

## Article

# Polyhydroxybutyrate-Natural Fiber Reinforcement Biocomposite Production and Their Biological Recyclability through Anaerobic Digestion

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**Abstract:** The existing recycling methods of PHA-based material are ineffective in terms of increasing resource efficiency and the production of high value end-of-life products. Therefore, in this study, a novel approach of acidogenic fermentation was proposed to recycle PHB-based composites reinforced with natural fibers such as cellulose, chitin, chitosan, orange waste, sawdust, soy protein, and starch. The inclusion of cellulose, chitosan, and sawdust improved the impact properties of the composites while other fillers had various effects on the mechanical properties. These three composites and neat PHB were subsequently subjected to biological degradation via acidogenic digestion to determine the possibility of converting PHB-based composites into volatile fatty acids (VFAs). Two different pH levels of 6 and 10 were applied to assess the effect of pH on the bioconversion and inhibition of the methanogenesis. The results showed promising PHB degradation, contributing to considerable VFA production of 2.5 g/L at pH 6 after 47 days. At pH 6, the presence of the natural fibers in the biocomposites promoted the degradation rate. On the contrary, pH 10 proved to be more suitable for the degradation of the fibers. The VFA which is produced can be recirculated into PHB production, fitting with the concept of a circulating bioeconomy.

**Keywords:** acidogenic fermentation; biocomposites; biological recycling; natural fillers; polyhydroxybutyrate; volatile fatty acids



**Citation:** Vu, D.H.; Mahboubi, A.; Ferreira, J.A.; Taherzadeh, M.J.; Åkesson, D. Polyhydroxybutyrate-Natural Fiber Reinforcement Biocomposite Production and Their Biological Recyclability through Anaerobic Digestion. *Energies* **2022**, *15*, 8934. <https://doi.org/10.3390/en15238934>

Academic Editors: Panagiotis Tsapekos and Merlin Alvarado-Morales

Received: 31 October 2022

Accepted: 24 November 2022

Published: 25 November 2022

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## 1. Introduction

The overwhelming negative environmental impacts of petroleum-derived plastics have been driving the development of bioplastics which, due their biodegradability, are considered promising alternatives for reducing the burden of conventional plastics on the environment. In this regard, polyhydroxyalkanoates (PHAs) have gained significant scientific and industrial attention by displaying, to some extent, comparable mechanical properties to those of petroleum-based plastics, in addition to their intrinsic biodegradability [1]. Within the PHA family, polyhydroxybutyrate (PHB) is the most studied, demonstrating beneficial properties for application in packaging, food service, agriculture, coatings, adhesives, etc. [2]. However, the share of PHB in the bioplastics market remains marginal due to shortcomings in their brittleness, rigidity, and low impact resistance, as well as prohibitive production costs which are mainly due to expensive fermentation substrates [3,4].

Despite high production costs, PHB-based products can still be considered competitive if they can be converted to biocomposites with the addition of natural fillers. Natural fillers possess numerous advantages, i.e., they are inexpensive, lightweight, and can modify technical properties such as tensile strength, abundance, renewability, and biodegradation [5,6]. The inclusion of natural fillers as reinforcement in PHB-based composites may improve mechanical properties and enhance biodegradability, while decreasing overall cost. Fillers can be waste or by-products from different processes. Using waste-derived fillers in biocomposites not only enhances the creation of value out of a low or negative value waste stream but also reduces the cost of PHB-based biocomposites. In fact, various natural fibers,

including almond shell [7], cellulose [8], coconut [9], coffee waste [10], flax [11], hemp [12], kenaf [13], lignin [14], rice husk [7], starch [15], etc., have been studied as PHB composite reinforcement and have shown promising outcomes.

Considering the current detrimental impact of conventional plastics on the environment, a competent strategy for the recycling of bioplastics and biocomposites should be established [16]. To the authors' knowledge, a proficient system of recycling end-of-life PHB-based products has not been fully established. While conventional methods of mechanical recycling could be considered for reusing the building blocks of plastics, research studies have shown a sensitivity of PHB to this recycling route, which significantly weakens PHB physical properties after two cycles [17]. Chemical recycling has also been considered; however, the original main compounds, crotonic acid, 2-pentenoic acid, and 2-decenoic acid, have limited practical applications [18]. Since biodegradability is a prominent characteristic of PHB, composting could be a potential method of recycling this material, and has been explored in previous studies [19–21]. However, according to the standard of ISO 14855-1:2012, a plastic is deemed compostable if 90% of its carbon atoms are converted into CO<sub>2</sub> within six months. Thus, most of the polymer is converted to CO<sub>2</sub> which is released into the atmosphere. It is, here, interesting to explore incineration; this end-of-life option also generates CO<sub>2</sub>, except the developed heat can be recovered. Composting is, therefore, not necessarily the best method of recycling biopolymers, which is consistent with the scarcity in studies of PHB biodegradation under aerobic conditions in the last decade [22].

Apart from composting, anaerobic digestion is another method which also utilizes the biodegradability of PHB to recycle PHB waste. In fact, the biodegradation of PHB-based products has been tested in anaerobic conditions, showing promising results with a considerable yield of biogas [23–25]. The major drawback of this method, however, is an end-product of biogas, which has low unit value, market appeal, and application range [26]. Instead of biogas, the anaerobic digestion process has recently been manipulated to enhance the accumulation of intermediate products such as volatile fatty acids (VFAs). This modified process is commonly referred to as acidogenic fermentation. VFAs have served as precursors to the production of biogas; however, they are increasingly being studied as a platform to produce biofuels and biochemicals which are more valuable than biogas [27]. VFAs have been proven to be potential substrates for the biosynthesis of PHAs, and, hence, VFAs produced from the acidogenic fermentation of organic waste have been studied extensively as an economical carbon source which lowers the cost of PHB production [22,28].

