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

Article Highlights

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

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 the 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.

During the COVID-19 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 µm) 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

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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 a good ability to degrade hydrocarbons and hydrophobic polymers [10]. They found that the majority of naturally occurring isolated bacteria from the genus *Pseudomonas* is 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 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 are 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.

MATERIAL AND METHODS

Materials

Initially, 4 different types of compost were studied first to determine their maximum activity. Compost used: Humovit univerzal, composition: white and black peat, coconut fiber, 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. Chemical composition of tested types of compost.

Content Element	Hummovit	Substratica	Bioplantella	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

The degradation of several different protective masks was tested. All were commercially available: made of polypropylene (PP); made of Himalayan hemp

fibers (HK); made of linen and cotton mixed fibers (LB), two layers: the inner layer was made from 100% cotton and the 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 a 12-channel respirometer for measurements in compost and in a 6-channel respirometer for measurements in aqueous media. Data handling in each reactor was automatic. An airflow 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 by following Eq. (2):

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

where w_d is the biodegradability (%), m_m is the mask mass (g), TOC is the total organic carbon (%), and r_M is the 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 the compost after 10 days (in mg) and m_{vs} is the mass of volatile solids (in g).

The mass of volatile solids is calculated according to Eq. (4):

$$m_{vs} = m_{compost} \cdot \frac{d_m}{100} \cdot \frac{vs}{100} \quad (4)$$

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

Respirometry experiments in compost

The sample composition in individual reactors is seen in 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 an 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, and 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 FeCl₂·H₂O 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 KH₂PO₄, 69.6 g of Na₂HPO₄, and 2 g of NH₄Cl 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 a 1 mm sieve and a 0.2 mm sieve. Finally, a 5% compost solution in the aqueous medium was prepared. The Blank was prepared without plastic material. Microcrystalline cellulose served as reference control

material. PP, HK, LB, and PC were tested for degradation with a mass of 2 g per 0.5 L solution. The respirometric experiments were performed in the same manner as described in the section Respirometric measurements at 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 to perform microscopic images in a magnification range of 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.

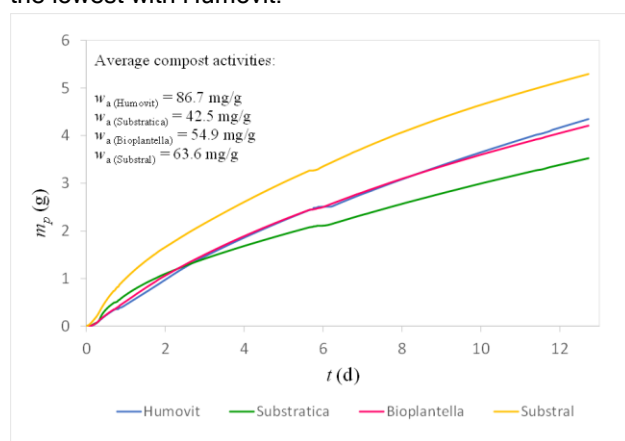


Figure 1. (a) Average values of produced CO_2 (m_p) in 12 days and calculated average activity values for the tested compost samples.

The activities were calculated according to Eq. 3. Compost activities were calculated the highest for Humovit $86.7 \pm 4.9\%$, followed by Substral $63.6 \pm 0.4\%$, Biopiantella $54.9 \pm 2.7\%$, and Substratica $42.5 \pm 3.3\%$.

Further, pH remained within demanded values until the end of the experiment, between pH 7 and pH 8, whereas Substratica and Biopiantella 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 Eq. (5):

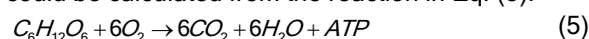


Fig. 2a shows the final measurement values of produced CO_2 for the two parallel samples of each tested polymer versus blank samples. We noticed that the produced CO_2 value is the highest for samples with added microcrystalline cellulose, which we used for reference. Very close values are reached with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

