

**BIODEGRADATION OF PROTECTIVE MASKS (COVID-19) IN COMPOST AND
AQUEOUS MEDIA**

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Abstract

The study of biodegradation of facial masks made of natural and synthetic materials was performed. The experimental part was performed with a respirometer from the Slovenian manufacturer ECHO Instruments. We conducted a preliminary test to select the compost with the highest activity for further work. The rate of biodegradation of the materials was calculated based on measurements of the carbon dioxide produced in the reactors. The biodegradability results of the materials were verified using FTIR spectroscopy and microscope images. Masks made of linen and cotton particles performed best biodegradation at 72 %, while particles of masks made of polypropylene (PP) did not degrade.

Since there are no studies on biodegradability in water, the second part of our study was to investigate the rate of degradation of the masks in wastewater. Based on the FTIR spectra obtained, the presence of new functional groups on the surface of the protective masks was confirmed as a result of the oxidation process. Humovit compost was chosen as the inoculum for the water medium. We determined the highest degree of biodegradation for the linen/cotton mask (67 % in 75 days).

Keywords: protective masks, linen/cotton, himalayan hemp, respirometry, FTIR

Highlights

- Self made respirometer showed excellent performance characteristics.
- Microcellulose reference material degrade at 58.4% in 75 days.
- The linen/cotton mask degradation at 67.1% in 75 days and is still increasing.
- The hemp mask degrade at 59.6% in 75 days.

INTRODUCTION

During the covid pandemic, demand led to an unprecedented increase in the global production of disposable face masks made from polymeric materials [1]. The most commonly used material for the production of surgical masks is petroleum-based polypropylene (PP) [2]. Surgical (medical) masks consist of at least three layers [3]. The inner layer consists of an absorbent material that absorbs moisture from the user's breathing air. The middle layer consists of a nonwoven material that provides effective filtration. The outer layer is made of a non-woven waterproof material. Surgical masks are designed to prevent the ingress of larger droplets ($> 100 \mu\text{m}$) and help reduce the spread of saliva and exhaled air into the environment. The SARS-CoV-2 virus has a spherical shape with a diameter between 60 and 140 μm , which means that a surgical mask cannot fully protect the user from infection [4]. Due to the increasing plastic waste pollution worldwide and the accumulation of microplastics in ecosystems, we urgently need to seek alternative, more sustainable methods for waste disposal. One of the promising solutions is to replace masks made of plastic polymers with biodegradable and reusable materials [5]. The global problem of plastic accumulation in the environment encourages us to apply and develop more effective methods [6]. Over time, fragments of plastic pollution transform into microplastics or nanoplastics [7]. Microplastics were also recently detected in hail that fell directly in Slovenia. It is the first case of its kind in the world and is definitely a cause for concern [8]. Researchers are currently focusing on acquiring knowledge in the field of aerobic and anaerobic biodegradation of (micro)plastics using microbial cultures and on the production of bioplastics [9].

Microorganisms belonging to the genus *Pseudomonas* are often the subject of research, because they have good abilities to degrade hydrocarbons and hydrophobic polymers [10]. They found that the majority of naturally occurring isolated bacteria from the genus *Pseudomonas* are capable of at least partially degrading the most common representatives of synthetic polymers, such as polyethylene, polypropylene, polystyrene, and polyvinyl chloride. A novel consortium was studied for PP degradation [11]. The study provided evidence that thermophilic *Pseudomonas* spp., *Stenotrophomonas* spp., *Bacillus* spp., and *Paenibacillus* spp. Exhibited increased rates of low-density polyethylene LDPE and high-density polyethylene HDPE degradation when formulated as consortia of all the four bacteria. The evaluation of respirometry-based control strategies for activated sludge processes was presented many years ago [12]. To date, a limited number of respirometric protocols is available in the scientific literature; however, most of them are related to long-term biodegradability evaluations (such as the measurement of biological oxygen demand BOD measurement). The advantages of using a respirometer is that no titration is required, the concentration of oxygen and/or carbon dioxide is continuously measured, and the simultaneous measurement of multiple samples to have the same experimental conditions for all samples throughout the experiment [13].

The biodegradation of commercial polyurethane formulations has been monitored by combining respirometry and Raman spectroscopy [14]. Respirometry tests on cheese showed a linear increase in oxygen consumption during respiration of polycaprolactone with α -

tocopherol addition [15]. The respirometry results obtained support the potential of using oleaginous craft paper as a food packaging material [16].

Many factors, such as pH and temperature, as well as polymer properties (e.g., polarity, glass transition temperature, and morphology) influence the efficient degradation and breakdown of different mask materials under industrial composting conditions. Thus the aim of the article was the study of face mask biodegradation through model respirometry experiments. The study is divided into two sections: First, the polymeric material was degraded in compost; in the second section the polymeric material was degraded in aqueous media. Bio-based materials, such as gluten fiber, cellulose, polylactic acid, banana stem fiber, chitosan whiskers, and polyhydroxyalkanoates, have been widely used to create biodegradable face masks [3]. To the best of our knowledge, the degradation of face masks made from linen/cotton blend and Himalayan hemp has not been studied yet. Based on organic carbon content measurements under controlled conditions the produced CO₂ was calculated. The microcrystalline cellulose was selected as a reference material according to ISO standards [17,18]. The biodegradation rate was calculated based on the measurements of produced CO₂. The results of the material after the respirometric measurements were verified using FTIR spectroscopy and microscope images.

