fibers mainly consists of cellulose, hemicellulose, pectin, lignin and wax [56,57]. Cellulose is the basic structural component found in all plant fibers and consists of glucose units linked together in long chains, (the repeating units of D-anhydro glucose  $C_6H_{11}O_5$  are joined by  $\beta$ -1,4 glycoside linkages), which in turn are linked together in bundles called microfibrils. The crystallinity in cellulose is determined by the hydrogen bonding in it, which regulates the physical properties of natural fibers. Hemicelluloses are polysaccharides bonded together in relatively short, branching chains. Lignin is a three-dimensional polymer with an amorphous structure and pectin is a compound of heteropolysaccharides. Wax consists of a mixture of substituted long chains of aliphatic hydrocarbons containing alkaline, fatty acids, primary and secondary alcohols, ketones, aldehydes, and other elements [14]. The FT-IR spectrum of the NR/natural fibers composites (Figures 13–16) shows: (a) a three-shoulder band with a maximum of  $2900\text{ cm}^{-1}$  assigned to deformation vibrations of C–H groups in methyl and methylene groups [ $CH_3$ ,  $CH_2$ ,  $CH_2-OH$ ] found in cellulose and lignin; these overlap onto the absorption peaks seen at  $2970\text{--}2830\text{ cm}^{-1}$  for NR which are assigned to  $\nu_{as}(CH_3)$ ,  $\nu_{as}(CH_2)$ , and  $\nu_s(CH_2)$ , respectively [53,58]; (b) an absorption band at  $1600\text{--}1720\text{ cm}^{-1}$  (overlapping absorption bands in the spectral region at  $1670\text{--}1640\text{ cm}^{-1}$  in the NR structure), due to the valence vibration of homogeneous double bonds ( $\nu_{C=C}$ ). Natural fibers degrade in favorable environmental conditions and according to the degradation capacity of the microbial population [56,59]. Enzymes such as cellulase produced by microorganisms reduce the cellulosic part of fibers to hexose, a nutrient for these microorganisms [56]. Comparing FT-IR spectra of composites containing natural fibers (Figures 13–16) with that of the control NR sample (Figure 12), it is shown that the intensity of characteristic bands of the natural rubber chain located between  $2970\text{ cm}^{-1}$  and  $2850\text{ cm}^{-1}$  is decreased in the FT-IR spectra of the NR/FN composites after inoculation. The peaks at  $2954$ ,  $2920$  and  $2854\text{ cm}^{-1}$  assigned to C–H asymmetric stretch in  $CH_3$ , C–H asymmetric stretch in  $-CH_2-$ , and C–H symmetric stretch in both  $-CH_2-$  and  $-CH_3$ , respectively, are less intense due to oxidation of terminal methyl group with formation of carboxylic acid derivatives group [54]. After inoculation with *Aspergillus niger*, the band associated with doubly bonded carbon ( $-C=C-$ ) located at  $1660\text{--}1640\text{ cm}^{-1}$  is also reduced in intensity for composites with 10 and 20 phr flax and sawdust. This is explained by hydration of double bond to tertiary alcohol (aldehyde and ketones) [54]. Composites containing flax fibers, with a higher cellulose content, show decrease of bands particularly at  $900\text{--}1400\text{ cm}^{-1}$  when moving from high crystalline to amorphous cellulose, which means that the samples are degraded [60].



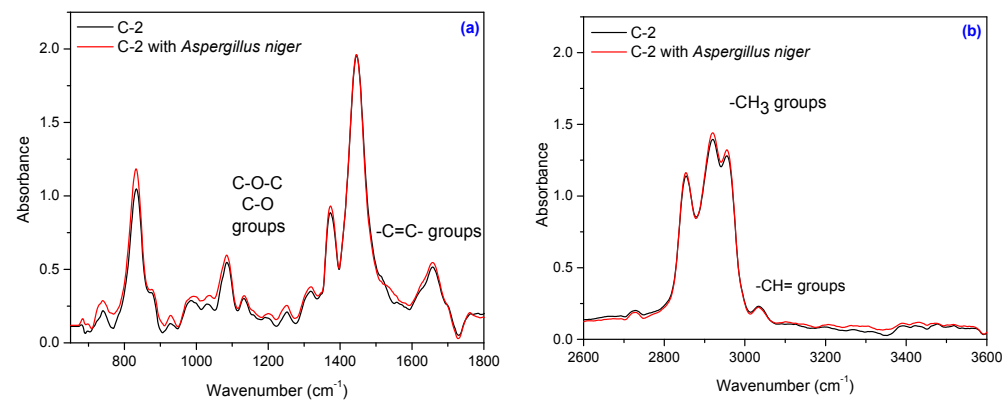
**Figure 13.** Infrared spectrum of the B-1 sample: (a) in the range of  $650\text{--}1800\text{ cm}^{-1}$  and (b) in the range of  $2600\text{--}3600\text{ cm}^{-1}$ .