Considering the potential of waste-derived VFAs as low value substrates for PHB production, the production of VFAs through the acidogenic fermentation of PHB waste should be considered. This would potentially allow for a future circular waste management where PHB waste could be valorized into VFAs and then back to PHB. This fully complies with the core concept of circular economy. To the best of the authors' knowledge, the anaerobic degradation of PHB to produce VFAs still lacks proper investigation. Therefore, the main purpose of this study was to conduct an acidogenic fermentation of PHB and PHB-based composites to produce VFAs. This strategy is believed to be a novel method of recycling the PHB products, which not only supports the PHAs-based waste management but also potentially makes the PHA production more sustainable.

## 2. Materials and Methods

### 2.1. Polymer and Natural Reinforcements

Polyhydroxybutyrate (PHB) pellet was purchased from ENMATTM (Ningbo TianAn Biologic Material Co., Ltd., Ningbo, China). Seven different components, namely Avicel (cellulose microcrystalline, Fisher, Hampton, New Hampshire, USA), chitin (Sigma-Aldrich), chitosan (Sigma-Aldrich, Missouri, USA), orange waste (Brämhults Juice AB, Borås, Sweden), sawdust (Borås, Sweden), soy protein (Borås, Sweden), and starch (ICA Borås, Sweden) were used as reinforcement (between 50 and 250 µm) in the biocomposites.

## 2.2. Biocomposites Preparation

PHB pellets and filler powders were dried at 50 °C for 16 h in a vacuum oven (Munchen, Germany) and stored in a desiccator for cooling down before the process. The biocomposites were prepared with a ratio of 70/30 (*w/w*) of PHB and natural fibers, respectively, by using a micro 12 cc injection molding machine (Xplore Instruments BV, Sittard, The Netherlands)—7.5 g of the mixture was fed into a micro 15 cc twin screw compounder (Xplore Instruments BV, Sittard, The Netherlands) which was set up at 180 °C with a screw speed of 100 rpm to achieve a homogenous combination. The polymer melt was then transferred to the injection molding machine, which shaped the exudate into 75 mm-long dog-bone samples, while rectangular specimens (75 mm long and 4 mm wide) were used for mechanical testing.

## 2.3. Inoculum

Anaerobic sludge from an upflow blanket reactor (UASB) for treating wastewater (Hammarby Sjöstad, Stockholm, Sweden) was used as an inoculum for the acidogenic fermentation. The inoculum consisted of  $9.54 \pm 0.32\%$  of total solid (TS) and  $6.50 \pm 0.15\%$  of volatile solid (VS).

## 2.4. Acidogenic Fermentation of PHB-Based Composites

The broken specimens from the mechanical tests were collected and ground into powder for the anaerobic digestion test. Each type of material was fed separately into a SM 100 cutting mill (RETSCH Mill, Haan, Germany) to obtain a mixture of particles with different sizes of between 0.2 and 2 mm. The particles were further sieved with a kitchen strainer with a pore size of 0.21 mm and then kept in a zip-lock bag for further use.

The acidogenic fermentation was conducted in 120 mL glass serum bottles with a working volume of 50 mL. The ratio of each sample to the inoculum was 1:1 (0.3 g vs. samples and 0.3 g vs. inoculum). After feeding the inoculum and substrates, ammonium sulfate (2 g/L) was supplied as a nitrogen source to support the bacterial growth. The serum bottles were then filled up to 50 mL with buffer solutions to inhibit methanogenesis and maintain the pH throughout fermentation. Two different pH levels of 6 and 10 were used to study the effect of pH on the substrate hydrolysis rates.

Afterward, an anaerobic condition was created by sparging a mixture of N<sub>2</sub> and CO<sub>2</sub> into the serum bottles for two minutes. The bottles were then placed in the water baths at 37 °C and 100 rpm for 48 days. All conditions were carried out in triplicate and three blank bottles containing only inoculum served as controls. Each natural fiber was also individually fermented in the same conditions to determine its contribution to the total VFA production of the biocomposites.

## 2.5. Analytic Methods

The mechanical properties of the biocomposites were determined by tensile and impact testing. The tensile test was done using a Tinius Olsen H10KT testing machine (Elastocon AB, Brämhult, Sweden) with a moving crosshead of 100 N load cell and a speed of 1 mm/s. Tensile strength (TS), elastic modulus (MPa), and elongation at break (E%) were calculated with QMat software. The impact properties were determined by using a Charpy impact testing machine (Cometech Testing Machines Co., Ltd., Taiwan). Ten specimens were tested and the final average Charpy energy (KJ/m<sup>2</sup>) was calculated with Meteorite software.

The thermal properties of the studied composites were determined by differential scanning calorimetry analysis (Q2000 TA instruments, Waters LLC, New Castle, DE, USA). Each sample (5–8 mg) was heated from −40 °C to 225 °C at a rate of 10 °C/min. The values were taken from the endothermic peak by linear peak integration of the second heating for the calculation of crystallinity according to the equation below:

$$\Delta X = \frac{1}{w_{\text{PHB}}} \times \frac{\Delta H}{\Delta H_m^0} \times 100$$

where  $w_{\text{PHB}}$  is the PHB weight fraction in the composites,  $\Delta H_m^0$  (146 J/g) is the enthalpy of fusion of a 100% crystalline PHB [29], and  $\Delta H$  is the specific enthalpy of fusion of the sample determined from the peak area.