EXPERIMENTAL

Materials

Initially, 4 different types of compost were studied first in order to determine their maximum activity. Compost used:

- »Humovit univerzal«, composition white and black peat, coconut fibre, organic foam, quartz sand and Hygromull.
- »Substratica Compact mix« made of humic acids, white and black peat, perlite, stone flour,
- »Bioplantella« made of white and black peat
- »Substral premium« made of peat, compost from plant residues, wood fiber, composted bark and fertilizer.

The chemical composition is presented in Table 1.

Table 1

The degradation of several different protective masks was tested. All were commercially available:

- made of polypropylene (PP),

- made of himalayan hemp fibres (HK),
- made of linen and cotton mixed fibres (LB); two layers: inner layer was made from 100 % cotton and outer from 50 % linen and 50 % cotton,
- made of blown cellulose (PC),
- microcrystalline cellulose (MC) served as control (Reference material, Ref), Sigma-Aldrich, mass was 32 g per reactor.

The masks were cut into particles with an area between 0.5 cm² and 2 cm².

Respirometry measurements

Mask polymer degradation was determined by carbon dioxide (CO₂) evolution and monitored using an ECHO Instruments respirometer (Slovenske Konjice, Slovenia). Each experimental set was carried out in 12-channel respirometer for measurements in compost and in 6-channel respirometer for measurements in aqueous media. Data handling in each reactor was automatic. An air flow of 50 mL min⁻¹ was pumped into each reactor, and the composition of exhausted gas was analyzed using the built-in gas sensors. All samples were performed in duplicate for two and a half months and compared against microcrystalline cellulose as the positive control at constant conditions. The mass of produced CO₂ for each tested sample (in g) was calculated in approx. 2 hours intervals by the difference in mass of produced CO₂ in each sample and mass of produced CO₂ in blanks, as described by eq. (1):

$$m_p = m_{r,s} - m_b \quad (1)$$

where

m_p is the mass of produced CO₂ (g) derived from the tested sample (or reference), $m_{r,s}$ is the mass of produced CO₂ from the reference or the tested sample, and m_b is the mass of produced CO₂ from the blanks.

Biodegradability was determined following equation (2):

$$w_d = \frac{m_p}{m_m \cdot TOC \cdot r_M} \times 100 \quad (2)$$

where

w_d = biodegradability (%)

m_m = mask mass (g)

TOC = total organic carbon (%)

r_M = ratio of molar masses CO₂ to C

Compost activity w_a is calculated according to eq. (3):

$$w_a = \frac{m_p}{m_{vs}} \quad (3)$$

where m_p is the mass of produced CO₂ in compost after 10 days (in mg) and m_{vs} is the mass of volatile solids (g).

Mass of volatile solids is calculated according to eq. (4):

$$m_{vs} = m_{compost} \times \frac{dm}{100} \times \frac{vs}{100} \quad (4)$$

where $m_{compost}$ is the mass of compost (in g), dm is the dry matter (in %) and vs is the volatile matter (in %).

Respirometry experiments in compost

The sample composition in individual reactor is seen from the next paragraph.

Reactor number	Content	Denotation
1 and 2	Compost	K
3 and 4	Compost + microcrystalline cellulose	KMC
5 and 6	Compost + polypropylene	KPP
7 and 8	Compost + linen/cotton	KLB
9 and 10	Compost + blown cellulose	KPC
11 and 12	Compost + himalayan hemp	KHK

Two replicate measurements were performed.

Respirometry experiments in aqueous medium

The aqueous medium was prepared according to the instructions in the standard (ISO 14852), which describes the preparation from four mineral solutions (B,C,D,E) and the compost inoculum.

Solution B: 1.10 g of MgSO₄ was dissolved in a 100 mL volumetric flask.

Solution C: 2.75 g of CaCl₂ was dissolved in a 100 mL volumetric flask.

Solution D: 0.125 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was dissolved in a 0.5 L volumetric flask. Solution D must always be prepared fresh or a drop of concentrated HCl.

Solution E: 37.5 g of KH_2PO_4 , 69.6 g of Na_2HPO_4 and 2 g of NH_4Cl were dissolved in a 1 L volumetric flask with deionized water. In the case of difficult solubility, the flask was placed in an ultrasonic bath for a few minutes. Other solutions can be stored for up to 6 months in a dark place. Test medium, which is a mixture of the previously listed solutions in a certain ratio, was prepared: 800 mL of deionized water, 100 mL of solution E in 1 mL of solution B, C and D were mixed. The pH of the test medium was then adjusted to 7.0 ± 0.2 .

Inoculum: 10 g of compost was suspended in 100 mL of the test medium by mixing well. After 30 min the solution was filtered through a 2 mm, followed by 1 mm sieve and a 0.2 mm sieve. Finally, 5% compost solution in the aqueous medium was prepared. Blank was prepared without plastic material. Microcrystalline cellulose served as reference control material. PP, HK, LB, and PC were tested for degradation with mass 2 g per 0.5 L solution. The respirometric experiments were performed at the same manner as described in section “Respirometric measurements” at temperature 303.15 K.

FTIR spectroscopy

The samples were analyzed with the spectrometer ATR FTIR Perkin Elmer Spectrum GX (Perkin Elmer FTIR, Omega, Ljubljana, Slovenia). The ATR accessory (supplied by Specac Ltd., Orpington, Kent, UK) contained a diamond crystal. A total of 16 scans were taken of each sample with a resolution of 4 cm^{-1} . All spectra were recorded at ambient temperature over a wavenumber range between 4000 and 650 cm^{-1} .

Microscopy

Microscope BRESSER Science Infinity 57-60700, equipped with camera BRESSER MikroCam SP 5.0 was used for perform microscopic images in magnification range 40-1000x.