**Figure 14.** Infrared spectrum of the B-2 sample: (a) in the range of 650–1800  $\text{cm}^{-1}$  and (b) in the range of 2600–3600  $\text{cm}^{-1}$ .



**Figure 15.** Infrared spectrum of the C-1 sample: (a) in the range of 650–1800  $\text{cm}^{-1}$  and (b) in the range of 2600–3600  $\text{cm}^{-1}$ .

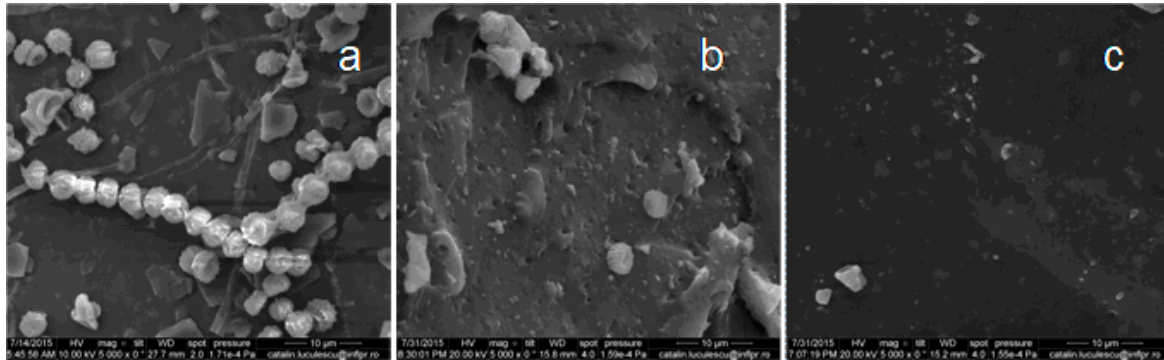


**Figure 16.** Infrared spectrum of the C-2 sample: (a) in the range of 650–1800  $\text{cm}^{-1}$  and (b) in the range of 2600–3600  $\text{cm}^{-1}$ .

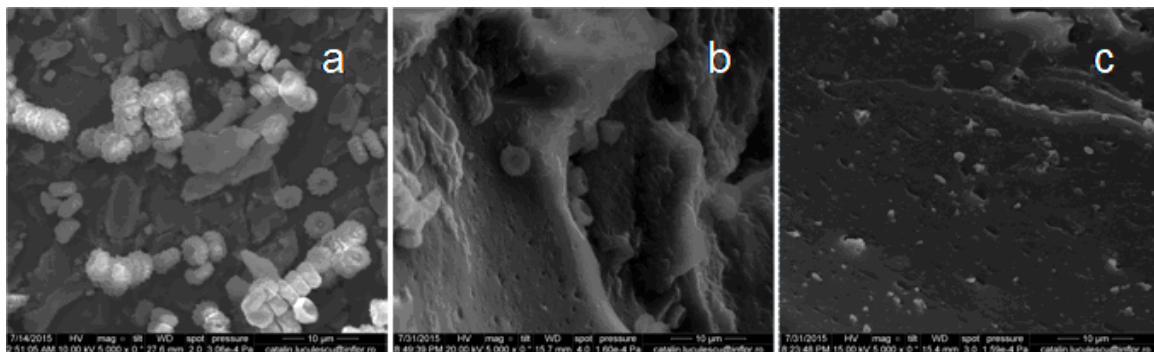
### 3.6. Scanning Electron Microscopy (SEM)

In recent years, most of the biodegradation studies on plastics or elastomers were carried out using various microorganisms, because they are capable of degrading both organic and inorganic materials. In our experiments, *Aspergillus niger* was used for biodegradation of natural rubber composites. *Aspergillus niger* or *A. niger* is a fungus and one of the most common species of the genus *Aspergillus*. The SEM analysis was performed in order to observe the changes on the surface of the composite

