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Sarah Lammi[1,2], Emmanuelle Gastaldi[1], Fabrice Gaubiac[1], Hélène Angellier-Coussy[1*]

[1]JRU IATE 1208 – CIRAD/INRA/Montpellier SupAgro/University of Montpelier, 2 place Pierre Viala, F-34060 Montpellier, France

[2]Department of Agronomic Sciences, University of Mouloud MAMMERI, BP 17, 15000 Tizi-Ouzou, Algeria.

*Corresponding author: helene.coussy@umontpellier.fr

Abstract

Polyhydroxybutyrate-co-valerate (PHBV) is a very promising bio-sourced and biodegradable bacterial polyester, the commercial development of which being limited by its high cost. On the other hand, olive oil extraction generates a large amount of lignocellulosic solid residue, called olive pomace (OP), whose disposal raises environmental concerns, including the inhibition of soil microbial populations and the reduction of seed germination. To address these issues, PHBV/OP based biocomposites were produced (at a filler content of 15 wt%) using three different fillers obtained by dry fractionation of OP, *i.e.* a stone-rich fraction (SF), a pulp-rich fraction (PF) and a crude pomace (F0). The objective of the present work was to investigate how the composition of OP-based fillers influenced the biodegradability of resulting biocomposites. The biodegradation of PHBV, OP-based fillers and OP/PHBV-based biocomposites was assessed by respirometric tests conducted in a standardized soil environment over 4 months. Results showed that the incorporation of OP-based fillers in PHBV favored the overall biodegradability of the materials. Indeed, 100% of biodegradation was achieved after 75, 79 and 87 days for PHBV-F0, PHBV-SF and PHBV-PF formulations

respectively, while a biodegradation rate of only 91% was obtained after 123 days in the case of neat PHBV. These results were discussed in the light of several parameters such as the material structure, the affinity of the fillers for the matrix, the composition and the degree of crystallinity of the filler, together with the antibacterial activity of the OP samples assessed by a direct contact technique on solid medium.

Keywords: Biodegradation; biocomposites; PHBV; olive pomace; soil; packaging; respirometric tests.

1. Introduction

Most of currently used synthetic polymers are produced from petrochemicals and are not biodegradable. Persistent polymers generate long-term negative impacts on environment and human health, due to their accumulation in the environment and to the diffusion of degraded plastic micro- and nano-particles towards the soil, the water and finally living organisms. That is why bio-sourced polymers that are fully biodegradable in natural conditions have raised great interest, particularly for short-term applications such as packaging and agriculture (Avérous and Pollet, 2012). It is worth noting that food packaging and agriculture sectors represent the largest plastic consumption sectors, with respectively 40% and 5% of the total consumption, and more than 75% of uncollectable and dispersed plastics.

Biodegradation is a process in which the molecular structure of materials is broken down through metabolic or enzymatic mechanisms. The decomposition process occurs via enzymes secreted by naturally occurring microorganisms such as bacteria, fungi or actinomycetes. Biodegradation can occur in aerobic (requiring oxygen) or anaerobic (without oxygen) conditions. Biomass (humus, compost) and gas (carbon dioxide and methane) are the main products of a biodegradation process, carbon dioxide being generated under aerobic conditions while in the case of anaerobic conditions methane was mainly produced (Andrady,

2011; Kumar et al., 2013; Stoleru, 2017). Besides surface properties (surface area, hydrophilic/ hydrophobic properties, roughness) other intrinsic chemical and physical factors are involved in the biodegradation of polymers like, first order structures (chemical structure, molecular weight and molecular weight distribution) and higher order structures (glass transition temperature, melting temperature, modulus of elasticity, crystallinity and crystal structure) (Tokiwa et al., 2009). The degree of crystallinity is a crucial factor affecting biodegradability, since enzymes mainly attack the amorphous domains of a polymer where polymer chains are loosely packed, and are thus more susceptible to degradation. The crystalline parts of the polymers are generally more resistant to biodegradation than the amorphous ones. Aliphatic polyesters [ester bond (-CO-O-)] are typical polymers that show high potential for use as biodegradable plastics, owing to their susceptibilities to lipolytic enzymes and microbial degradation (Tokiwa et al., 2009).

Among the bio-sourced polymers able to biodegrade in natural conditions, polyhydroxyalkanoates (PHAs) are a class of polyesters that present the advantage to be naturally produced by a wide variety of bacteria able to accumulate from 30 to 80 % of their dry weight in the form of intracellular granules as carbon and energy reserves (Madison and Huisman, 1999; Singh Saharan et al., 2014). The copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is one of the most well-known PHA since already commercially available for the production of disposable items and food packaging materials (Kourmentza et al., 2017). However, as compared to conventional plastics, PHBV is still more expensive (around 5 €/kg), which limits its use to a narrow range of applications. To decrease the overall cost of materials while modulating their functional properties and maintaining their biodegradability, the incorporation of lignocellulosic fillers stemming from agricultural residues has become an attractive strategy (Barkoula et al., 2010; Berthet et al., 2016; Berthet et al., 2017; Mousavioun et al., 2013; Lammi et al., 2018b).

Olive pomace (OP) is an agro-waste that has already been combined with PHBV to produce biocomposites (Berthet et al., 2015; Dufresne et al., 2003; Hassaini et al., 2017; Lammi et al., 2018b). OP is a solid lignocellulosic residue representing 30 to 40% of the olive oil extraction by-products. It is mainly composed of skin, pulp and fragments of crushed stone (Gharbi et al., 2014; Gómez-Muñoz et al., 2012). OP represents an important environmental issue mostly in Mediterranean countries where they are generated in huge quantities, reaching 2,881,500 tons/year worldwide, in a short period of time (Ravindran and Jaiswal, 2016). Moreover, they cannot be used as agricultural spreading since susceptible to cause harmful impacts on the environment when let on soil. Such negative effects are mainly due to their high content in phenolic and lipidic constituents, organic acid, low pH, relatively high salinity, which confer them phytotoxic and antimicrobial properties. They also may be contaminated by fungi, whose fungal toxins combined with polyphenolic compounds are resistant to bacterial degradation, and consequently makes OP a significant source of environmental pollution (Cardelli and Benitez, 1998; Gómez-Muñoz et al., 2012; Niaounakis and Halvadakis, 2004). For all the above considerations related to the waste management issues generated by olive pomace, there is an urgent need to explore alternatives to convert them into valuable resources.