The total solids (TS) and volatile solids (VS) were analyzed by following the procedures of the American Public Health Association, the American Water Works Association, and the Water Environment Federation (APHA-AWWA-WEF).

The VFA production was determined by gas chromatograph (GC; Clarus 550, Perkin-Elmer, Norwalk, CT, USA) using a capillary column (Elite-WAX ETR, 30 m  $\times$  0.32 mm  $\times$  1.00  $\mu\text{m}$ , Perkin-Elmer, Shelton, CT, USA) and a flame ionized detector (PerkinElmer, Shelton, CT, USA). Nitrogen was used as the carrier gas at a flow rate of 2 mL/min under 20 psi. The temperatures of injection and detection were 250  $^{\circ}\text{C}$  and 300  $^{\circ}\text{C}$ , respectively.

Characterization by Scanning Electron Microscopy (SEM) of the fracture surfaces from the tensile testing was done by an external institute.

### 3. Results and Discussion

To evaluate the potentials in the circular bioproduction and application of PHB and its natural composites, PHB-based natural composites were produced using different natural fibers. Several thermal and mechanical properties of the produced composites were analyzed to determine the potential fibers for future studies of PHB composites. Finally, the produced composites and pure PHB were subjected to acidogenic fermentation to investigate the possibility of the production of VFAs.

#### 3.1. Thermal Characteristics of PHB-Based Biocomposites

Differential scanning calorimetry analysis was performed to determine changes in the thermal properties. The enthalpies obtained were used to calculate the crystallinity of the neat PHB reinforced with different natural fibers, the results of which are summarized in Table 1.

**Table 1.** Thermal properties of neat PHB and its biocomposites. Single measurements were carried out for each sample.

Samples	$T_m$ ( $^{\circ}\text{C}$ )	$\Delta H$ (J/g)	$X_c$ (%)
Neat PHB	169.26	78.13	53.51
PHB/cellulose	167.77	54.33	53.15
PHB/chitin	167.54	62.01	60.67
PHB/chitosan	166.58	49.88	48.80
PHB/orange waste	165.41	56.86	55.63
PHB/sawdust	162.07	48.04	47.00
PHB/soy protein	152.26	14.82	14.50
PHB/starch	167.40	56.07	54.86

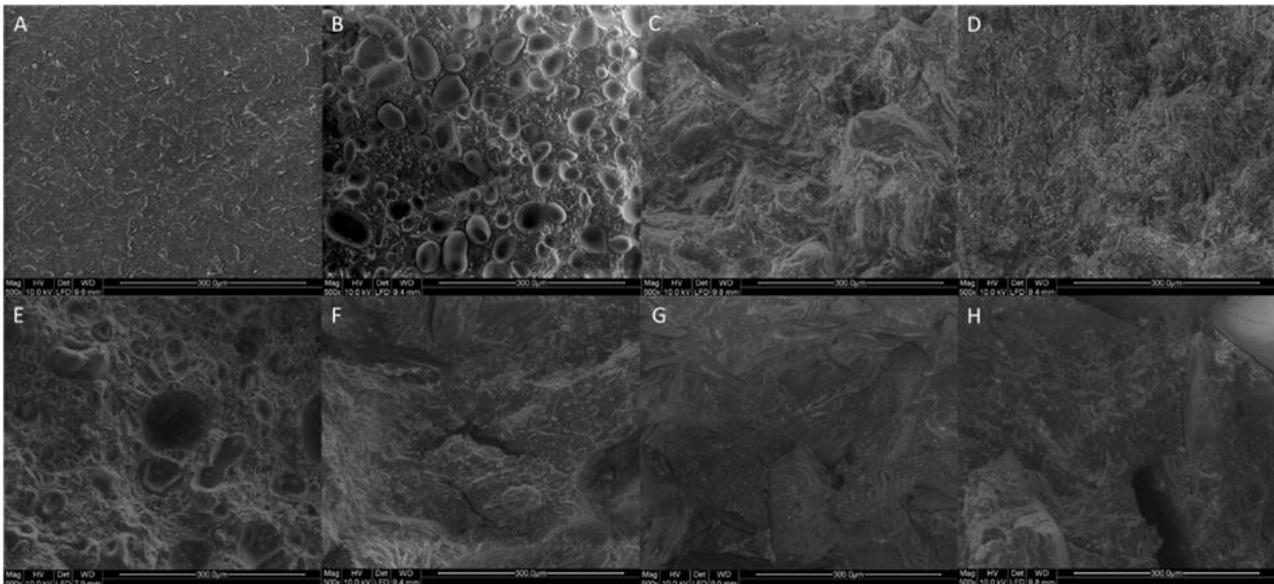
The inclusion of soy protein significantly decreased the melting temperature ( $T_m$ ) of the neat PHB from 169.26  $^{\circ}\text{C}$  to 152.26  $^{\circ}\text{C}$ . This reduction is presumed to be caused by the polar groups in the soy protein which triggers an irreversible denaturation from the first heating [30]. Natural fibers, however, only decreased slightly (between 2  $^{\circ}\text{C}$  and 7  $^{\circ}\text{C}$ ) compared to the neat PHB. Moreover, the heat of enthalpy ( $\Delta H$ ) of the PHB-based composites and crystallinity ( $X_c$ ) of some PHB content were also reduced. This phenomenon is predictable since the fibers act as a nucleating agent to restrict the mobility of PHB chains and hinder reorganization. The same behavior was also observed in other studies of PHB reinforced with natural fibers [8,31,32]; however, the reduction in this study was, relatively, higher due to the absence of coupling agents.