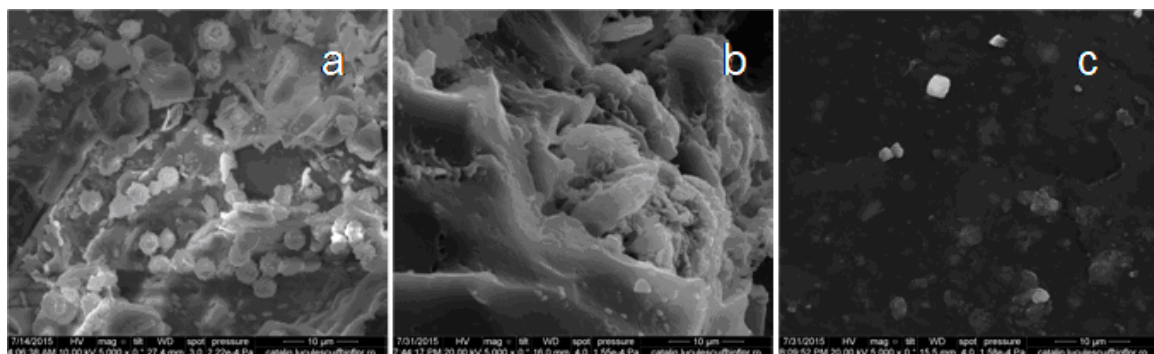
films. Figures 17–21 show the SEM micrographs of the natural rubber with and without flax and sawdust (natural rubber composites), as follows: (a) after 60 days of incubation with *Aspergillus niger*; (b) after 60 days of incubation with *Aspergillus niger* and after 72 h immersed in toluene; (c) the blanks (before incubation with the *Aspergillus niger*) and after 72 h immersed in toluene. Samples were immersed in toluene before and after incubation with the *Aspergillus* in order to determine the degree of cross-linking.



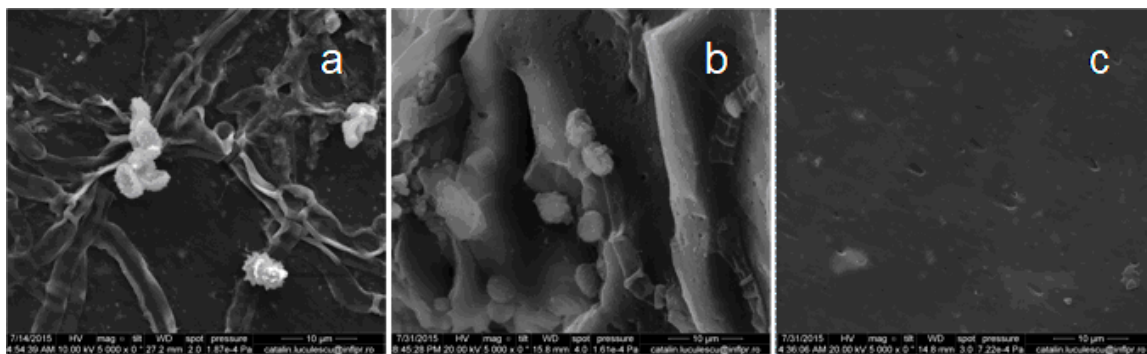
**Figure 17.** SEM of the natural rubber vulcanizate (A sample). (a) after 60 days of incubation with *Aspergillus niger*; (b) after 60 days of incubation with *Aspergillus niger* and after 72 h immersed in toluene; (c) the blanks (before incubation with the *Aspergillus niger*) and after 72 h immersed in toluene.



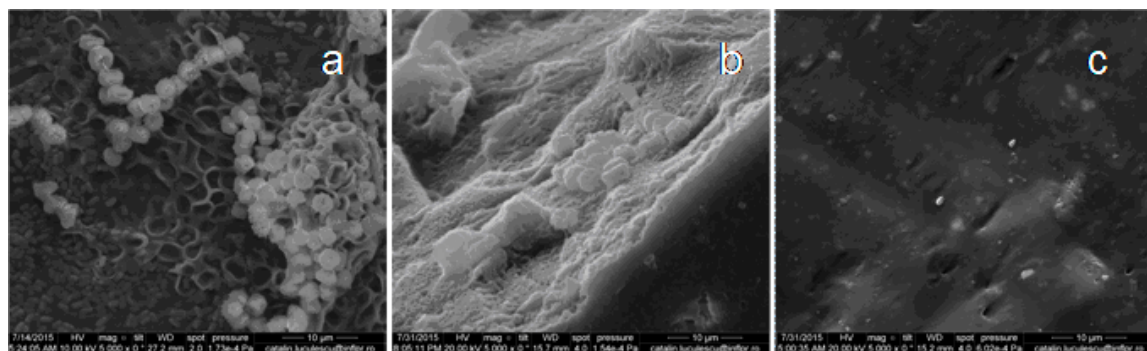
**Figure 18.** SEM of the B-1 composite. (a) after 60 days of incubation with *Aspergillus niger*; (b) after 60 days of incubation with *Aspergillus niger* and after 72 h immersed in toluene; (c) the blanks (before incubation with the *Aspergillus niger*) and after 72 h immersed in toluene.



**Figure 19.** SEM of the B-2 composite. (a) after 60 days of incubation with *Aspergillus niger*; (b) after 60 days of incubation with *Aspergillus niger* and after 72 h immersed in toluene; (c) the blanks (before incubation with the *Aspergillus niger*) and after 72 h immersed in toluene.



**Figure 20.** SEM of the C-1 composite. (a) after 60 days of incubation with *Aspergillus niger*; (b) after 60 days of incubation with *Aspergillus niger* and after 72 h immersed in toluene; (c) the blanks (before incubation with the *Aspergillus niger*) and after 72 h immersed in toluene.