To date, most of available studies dealing with the incorporation of OP in polymer matrices have specifically focused on the use of olive stone, which is a fraction very rich in cellulose. In a previous study, it has been demonstrated that the use of either the whole OP or a pulp-rich fraction, richer in lignin, could be interesting in terms of final performance of materials (Lammi et al., 2018b). A better preservation of mechanical characteristics was achieved in the case of pulp-rich filler (PF), due to better filler/matrix interactions and hence higher interfacial adhesion. A range of water vapor and oxygen permeabilities could be reached, which makes it possible to consider the use of OP-based biocomposites as food packaging

(rigid trays) for various types of food products, but also for horticulture applications. However, it has never been checked if the presence of lignin could have a negative impact on the biodegradability of resulting composite materials.

In this context, the objective of the present work is to investigate for the first time the impact of the composition of OP fillers on the capability of PHBV/OP-based biocomposites to biodegrade in soil. This is of prime importance since such materials are intended to be used to short-term use applications, such as pots or trays. For that purpose, three types of fillers displaying contrasted biochemical composition have been produced by following a dry fractionation process already reported by Lammi et al., 2018a, *i.e*. a stone-rich filler, a pulp-rich filler and a filler produced from the whole OP residue. PHBV/OP-based biocomposites were prepared by thermomolding, with filler content up to 15 wt%. Biodegradation was evaluated through respirometric tests conducted in soil, at 28°C and in aerobic conditions during 4 months. The biodegradation rate of the different OP fractions and the resulting PHBV-based composites was discussed in relation to the material structure, as well as to the antibacterial activity of the OP samples assessed by a direct contact technique on solid medium.

2. Materials and methods

2.1. Materials

Olive pomace (OP) was kindly supplied by a local olive producer in the region of Azeffoun (Tizi-Ouzou, north-center of Algeria). It was obtained from the olive oil extraction of the *Chemlal* variety. Raw olive pomace was dried in an oven at 60°C for 24 hours before further usage.

A commercial grade of polyhydroxy-(butyrate-co-valerate) (PHBV) containing 3% of valerate was purchased from NaturePlast (France) in the form of pellets (PHI002 grade).

Sulfuric acid, arabinose, xylose and glucose (Sigma-Aldrich), formamide, diiodomethane (Acros Organics, Geel, Belgium), ethylene glycol (Aldrich chemical Co. Inc., Milwaukee, USA) and glycerol (Merck, Darmstadt, Germany) were used for characterization of OP fillers. Barium chloride-Dihydrat (VWR Chemicals, Germany), sodium hydroxide (Grosseron, France), thymolphtalein (Alfa Aesar, Germany), hydrochloric acid and microcrystalline cellulose (Merck, Germany) were used for biodegradation tests.

2.2. Preparation and characterization of olive pomace-based fillers

Pulp-rich and stone-rich fractions were isolated from the raw olive pomace sample by dry fractionation with friction solicitations in a ball mill, as described by Lammi et al. (2018a). resulting fractions were then sieved and reduced in size using successive dry grinding steps (Lammi et al., 2018a). The obtained powders, *i.e.* the pulp-rich fraction (PF), the stone-rich fraction (SF) and the crude OP fraction (F0), displayed a median apparent diameter of respectively 55, 59 and 85 µm (Lammi et al., 2018b).

The cellulose, hemicellulose and lignin contents of OP-based fillers were characterized in a two-stage acid hydrolysis. The first hydrolysis was carried out using a 72 wt% H_2SO_4 solution at 30°C for 1 hour, while the second one was performed using a 4 wt% H_2SO_4 solution at 121°C for 1 hour following the protocol described by Barakat et al. (2014). The lignin content was determined by the Klason method (Nicholson et al., 2014). The protein content was estimated according to the formula (N x 6.25) (Jones, 1941), after the determination of nitrogen content (N) by elementary analysis. The moisture content was determined by drying about two grams of sample in an oven at 110°C until reaching weight equilibrium. The ashes content was obtained by igniting about one gram of sample in a muffle furnace at 600°C for 5 hours. pH measurements were carried out with a pH electrode (VWR 1100L, Germany) on an aqueous suspension (five grams of sample mixed with 50 mL of distilled water) put under

magnetic stirring during 30 min (Haddadin et al., 2009). All the analyses were performed in triplicate.

2.3. Preparation and characterization of OP/PHBV-based biocomposites

PHBV pellets and OP-based fillers (15 wt%) were blended in a HAAKE Rheomix internal mixer operating at a rotor speed of 60 rpm and 170°C during 5 min. Obtained compounds were cooled to room temperature and then, ground in a knife mill (SM 300, Retch, Germany) at a speed of 2200 rpm through a 4 mm grid to obtain composite pellets. Compounds were dried at 60°C for at least 8 hours before the preparation of materials. Biocomposite films (10.5 cm wide squares, average thickness about 250 µm) were prepared by hot pressing the pellets between two Teflon coated plates at 170°C, using a hydraulic thermopress (PLM 10 T, Techmo, Nazelles, France). Samples were first allowed to melt for 3 min. Then, a pressure of 150 bars was applied for 2 min. The films were cooled at room temperature before being removed from the mould.

The percentage of carbon of OP fillers, neat PHBV, biocomposites and microcrystalline cellulose was determined using an elemental analyzer Vario Micro Cube (Elementar, Germany).

The surface morphology of the OP-fillers and the cross-section of the PHBV-based films (obtained after cryo-fracturing under liquid nitrogen) were observed by scanning electron microscopy (SEM) using a scanning electron microscope (SEM S-4500, Hitachi, Japan) with an acceleration voltage of 2 kV and a detector for secondary electrons. The samples were previously coated with gold/palladium by ion beam sputtering.

Antibacterial tests were carried out to evaluate the antibacterial effect of both fillers and composites films following the direct contact technique on Mueller-Hinton agar against two

selected bacterial strains (*Staphylococcus aureus* CIP 53156 and *Escherichia coli* 55B5 CIP 52170). The tests were performed on young pre-cultures of both bacteria in a Mueller-Hinton broth. Bacterial suspensions turbidity was standardized to 0.5 McFarland. The OP-based fillers (in the form of powders) were deposited in wells of 10 mm in diameter obtained with a piece-cutter on the agar medium. In the case of materials, discs of 10 mm diameter precut from the composite films were putted on the surface of the agar previously inoculated with the target bacteria. The incubation of Petri dishes was carried out at 30°C for 24 hours. The occurrence of an antibacterial effect of both materials was evidenced by the appearance a clear inhibition zone around the wells/discs.