### 3.2. Mechanical Properties of the PHB/Natural Reinforcement Biocomposites

#### 3.2.1. The Morphology of Natural Filler-Reinforced PHB Composites

SEM was applied to determine the morphology of PHB-based composites by observing the cross-section of the fractured surface to better understand the interfacial adhesion between the filler and PHB matrix.

Compared to neat PHB, which showed a homogenous and compact appearance without visible flaws, the obtained micrographs generally showed poor adhesion between the fibers and PHB, revealing that the main role of the fibers in the composites is that of filler. As expected, starch (Figure 1B) and soy protein (Figure 1E) displayed a biphasic morphology, meaning that they are not blendable with PHB. Small voids can also be observed. Orange waste (Figure 1F), chitosan (Figure 1G), and sawdust (Figure 1H), however, presented in longer shapes, showing the pulled-out fibers and voids in the system. Similar poor interfacial bonding behavior in PHB composites has been reported in previous studies where starch [15] and lignocellulosic materials [11,33,34] were used as filler. By contrast, the structure of PHB reinforced with chitin and cellulose is preferable since the fibers were well blended in the PHB matrix, forming a homogenous agglomeration and distribution.



**Figure 1.** SEM micrographs of the fractured surfaces of neat PHB (A) and PHB composites reinforced with starch (B), chitin (C), cellulose (D), soy protein (E), orange waste (F), chitosan (G), and sawdust (H). All micrographs have the same magnification of 500 $\times$ .

The obtained SEM micrographs, in this study, exposed the weak interfacial bonding and inferior interaction within the composite networks which, consequently, entail negative impacts on the mechanical properties compared to the neat PHB. In particular, the imperfect bonding between fiber and matrix, where the fiber heads play the role of stress concentrator and crack initiation facilitator rather than reinforcement when it comes to application, functions with a decrease in tensile loading [11]. Furthermore, in the discontinuous fiber-reinforced composites, fiber length is also an important factor for effectively utilizing the intrinsic properties of the fiber [35]. During the process of mixing and injection molding, the fiber may become partly degraded, leading to a decrease in fiber length, thus weakening the reinforcing effect of the fiber in the PHB matrix. This problem could have been worse, as the studied fibres were used in powder form, the damage of which could be exacerbated during the processing.

### 3.2.2. The Effect of Type of Natural Fillers on the Mechanical Properties of a PHB-Based Composite

The mechanical behavior of PHB and its composites were determined by tensile and impact tests, and the obtained results are summarized in Table 2.

**Table 2.** Summary of the mechanical properties of neat PHB and its composites.

Materials (Ratio of 70/30)	Tensile Strength (MPa)	E-Modulus (GPa)	Strain at Break (%)	Impact Strength (kJ/m <sup>2</sup> )
Neat PHB	34.6 ± 1.1	4.7 ± 0.19	0.84 ± 0.05	4.61 ± 1.23
PHB/cellulose	31.6 ± 1.7	6.7 ± 0.12	0.63 ± 0.04	5.80 ± 1.03
PHB/chitin	31.4 ± 1.1	5.9 ± 0.07	0.67 ± 0.06	4.30 ± 0.70
PHB/chitosan	29.7 ± 2.4	5.7 ± 0.16	0.67 ± 0.08	4.98 ± 0.74
PHB/orange waste	28.2 ± 1.1	5.2 ± 110	0.71 ± 0.04	4.43 ± 0.62
PHB/sawdust	34.3 ± 0.3	6.7 ± 0.09	0.7 ± 0.01	5.60 ± 0.52
PHB/soy protein	18.4 ± 0.5	4.1 ± 0.06	0.55 ± 0.02	3.37 ± 0.64
PHB/starch	24.2 ± 1.8	4.6 ± 0.16	0.56 ± 0.07	3.23 ± 0.99

Compared to the neat PHB, the presence of different natural fillers varyingly influenced the mechanical properties. Overall, none of the reinforcements had the ability to significantly improve tensile strength. Sawdust had the best results, with a tensile strength of 34 MPa and clear improvement in the modulus. Since sawdust is a cheap and biodegradable byproduct, it could be a good filler for PHB.

A similar decline was also observed in the strain-at-break data—the results show that the fracture strain of biocomposites is 15–35% lower than in the pure PHB (0.84%), reflecting the stiffening effect induced by the presence of the natural fillers. The increased stiffness was also confirmed by the increase in the E-modulus of the biocomposites corresponding to an increase of 12–43% compared to 4.7 GPa from the neat PHB. However, the addition of starch and soy protein significantly decreased the mechanical performance of the biocomposites across all perspectives. Similar results relating to poor physical properties can be found in the studies of Fang et al. [36] and Zhang and Thomas [15], where bioplastics were reinforced with starch and soy protein.

Regarding impact strength, the inclusion of the fillers only caused an increase of 5.80, 4.98, and 5.60 kJ/m with respect to cellulose, chitosan, and sawdust, respectively. However, only the addition of cellulose resulted in a significantly different ( $p$ -value = 0.032) impact strength. The decrease in the impact strength results of other biocomposites can be predicted from the SEM micrographs (Figure 1). There is an uneven distribution of starch and soy protein granules as well as the voids from the pull-out fibers of orange waste, leading to poor interaction and low compatibility between the biopolymer matrix and the natural fibers.