RESULTS AND DISCUSSION

A preliminary test with all compost types was performed first to determine the activity of all compost samples according to ISO 14855. The pH was maintained between 7 and 8. The results are shown in Fig. 1. The condition for compost application is its activity between 50 and 150 mg/g reached in about 10 days. From Fig.1 it is seen that the highest activity was obtained using Humovit. The results are in agreement with the measurement of dry residue lost, which was the lowest with Humovit.

Fig. 1

The activities were calculated according to Eq. 3. Compost activities were calculated the highest for Humovit 86.7 ± 4.9 %, followed by Substral 63.6 ± 0.4 %, Bioplantella 54.9 ± 2.7 %, and Substratica 42.5 ± 3.3 %. Further, pH remained within demanded values until the end of experiment, between pH 7 and 8, whereas Substratica and Bioplantella composts showed values between pH 5.6 and 6.6, respectively, – this is out of the demanded pH range. Therefore, Humovit was chosen for further experiments.

Throughout the time interval of samples' incubation in the compost, we carefully monitored the activity of microorganisms. The functioning of microorganisms is also reflected in the consumption of oxygen, which microorganisms need for cellular respiration. Regarding aerobic cell respiration (reaction 4), the moles of consumed O_2 are linear to moles of produced CO_2 . Based on this assumption, the produced CO_2 could be calculated from the reaction in equation (5):

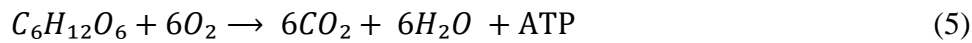


Fig. 2a) shows final measurement values of produced CO_2 for the two parallel samples of each tested polymer versus blank samples. We noticed that produced CO_2 value is the highest for samples with added microcrystalline cellulose, which we used for reference. Very close values are reached with LB mixture, little lower with PC, even lower with HK, while PP showed the m_p values comparable with blank as reported by others [19,20,21].

Fig. 2

Fig. 2b) presents the average cumulative CO_2 production (in mg) for the tested samples deducted for blank samples during the whole respirometric test in compost, where the produced CO_2 in compost was considered. The calculated biodegradability was the highest in the case of reference MC, about 76 %, followed by LB and PC, which reached 72 % and 59 % biodegradation rate, respectively. High biodegradability was confirmed with LB. The disintegration of linen fabric under composting conditions was reported at lower value of 55 % [22]. Similar results were achieved in the present study and this could be explained by enzyme attack of the cellulose chain and break-down of the cellulose internal bands. The main difference is between the shape of the LB and PC curves. LB seemed to still degrade, while PC reached a plateau. Similar results were obtained by others – In this time period after 50th day the disintegration of linen can still proceed [22]. Validity of the test or measurement according to the standard was also achieved, as the percentage of biodegradation for microcrystalline-cellulose was above 70 % in 45 days [17]. TOC represents the nutrients for microorganisms; however, carbon could be unavailable for biodegradation. The results showed the highest share of TOC in PP (almost 79 %), while in PC, LB and HK the values were similar, between 42 and 44 %. Many compostable materials need extended periods above 45 days for composting [23]. The HK was shown as not readily biodegradable, reaching only 31 % rate. According to

Himalayan hemp amorph structure, it could result to be more biodegradable, but on the other hand, HK is known for its high mechanical strength [24], which presumably slowdown the biodegradation process.

A comparison between the PP degradation with pretreatment methods showed similar non-degradability as in our study. The degradation of polypropylene was previously investigated in several studies, where it was exposed to several types of microbial cultures under different conditions. The degradation of PP was not improved by using *Lysinibacillus*, while only 3 % dry residue loss was observed [25]. Similarly, Flexus did not improve PP degradation after 365 days, as only 1.95 and 1.45 % degradation was measured [20, 21]. If the PP was pre-treated by the UV, the results were much better only in one study, where more than 60 % of PP was degraded.

Microscopy results

Pieces of protective masks were recorded under a microscope with 40x magnification. The micrographs were used to visually compare the mask material after the composting experiment and observe possible changes on the surface of the sample (fibrillation).

Fig. 3

All materials showed some increased pore size after composting. Among them the difference was most obvious in the LB material, as shown in Fig. 3a) and 3b) (before and after composting, respectively). Comparing the micrographs of HK (Fig. 3c) and 3d)), we also noticed some fibrillation of the fibers after composting (Fig. 3d), as well as an increase of pore size compared with raw fibers. Minimal changes in pore size were observed at PP (Fig. 3f) compared to PP before composting (Fig. 3e)). Also, the pore size was the smallest in the PP sample (Fig. 3e).

FTIR spectroscopy results

There were not observed major differences in the spectra of PP, HK and PC. Small differences were seen from FTIR spectra for LB. The broad signal at the wavenumber of 3333 cm^{-1} is an indication of the existence of a hydrogen bond in the molecule and the longitudinal valence fluctuations of the OH-bond. Stretching vibration of the CH-bond appear as a medium-strong signal at a wave number of 2900 cm^{-1} . According to our predictions, the signal at 1630 cm^{-1} is the result of bending vibrations of the OH-bond of adsorbed water. At the wavenumber of 1315 cm^{-1} , a smaller signal is observed, which is the result of vibration due to the bending of the CO bond. The vibration of CO- and OH-bonds is the cause of the most intensely expressed signal at the wave number of 1030 cm^{-1} [26]. Another study describes the degradation of linen by composting and similar spectra before and after the biodegradation experiment were obtained [22].