2.4. Biodegradation tests

Respirometric tests were conducted in aerobic conditions to evaluate the biodegradation rate of OP-based fillers, PHBV matrix and PHBV/OP based biocomposites in soil medium. The method was adapted from the US standard ASTM D5988-96, which is a Standard Test Method for Determining Aerobic Biodegradation in Soil of Plastic Materials. The released CO_2 being proportional to the percentage of biodegraded substrate, the CO_2 evolution measures ultimate degradation (*i.e.* mineralization) in which a substance is broken down to its final products. Beforehand, film samples were frozen under liquid nitrogen and then ground with a domestic grinder (Moulinex type DPA1, France) to obtain particles around 1–2 mm.

The soil used in this study was taken from the park of the University of Montpellier (France) in April 2017, after removal of the superficial vegetation layer. The big pebbles and foreign objects were removed manually to obtain a homogeneous sample. The soil was dried in open air for one week and then sieved through a 2 mm mesh. The total dry solids content was determined by drying the soil at 105 °C until a constant weight. Soil characteristics were as follows: pH 6.8 (H_2O), 2.3 wt% of organic matter, 16.85 wt% of clay, 26.85 wt% of lime and 56.3 wt% of sand.

195 Biodegradation tests were carried out in cylindrical hermetic glass vessels (1000 mL capacity)
196 containing three small open polypropylene flasks (60 mL capacity) as described by Chevillard
197 et al. (2012). The first flask contained 25 g of dry soil mixed with samples whose weight
198 corresponded to 50 mg of carbon. The water content of soil samples was adjusted to reach
199 80% of the soil water retention capacity (410µl/g wet basis). The second flask contained 10
200 mL NaOH solution (0.1 M) to trap the CO_2 produced by microorganisms. The third flask
201 contained distilled water, in order to maintain the relative humidity at 100% inside the vessel.
202 The glass vessels were hermetically closed and incubated in the dark at 28 ± 1°C. Every
203 week, glass vessels were open to ensure the back titration of the excess of NaOH, which has
204 not reacted with CO_2. Before titrating the residual NaOH with HCl solution (0.1 M) in the
205 presence of thymophthaleine 0.10% (prepared in ethanol 95.5% (v/v)), 5 mL of barium
206 chloride solution (20% (w/v) in water) were added in each flask to precipitate carbonate ions.
207 The flasks containing soil were weighted and appropriate amount of water was added in order
208 to keep constant the water content initially fixed at 80% of the soil water retention capacity.
209 During this procedure, the glass vessels were left open during 2 min in order to be aerated. At
210 each dosage, a new flask containing 10 mL NaOH (0.1 M) was placed in each glass vessel
211 before being closed and put in the dark at 28°C until the next measurement. Biodegradation
212 experiments included control and blank samples. The control samples were microcrystalline
213 cellulose, a positive reference material well known for its biodegradation properties. The
214 blank samples corresponded to the soil alone, without any addition of an external carbon
215 source to measure both the CO_2 produced by the soil carbon substrate and the CO_2 present in
216 the air of the glass vessel. All experiments were run in triplicate.
217 Results were calculated by subtracting the CO_2 production of the blank. The theoretical
218 maximum CO_2 potential ($CO_{2\ max}$ (mg)) produced by total oxidation of the material is
219 calculated using the following equation:

$$CO_{2\,max} = C \times \frac{44.01}{12.01} \quad \text{(equation 1)}$$

Where C is the amount of carbon of the sample introduced in the soil for the test (mg).

The percentage of biodegradation (B) is calculated by equation (2):

$$B = \frac{CO2\ material - CO2\ blank}{CO2\ max} \quad \text{(equation 2)}$$

As required by the ASTM D5988-96 standard, cellulose is used as a reference substance in order to check the activity of the soil and its biodegradation percentage should be higher than 70 % after six months. Otherwise, the test must be regarded as invalid and should be repeated using another fresh soil.

The experimental degradation data were modeled with the Hill equation:

$$Deg = Deg_{max} \cdot \frac{t^n}{(k^n + t^n)} \quad \text{(equation 3)}$$

where Deg [%] is the percentage of degradation at time t [days], Degmax [%] the percentage of degradation at infinite time, k [days], the time for which Deg = ½ Deg_{max} and n the curve radius of the sigmoid function.

3. Results and discussion

3.1. Biodegradability of OP-based fillers

Respirometric tests were used to evaluate the degree and rate of aerobic biodegradation of OP-based fillers and related PHBV composites in contact with soil. CO_2 evolution provides an indicator of the ultimate biodegradability ascribed to the mineralization of the test samples. The biodegradation kinetic of the three OP-based fillers and corresponding Hill parameters are presented in Figure 1 and Table 1, respectively. Respirometric tests conducted in the present study were validated since the biodegradation rate of micronized cellulose selected here as a reference material, reached 70% after only 45 days, while this threshold should be

obtained in less than 183 days (6 months) to consider the test as valid according to the requirements of ASTM D5988-96 standard.

The characteristic Hill sigmoidal shape of the biodegradation curves is known to reflect the two phases commonly ascribed to the polymer degradation process involving microorganisms. Firstly, the polymer chains are cleaved in small fragments due the action of extracellular enzymes, and then, these latter are transported into the cell where they are ultimately mineralized mainly in CO_2, water vapour, minerals, and biomass (Bastiolli, 2005).

As compared to cellulose known to rapidly biodegrade in soil, all the OP-based fillers biodegraded rather well, ranking as follows F0<SF<PF, with a biodegradation threshold superior to 70% (relative to cellulose) reached in 65, 43 and 37 days respectively. As evidenced in Table 1, they all reached their rate of maximal degradation ($Time_{rate\ max}$) in less than 20 days, and it took less than 6 months for F0 and SF samples to reach 50% of Deg_{max} as indicated by *k* values. Regarding the pulp-rich fraction (PF), which exhibited the highest maximum rate of biodegradation (with a $Deg_{rate\ max}$ value twice higher than these observed for the two others fillers), it is worth noting that this sample displayed a peculiar biodegradation curve shape reflected by a low radius of the sigmoid function.

Figure 1.

Table 1.

