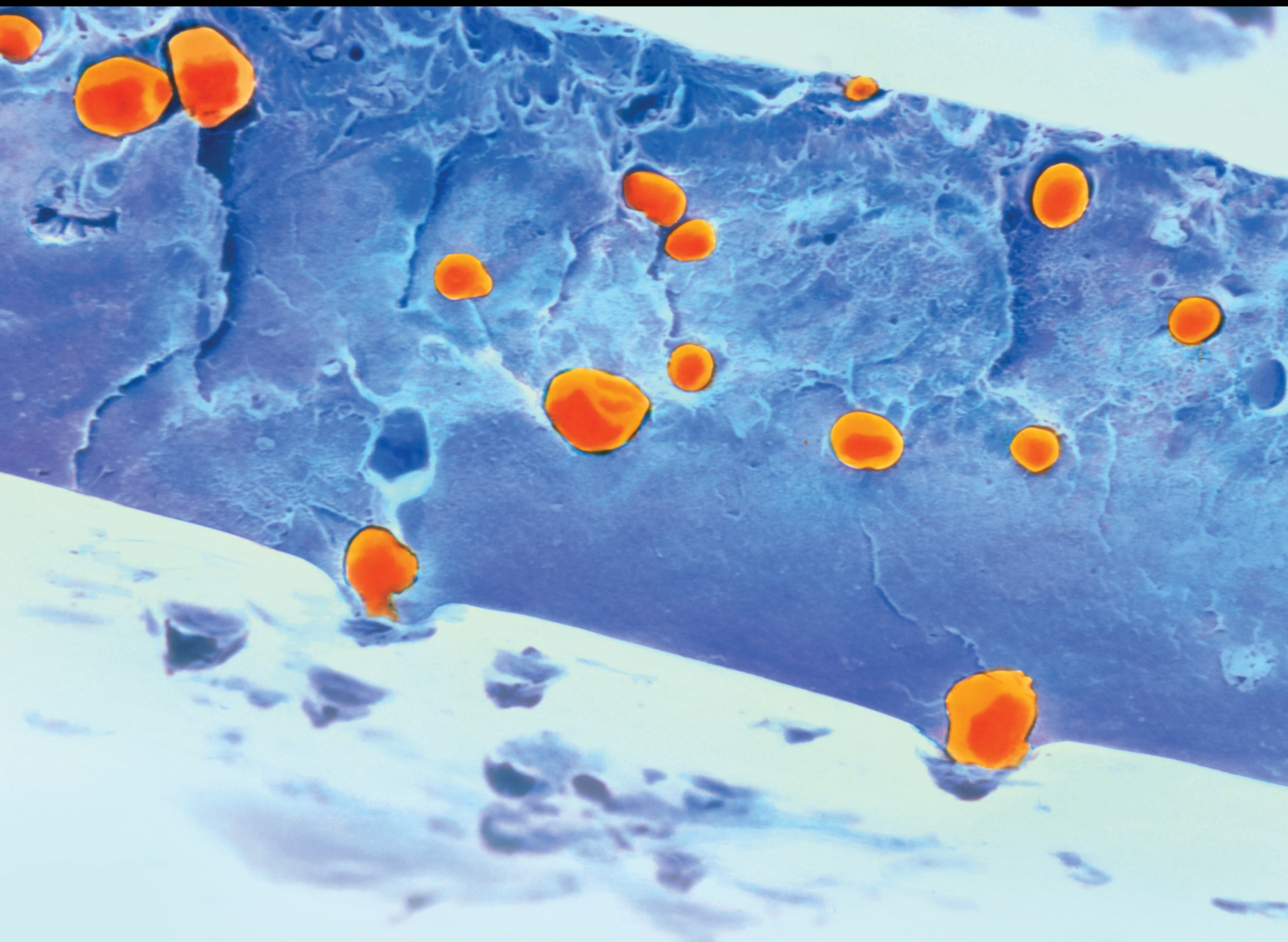


# Polymeric Materials from Renewable Resources for Environmentally Friendly Applications

Lead Guest Editor: Maria Râpă

Guest Editors: Joanna Ludwiczak and Raluca Nicoleta Darie-Nita





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International Journal of Polymer Science

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## Research Article

# Reaction Mechanisms Applied to Starch Modification for Biodegradable Plastics: Etherification and Esterification

Yongun Kim and Cheolsoo Jung 

*Department of Chemical Engineering, University of Seoul, 163-60 Seoulsiripdae-ro, Dongdaemun-gu, Seoul 163-60, Republic of Korea*

Correspondence should be addressed to Cheolsoo Jung; [csjung@uos.ac.kr](mailto:csjung@uos.ac.kr)

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Although many studies are being actively conducted to develop biodegradable plastic materials, most of these reports focused more on efficiency or performance improvement than on the reaction mechanism. This paper discussed the reaction mechanism applied to starch modification by etherification and esterification, which are the most studied in the field of biodegradable plastics. In the starch-reforming reaction by etherification, the effects of the reaction temperature, pH, solvent, and by-products on the chemical structure and physical properties of biodegradable plastics were discussed. In esterification, the structure of the substituents and the reaction solvents were examined. As a material that can replace plastics, the aim is to help derive new ideas on the design of reaction condition that can expand the use of starch.

## 1. Introduction

The mass drilling of petroleum, which began in Titusville, Pennsylvania, in 1859, caused a boom in the research of polymeric materials in the 1900s. As a result, a new material called plastic began to appear in human technological civilization. Plastic refers to the properties or characteristics of a material composed of polymers. Plasticity has the dictionary meaning of “easy to shape and easy to process, etc.” Plastic is a term coined by reflecting this concept in material engineering, and its general process can be explained, as shown in Figure 1 [1]. The addition of a plasticizer that can reduce the attraction among polymer chains can make the polymer properties more flexible. Unlike ceramic materials or metallic materials, polymers can be molded at an appropriate temperature without a plasticizer because of their unique thermal properties, such as glass transition ( $T_g$ ), crystallization ( $T_c$ ), melting ( $T_m$ ), and decomposition ( $T_d$ ). These materials made without a plasticizer are also included in the category of plastics [2].

Up to date, plastics are being developed as high-performance materials with sufficient physical properties to replace wood, metal, and ceramics, but the developed plastics are not environmentally friendly compared to those traditional materials. They do not degrade easily and remain in nature as microplastics. Therefore, the marine and land environments have been seriously polluted by waste plastics since the mid-20<sup>th</sup> century, when the amount used increased rapidly. The recycling movement of commodity plastics has been promoted internationally since 1988 to suppress this environmental pollution caused by discarded plastics. Owing to the economic feasibility and convenience of using plastics, however it has not yet reached the level to suppress the increased use of plastics.

Meanwhile, research on biodegradable plastics that can replace commodity plastics has increased rapidly since 2008, as shown in Figure 2 that was searched on the keywords of biodegradability within the plastic category through the Scopus. The published papers on the seriousness of environmental problems caused by plastics began to

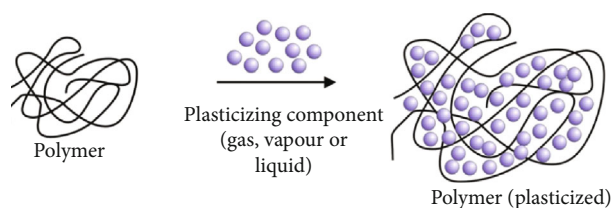


FIGURE 1: Plasticization phenomenon [1].

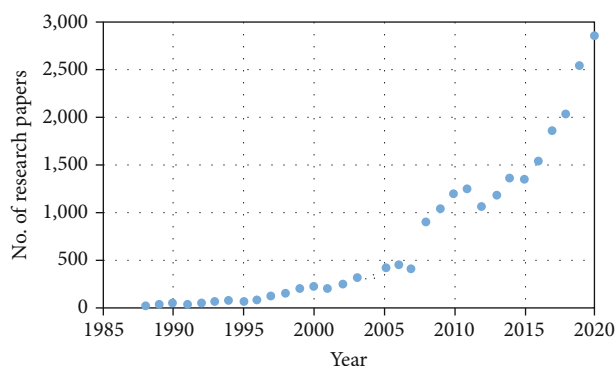


FIGURE 2: Number of research papers related to biodegradable plastics.

attract the international community's attention around this time [3–7].

The chemical structures used most widely as biodegradable plastic materials are  $\beta$ -glucose chain present in cellulose, such as wood and cotton fiber, and  $\alpha$ -glucose chain structure in starch. Cellulose, a polymeric material, comprises 40–50% of wood and 90% of cotton fiber and is an environmentally friendly material used widely. On the other hand, the cellulose present in wood has a structure with lignin and hemicellulose intertwined, and  $\beta$ -glucose is linked in a linear structure with *cis*-shaped chains connected by high-density hydrogen bonds. Therefore,  $\beta$ -glucose chain is a polymeric material that is difficult to process [8].

Cellulose structures in wood and fibers were discovered by the French chemist Anselme Payen in 1838. In 1870, John Wesley Hyatt of the United States developed a thermoplastic material called celluloid by applying heat to cellulose. In 1908, the Swiss chemist Jacques Brandenberger developed a chemical treatment process for manufacturing a transparent cellophane sheet by extracting cellulose from wood through treatment with an aqueous NaOH solution and a reaction with  $\text{CS}_2$  to make cellulose substituted with xanthate (Scheme 1) [9].

This process was a method that could produce a transparent and excellent cellophane sheet without destroying the basic structure of cellulose, which is still being used in packaging fields, such as food, cosmetics, and gifts. On the other hand, because the synthetic route of phenol resin was reported in 1907 (Scheme 2), plastics with various structures, such as PP, PE, and PVC, have been developed. The interest in biodegradable plastic materials has weakened because of the flow of technological development that places

importance on convenience and economy rather than eco-friendly concepts.