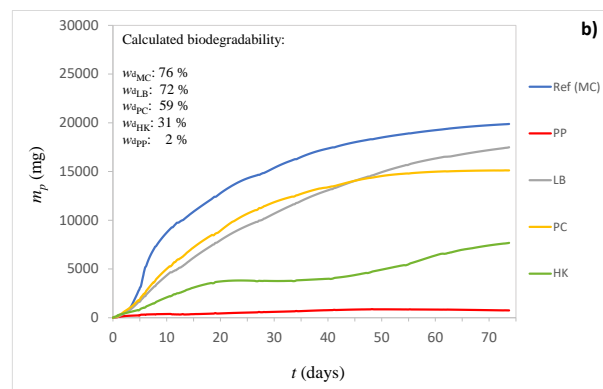
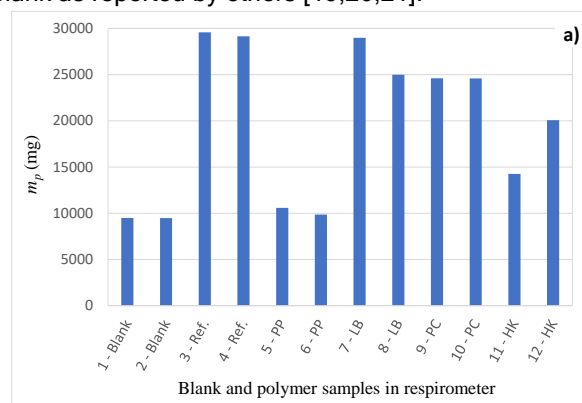


Figure 2. (a) Final production of CO_2 for all tested samples in the respirometer; (b) Production of CO_2 deducted for blank samples for MC, LB, PC, HK, and PP, respectively; each graph represents the mean values of 2 replicates. All samples in (a) and (b) are measured in Humovit compost during 73 days of respirometric measurements.

Fig. 2b presents the average cumulative CO₂ production (in mg) for the tested samples deducted for blank samples during the whole respirometric test in compost, where the produced CO₂ 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 rates, respectively. High biodegradability was confirmed with LB. The disintegration of linen fabric under composting conditions was reported at a 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 period after the 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 a 31% rate. According to Himalayan hemp amorph structure, it could result in being more biodegradable, but on the other hand, HK is known for its high mechanical strength [24], which presumably slows down 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 were 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).

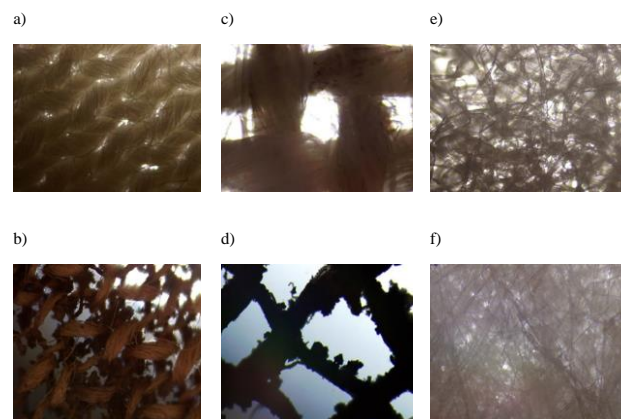


Figure 3. Micrographs of linen/cotton, hemp, and polypropylene before LB (a), HK (c), PP (e), and after respirometric measurements in Humovit compost LB (b), HK (d), PP (f) recorded at 40-times magnitude.

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 in 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 no 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. The stretching vibration of the CH-bond appears 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.

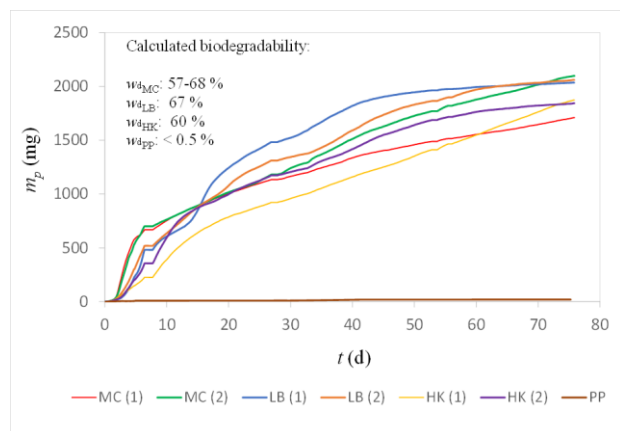


Figure 4. Production of CO_2 during two months of respirometric measurements for MC, LB, HK, and PP, respectively, in water media; each graph represents the mean values of 2 replicates.

LB blend seemed to reach the plateau after 60 days of 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 to 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 a further increase in CO_2 production. The protective mask made of PP was not biodegradable, with calculated biodegradation below 0.5%, which is in accordance with the literature [28]. As seen from Fig. 4 the prolonged time might contribute to a further increase in CO_2 production.

Fig. 5 presents the microscopic graphs of LB and HK at 40 times magnitude. The 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 as the disintegrated material, as fibers are not visible anymore. Similar was observed with HK as seen in Fig. 5c and 5d.