The biodegradation curve of the stone-rich fraction (SF) was almost superimposed on that of the cellulose reference, which could be ascribed to its richer cellulose content as compared to the other fillers (around 10, 12 and 17% for PF, F0 and SF respectively) (Table 2). Contrary to our expectations, the highest rate of biodegradation was recorded for the pulp-rich sample (PF), despite its highest lignin content (48.9 wt%) as compared to F0 (44.2 wt%) and SF (37.7 wt%) samples (Table 2). Indeed, lignin is a complex polyphenolic polymer that is known for

its resistance to microbial degradation (Dashtban et al., 2010; Singh et al., 2016; Taherzadeh and Karimi, 2008). Furthermore, the biodegradation rate of organic substances was demonstrated to follow this order: carbohydrates > hemicellulose > cellulose > lignin (Cooperband, 2002). In addition, as already evidenced by contact angle measurements (Lammi et al., 2018b), lignin was shown to confer a greater hydrophobicity to the PF fraction compared to the other OP-based ones. Thus, the hydrophobic character of PF should theoretically have reduced its affinity for soil water and therefore its contact with microorganisms. As a consequence, another reason is here proposed to explain the highest biodegradation rate displayed by the pulp-rich fraction (PF), related to the higher protein content of PF (8.7%) than the one of F0 (4.9%) and SF (0.6%) (Table 2). Recent researches have highlighted that factors such as organic carbon (C), nitrogen (N) content and carbon to nitrogen ratio (C/N) could be key elements to improve the biodegradation process, since they ensure optimal conditions for the growth and development of microbial communities involved in the decomposition of organic matter (De Bertoldi et al., 1983; Muktadirul Bari Chowdhury et al., 2013). Indeed, the optimal C/N ratio generally found in a compost medium ranged from 25 to 35, since it is assumed that microorganisms require 30 parts of C per unit of N for the composting process. Medium exhibiting high C/N ratios are known to delay the composting process due to a surplus of degradable substrate for the microorganisms (Bishop Godfrey, 1983). In the soil medium used in the present study, SF sample exhibited a C/N ratio of about 555 against 37 and 65 for PF and F0 respectively (Table 2), which may explain the fact that PF biodegraded at a higher rate than F0 and SF.

Given that the crude OP sample (F0) consisted of a mixture of the pulp and stone tissues, composed of about 31% of PF and 56% of SF (Table 2), it was expected to display an intermediate biodegradation kinetic. However, results showed that F0 exhibited a slower biodegradation rate than these two fractions. It is worth noting that all the samples underwent

291 the same grinding route in such a way to obtain similar average particle sizes (Lammi et al.,
292 2018b) (Figure 2). The slow biodegradation kinetic of F0 was probably due to a more
293 complex organization of the lignocellulosic structure within the particles, making them less
294 accessible to the soil microorganisms, as compared to the two other pure and contrasted
295 fractions (PF and SF). As reported by Taherzadeh and Karimi (2008), lignin and
296 hemicellulose contents could also influence the accessibility of surface for enzymatic attack.
297 In the same context, Mais et al. (2002) indicated that milling could be employed to alter the
298 inherent ultra-structure of lignocellulose and decrease the degree of crystallinity, and
299 suggested that reducing the size of the material by milling would make it more amenable to
300 enzymatic degradation. However, this hypothesis would deserve to be further explored and
301 validated through crystallinity measurements since, in the present study, the physical
302 treatments applied to mill the pomace fractions were probably too superficial and mild to
303 induce such structural changes.

304 **Figure 2.**

305 **Table 2.**

306 After 123 days, the biodegradation rates reached 88.6, 111.8, 117.2 and 162.8% for F0,
307 cellulose, SF and PF samples respectively (Figure 1A). Reaching biodegradation rate higher
308 than 100% has already been reported and is known as priming effect. It generally occurs when
309 the soil inoculum of the test reactor containing the samples produces more CO_2 than the soil
310 inoculum in the blank reactors. This peculiar behavior results from a stimulation of the
311 organic matter mineralization due to easily decomposable organic matter that would be
312 released in the medium during the biodegradation of specific materials. This phenomenon
313 would arise from particular interactions occurring between the transformation of the added
314 substances and the natural soil cycle of carbon (Kuzyakov et al., 2000).

Based on our results, the biodegradation patterns of the OP-fillers (Figure 1A) did not support the different literature data reporting that crude olive pomace would be unable to biodegrade in soil, being consequently at the origin of environmental pollution. According to Cardoso et al. (2005), the phenolic compounds being not degraded during olive oil extraction, olive pomace, like olive pulp, would be expected to be a good source of those compounds.

To verify this assumption, antibiogram tests have been conducted on the OP-based fillers using two targeted bacterial strains (*S. aureus* and *E. coli*). No antibacterial activity was evidenced, as clearly shown in Figure 3 by the absence of bacterial growth inhibition zone around the wells of the different samples. The dark color was due to the diffusion of chlorophyll pigments in Mueller-Hinton agar. These pigments responsible for the olive fruit color were more concentrated in the particles of F0 and PF powders, as already demonstrated by Lammi et al. (2018a). Even if these results appeared rather unexpected with respect to literature, they underpinned our biodegradation results. To explain the lack of antibacterial effect, it could be assumed that the preliminary stage consisting in the drying of the raw biomass for 24 hours at 60°C would cause a thermal denaturation of the active compounds resulting in a loss of any expected inhibiting effect. In addition, during the successive grinding operations, the various OP-based samples were exposed to frictional heating when in contact with grinder. Exposure to such a temperature increase would also contribute to denature the bioactive substances involved in the targeted antibacterial effect. To finish with, it cannot be excluded that the OP-based fillers were also probably already microbiologically contaminated. This contamination could occur after the drying of the crude pomace or during the grinding and sieving operations, both stages being done at room temperature in contact with the ambient air. These microorganisms might enhance the biodegradation of the OP-based fillers acting in cooperation with the soil microorganisms, both contributing to a faster biodegradation rate.

Figure 3.

3.2. Biodegradability of OP-based biocomposites

The biodegradation kinetic of neat PHBV and OP/PHBV-based composites are presented in Figure 4, and the respective Hill's parameters obtained by modeling experimental data according to Hill equation are gathered in Table 1.

Neat PHBV exhibited slower degradation kinetic than OP/PHBV-based composites whatever the OP fraction used as filler. Hill's parameters clearly confirmed that incorporation of OP fillers up to 15wt% in PHBV accelerated the biodegradation of the composite materials compared to the neat polymer. As indicated in Table 1, this latter recorded only 163% of degradation at infinite time and reached 50% (*k*) of this degradation after about 109 days. By contrast, all the OP-based composites exceeded 200%, with *k* values being reached in less than 85 days. It is interesting to note that the nature of the filler used had no significant influence on the degradation profile of composites. In fact, the neat PHBV matrix recorded the lowest rate of degradation with a maximum ($Deg_{rate\ max}$) reached after 95 days ($Time_{rate\ max}$) whereas all the composite formulations displayed twice the maximum degradation rate after only 68 days (Table 1).