Results of experiments in aqueous media

Fig. 4 represents the production of CO₂ (in mg) for LB, HK and PP, comparably with the reference MC. MC was set as reference material in two replicates. The produced CO₂ was above 2000 mg in 70 days. It did not reach the plateau and was still increasing after 70 days. The tests should be prolonged to achieve the final degradation rate.

Fig. 4

LB blend seemed to reach the plateau after 60 days respirometric measurements, which means the degradation rate slowed down. The biodegradability was calculated at 67 %. The results showed slightly lower biodegradation as reported for cotton, 89 % [27]. In our case, the result might suggest that some chemical additions into mask material to improve the mask strength might interfere with the degradation process.

The protective mask made of HK was degrading somehow similarly and reached about 60 % biodegradation after 70 days; however, from the recorded graphs it is seen that prolonged time could be able to contribute to further increase in CO₂ production. The protective mask made of PP was not biodegradable, with calculated biodegradation below 0.5 %, which is in accordance with literature [28]. ~~HK did not degrade as well as expected for natural material, but after 70 days the slope was the same as a month before and did not show slower decay approaching the plateau.~~ As seen from Fig. 4 the prolonged time might contribute for further increase in CO₂ production.

Microscopy results

Fig. 5 presents the microscopic graphs of LB and HK at 40 times magnitude. Clearly woven linen/cotton structure is seen in Fig. 5a (before starting the respirometric test), whereas in Fig. 5b (after finishing the test) it is seen the clearly disintegrated material, as fibers are not visible anymore. Similar was observed with HK as seen from Fig. 5c and 5d.

Fig. 5

FTIR spectroscopy results

Fig. 6 shows the FTIR spectra for a PP medical mask before and after the experiment according to ISO 14852. Spectra a) and c) represent the outer layer of the mask before and after the experiment, while spectra b) and d) represent the inner layer of the medical mask, which is slightly thinner and more transparent. The spectra of outer and inner layer (6a and 6b) showed the following signals: multiple signals in the wavenumber range from 3000 to 2800 cm⁻¹ and

two large signals in the range from 1456 to 1375 cm^{-1} . The signals in the range from 3000 to 2800 cm^{-1} were attributable to asymmetric and symmetric stretching vibrations of CH_2 groups, while the signals at 2950 and 2850 cm^{-1} were due to the asymmetric and symmetric stretching vibrations of CH_3 . The signal at 1456 cm^{-1} indicates the asymmetric CH_3 vibrations or CH_2 scissor vibrations, while the signal at 1375 cm^{-1} was the result of the symmetric CH_3 deformation. All mentioned signals represent typical signals for PP materials. We found from the spectra that there really is no difference between the materials of the outer layer and the inner layer, but the inner layer is made of finer fibers to serve as a filter. The same spectrum for PP was also recorded by other authors [29], who investigated the release of microplastics from medical masks in seawater. The FTIR spectra of the outer and inner layer after the experiment (6c and 6d) show new signals around 3300 cm^{-1} , 1650 cm^{-1} and at 1007 cm^{-1} . Listed signals at corresponding wavenumbers are typical for O-H, C=O, C-O, etc. vibrations. The latter indicates that PP undergoes oxidation/degradation reactions.

Fig. 6

Even with the linen/cotton mask, we did a FTIR analysis of two parts of the mask, i.e. the inner one, which is supposed to be made of 100 % cotton, and the outer one, which is composed of 50 % linen and 50 % cotton. FTIR spectra are identical, since both linen and cotton, as natural polymers, are mostly composed of cellulose. The spectra are also unsurprisingly similar to the spectra of blown cellulose and Himalayan hemp (not presented here). The FTIR spectra of the light layer of the linen/cotton mask before and after the experiment were compared, but there were no differences between them. For the FTIR analysis of the state of the bright layer of the linen/cotton mask after the experiment, a slightly more preserved piece of string was used, which could be the reason for the unchanged spectrum result compared to the spectrum of the material before the experiment.

CONCLUSUON

Protective face masks made of linen/cotton basically met our expectations regarding biodegradation in compost media. The masks made of Himalayan hemp fibers were not as good biodegradable as expected, reaching only 31 % degradation. From the results we can conclude that longer monitoring time is required, since the biodegradation rate began to increase significantly only after 1 month. Validity of the test or measurement according to ISO 14855 standard was achieved, since the biodegradation percentage of 70 % for microcellulose was achieved in 45 days.

From the results in aqueous media, we can summarize the findings that protective face masks were readily biodegradable, while the medical mask made of polypropylene was not. Reference material degradation was measured between 57 % and 68 %. Thus, it was proven that the large-scale use of linen/cotton face masks would reduce the consumption of non-renewable

resources, thus reducing the emission of greenhouse gases and other toxic pollutants, as well as conventional plastic pollution.

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TABLE CAPTION

Table 1. Chemical composition of tested types of compost.

Table 1.

Content Element	Hummovit	Substratica	Biopiantella	Substral
Organic matter (%)	35 – 50	-	-	20
P ₂ O ₅ (mg/100 g)	100 – 200	200 – 500	150 – 250	-
K ₂ O (mg/100 g)	150 – 250	200 – 500	200 – 300	-
Total N (mg/100 g)	600 – 1200	20 – 50	130 – 180	-
pH	6.5 – 7.2	6.5 – 7.2	6.2 – 6.8	5.2 – 6.8

FIGURE CAPTION

Fig. 1 a) Average values of produced CO₂ (m_p) in 12 days' time and calculated average activity values for the tested compost samples.