As of 2021, many materials have been studied as biodegradable plastics, including cellulose, starch, poly(lactic acid) (PLA), and poly(butylene adipate-co-terephthalate) (PBAT). In this report, the mechanisms of etherification and esterification, which are the most widely applied reactions in the development of biodegradable plastics for modifying the hydroxyl group of starch, are discussed for novice researchers in this field.

Starch comprises 10 to 35% of amylose, in which 100 to 10,000  $\alpha$ -glucose units are linked spirally, and 65 to 90% of amylopectin, in which 19 to 30  $\alpha$ -glucose units are linked as branched structures (Figure 3(a)). Because the branched amylopectin cannot be formed into a denser structure than helical linear amylose, a higher content of amylopectin results in higher solubility in water [10–12].

Starch is much easier to materialize into biodegradable plastics than cellulose because the  $\alpha$ -1,4-glucosidic bond of starch is weaker than the  $\beta$ -1,4-glucosidic bond of cellulose, and starch has lower crystallinity structurally (Figure 3(b)) and excellent solubility in water. The three hydroxyl groups present in each glucose unit have the greatest influence on the durability and biodegradability of materials manufactured from starch and cellulose. Therefore, the conversion process from starch and cellulose to the materials with improved mechanical properties or durability that can replace commodity plastics is an organic synthesis technology that transforms these hydroxyl groups. The conversion of the hydroxyl group of starch and cellulose proceeds through crosslinking and substitution reactions, but the most converted chemical structure is a reaction mechanism that proceeds through esterification and etherification [13, 14].

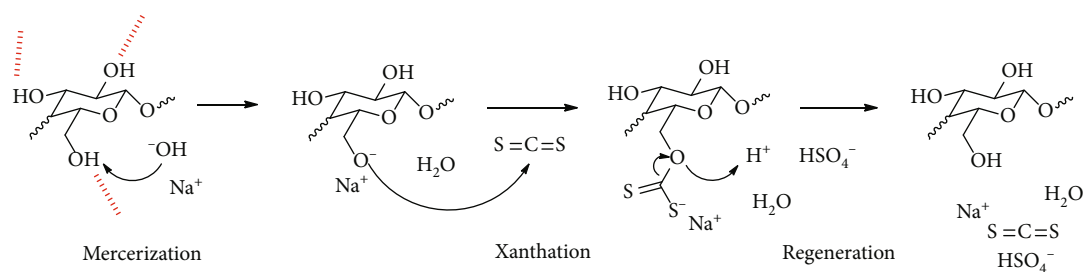
## 2. Etherification of Starch

The etherification for the hydroxyl groups of starch can be carried out without difficulty by a nucleophilic substitution reaction between an alkoxide and an alkyl halide developed by Alexander Williamson in 1850 (Scheme 3).

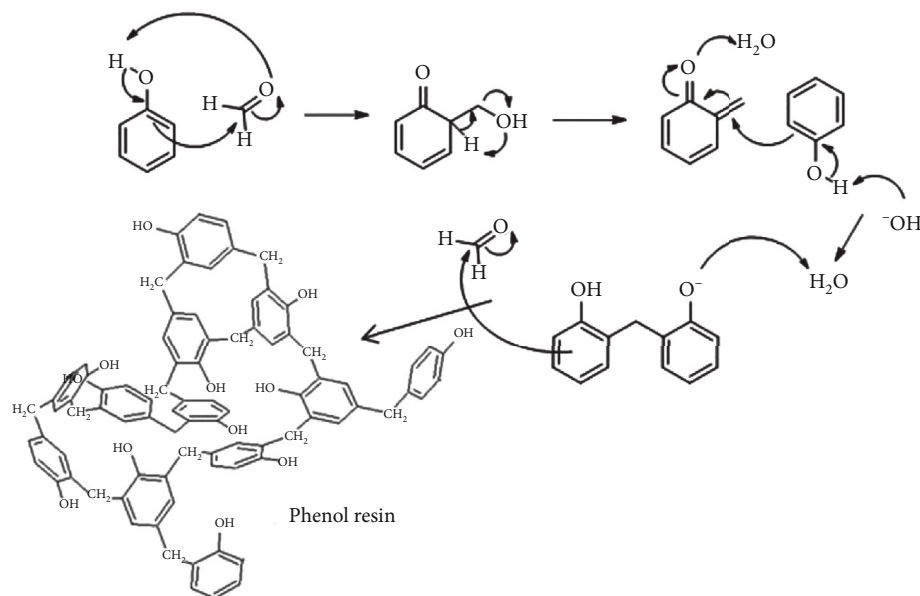
Because the electronegativity of oxygen is higher than that of carbon, ether compounds containing oxygen have higher polarity than chloroform (diethyl ether 1.15 D, chloroform 1.04 D), which is an aprotic polar substance that does not form hydrogen bonds itself. These structural characteristics give ether a lower boiling point and excellent solubility in polar substances than other compounds with a similar molecular weight. Therefore, when the hydroxyl group of starch is converted to an ether structure, the solubility of starch is increased to improve its processability. The improved performance can be carried out in terms of thermal stability and mechanical strength of the modified materials.

Alkoxide, which acts as a nucleophile in Williamson's etherification reaction, can be prepared by a reaction in which alkoxide, a conjugate base of alcohol, and  $\text{H}_2\text{O}$ , a conjugate acid of the  $\text{OH}^-$  ion, are synthesized by reaction of alcohol with NaOH, a typical acid-base reaction. In this





SCHEME 1



SCHEME 2

reaction, a comparison of the  $pK_a$  and  $pK_b$  of the reagents involved in the acid-base reaction proceeding in the etherification of starch shows that the reverse reaction can proceed better (Scheme 4). Therefore, some energy must be applied to increase the efficiency of the forward reaction [15].

The etherification for starch reforming can be carried out by nucleophilic substitution with an alkyl halide after forming alkoxide-structured starch under alkaline conditions. To increase the efficiency in this reaction step, it is necessary to maintain favorable reaction conditions for the forward reaction, such as heating of the reactor or the elimination of the generated products, water, and NaCl, during the reaction process (Scheme 5) [16].

The Hamid group published the experimental results on modifying the physical properties of starch, such as thermal behavior, mechanical endurance, and processability, by the etherification of the hydroxyl groups of starch extracted from palm trees with benzyl chloride [17]. In their study, as discussed in Scheme 4, the reaction proceeded with using NaOH at 60°C to increase the conversion rate of hydroxyl groups to methoxy (Scheme 6).

However, what was intensively discussed in Hamid's paper is the change of the physical properties by changing the starch modification process from the commonly used

water-based condition to the ethanol condition. In other words, etherification of the hydroxyl group of starch produced a significantly different result depending on the reaction solvent used to evaluate the size of starch particles, substitution rate, and deformation force. The change in the mechanical properties of starch suggests replacing commodity plastics used in various application fields only by changing the reaction solvent for modifying starch [18]. In this reaction, the solvents capable of forming hydrogen bond, such as ethanol or water, and the polar solvents, such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF), may be used as the reaction solvent [19, 20]. The effect of the reaction solvent may be greater in the starch reforming reaction because starch composed of  $\alpha$ -1,4-glucose bonds has a lower density of hydrogen bonds than cellulose composed of  $\beta$ -1,4-glucose bonds.

The question remains what causes these changes. As mentioned above, the etherification of starch proceeds with an acid-base reaction followed by a nucleophilic substitution reaction. To understand the flow of the first acid-base reaction in Scheme 6, it is necessary to consider the acidity of substances that may exist in the etherification reaction of starch, such as water, ethanol, and glucose. In this paper, the acidity of hydroxyl groups in the substances was

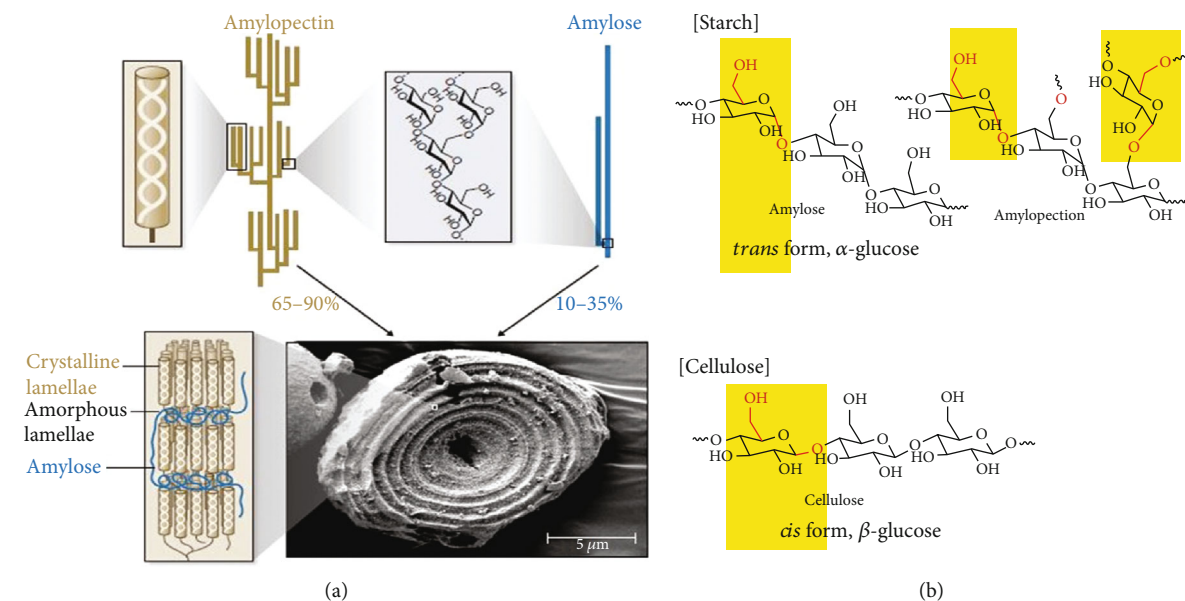
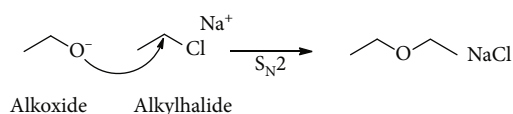


FIGURE 3: Structure of the starch granule (a) [8] and structural difference of starch and cellulose (b).