Finally, 4 months of incubation at 28°C in the soil and under aerobic conditions, PHBV biodegradation reached 91% (*i.e.* about 83% relative to cellulose) without attaining the plateau of carbon dioxide released. This indicated that respirometric measurements should have been prolonged a few months more for PHBV without however questioning its biodegradability in soil. As claimed by the recent specification standard NF EN 17033, a biodegradation level of 90% relative to cellulose should be reached no later than 2 years. According to Boopathy (2000), biodegradation is dependent on a number of factors such as microbial activity of the environment, exposed surface area, temperature, pH and molecular

364 weight. It is worth specifying that in the present study all the material samples were
365 previously ground as particles around 1–2 mm before being tested. As a consequence, it could
366 be attested that all the respirometric tests have been performed in the same standardized
367 conditions, the results obtained being therefore comparable.

368 To conclude, the PHBV-based biocomposites could be considered as significantly much more
369 biodegradable than the virgin PHBV matrix whatever the OP fractions used. Monitoring the
370 evolution of the carbon dioxide over time through respirometric revealed that the
371 biodegradation kinetics of the biocomposite materials followed the order: PHBV<PHBV-
372 PF<PHBV-SF<PHBV-F0. After 123 days of soil incubation, PHBV-PF and PHBV-SF
373 formulations overpassed 134% of biodegradation, and the PHBV-F0 film exceeded 143%.
374 This peculiar behavior resulted from the priming effect already mentioned above for the OP-
375 fractions which all reached biodegradation rates greater than 100%. This synergistic effect
376 was certainly related to the presence of OP-fillers, which further boost the biodegradation
377 process of the composites compared to the PHBV matrix alone. Biodegradation rates of
378 biocomposites exceeding 100% have already been reported for films made of gliadins cross-
379 linked by cinnamaldehyde (Balaguer et al., 2015), starch-polystyrene foams (Pushpadass et
380 al., 2010), or L-polylactide-polycaprolactone co-polymer films (Plackett, 2006) under
381 composting conditions.

382 **Figure 4.**

383 The increased biodegradability of composite materials might be the result of three
384 concomitant effects. Firstly, it could be due to the increased hydrophilicity of biocomposites
385 compared to virgin PHBV due to the incorporation of fillers that are much more hydrophilic
386 than the matrix. This assumption was supported by results reported by Lammi et al. (2018b)
387 indicating a significant increase of the water vapor permeability of PHBV films after

388 introducing OP-fillers, and especially in the case of F0 and SF fractions. A higher
389 hydrophilicity would favor the adsorption and then the transport of water within the
390 composite material, which in turn would promote the microbial degradation of material
391 (Chevillard et al., 2012).

392 Secondly, differences in biodegradation patterns between composite materials could be
393 attributed to differences in the microstructure of materials. A poor affinity between the filler
394 and the polymer matrix would result in a poor dispersion of the fillers and the formation of
395 aggregates as well as a poor filler/matrix adhesion that would induce the creation of voids
396 between the filler and the matrix. These two phenomena would lead to the creation of a bulk
397 porosity and therefore to the creation of a preferential pathway for the transport of water
398 within the composite material. Such microstructural defects would also be prone to increase
399 the fragility of materials favoring their physical disintegration, and thus resulting in a higher
400 contact surface for ensuring microorganisms colonization. This hypothesis was supported by
401 the microstructural investigation of the composite materials characterized by SEM after cryo-
402 fracture (Figure 5). Although PHBV cross-section displayed a homogeneous and smooth
403 surface, contrariwise, the cross sections of PHBV-F0 and PHBV-SF films were rough, with
404 the evidence of cracks and voids at the filler/matrix interface probably due to the debonding
405 of fillers. As described above, this would allow microorganisms to attach and form biofilms
406 colonizing the material surface. In the case of PHBV/PF composites, the particles were
407 entirely wetted by the polymer matrix, indicating a good filler/matrix interfacial adhesion.
408 This was attributed to the great affinity between PF fillers and PHBV matrix as confirmed by
409 similar values of surface energy, previously demonstrated (Lammi et al., 2018b). This more
410 homogenous and compact microstructure would hinder the diffusion of water and thus the
411 accessibility to microorganisms, as compared to PHBV/F0 and PHBV/SF composites. As a

412 consequence, PHBV-PF composite material biodegraded at a lower rate than the two other
413 composites containing F0 and SF fillers.

414 **Figure 5.**

415 Difference in crystallinity between the materials might also be at the origin of changes in their
416 biodegradation patterns. Indeed, the kinetic of enzymatic degradation is known to depend on
417 the overall crystallinity of the material (El-Hadi et al., 2002). An increase in polymer
418 crystallinity is known to be detrimental for the polymer biodegradability since crystalline
419 zones are less accessible for the microorganisms (Wei et al., 2015). Indeed, as previously
420 demonstrated by the authors Lammi et al. (2018b), the presence of F0 and SF as fillers in the
421 PHBV led to a decrease in the crystallinity degree (Xc) of the neat matrix whereas the
422 crystallinity of the PHBV/PF was increased. The recorded degrees of crystallinity Xc (%)
423 were 65, 68, 75 and 78% for PHBV-F0, PHBV-SF, PHBV and PHBV-PF films, respectively
424 (Lammi et al., 2018b). Such an anti-nucleating effect might contribute to facilitate the
425 biodegradability of PHBV/F0 and PHBV/SF composites as compared to PHBV/PF. This
426 phenomenon was previously observed for biocomposites based on polyhydroxybutyrate and
427 fermentation residue coming from potato peel waste (Wei et al., 2015). In the case of the
428 PHBV/PF composite formulation, it is worth noting that the effect of the increased
429 crystallinity was compensated by the opposite effect resulting from the increased
430 hydrophilicity and the more heterogenous microstructure as compared to the neat PHBV
431 matrix.

432 Finally, biodegradation performance of all the composite materials was underpinned by the
433 antibiogram tests undertaken on composite films and neat PHBV chosen as control. As shown
434 in Figure 3, no inhibition zone was evidenced around the different disks, which was in full
435 agreement with their fast biodegradation in the soil medium. Such positive results enabled to

envisage other routes for valorizing the different fractions stemming from olive pomace wastes and justified the approach consisting in introducing them as fillers for developing biodegradable composites based on PHBV.

4. Conclusion

The present work fits into a larger environmental context aiming at reducing the impact of the agro-residues by valorising them to design biodegradable materials. With a view to achieve a complete valorisation of olive pomace, all its components (including the constitutive tissues of the olive fruit, namely the pulp and the stone) were transformed in powders using a rationale dry fractionation process before being used as fillers to develop PHBV-based biocomposites. The impact of such fillers on the biodegradability in soil of the resulting composite materials was investigated in relation with their respective chemical composition. Results revealed that the incorporation of OP-based fillers in PHBV accelerated the biodegradation kinetics of the composites, which exceeded 100% after 4 months. These results were supported by measurements of crystallinity as well as SEM observations. Moreover, the absence of any antibacterial effect of both the olive pomace-based fillers and the resulting biocomposites towards the target bacteria (*Staphylococcus aureus* and *Escherichia coli*) confirmed the capability of such materials to biodegrade in soil and fully justified the relevance of this study. To conclude, this research presented a promising track for the valorisation of olive pomace through the elaboration of biocomposites materials that might be notably intended for short-term use such as packaging.

on the other hand, these results clearly showed the interest of crude OP fractionation and led us to propose another exploitation field for the SF and PF fractions as natural and biodegradable fertilizers for the agricultural grounds.