The poor interfacial adhesion can also be attributed to the dissimilarity characteristic of the composition, where the fiber is known to be actively polar and highly hydrophilic in contrast with the non-polar and considerable hydrophobicity of the biopolymer. This behavior, therefore, results in significantly inefficient load transfer in the biocomposite system, worsening the overall mechanical properties, which is considered a typical phenomenon when embedding fibers not only in PHB but also in several polymer matrices [10]. Future studies should consider other factors such as the average particle size, shape, and density of each filler to gain better insight into the relationship between a matrix and natural fillers. The addition of surface modifiers or compatibilizers could also be employed to improve the morphology of the PHB-based composites and, consequently, enhance their mechanical properties.

### 3.3. Acidogenic Fermentation of the PHB-Based Biocomposites

The biodegradability and biological recyclability of PHB are two prominent characteristics that make it preferable to conventional plastic. The investigation of PHB biodegradation has been conducted under different conditions, including aerobic, anaerobic, and natural environments [16]. The results obtained showed a faster degradation of PHB under

anaerobic conditions than under aerobic conditions, with the former accompanied by the more valuable final product of biogas [37,38]. Acidogenic fermentation is the third stage of anaerobic digestion and is achieved by manipulating the final step of anaerobic digestion, specifically inhibiting methanogenesis, which prevents the VFAs from being converted into methane. Current research in the field focuses on the use of VFAs as a sole carbon source in bacterial fermentation for PHB production [39]. Altogether, the combination of anaerobic digestion and bacterial fermentation offers the possibility of establishing closed-loop PHB production from PHB-based waste. After considering the results of mechanical tests, three PHB-based biocomposites of PHB/cellulose, PHB/chitosan, and PHB/sawdust with enhanced mechanical properties were, therefore, selected for further investigation of acidogenic fermentation for VFA production.

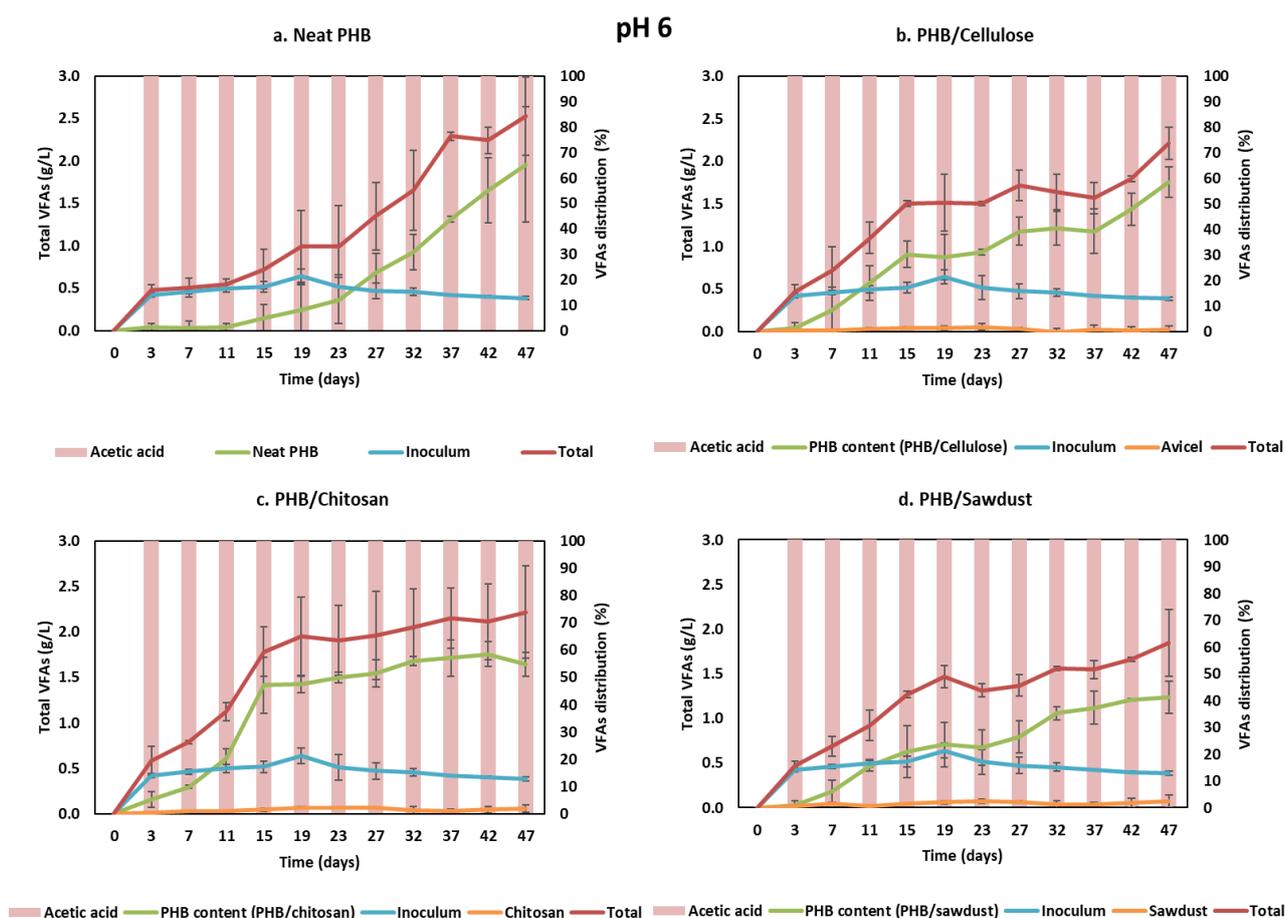
### 3.3.1. The Effect of pH on the Bioconversion of PHB-Based Composites to VFAs