Fig. 2 a) Final production of CO₂ for all tested samples in the respirometer; b) Production of CO₂ deducted for blank samples for MC, LB, PC, HK, and PP, respectively; each graph represents the mean values of 2 replicates. All samples in a) and b) are measured in Humovit compost during 73 days of respirometric measurements.

Fig. 3 Micrographs of linen/cotton, hemp and polypropylene before (LB (a), HK (c), PP e)), and after respirometric measurements in Humovit compost (LB (b), HK (d), PP (f)) recorded at 40-times magnitude.

Fig. 4 Production of CO₂ during two months of respirometric measurements for MC, LB, HK and PP, respectively, in water media; each graph represents the mean values of 2 replicates.

Fig. 5 Micrographs of linen/cotton and hemp before (LB (a), HK (c)), and after respirometric measurements in aqueous media (LB (b), HK (d)) recorded at 40-times magnitude.

Fig. 6 FTIR spectra for a polypropylene medical surgical mask; a) outer layer before respirometric measurements in compost/aqueous media, b) inner layer, layer before the experiment c) outer layer after the experiment, d) inner layer after the experiment.

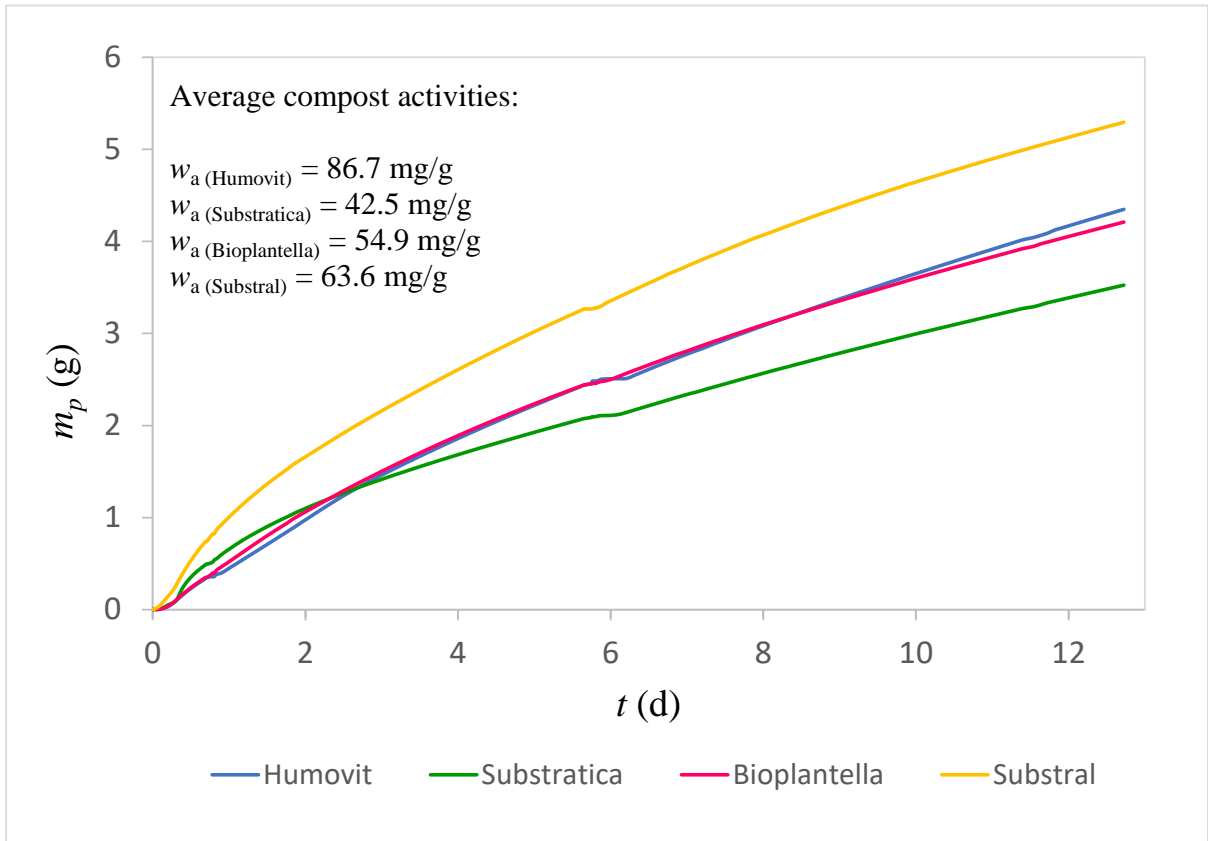


Fig. 1

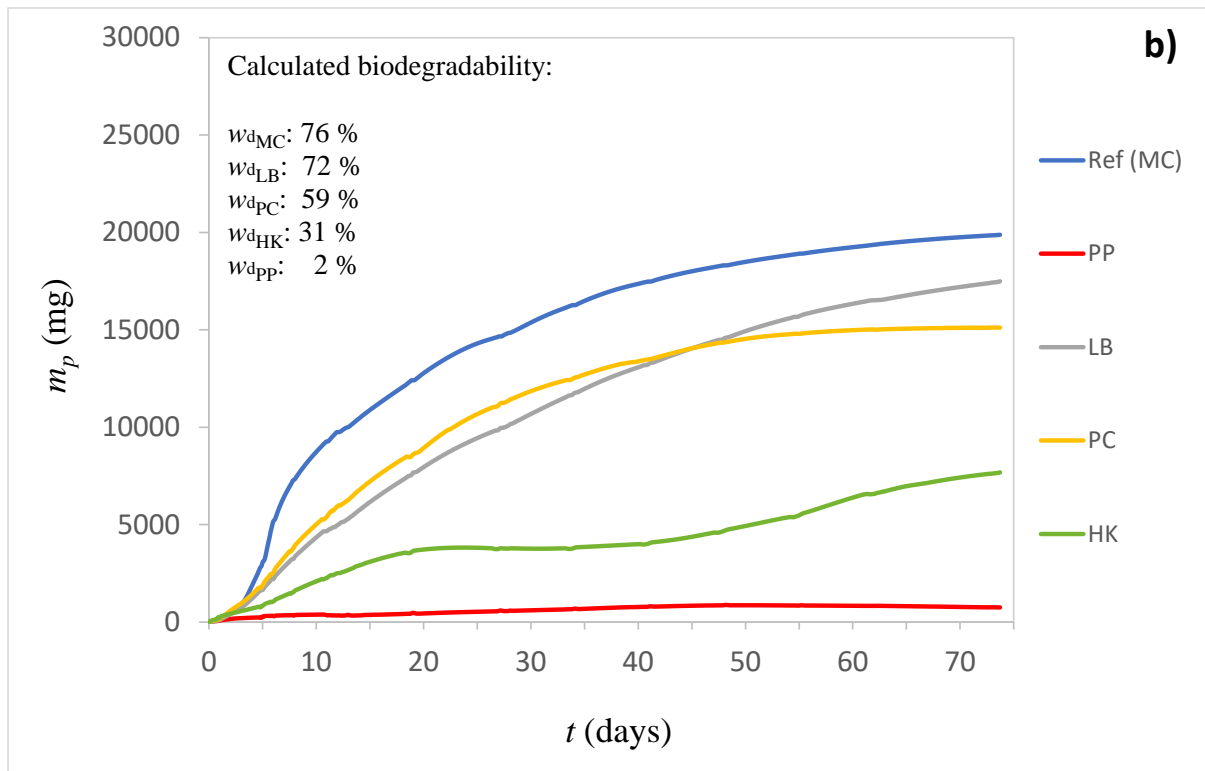
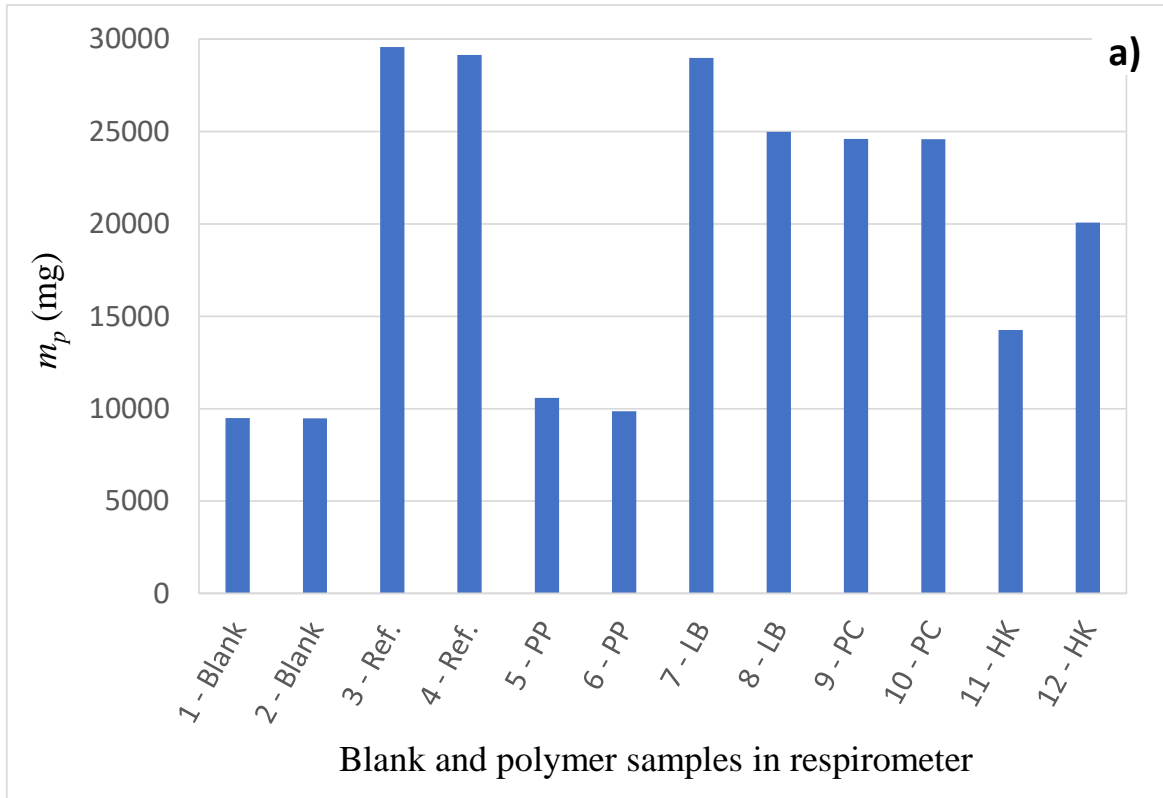
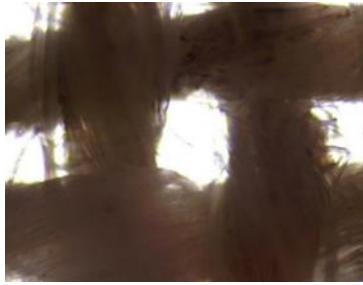