SCHEME 3: Williamson's etherification.

compared using the <sup>1</sup>H-nuclear magnetic resonance (NMR) data calculated from ChemOffice Suite 20.1. The <sup>1</sup>H-NMR data of the three hydroxyl groups in the glucose unit of starch were compared according to the presence or absence of an ether structure (Figure 4).

By analyzing the electron density of hydrogen atoms between the hydroxyl groups in glucose and cyclohexane, the acidity of the hydroxyl groups in glucose can be compared with that in ethanol indirectly. As shown in <sup>1</sup>H-NMR data of Figure 4, the hydrogen atom of the hydroxyl group in glucose (<sup>1</sup>H-NMR-b) was measured at a higher magnetic field than the hydrogen atom of the hydroxyl group in cyclohexane (<sup>1</sup>H-NMR-a). This result means that the electron density is higher at the hydrogen atom of the hydroxyl group in glucose, and the adjacent ether structure reduces the acidity of the hydroxyl group. On the other hand, the proton of the hydroxyl group of ethanol was calculated to be present at 4.7 ppm under the same conditions. Because the electron density of the protons of the hydroxyl groups is higher in glucose than in ethanol, the acidity of the substances that may exist in the reactor for the etherification reaction of starch is higher in the order of water > ethanol > hydroxyl group of glucose.

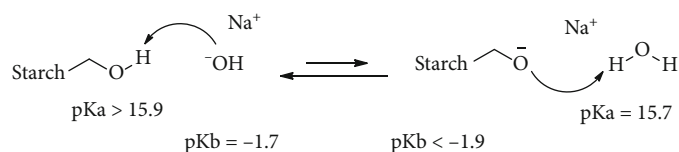
Then, the cause of the difference in the etherification reaction depending on the reaction solvent with such a subtle difference in acidity needs to be determined [21]. First, under experimental conditions in which water is used in excess, the following reaction mechanism may proceed, in which starch is decomposed and converted to glucose, a

monosaccharide (Scheme 7). However, this reaction is easy to proceed under acidic conditions, and it is not easy to proceed under alkaline reaction conditions, as in Scheme 6.

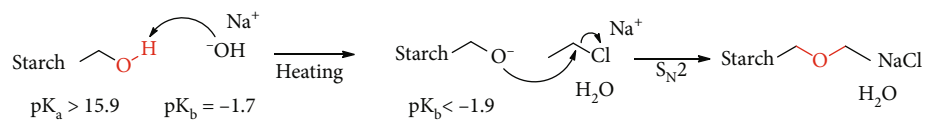
A second possibility to consider is the difference in pK<sub>a</sub> of the substances under the reaction conditions. The pK<sub>a</sub> of the -OH group differs according to the chemical structure, as shown in Figure 4. The electron density of the -OH group of glucose is higher than that of the compound without an ether structure. Even in this case, the OH<sup>-</sup> ion of NaOH dissolved in the reaction solvent, water or ethanol, acts as a base, and the reaction proceeds to become alkoxide or water (Scheme 8). A reaction of a substituent, such as benzyl chloride with the generated alkoxide, is the basic reaction of the starch reformation by the nucleophilic substitution.

On the other hand, when water is used as the reaction solvent in this process, the desorption reaction of the substituent by the oxidative decomposition of the ether structure (Scheme 7 or 8) can be actively carried out because the hydration state (H<sub>3</sub>O<sup>+</sup> and OH<sup>-</sup>) may exist in excess compared to the ethanol usage conditions (CH<sub>3</sub>CH<sub>2</sub>OH<sub>2</sub><sup>+</sup> and OH<sup>-</sup>). As a result, the substitution rate of the benzyl group may be lowered. However, when ethanol is used as the reaction solvent, the concentration of hydrated ions (H<sub>3</sub>O<sup>+</sup>, OH<sup>-</sup>) may be lowered. Hence, the probability of proceeding to the reaction by the mechanism shown in Scheme 6 rather than that in Scheme 7 may increase. A previous study [17] reported that it possible to increase the efficiency of the etherification reaction of starch, as shown in Scheme 6.

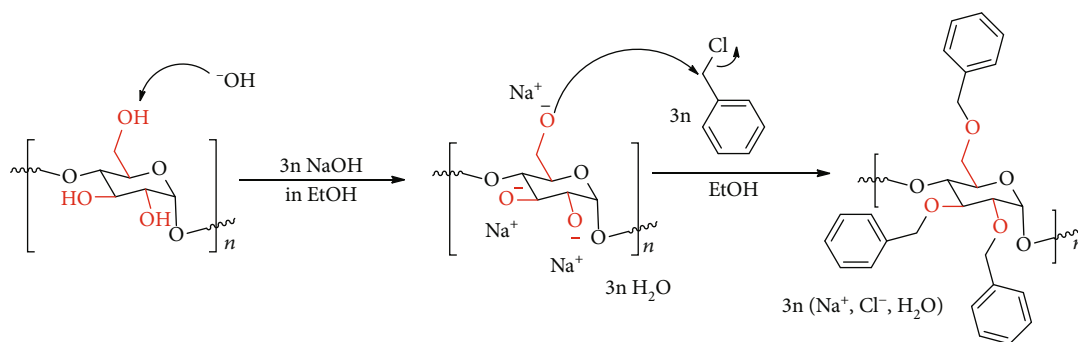
On the other hand, the most important step in the etherification of starch is the nucleophilic substitution reaction between the alkoxide and halide compounds, and this reaction step is most affected by the concentration of the alkoxide. Pieters et al.'s group reported an experimental condition in which the hydroxy groups were substituted by the benzyl groups almost completely by removing the water produced as a by-product in this reaction step [21]. As



SCHEME 4



SCHEME 5



SCHEME 6

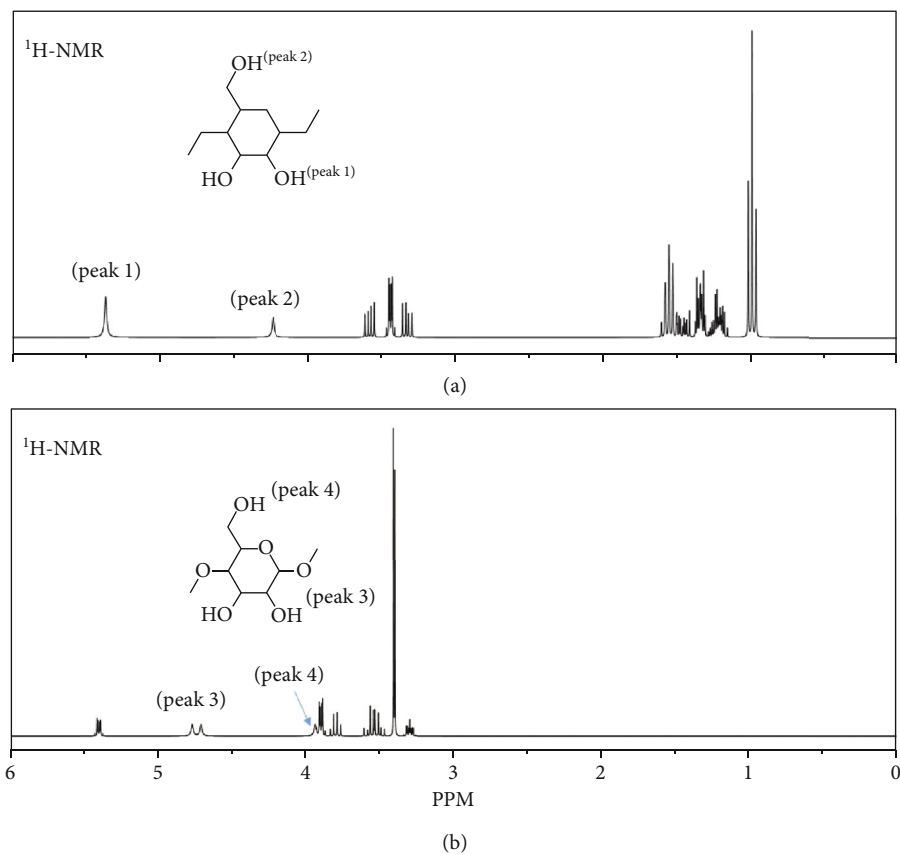
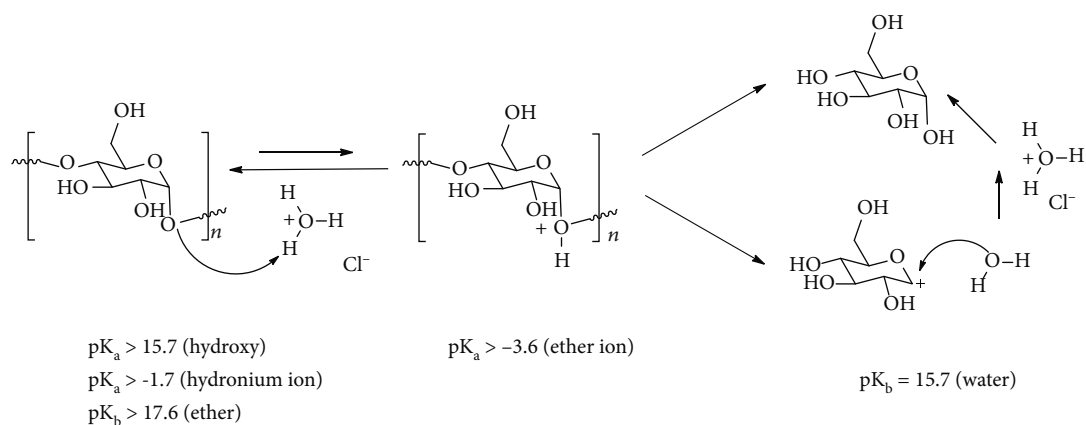
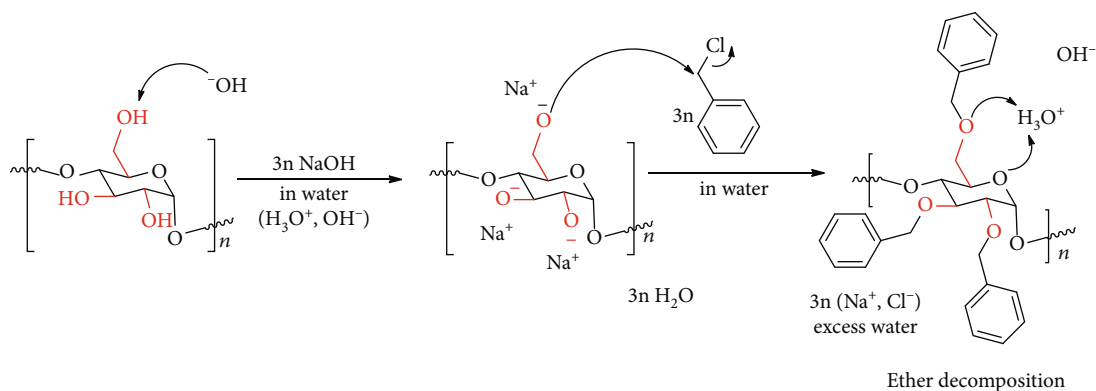