pH is one of the most important operational parameters in acidogenic fermentation, influencing the hydrolysis rate and the inhibition of methanogenesis, both of which have a direct effect on VFA production [40]. In general, a neutral pH state has been proven to be preferable for acidogenesis, while alkaline pH is more suitable for complex organic materials requiring extensive hydrolysis [41]. This is in line with Zhang et al. [42], in which pH 7 provided a better VFA yield compared to other pH levels of 5, 9, and 11 in the anaerobic digestion of kitchen wastes. In another study, by Parchami et al. [43], a more complicated food waste sample, collected from both households and retailers, yielded the highest total VFA production of 14.73 g/L at pH 12 after 25 days of acidogenic fermentation. Additionally, it should be noted that sharp pH reduction will occur during acidogenesis due to the accumulation of VFAs, causing toxicity to the microorganism by the time the undissociated form is reached [40]. Therefore, buffer solutions with two different pH levels of 6 and 10 were applied to inhibit the activities of methanogens and to determine the effect of pH on the bioconversion of PHB-based composites. In this study, bioconversion of the biocomposites can be attributed to the VFA production. The behavior of the PHB and fillers in composite form during acidogenic fermentation is determined by the comparison between the VFA generation from biocomposites and the VFAs produced from blank. The results obtained on the total VFAs production and VFA distribution are depicted in Figures 2 and 3.

At pH 6, the highest total amount of accumulated VFAs of 2.5 g/L was achieved from the neat PHB, with an increasing trend during the 47 days of fermentation (Figure 2a). The degradation of the biocomposites also showed similar behavior to the production of VFAs in a range of 1.8–2.2 g/L. However, after a noticeable increase in the first 15 days, fluctuations were observed in the next period (days 19 to 37) before the rising trend resumed. The VFA production at this pH level relied heavily on the degradation of the PHB content, which accounted for approximately 52%, 65%, 74%, and 40% of the total VFA production of respective samples of neat PHB, PHB/cellulose, PHB/chitosan, and PHB/sawdust. The inoculum, however, produced an average amount of 0.43 g/L, accounting for 27–37% of the total produced VFAs, which gradually decreased toward the end of the experiment. At the same time, the VFAs share from the fillers in all samples was significantly low, measured at about 0.02–0.04 g/L, contributing in the range of 1.5–3.4% of total VFA generation.

Generally, in this study, the total VFA production at pH level 10 (Figure 3) was lower than at pH 6 and was similar to the results of Cheah et al. [44], where municipal solid waste and food waste were anaerobically digested, producing 8.5 g/L of VFAs at pH 6 compared to 7.4 g/L at pH 10. In another study, by Jiang et al. [45], an acid-neutral condition of between pH 6 and 7 was also found to be favorable for acidogenic fermentation due to an enhancement of the hydrolysis rate of approximately 20%. Particularly, the maximum VFA production of all samples at pH 10 was in the range of 1.08–1.2 g/L, which is almost half that which was produced at pH 6. At pH 10, the trend shifts to favor the degradation of reinforcements and inoculum rather than PHB. This can be seen from the maximum VFA production from neat PHB which was 1.16 g/L and which equals only approximately half

of the amount obtained at pH 6. Noticeably, in the neat PHB sample, the VFA production from PHB content only accounted for a modest 7.57%, while the highest fraction was the proportion of inoculum, with 88.3% of the total production of VFAs. Further, 82–90% was also the share of the inoculum in the VFA contributions from the composites and the rest can be attributed to the fillers fractions. In Figure 3b,c, note that more VFAs are produced from cellulose and chitosan than the total VFA production from the composites. The content of VFAs produced from cellulose and chitosan powder, when being used as blank samples, was moderately higher than VFA content in the composites, which is only between 8 and 17%. This is because their original state is free powder which is not covered by the PHB and fully ready for hydrolysis by the bacteria. The contribution of PHB content, in contrast, was particularly low (less than 0.1 g/L) throughout fermentation.