Fig. 2

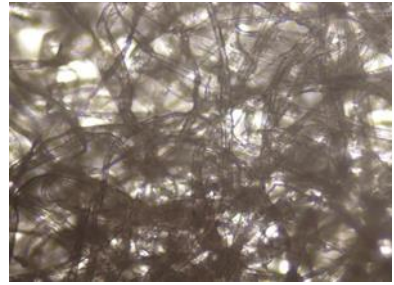
a)



c)



e)



b)



d)



f)

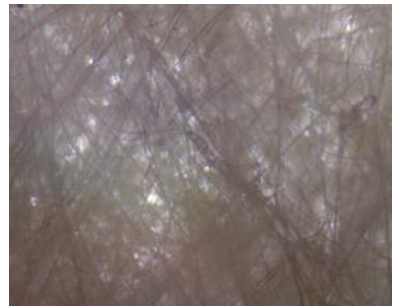


Fig. 3

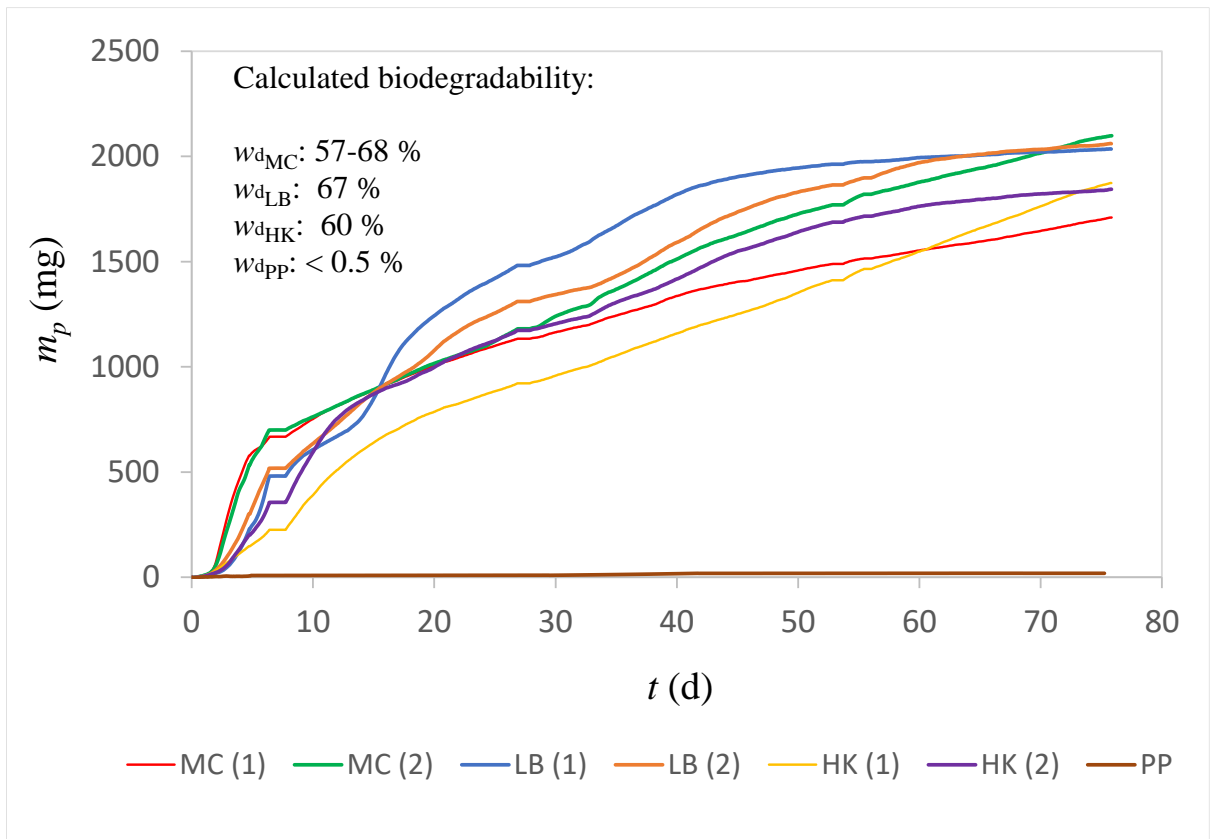
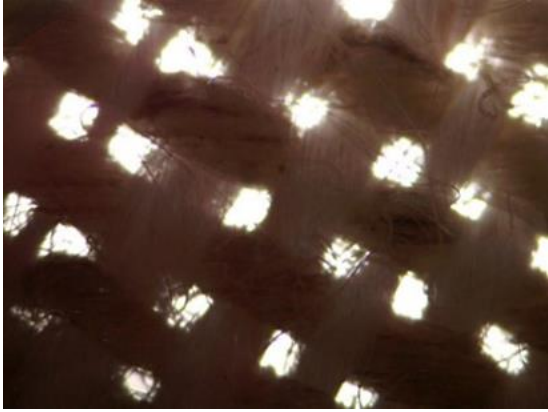
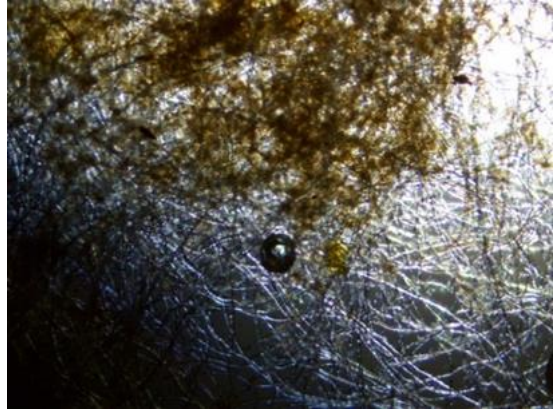