FIGURE 4: Simulated  $^1\text{H-NMR}$  data on the protons of the hydroxyl groups of cyclohexane without an ether structure (a) and glucose structure (b).

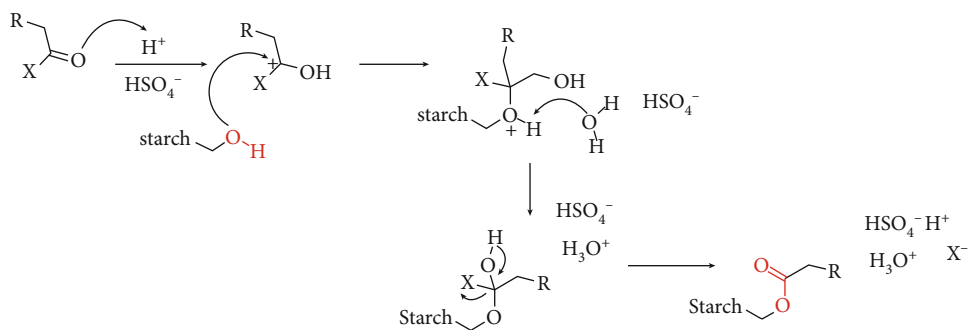




SCHEME 7



SCHEME 8



SCHEME 9: Fischer's esterification.

described in Scheme 8, these experimental results are interpreted as the effect of suppressing the cause of oxidation of ether by  $\text{H}_3\text{O}^+$  present in hydration.

Therefore, the reverse reaction can be inhibited if the moisture formed when the hydroxyl group of starch reacts with a  $\text{OH}^-$  ion is removed, resulting in an increase in the yield for the etherification reaction. This process can be explained by Le Châtelier's law, in that more product can be produced by removing product from the reaction. In addition, a comparison of the concentration of by-products

and the  $pK_a$  or  $pK_b$  of the substances before and after the reaction can be useful for designing the optimal reaction conditions and improving the product quality and yield [22].

### 3. Esterification of Starch

The hydroxyl group of starch can be esterified using the Fischer esterification reaction, which is a substitution reaction of a nucleophilic acyl compound with a compound

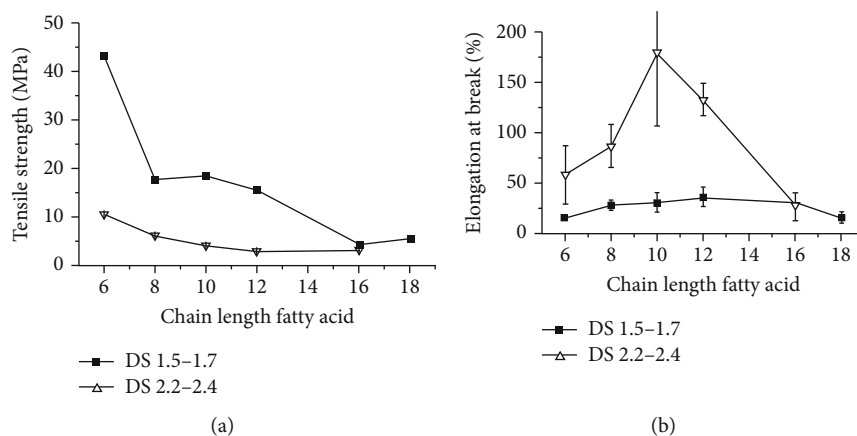
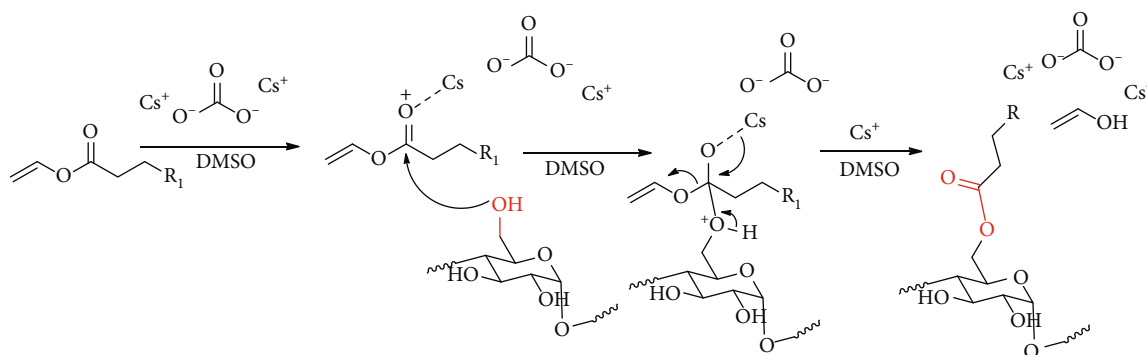
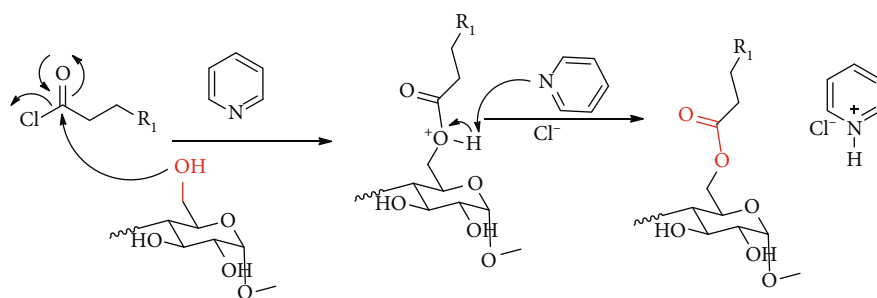


FIGURE 5: Tensile strength (a) and elongation at break (b) depending on the degree of substitution (DS) and type of fatty acid [25].



SCHEME 10



SCHEME 11

containing an acid anhydride, acid chloride, or carboxylic acid structure under an acid catalyst (Scheme 9).

As the hydroxyl group of glucose is esterified by this reaction mechanism, the hydrogen bonding ability of amylose or amylopectin is weakened. This structural change in starch can lower its reactivity with moisture, thereby enhancing its durability [23]. In addition, the basic structure of starch is converted to a thermoplastic material that prevents crosslinking at high temperatures by esterifying the hydroxyl group of the reactive glucose. This is effective when the ester structure is applied to a bioplastic that requires

properties, such as flexibility, elasticity, impact resistance, and chemical resistance [24].

Some studies have reported the production of fatty acid starch esters by reacting 80% amylose-containing starch with vinyl carbonates, such as vinyl laurate (CH<sub>3</sub>(CH<sub>2</sub>)<sub>10</sub>COOCH=CH<sub>2</sub>) or vinyl stearate (CH<sub>3</sub>(CH<sub>2</sub>)<sub>16</sub>COOCH=CH<sub>2</sub>). In these reports, the potential for use as a biodegradable packaging material was presented by examining the effects of the starch hydrophobicity, material flexibility at high temperatures, and film properties depending on the length of the alkyl group (Figure 5) [25, 26].