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Figure Captions.

Figure 1. Kinetic of biodegradation (A) and biodegradation rate (B) of OP-based fillers in sol.

Figure 2. SEM pictures of olive pomace-based fillers (scale bar = 300 μm).

Figure 3. Antibiogram results of OP-based fillers and OP-based composites after incubation at 37°C during 24h.

Figure 4. Kinetic of biodegradation (A) and biodegradation rate (B) of OP/PHBV-based composites in sol.

Figure 5. SEM pictures of OP/PHBV-based biocomposites surfaces (x 500: scale bar = 60 μm, x 1000: scale bar = 30 μm).

Figures.

Figure 1.

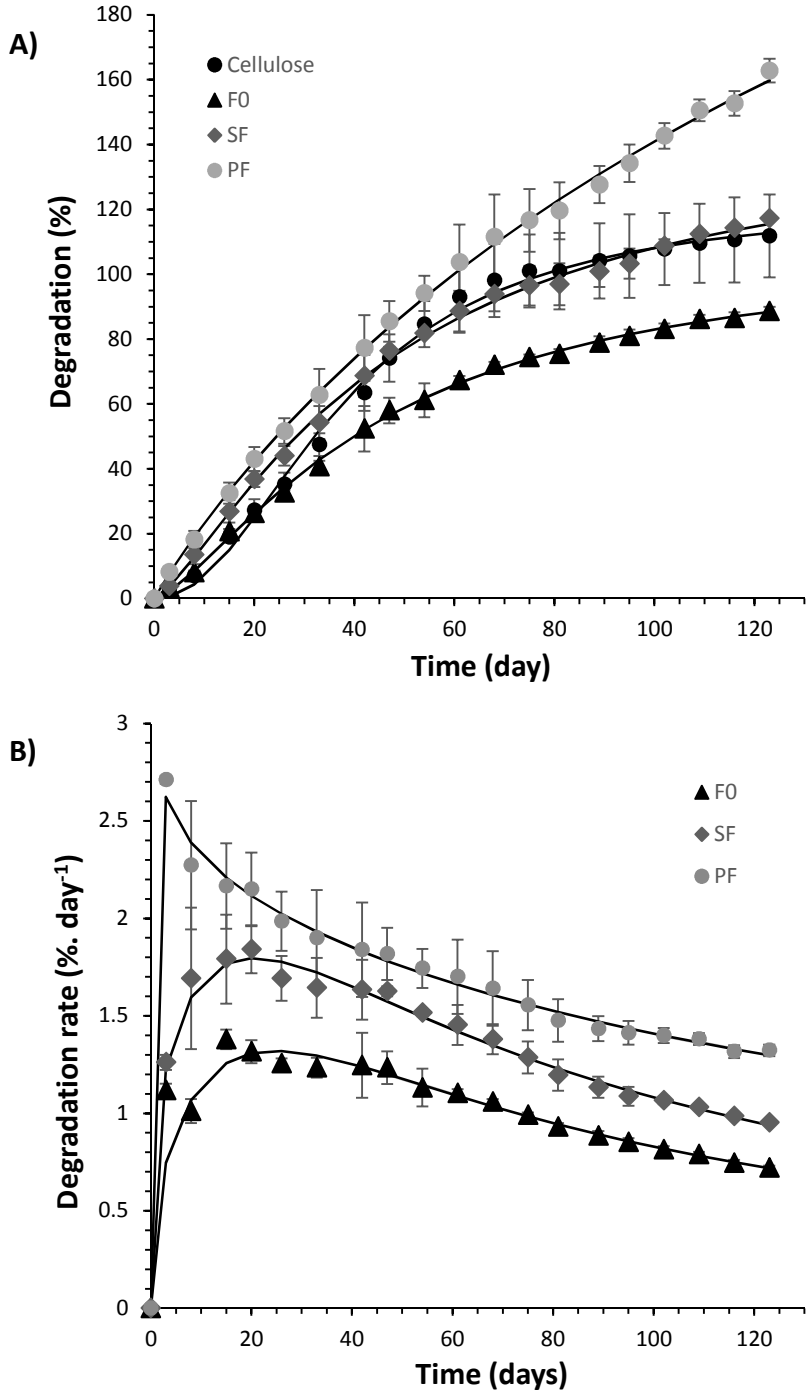


Figure 2.

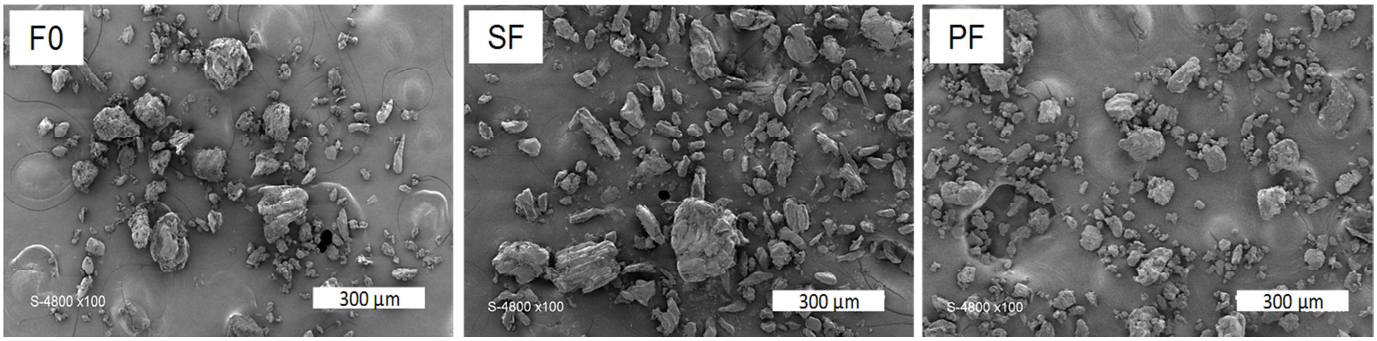


Figure 3.