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 the outer and inner layers (6a and 6b)

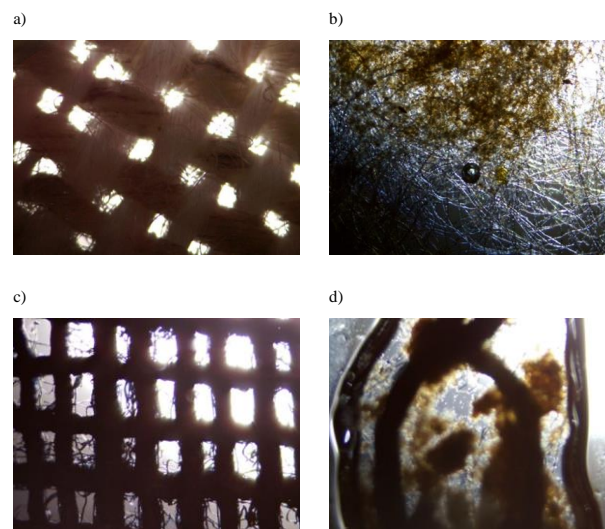


Figure 5. Micrographs of linen/cotton and hemp before LB (a), HK (c), and after respirometric measurements in aqueous media LB (b), HK (d) recorded at 40-times magnitude.

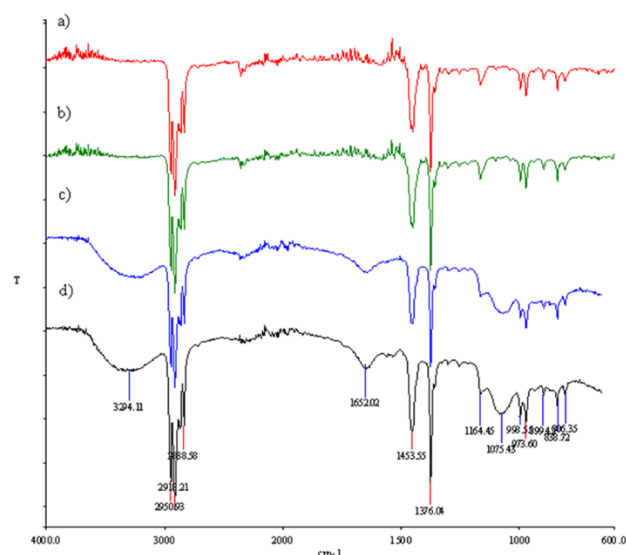


Figure 6. FTIR spectra for a polypropylene medical surgical mask: (a) outer layer before respirometric measurements in compost/aqueous media; (b) inner layer, layer before the experiment; (c) outer layer after the experiment; (d) inner layer after the experiment.

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 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 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.

Even with the linen/cotton mask, we did an 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.

CONCLUSION

Protective face masks made of linen/cotton met our expectations regarding biodegradation in compost media. The masks made of Himalayan hemp fibers were not as 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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NAUČNI RAD

BIORAZGRADNJA ZAŠTITNIH MASKI (COVID-19) U KOMPOSTU I VODENIM MEDIJIMA

Izvršena su istraživanja biorazgradnje maski za lice od prirodnih i sintetičkih materijala. Eksperimentalni deo je izveden sa respirometrom slovenačkog proizvođača ECHO Instruments. Izvršili je preliminarni test za odabir komposta sa najvećom aktivnošću za dalji rad. Brzina biorazgradnje materijala izračunata je na osnovu merenja ugljen(IV)-oksida proizvedenog u reaktorima. Rezultati biorazgradljivosti materijala su verifikovani korišćenjem FTIR spektroskopije i mikroskopskih slika. Maske napravljene od čestica lana i pamuka imale su najbolju biorazgradnju od 72%, dok čestice maski od polipropilena (PP) nisu degradirale. Budući da ne postoje studije o biorazgradivosti u vodi, drugi deo istraživanja odnosio se na brzinu razgradnje maski u otpadnoj vodi. Na osnovu dobijenih FTIR spektara, potvrđeno je prisustvo novih funkcionalnih grupa na površini zaštitnih maski kao rezultat procesa oksidacije. Kompost Humovit je izabran kao inokulum za vodeni medijum. Najveći stepen biorazgradnje je utvrđen za masku od lana i pamuka (67% za 75 dana).

Ključne reči: zaštitne maske; lan/pamuk; himalajska konoplja; respirometrija; FTIR.