Fig. 4

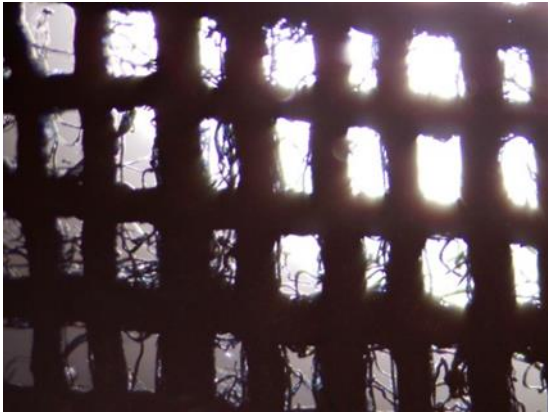
a)



b)



c)



d)

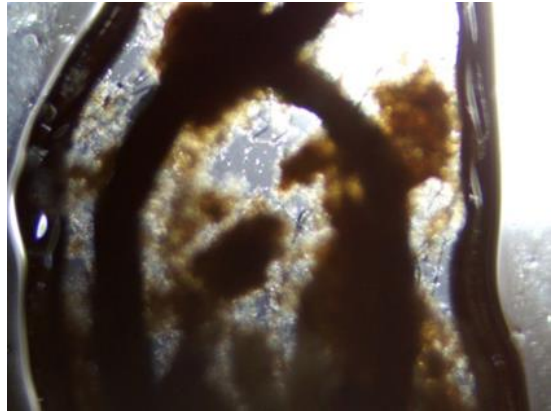


Fig. 5

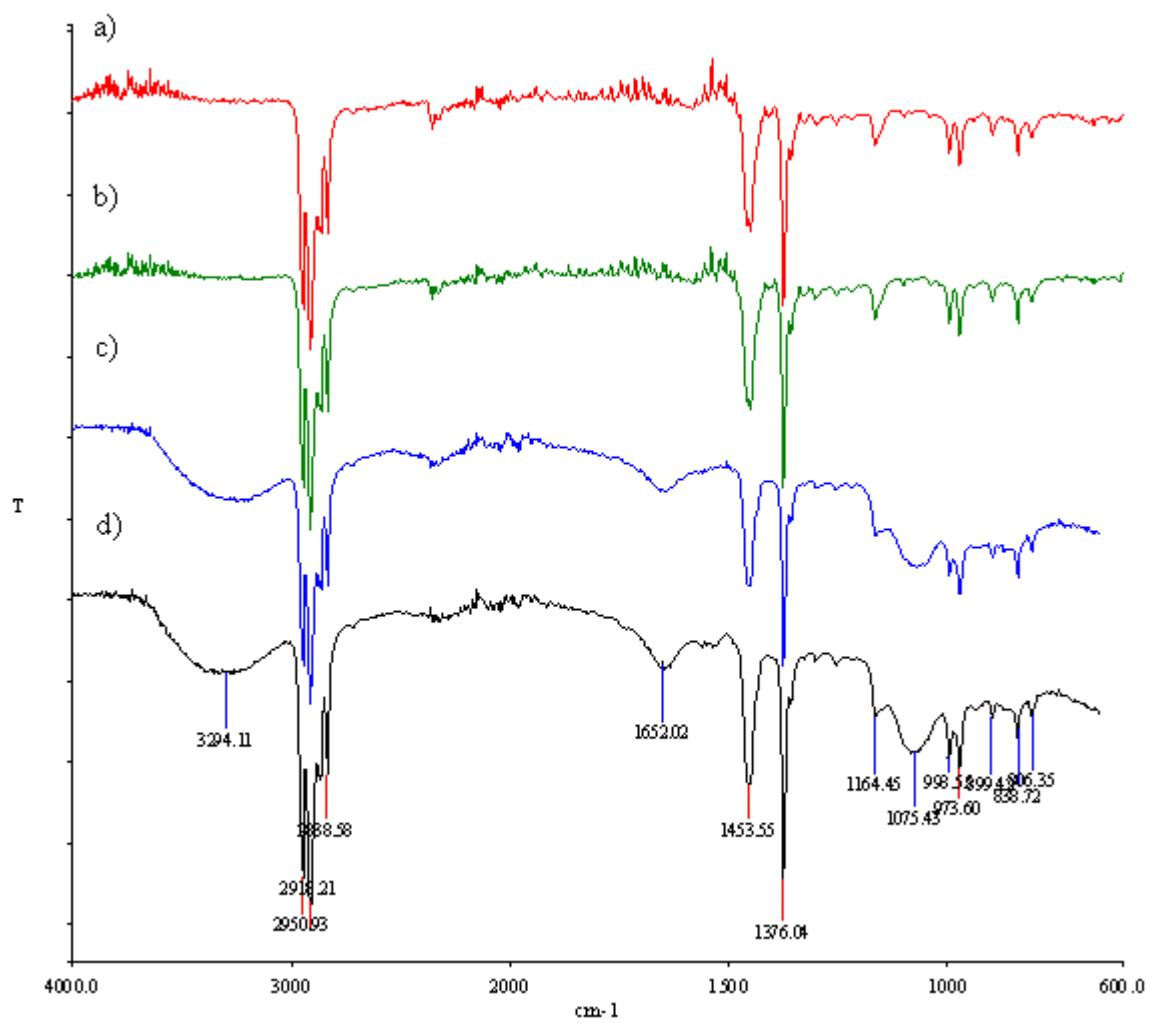


Fig. 6