**Figure 21.** SEM of the C-2 composite. (a) after 60 days of incubation with *Aspergillus niger*; (b) after 60 days of incubation with *Aspergillus niger* and after 72 h immersed in toluene; (c) the blanks (before incubation with the *Aspergillus niger*) and after 72 h immersed in toluene.

The biodegradation process relies on the adhesion of microorganisms to the surface of rubber composites. Figures 17a, 18a, 19a, 20a and 21a show formation of bacterial colonies and elongating hyphae surrounded by a fibrillar extracellular matrix. After incubation with *Aspergillus niger*, the surface appears to be eroded, while holes and cavities are formed on the surface of composites, as shown in Figures 17b, 18b, 19b, 20b and 21b. Also, the SEM micrographs indicate the appearance of pits and erosions after treatment with *Aspergillus niger*. The presence of holes and cavities may be due to biodegradation process suggesting that fungi have penetrated into the composites matrix. In Figures 17c, 18c, 19c, 20c and 21c are presented the composites before incubation, and from these SEM micrographs, it can be seen that the samples had a smooth surface with few defects. Materials exhibiting a damaged or rough surface are more susceptible to fungi attack compared to materials with smooth surfaces, because propagules are retained more easily at the surface [30,61]. Rough or cracked surfaces provide favorable conditions for fungal attachment and growth and, as fungi take their nutrients from the material or from debris accumulated at the surface, they continue to penetrate deeper layers through micro-cracks or damaged areas [30,62]. The degradation was also confirmed by comparing the FTIR results of both types of samples, NR/filler (flax or sawdust) composite and NR. Presence of aldehydic or ketone group in FTIR spectra (peaks at  $1550\text{--}1690\text{ cm}^{-1}$ ), or polar groups such as carbonyl and hydroxyl compound group frequencies ( $1260\text{--}1400\text{ cm}^{-1}$ ) along with increased absorption peaks at  $1370\text{--}1380\text{ cm}^{-1}$ ,  $2850\text{--}2880\text{ cm}^{-1}$ ,  $2950\text{--}2980\text{ cm}^{-1}$ , corresponding to  $\text{--CH}_3$  groups which may be due to cleavage of macromolecules with the decreased molecular masses or the cross-linking degree, which indicates the degradation of rubber composites [30,63].



#### 4. Conclusions

The results show that, after incubation with microorganisms, there is a weight loss of NR/FN composites, influenced by the amount of FN in the mixtures. A higher weight loss is also seen in mixtures containing flax fibers compared to those containing sawdust. These results have been correlated with those obtained for gel fraction, cross-linking degree, rubber-fiber interaction and water uptake. It is observed that for incubated samples, gel fraction shows lower values and that the cross-linking density is lower than the non-incubated samples. These results indicate a direct biological attack on the vulcanizate network. Analysing the rubber-fiber interaction, for all samples incubated with *Aspergillus niger*, it was seen that the reinforcement effect decreases. Decreasing of the reinforcement effect can be explained by some broken bonds, both from the vulcanized network of natural rubber and from the natural rubber-fiber network, due to the biodegradation effect. Increased fiber content resulted in a higher water uptake. After incubation with *Aspergillus niger*, an increased water uptake is seen, the highest value being found in NR samples, followed by NR/flax, and NR/sawdust, respectively. This may be due to biodegradation of composites that might lead to: (a) reduction of molecular mass (with the effect of decreased hydrophobicity) or (b) the formation of several functional polar groups:  $-\text{OH}$ ,  $-\text{O}^-$ ,  $-\text{COOH}$ ,  $-\text{COO}^-$  etc., (that increase hydrophilicity of materials). These observations are confirmed by the FT-IR spectrum of NR by the fact that after inoculation the following are seen: (a) an increase of absorption peaks due to formation of polar groups such as carbonyl and hydroxyl compound group frequencies ( $1260\text{--}1400\text{ cm}^{-1}$ ), carboxylate or conjugated ketone ( $1550\text{--}1690\text{ cm}^{-1}$ ), respectively; (b) cleavage of macromolecules with the decrease of molecular masses or the cross-linking degree, highlighted by the increased absorption peaks at  $1370\text{--}1380\text{ cm}^{-1}$ ,  $2850\text{--}2880\text{ cm}^{-1}$ ,  $2950\text{--}2980\text{ cm}^{-1}$ , corresponding to  $-\text{CH}_3$  groups. The SEM micrographs of the natural rubber with and without flax and sawdust after incubation with *Aspergillus niger* clearly show surface erosion, as well as the formation of holes and cavities on the surface of the composites, all of which are due to the biodegradation process.

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**Conflicts of Interest:** The authors declare no conflict of interest".

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