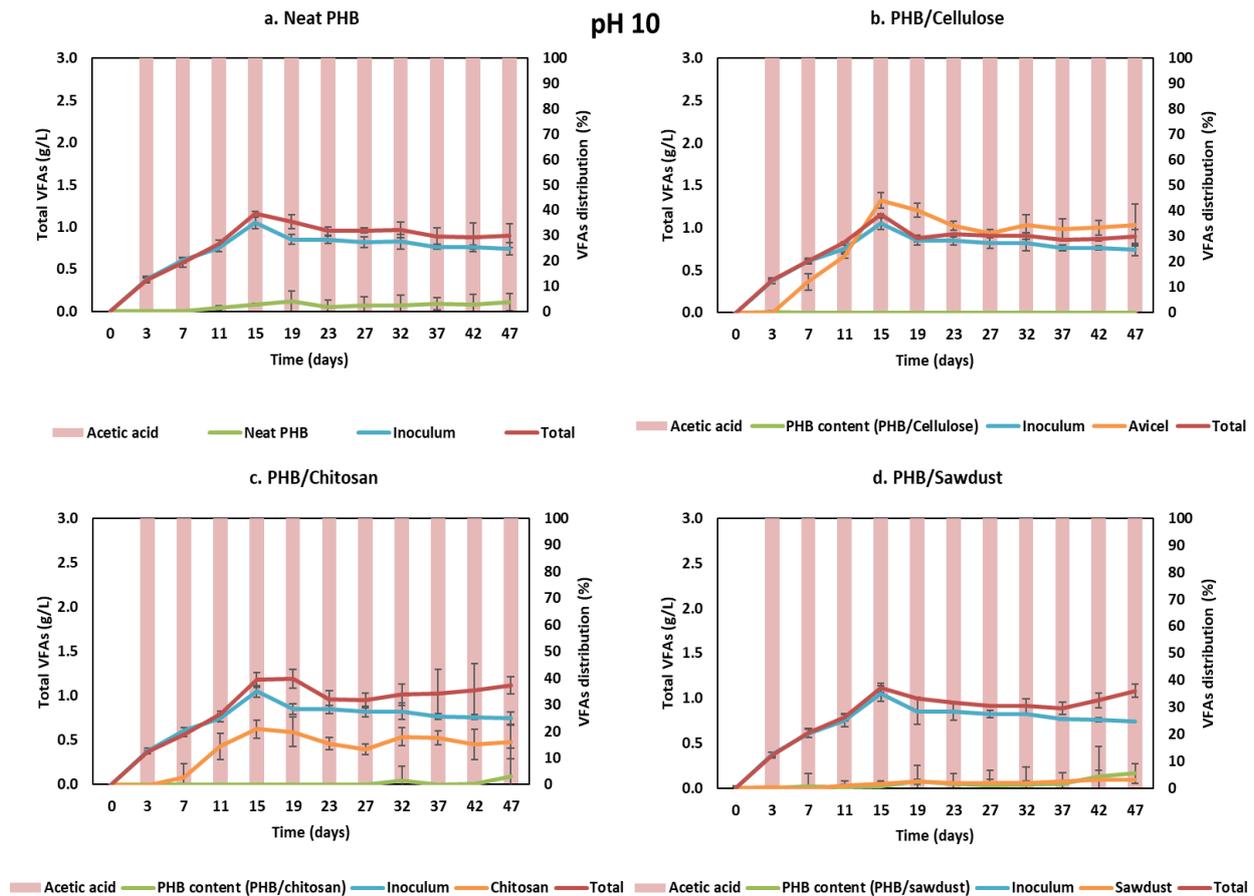


**Figure 2.** Concentration and percentage distribution of VFAs during acidogenic fermentation of neat PHB (a) and PHB composites of PHB/cellulose (b), PHB/chitosan (c), and PHB/sawdust (d) at pH 6.

During the experiment, changes in the volume and composition of the gas released in the glass bottles were also monitored. Only CO<sub>2</sub> was generated, with an average range of 5–8 mL at the end of the digestion (data not shown). These data show the stability of the acidogenic fermentation, where almost all the carbon content was directed to the production of VFAs and, to some extent, CO<sub>2</sub> generation.

The acidogenic fermentation results show the important influence of an environmental factor such as pH on the bioconversion of the biocomposite due to how it affects the variety of the microbial community [40]. In fact, pH has a remarkable impact on reactions such as redox potential, mineral dissolution, precipitation, etc., which regulate the availability of nutrients and trace elements in the environment [46]. A change in pH can impact the activities of enzyme systems and related bio-interactions, shifting the metabolic system of microbial communities for adaptation [47,48]. The abundance of microorganisms, therefore,

would be affected and decline, since the optimal pH is not afforded or shifted [49]. This, in turn, leads to a decrease in enzyme secretion by the microbial system, reducing its activities in line with bioplastic biodegradation, which mainly relies on extracellular enzymes. In this study, pH changes were also observed at both pH levels (Table 3), which, it is assumed, leads to a halt in biodegradation at pH 10 or fluctuations at pH 6. This hypothesis is supported by the results of Zhang [42], where a higher VFA yield was obtained when the anaerobic digestion was conducted under controlled pH conditions. The importance of controlled pH during the acidogenic fermentation of PHB composites should therefore be a matter of concern in future studies.



**Figure 3.** Concentration and percentage distribution of VFAs during acidogenic fermentation of neat PHB (a) and PHB composites of PHB/cellulose (b), PHB/chitosan (c), and PHB/sawdust (d) at pH 10.

**Table 3.** The final pH of each sample at pH levels 6 and 10 after acidogenic fermentation.

Samples	Final pH (with Initial pH of 6)	Final pH (with Initial pH of 10)
Inoculum	6.61 ± 0.03	8.15 ± 0.09
Neat PHB	5.84 ± 0.81	8.05 ± 0.04
PHB/Cellulose	5.81 ± 0.64	8.04 ± 0.04
PHB/Chitosan	5.35 ± 0.15	7.84 ± 0.24
PHB/Sawdust	6.14 ± 0.48	7.96 ± 0.18
Cellulose	6.58 ± 0.09	6.29 ± 0.14
Chitosan	6.57 ± 0.08	7.36 ± 0.53
Sawdust	6.55 ± 0.20	7.89 ± 0.01

### 3.3.2. The Effect of the Type of Natural Fillers on the Degradation of PHB-Based Composites

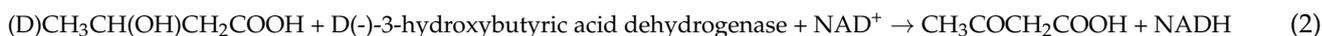
The introduction of natural fillers into PHB is believed to not only improve the mechanical properties of the polymer matrix, but also to enhance the biodegradability of the

whole system [6]. In this study, the influence of fibers on PHB-based composite degradation was utterly different and depended significantly on pH level.