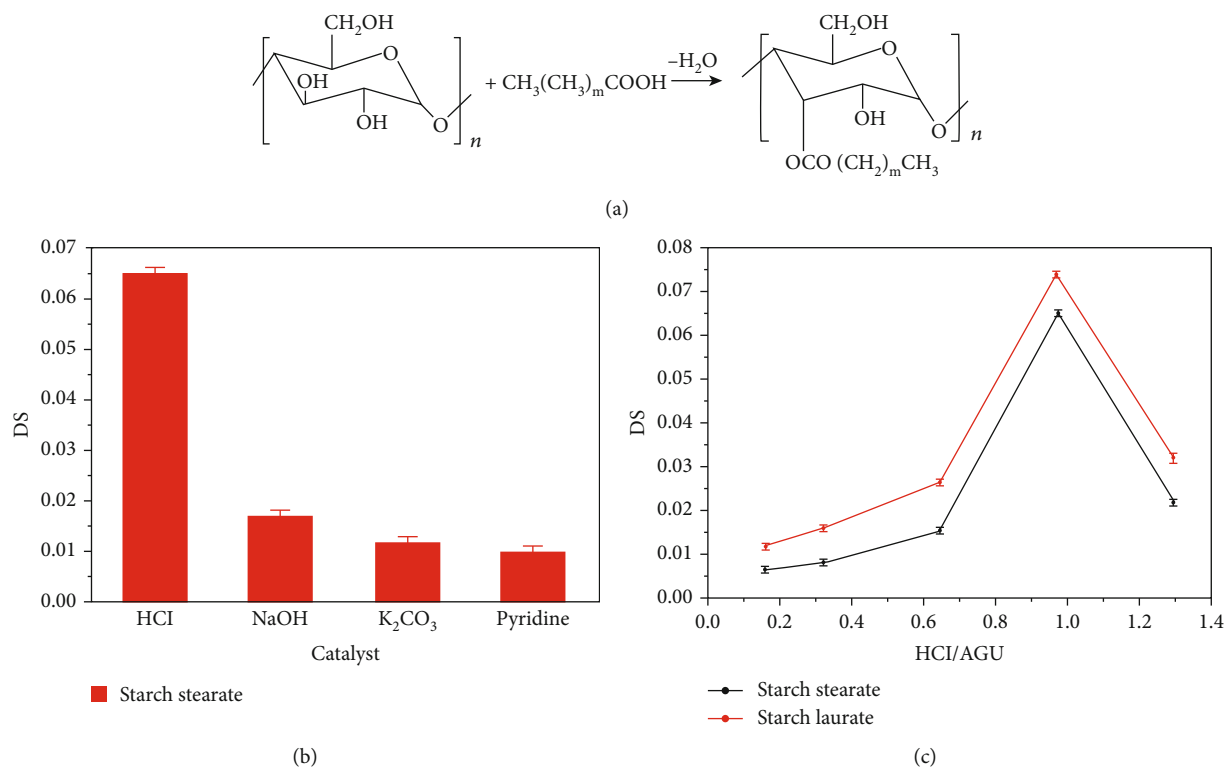


FIGURE 6: Starch esterification by saturated alkyl carboxylic acid (a) and effect of the catalyst on the degree of substitution of starch stearate (b) and HCl dose (c) [29].

The synthesis of fatty acid starch esters in these studies proceeded by a nucleophilic substitution reaction between the electrophilic carbonyl carbon of vinyl carbonate and the hydroxyl group of starch, a nucleophilic functional group, *via* an acid-base reaction between cesium carbonate and vinyl carbonate (Scheme 10). Cesium carbonate was used as a catalyst in many studies related to the esterification of starch because of its higher solubility in polar solvents, such as *N,N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO), which can dissolve the reactant starch and carbonate compounds, compared to potassium carbonate or sodium carbonate [27].

A material with enhanced hydrophobicity of starch can be made by introducing a hydrophobic alkyl group through esterification with an acyl halide compound, such as *n*-dodecanoyl chloride. In such a reaction, the use of a basic solvent, such as pyridine, which can remove the acid generated after the reaction, can improve the efficiency of the forward reaction. As shown in Scheme 11, this reaction can proceed smoothly by the basic nitrogen of pyridine, which removes protons of the cationic ether [25].

In the esterification reaction of starch with saturated fatty acids, various acids and bases, such as pyridine, Na<sub>2</sub>HPO<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>COONa, potassium silicate, HCl, and NaOH, are used as reaction catalysts [28]. These catalysts weaken the bond strength between the starch chains that are spirally connected by hydrogen bonding and improve the reaction efficiency in the process of inducing a nucleophilic substitution reaction (Fischer's esterification) with the starch hydroxyl group by carbocation formation (Scheme 11).

In the case of a reaction with stearic acid, the substitution rate is higher under acid conditions than under base conditions, as shown in Figure 6. Even under acid conditions, the ratio of the catalyst and glucose monomer, reaction temperature, and reaction time affect the substitution rate of ester groups [29]. On the other hand, the hydrophobicity of starch increases as the length of the alkyl group substituted with the hydroxyl group of starch increases, but many experimental results have shown that the substitution rate is lowered by its steric hindrance [30]. These results mean that it is also necessary to consider the structure of the substituted alkyl carbonyl to synthesize the optimal biodegradable plastic according to the application.

As described above, the hydroxyl group of glucose is a functional group that dominates the physical and chemical properties of starch or cellulose. The physical property of starch and its biodegradability in the presence of moisture can be controlled by substituting the hydroxyl group with ether, ester, or alkyl group. In addition, for plastic materials made from starch or cellulose to replace high-performance plastics, such as PE and PVC, while maintaining biodegradation properties, it is necessary to consider the optimal synthesis conditions through a study of the mechanism of the substitution reaction.

## 4. Conclusions

The production of biodegradable plastics using starch for developing alternative materials to plastics has attracted considerable attention. This study examined the reaction



factors that can affect the development of biodegradable plastics through starch modification by etherification and esterification. The reaction conditions with high efficiency were suggested after examining the reaction temperature, pH, solvent, and by-products involved in the etherification reaction. Regarding the ester reaction, the structure of the substituent and the reaction solvent affecting the reaction efficiency were examined. Overall, this review would serve as an opportunity to increase the use of starch as a material that can replace commodity plastics for many purposes.

## Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

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## Research Article

# In Vitro Degradation of PHB/Bacterial Cellulose Biocomposite Scaffolds

**Maria Râpă,<sup>1</sup> Cătălin Zaharia<sup>1</sup> ,<sup>2</sup> Paul Octavian Stănescu,<sup>2</sup> Angela Cășărică,<sup>3</sup> Ecaterina Matei,<sup>1</sup> Andra Mihaela Predescu,<sup>1</sup> Mircea Cristian Pantilimon,<sup>1</sup> Ruxandra Vidu,<sup>1,4</sup> Cristian Predescu,<sup>1</sup> and Horia Cioflan<sup>5</sup>**

<sup>1</sup>University Politehnica of Bucharest, Faculty of Materials Science and Engineering, 313 Splaiul Independentei, 060042 Bucharest, Romania

<sup>2</sup>University Politehnica of Bucharest, Advanced Polymer Materials Group, 1-7 Gh. Polizu Str., 011061 Bucharest, Romania

<sup>3</sup>National Chemical-Pharmaceutical for Research and Development Institute, Vitan Avenue, No. 112, District 3, Bucharest, Romania

<sup>4</sup>University of California Davis, Department of Electrical Engineering, One Shields Avenue, Davis, CA 95616, USA

<sup>5</sup>Sanador Clinic Hospital, Sevastopol Street No. 9 Sector 1, Bucharest, Romania

Correspondence should be addressed to Cătălin Zaharia; [zaharia.catalin@gmail.com](mailto:zaharia.catalin@gmail.com)

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The present work reported the preparation of biocomposites based on poly(3-hydroxybutyrate) (PHB), plasticizer, and bacterial cellulose (BC) by melt processing and their testing by means of thermal properties (DSC), water absorption, and in vitro degradation. The surface of the biocomposites was analyzed via atomic force microscopy (AFM). In vitro degradation of the biocomposites was evaluated by weight loss and thermal properties (DSC) assessment after the immersion of the specimens in phosphate-buffered saline solution (PBS of pH 7.4) over 20 days. The results showed that the BC can reduce the PHB crystallinity and promote its degradation under PBS medium. Moreover, it was found that the water absorption increased as the percentage of BC increased.

## 1. Introduction

Poly(3-hydroxybutyrate) (PHB) is a bacterial polyester produced as an intracellular energy storage material under growth conditions characterized by a good biocompatibility and biodegradability. It has a similar degree of crystallinity, glass transition temperature, melting temperature, Young's modulus, and tensile strength as polypropylene (PP). In addition, PHB is not toxic for the cells [1], in part due to the fact that it degrades *in vivo* to 3-hydroxybutyric acid, which is a common metabolite that occurs in living organisms [2]. However, PHB alone does not fulfill all technical specifications for use as a biomaterial in medical and tissue-engineering applications and has low melt elasticity and a narrow thermal processing window [3], which is due to its high crystallinity and high melting temperature of

the crystalline domains. As a consequence, mixing PHB with other polymers and additives is a good strategy to obtain the desired properties. It was found that the reinforcement of PHA with cellulosic fibers gives the polymer composite good mechanical properties (stiffness, strength, and toughness) as well as a low cost [4–6].

Bacterial cellulose (BC) is mainly produced by Gram-negative bacteria of the *Gluconacetobacter* genus [7]. It is a highly flexible biomaterial used to treat chronic wounds and burns, such as artificial skin or wound dressings, to obtain artificial vessels and scaffolds for tissue engineering [8, 9]. BC differs from plant cellulose by high purity, crystallinity, degree of polymerization, tensile strength [10, 11], and high water-absorbing capacity [12]. In a previous paper, we reported the good biocompatibility of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

loaded with bacterial cellulose and microcrystalline cellulose via melt processing [13].