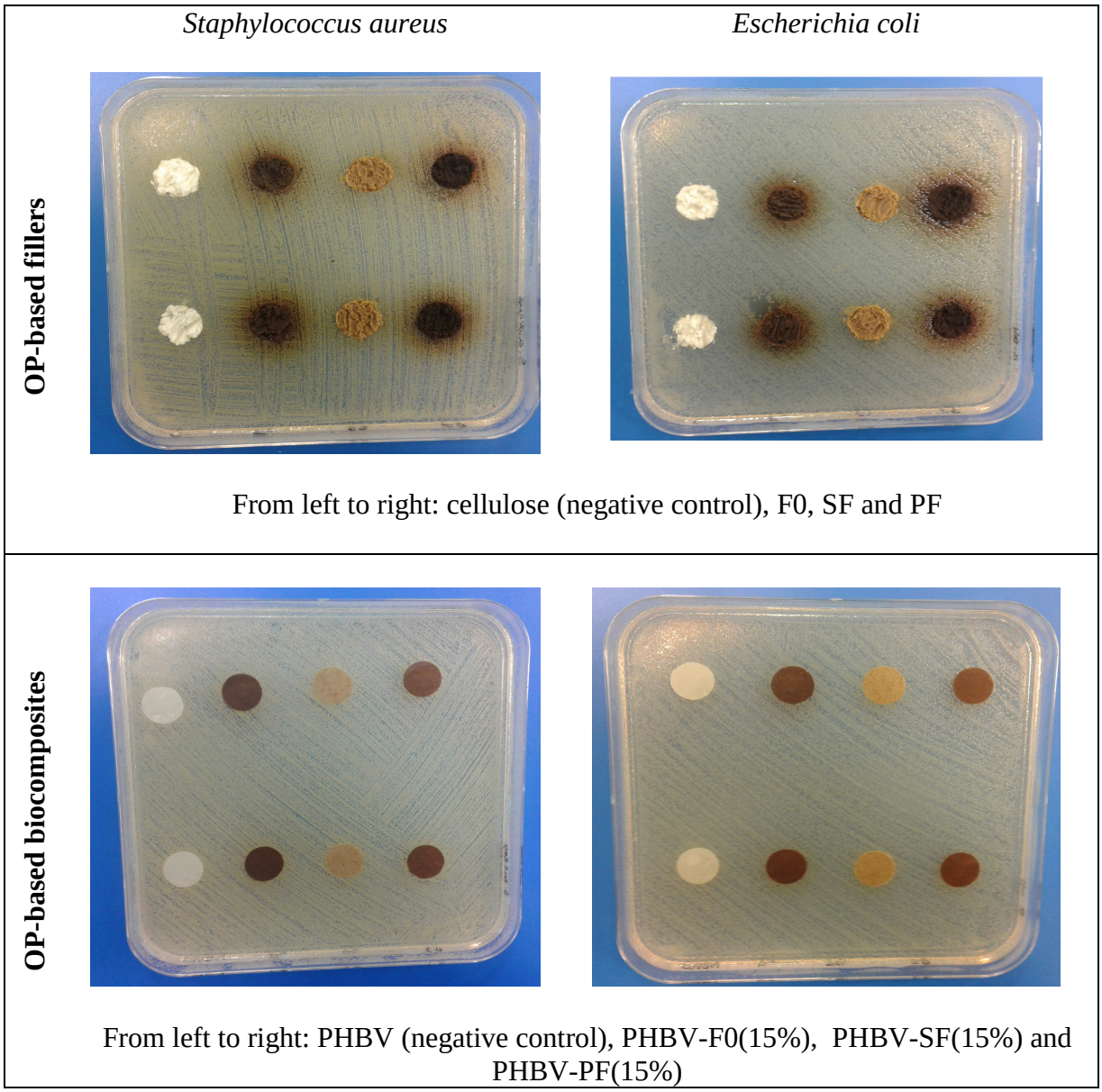

Staphylococcus aureus
Escherichia coli
OP-based fillers
From left to right: cellulose (negative control), F0, SF and PF
OP-based biocomposites
From left to right: PHBV (negative control), PHBV-F0(15%), PHBV-SF(15%) and PHBV-PF(15%)

Figure 4.