This can be seen at pH 6 (Figure 2a), where it took the neat PHB 11 days to produce VFAs before the production began to accelerate from day 23. Nevertheless, this long lag phase can potentially be tackled with the addition of natural fibers that are highly biodegradable and which can notably promote the composite degradation. The disintegration of natural fibers, moreover, results in a rougher surface and higher surface area-to-volume ratio that facilitates the aggregation and growth of the microbial community, thereby assisting advanced depolymerase accumulation which enhances the PHB degradation [50].

This assumption is strengthened by the results from pH 6, where, with the presence of natural fillers, the PHB content in the biocomposites tended to degrade in the earlier stages of the fermentation (from day three [chitosan] and day seven (cellulose and sawdust)) compared to the longer time of 15 days in the neat PHB sample. However, with the intensive hydrolysis effect of pH 10, the fibers become more vulnerable and ready for digestion, resulting in the dominance of fiber degradation throughout the experiment compared to a low degradation of PHB. In this study, pH 10 proved to be more suitable for the degradation of the fillers, which is in line with results obtained by Lu et al. [51] where a better hydrolysis efficiency of organic particles was achieved in alkaline pH. In a study by Chen, et al. [52], considerable VFA recovery was obtained under alkaline fermentation where the accessibility of cellulose surface and cellulose activity were significantly enhanced, facilitating an increase in the bio-release of carbohydrate from lignocellulosic wastes.

For a better understanding of the fiber impact on PHB degradation, the degradation mechanism of the PHB should be considered. The physical properties of the PHB and microbial enzyme secretion define its degradability [53]. The enzyme secretion can be divided into two groups, intracellular and extracellular PHB depolymerases, where the second type is responsible for polymer hydrolysis in this circumstance. The secretion of depolymerases, however, is a time-consuming process where the microbial community needs time to adapt, and where anaerobic stress leads to an initially slow PHB degradation that later accelerates [54]. The biological processes of PHB degradation are generally described in following equations:



In fact, the extracellular PHB is biodegraded by surface erosion from the colonization of the microorganisms, where the exogenous depolymerases are secreted to hydrolyze the exposed areas, leading to the formation of D(-)-3-hydroxybutyric acid. D(-)-3-hydroxybutyric acid is further oxidized by NAD-specific dehydrogenase to create acetoacetyl-CoA, which is, finally, converted to acetyl.CoA [54,55]. Acetyl-CoA, under an anaerobic condition, is a precursor for the production of acetic and butyric acid [40]. However, only acetic acid was produced in this study. The VFAs produced, furthermore, can be used as chemical building blocks for the production of PHAs, creating an efficient strategy to recycle the PHB-based production and which fits the concept of circular bioeconomy.

Another factor which affects the influence of natural fillers on biocomposite degradation is the presence of corresponding hydrolysis products. Compared to sawdust, cellulose and chitosan are refined materials obtained from the processes of wood pulp hydrolysis and chitin deacetylation, respectively [56,57]. Cellulose and chitosan, therefore, are readily available for further hydrolysis, leading to the production of corresponding simple sugars of glucose and glucosamine which can be used as a carbon source for the microbial

growth [58,59]. By contrast, hydrolysis of the lignocellulosic material sawdust can normally produce inhibitory chemicals of phenolic compounds, furfural, etc., which is not preferable for microorganism metabolism [60]. These factors, taken together, explain the modest degradation of sawdust, which thus resulted in sawdust contributing less to total VFA production than cellulose and chitosan. The depletion of nitrogen source and the insufficiency of other nutrients should also be hypothesized as a reason for the termination of the biodegradation.

#### 4. Conclusions

This study successfully converts PHB-based composites into VFAs through acidogenic fermentation. In this regard, pH and the type of fillers are the key factors in defining the final outcome. In particular, pH 6 is suitable for PHB degradation, providing a maximum VFA production of up to 2.5 g/L after 47 days of fermentation. pH 10, in contrast, is more advisable for fiber degradation, as the VFAs obtained from PHB deterioration were negligible. The presence of natural fibers, in general, positively accelerated the degradation of PHB at pH 6. In terms of mechanical properties, only the inclusion of cellulose, chitosan, and sawdust presented an increase in impact strength. The different results at pH levels 6 and 10, therefore, contribute two potential recycling routes for PHAs-based biocomposites, including VFA production and PHB recovery, respectively. The addition of compatibilizer or surface modification and fiber-matrix ratios should be considered in future studies to obtain a better balance in mechanical property improvement and the biodegradation of PHB-based composites.

**Author Contributions:** Conceptualization, D.H.V., J.A.F. and A.M.; methodology, D.H.V., J.A.F. and A.M.; software, D.H.V.; validation, A.M., J.A.F. and D.Å.; writing—original draft preparation, D.H.V.; writing—review and editing, D.H.V., A.M., J.A.F., D.Å. and M.J.T.; supervision, A.M., J.A.F. and D.Å.; funding acquisition, A.M., D.Å. and M.J.T. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received partial funding from Sweden’s Innovation Agency.

**Acknowledgments:** The authors gratefully acknowledge the technical and financial support of Sparbanksstiftelsen Sjuhärad, Sweden’s Innovation Agency, and University of Borås.

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

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