Conventional techniques to fabricate open porous scaffolds include solvent casting/salt leaching, phase separation, gel casting, precipitation, emulsion freeze-drying, and electrospinning methods. There are known scaffolds, mostly prepared by casting method, based on PHB blended with hydroxyapatite (HAP) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) using salt-leaching method [14], bacterial cellulose (BC)/polyethylene glycol (PEG) [15], polyacrylamide (PAm)/hydroxyapatite (HAP)/poly(vinyl alcohol) (PVA) [16], poly(lactic acid) (PLLA) [17], poly(hydroxyalkanoates) (PHA) [18–22], and polycaprolactone (PCL) [23]. The designed polymers for the production of scaffolds should have appropriate surface chemistry to favor cellular attachment, porous structure, favorable mechanical properties, a controlled degradation rate, preferably less than one year under physiological conditions, biodegradability or bioresorbability, and a good biocompatibility [24]. The degradation rates of polymers can be designed through the addition of various components to the polymeric composition, as well as selection of the chemical composition, molecular weight, and processing conditions. *In vitro* degradation of biodegradable polymers has been largely investigated and monitored by using various techniques to evaluate the changes of weight loss, water absorption, thermal properties, and chemical composition [25, 26].

This paper describes the synthesis of bacterial cellulose, preparing of PHB/BC formulations by melt processing and, subsequently, their characterization by surface examination, thermal analysis (DSC), water absorption technique, and *in vitro* degradation of scaffolds occurred after immersion of specimens in phosphate buffered saline (PBS, pH = 7.4) over 20 days.

## 2. Materials and Methods

**2.1. Materials.** Poly(3-hydroxybutyrate) (PHB, T19 grade) with size dimensions in the range 0.5–50  $\mu$  was purchased from BIOMER, Germany. Bacterial cellulose (BC) was synthesized by The National Chemical-Pharmaceutical for Research and Development Institute (ICCF Bucharest, Romania). Tributyl citrate (TBC) supplied by Sigma-Aldrich was used as bioplasticizer. Sodium chloride (NaCl) with the size in the range 420–500  $\mu$ m was used as a porogen. Phosphate buffered saline (PBS) medium (pH 7.4) was prepared in laboratory.

**2.2. Preparation of PHB/BC Composite Scaffolds.** PHB/BC composite scaffolds were obtained following the salt leaching procedure [27]. PHB powder and BC powder containing 1% and 2% by weight, respectively, were melted in a Brabender Plastograph at 180°C, for 6 minutes and a rotor speed of 40 rpm, respectively. Then 3 wt% NaCl particles were introduced into the melted mixtures, and the process continued until 6 minutes. The plasticizer was added to each formulation at the beginning of mixing to provide 4:1 ratio between PHB and TBC. The samples were labeled PHB/BC1% and

TABLE 1: Characteristics for melt processing of PHB/TBC and PHB/BC samples.

Sample	$M_{1min}$ (nm)	$M_{4min}$ (nm)	$M_{6min}$ (nm)	(nm/rpm)
PHB/TBC	29.5	20	11	0.275
PHB/BC 1%	36	22	12	0.300
PHB/BC 2%	34	23	12	0.300

PHB/BC2% according to their composition. PHB/TBC signifies the sample containing only PHB and plasticizer.

Before processing, both PHB and BC were oven dried at 60°C overnight and at 105°C, for 2 h, respectively. The melted biocomposites were pressed into thin films by compression molding using a laboratory press type POLYSTAT 200 for 5 minutes pressing time, at a temperature of 185°C and a pressure of 200 bars. The investigated methods were carried out on the films subjected to salt-leaching process to remove the NaCl.

### 2.3. Investigation Methods

**2.3.1. Processing Behavior.** Processing behavior of plasticized PHB and PHB/BC biocomposites was evaluated by torque at different times up to 6 minutes and 40 rpm and melt viscosity obtained from torque-time Brabender diagrams.

**2.3.2. Microscopic Characterization.** The surface morphology of the scaffolds was evaluated with a 4000 SPM Multi-View/NSOM atomic force microscope system (AFM) (Nanonics Imaging LTD). SPM images were acquired in air, noncontact mode (at a distance between 0.1–10 nm of sample). Analyzed surface area was 80  $\mu$ m<sup>2</sup>, and the measurement speed used was 12 ms/point. The needle used for surface analysis showed a peak of Cr. Interpretation of the analysis was performed using the WSxM 4.0 software. Surface roughness of the PHB/BC biocomposites was expressed as the root mean square (RMS) of the vertical Z-dimension values within the examined areas, according to Selli et al. [28].

**2.3.3. Differential Scanning Calorimetry (DSC) Measurements.** Thermal properties of the biocomposites were determined directly after melting processing and after the degradation process using a calorimeter (Mettler Toledo, Model DSC 823e, Switzerland). The melting temperature ( $T_m$ ), melting enthalpy ( $\Delta H_m$ ), and the degree of crystallinity ( $\chi_c$ ) were investigated for all samples, from the first heating scan, with the aid of the software STARE 9.10 from Mettler Toledo. The degree of crystallinity ( $\chi_c$  (%)) of the PHB from the samples was determined according to the following equation:

$$\chi_c = \frac{\Delta H_m}{\Delta H_{100\%,PHB} \cdot w} \times 100\%, \quad (1)$$

where  $\Delta H_m$  is the measured enthalpy of the melted blends (J/g),  $\Delta H_{100\%,PHB}$  is the theoretical heat of fusion for a fully crystalline PHB (146 J/g), and  $w$  is the weight fraction of

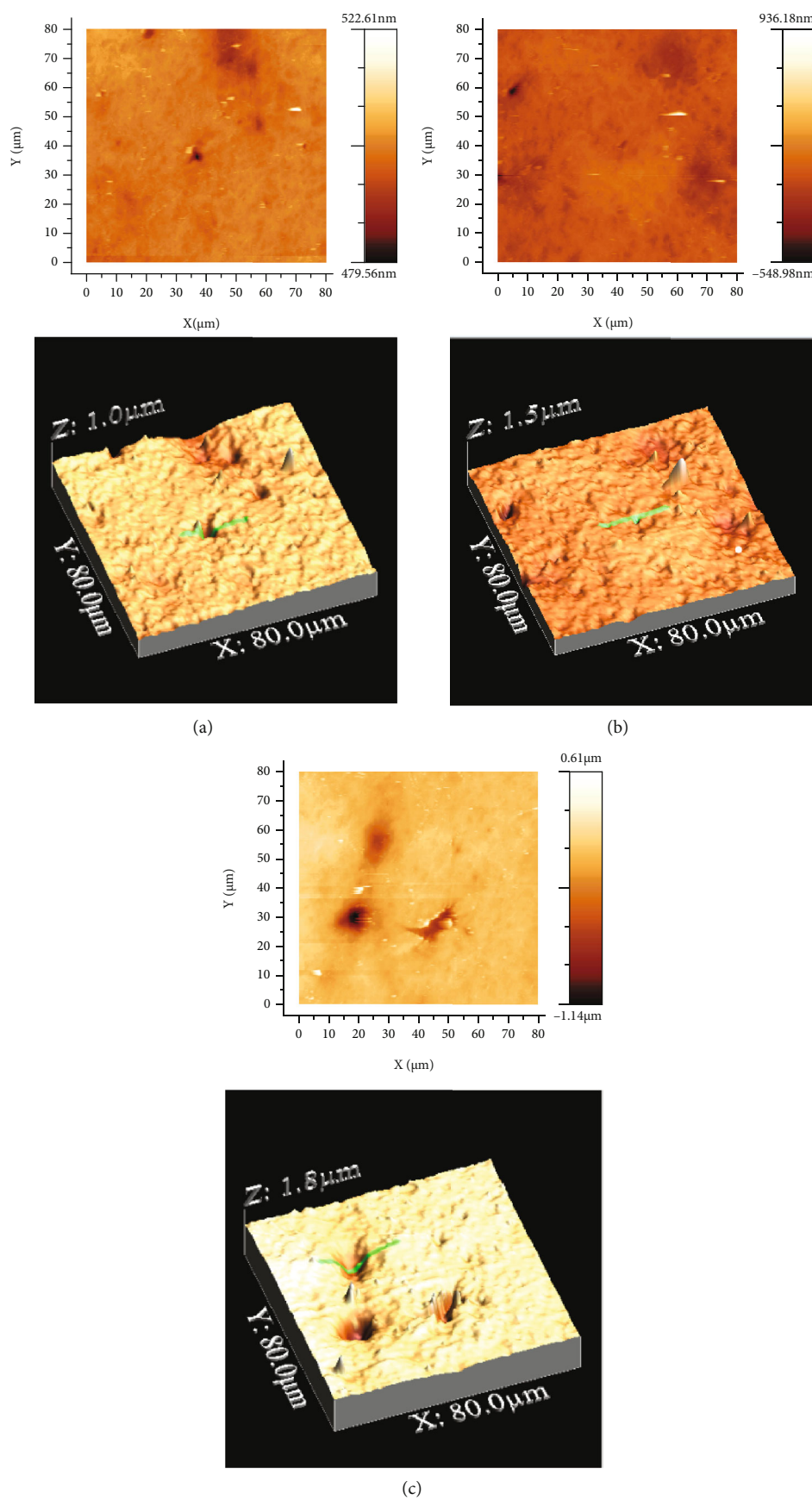


FIGURE 1: AFM topography images for the plasticized PHB and PHB biocomposites. (a) PHB/TBC, (b) PHB/BC1%, and (c) PHB/BC2%.



TABLE 2: Roughness (RMS) values for the samples calculated from AFM analysis.