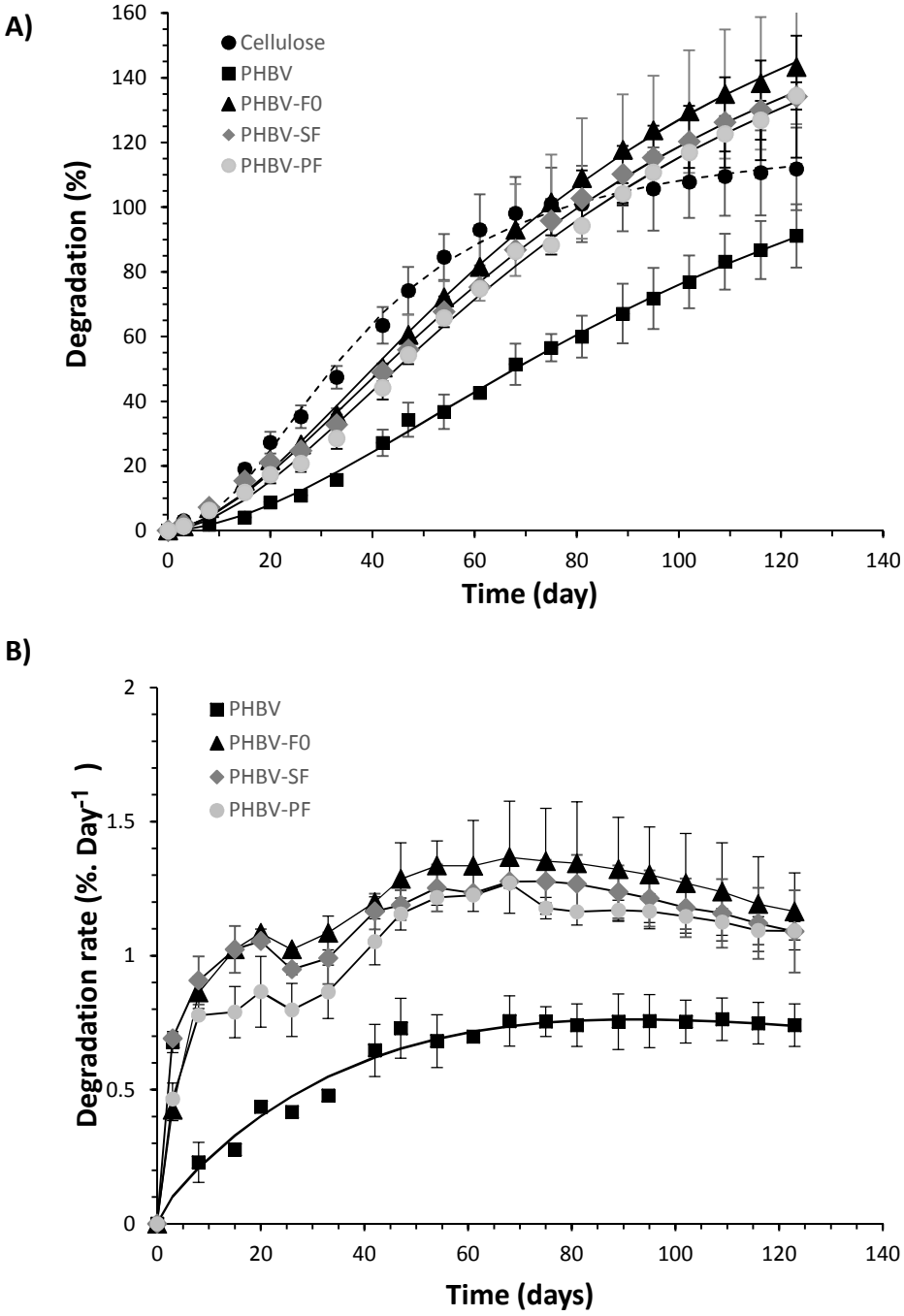
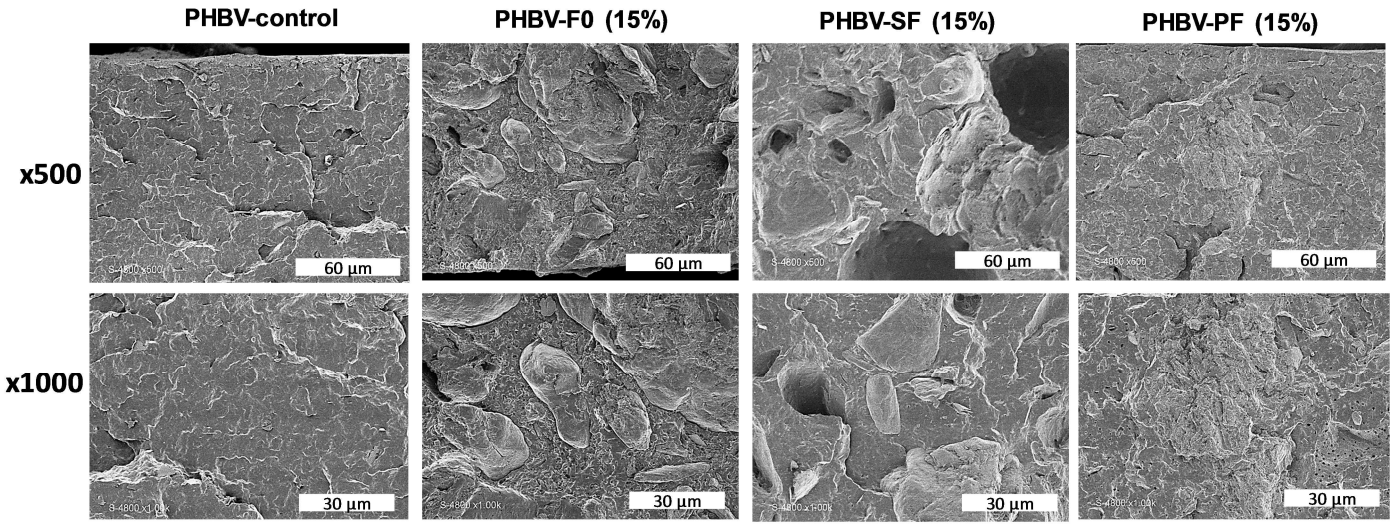

A)
Cellulose
PHBV
PHBV-F0
PHBV-SF
PHBV-PF
Degradation (%)
Time (day)
B)
PHBV
PHBV-F0
PHBV-SF
PHBV-PF
Degradation rate (%. Day-1)
Time (days)

Figure 5.


PHBV-control
PHBV-F0 (15%)
PHBV-SF (15%)
PHBV-PF (15%)
x500
x1000
60 µm
30 µm

Tables.

Table 1: Hill parameters (Deg_{max}, *k*, *n*) and related biodegradation indicators ($Time_{rate\ max}$, $Deg_{rate\ max}$) of OP-based fillers and OP/PHBV-based composites in respirometric test.

Sample	Hill parameters				$Time_{rate\ max}$[b]	$Deg_{rate\ max}$[c]
	Deg_{max}[a] [%]	*k* [days]	*n*	R^2	[days]	[% day^{-1}]
Control(cellulose)	122 (±3.1)	38.2 (±1.3)	2.1 (±0.1)	0.99	42	1.6
F0	109 (±3.0)	44.9 (±2.1)	1.4 (±0.0)	0.99	15	1.3
SF	147 (±5.6)	46.7 (±3.2)	1.3 (±0.0)	0.99	20	1.8
PF	426 (±66.5)	213.1 (±60.9)	0.9 (±0.0)	0.99	3	2.6
PHBV	163 (±15.5)	108.2 (±11.5)	1.7 (±0.1)	0.99	95	0.7
PHBV-F0(15%)	219 (±12.0)	82.6 (±5.8)	1.7 (±0.1)	0.99	68	1.3
PHBV-SF(15%)	209 (±14.3)	84.9 (±7.5)	1.6 (±0.1)	0.99	68	1.3
PHBV-PF(15%)	204 (±15.7)	85.6 (±8.1)	1.7 (±0.1)	0.99	68	1.2

Values in parentheses represent the confident intervals.

[a]Deg_{max}: percentage of degradation at infinite time;

k: constant of the Hill equation representing the time for which Deg =½ Deg_{max};

n: constant of the Hill equation representing the curve radius of the sigmoid function.

[b]$Time_{rate\ max}$: time to reach the maximum biodegradation rate.

[c]$Deg_{rate\ max}$: maximum degradation rate.

Table 2: Biochemical composition (% dry basis) of olive pomace fractions

	Yield	pH	Humidity	Ashes	Cellulose	Hemicellulose	Lignin	Proteins	C	N
F0	100	4.9± 0.0	4.5± 0.0	2.6± 0.2	12.8±3.1	11.9±2.6	44.2±4.3	4.9	51.07	0.78
SF	56.4	4.9± 0.0	3.3± 0.0	0.8 ±0.0	17.4±1.1	18.5±0.4	37.7±3.4	0.6	49.97	0.09
PF	31.3	5.0± 0.0	7.2 ±0.0	4.6± 0.1	10.8±0.9	7.9±0.4	48.9±5.9	8.7	52.05	1.38