Sample	RMS (nm)
PHB/TBC	51.1
PHB/BC1%	67.3
PHB/BC2%	129.0

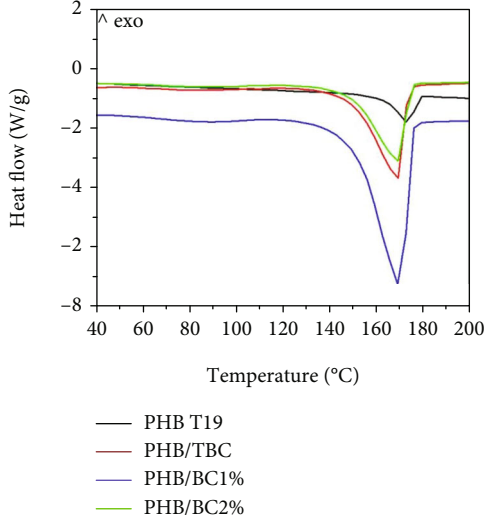


FIGURE 2: DSC thermograms for neat PHB, plasticized PHB, and PHB biocomposites (first heating scan).

PHB from samples. PHB powder was used as a control sample.

**2.3.4. Water Absorption.** The water absorption of each scaffold was measured after immersion of the round-shaped specimens in distilled water at 23°C for four different periods: 24 h, 168 h, 336 h, and 504 h, according to ISO 62. The test specimens were kept out in an oven at 50°C for 24 h, cooled, and weighed until the mass was constant ( $m_1$ ) with an accuracy of 0.1 mg. Then, the specimens were submerged into distilled water and, at target period, they were removed from water and reweighing ( $m_2$ ). The average content of absorbed water was calculated by using the appropriate formula:

$$\text{Water absorption} = \frac{m_2 - m_1}{m_1} \times 100\%, \quad (2)$$

where  $m_1$  is the mass of the test specimen in mg, after initial drying and before immersion;  $m_2$  is the mass of the test specimen in mg, after immersion.

**2.3.5. In Vitro Degradation Study.** *In vitro* degradation study was performed by immersion of specimens in phosphate-buffered saline medium (pH 7.4) at 37°C, to simulate the human physiological medium [29]. The dried scaffolds subjected to degradation were investigated by their weight loss, as follows: round pieces of 20 mm diameter were cut from samples, weighed ( $w_0$ ) and placed in sealed test tubes con-

TABLE 3: Thermal properties of PHB, PHB/TBC, and PHB/BC samples.

Sample	$\Delta H_m$ (J/g)	$T_m$ (°C)	$\chi_{c,PHB}$ (%)
Neat PHB	$90.3 \pm 0.6$	$174.0 \pm 2.1$	$61.8 \pm 0.6$
PHB/TBC	$61.0 \pm 0.9$	$170.8 \pm 1.7$	$52.2 \pm 0.9$
PHB/BC1%	$58.4 \pm 1.1$	$170.8 \pm 1.0$	$52.0 \pm 1.1$
PHB/BC2%	$57.1 \pm 1.1$	$169.5 \pm 0.8$	$51.4 \pm 1.1$

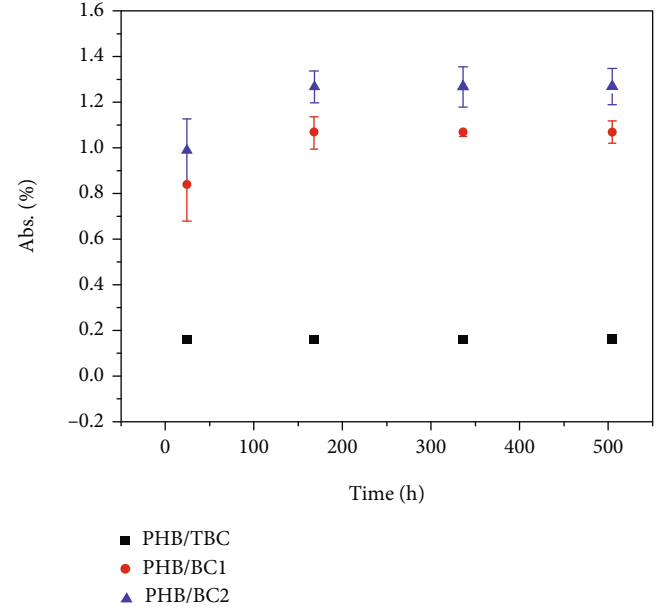


FIGURE 3: Water absorption for plasticized PHB/TBC sample and its biocomposites at different times.

taining 2 mL PBS for up to 20 days. At predetermined degradation intervals (i.e., 1 day, 5 days, 10 days, 15 days, and 20 days), the specimens were taken out, washed with distilled water, and dried in an oven at 37°C for 72 h and then reweighed ( $w_1$ ). The degree of degradation was assessed by weight loss evaluation using the following equation [30]:

$$\text{Weight loss\%} = \frac{w_0 - w_1}{w_0} \times 100\%, \quad (3)$$

where  $w_0$  and  $w_1$  are the weights of the scaffolds before and after hydrolytic degradation, respectively.

The degraded scaffolds were also examined by DSC to find any detectable differences in their thermal profile.

### 3. Results and Discussions

**3.1. The Processing Behavior.** The processing behavior of the obtained blends was evaluated from the torque-time curves, and it is expressed as torque ( $M$ ) at 1 minute, 4 minutes, and 6 minutes, respectively, and melt viscosity ( $\eta$ ).

Table 1 shows that the samples can be easily processed by melt blending. As the BC agent was incorporated into

the plasticized PHB matrix, an increase in torque was obtained up to 4 minutes compared to that for the plasticized PHB. However, after 6 minutes of processing, there are no significant differences between the biocomposites and the plasticized PHB torque (i.e., values of 11–12 nm) and their melting viscosity (i.e., values of 0.27–0.3 nm/rpm). The advantages of this blending technique are its compatibility with the processing methods for conventional polymers and the fact that it does not use solvents.

**3.2. AFM Analysis.** AFM analysis reveals the rough surface of the PHB biocomposites (Figures 1(a)–1(c)) on which numerous holes and topographic features having relatively high depths and peaks ranging from 100 to 700 nm can be observed. The roughness of the samples characterized by AFM is listed in Table 2.

The roughness surface of PHB composites increased with the incorporation of BC into the plasticized matrix, due to the interaction between the polymeric matrix and cellulose. These results are in agreement with the melt viscosity reported in Table 1.

**3.3. Differential Scanning Calorimetry (DSC).** DSC curves for the neat PHB, plasticized PHB, and PHB biocomposites are shown in Figure 2.

The melting enthalpy ( $\Delta H_m$ ), melting temperature ( $T_m$ ), and degree of crystallinity ( $\chi_c$ ) of the samples evaluated from DSC curves are shown in Table 3. Data were expressed as mean values  $\pm$  standard deviation ( $n = 3$ ).

Data presented in Table 3 reveal that the melting temperature ( $T_m$ ) of PHB decreases with the addition of plasticizer and bacterial cellulose. Thus, the PHB/BC2% biocomposite shows a decrease of  $T_m$  to 4.5°C compared to the neat PHB. The reduction of  $T_m$  could be the effect of the introduction of plasticizer and the good dispersion of BC inside the polymeric matrix. Several studies showed that adding of plasticizer to the PHB matrix can lead to an increase in the thermal processing window [31–34]. Furthermore, the melting enthalpy ( $\Delta H_m$ ) was reduced from 90.3 J/g for neat PHB to 57.1 J/g for PHB/BC2%, and as a consequence, the crystallinity of PHB ( $\chi_c$ ) decreased from 61.8% to 51.4%. This can be explained by disruption of the regularity of PHB chains following the interactions with BC, which resulted in a decrease of the crystallinity. A similar mechanism was observed in green nanocomposites based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with various cellulose nanocrystals [35], PHB/BC composite membranes [36], and PHB blends [37].

**3.4. Water Absorption.** The water absorption capacity of scaffolds is an important measure of scaffold hydrophilicity, as it can affect the rate of scaffold degradation as well as the attachment of cells [38].

Figure 3 shows the percentage of water absorption for PHB/TBC and its corresponding PHB/BC scaffolds measured at four time periods.

The PHB/TBC sample showed that the water absorption is very low ( $0.16\% \pm 0.003\%$ ) during 504 h, due to its initial high crystallinity. The PHB/BC1% and PHB/BC2% scaffolds

TABLE 4: Thermal properties of PHB/TBC and PHB/BC composite materials after *in vitro* degradation.

Sample	Period of exposure to PHB medium	$\Delta H_m$ (J/g)	$T_m$ (°C)	$\chi_c$ (%)
PHB/TBC	5 days	$55.9 \pm 0.3$	$168.9 \pm 1.3$	$47.8 \pm 0.3$
	10 days	$57.5 \pm 0.3$	$166.9 \pm 0.9$	$49.2 \pm 0.2$
	15 days	$63.0 \pm 0.4$	$168.1 \pm 0.8$	$53.9 \pm 0.4$
	20 days	$62.4 \pm 0.3$	$168.0 \pm 0.9$	$53.4 \pm 0.3$
PHB/BC1%	5 days	$54.0 \pm 0.3$	$167.6 \pm 0.6$	$48.1 \pm 0.3$
	10 days	$56.3 \pm 0.2$	$168.3 \pm 0.4$	$50.2 \pm 0.2$
	15 days	$58.1 \pm 0.3$	$168.9 \pm 0.3$	$51.8 \pm 0.3$
	20 days	$59.3 \pm 0.4$	$167.1 \pm 0.6$	$52.8 \pm 0.4$
PHB/BC2%	5 days	$59.0 \pm 0.3$	$168.3 \pm 0.3$	$53.1 \pm 0.3$
	10 days	$60.3 \pm 0.3$	$167.6 \pm 1.0$	$54.3 \pm 0.3$
	15 days	$60.4 \pm 0.2$	$168.2 \pm 0.8$	$54.4 \pm 0.2$
	20 days	$56.5 \pm 0.3$	$168.9 \pm 0.6$	$50.9 \pm 0.3$

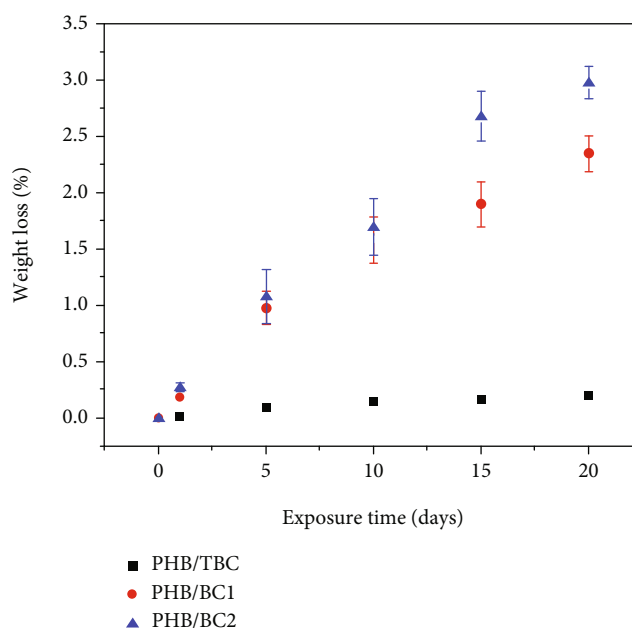


FIGURE 4: Weight loss of plasticized PHB sample and its biocomposites during 20 days of exposure to PBS medium.

exhibited higher water absorption than that of PHB/TBC sample. Introduction of BC in the polymer scaffolds significantly enhanced the water absorption content. Thus, the PHB/BC2% scaffold shows the maximum hydration ( $1.27\% \pm 0.07\%$ ) at 168 h. This may be attributed to the addition of BC in the polymeric matrix that the decreased crystallinity of PHB (decreased  $\Delta H_m$  from Table 4) allowing the mobility of PHB chains and as consequence the production of more hydrophilic hydroxyl and carboxyl end groups due to ester bond cleavage [25, 37]. It is worth noting that after 168 h immersion in distilled water, the scaffolds



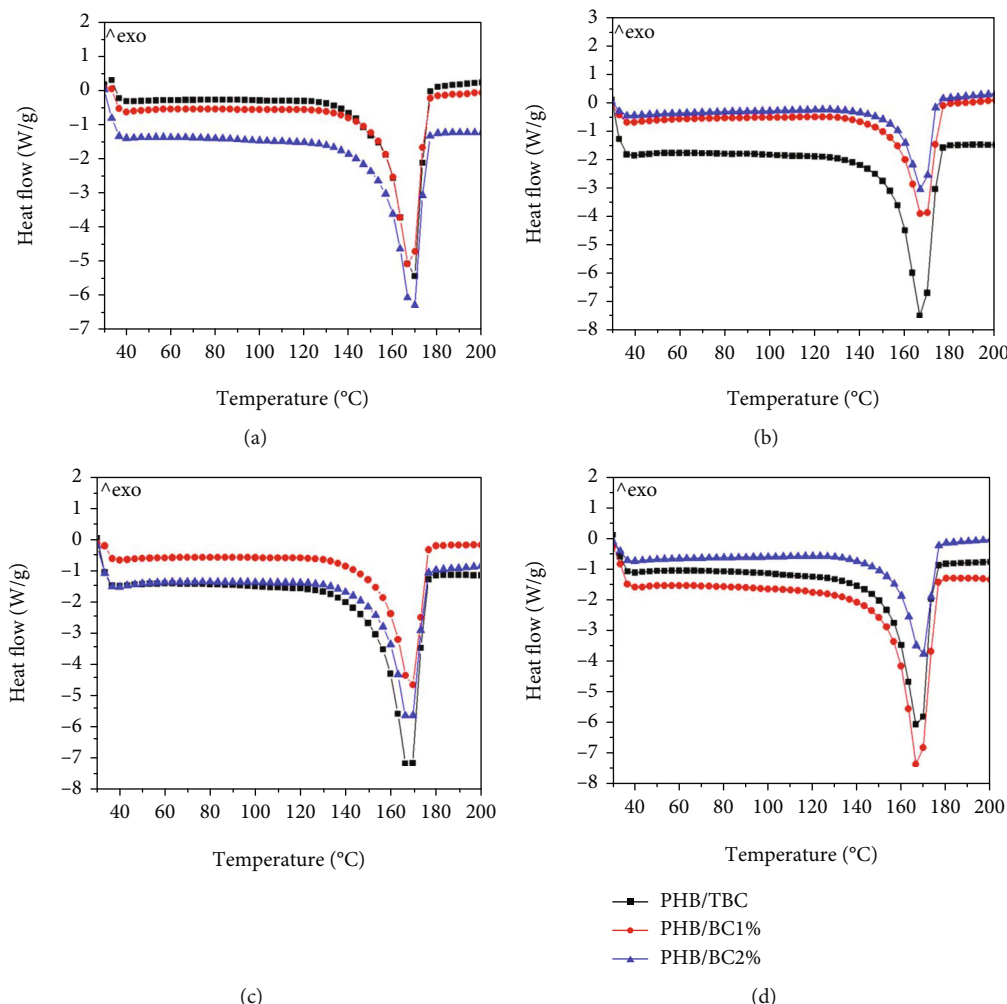


FIGURE 5: DSC thermograms for plasticized PHB sample and its biocomposites subjected in PBS medium at different time periods: (a) 5 days, (b) 10 days, (c) 15 days, and (d) 20 days.

reached the highest values for water absorption, which remained constant during 504 hours of testing.

### 3.5. In Vitro Degradation

**3.5.1. Weight Loss.** Weight loss profiles of PHB/TBC and PHB/BC scaffolds as a function of the degradation time are shown in Figure 4.

Plasticized PHB blend presented a negligible weight loss during the whole 20-day degradation period due to its high crystallinity (Table 3). The weight loss was 0.02% at one day exposure to PBS medium, and it slowly increased until 0.2% at the end of exposure time. It is well recognized the low degradation of PHB under physiological conditions, for example, PHB films had lost approximately 3.5% of their initial weight after 105 days incubation in PBS [37].

Data from Figure 4 clearly show that the increase of the BC content in the biocomposite samples leads to the increase of their degradation. Thus, the PHB/BC2% scaffold exhibits a higher weight loss ( $2.98 \pm 0.14\%$ ) than the PHB/BC1% scaffold ( $2.35 \pm 0.16\%$ ) at 20 days. This behavior is in agree-

ment with the water absorption test (Figure 3) and the decrease in crystallinity (Table 3).

**3.5.2. DSC Analysis for the Degraded Samples.** The DSC technique was used to evaluate the change of thermal parameters for the PHB/TBC and PHB/BC samples after *in vitro* degradation in PBS solution at 37°C and targeted periods (Figures 5(a)–5(d)).

The melting enthalpy ( $\Delta H_m$ ), melting temperature ( $T_m$ ), and degree of crystallinity of PHB ( $\chi_c$ ) evaluated from DSC curves could be observed in Table 4. Data were expressed as mean values  $\pm$  standard deviation ( $n = 3$ ).

Table 4 shows a general trend of the  $T_m$  to decrease for blends after subjected to PBS solution in comparison with the unexposed blends. A decrease of melting temperature ( $T_m$ ) from 170.8°C to 168.0°C was recorded for the PHB/TBC blend during the degradation with the lowest value at 10 days of exposure in PBS (166.9°C). The PHB/BC1% and PHB/BC 2% scaffolds showed a decrease of  $T_m$  from 170.8°C to 167.1°C after exposure in PBS.

The degree of crystallinity ( $\chi_c$ ) of PHB shows an increased trend up to 20 days, being more pronounced for

the PHB/TBC blend, which became more rigid, maybe due to the loss of plasticizer. While the amorphous regions of the samples were attacked, the degradation continues in the crystalline regions of the material, which are less susceptible to attack because of their orderly structure [29]. As a consequence, the crystallinity of all blends exposed to PBS medium increased, which points to their degradability. Indeed, up to 20 days, an increase of  $\chi_c$  is detected in the case of PHB/TBC (from 41.8% to 47.6%), PHB/BC1% (from 40% to 46.9%), and PHB/BC2% samples (from 39% to 45.6%), respectively. These data are well correlated with the weight loss of PHB blends (Figure 4). The burial degradation of PHB reinforced with cellulose fibers also showed an increased degree of crystallinity (65%) after 90 days of exposure [39].

#### 4. Conclusions

The present work investigated the effect of bacterial cellulose on thermal properties, water absorption, and *in vitro* degradation of scaffolds based on poly (3-hydroxybutyrate) (PHB) prepared by melt processing using salt-leaching technique.

It was found that the prepared scaffolds show a good processing and increased roughness of the surfaces.

The *in vitro* degradation study of biocomposites in PBS medium for twenty days was performed, and the occurring changes in comparison with unexposed samples were investigated by evaluation of weight loss and thermal properties. The obtained results revealed that all samples showed the increased degree of crystallinity after 20-day immersion in PBS, in good agreement with weight loss, which denoted their degradation under PBS medium.

Further interdisciplinary research is needed regarding the *in vitro* biocompatibility and clinical studies of the PHB/BC composites together with the production of bacterial cellulose at a large scale.

#### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

#### Conflicts of Interest

The authors declare no conflict of interest.

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