National Research Programme "to mitigate consequences of COVID-19"

Project "Integration of reliable technologies for protection against Covid-19 in healthcare and high-risk areas"

Deliverable of Task 2.2.

Prototype description

Fungal hyphae and cellulosic fibre composite material membrane KF FF 50/50

December, 2020

Summary

Fungal hyphae and cellulosic fibre (w/w 50:50) composite material KF FF 50/50 membrane with grammage <60 g/m^2 was chosen from series of different composites consisting from Kraft fibres (KF), hemp fibres (HF) and fungal fibres (FF) in different ratios. KF FF 50/50 was proposed as material applicable in the face mask as a natural, biodegradable filter layer. Commercially available Kraft fibres were used, however fungal hyphae were obtained from fruiting bodies of lignicolous basidiomycetes growing in nature. Material was obtained using paper producing technique and appropriate laboratory scale equipment. Air permeability was close to the standard breathability number needed for face masks according to EN 14683+AC:2020. Mechanical performance was appropriate to handle the samples in order to characterise, evaluate and integrate in mask/respirator. Chemical composition analysis (FTIR) revealed presence of natural polysaccharides, mainly – cellulose and chitin as well as appropriate elemental components distribution of C, H and N. Potential biodegradability was proven by fast biodegradation assessment via measurements of biochemical oxygen demand changes in mixture of sample and composting substrate as well as the detected 54% decrease of C/N amount ratio after the test.

Raw materials

Bleached softwood Kraft fibres were kindly provided by Metsä Fibre (Finland) as pressed sheets (Fig. 1) and used without specific pre-treatment. Av. length 2,1 mm, av. width 29,7 µm.

Fungal hyphae were obtained from naturally growing fruiting bodies of basidiomycete *Ganoderma applanatum* (Fig. 2). The fruiting bodies were dried in room temperature and cut in smaller pieces (3 x 3 cm). Sample pieces were kept in 4% NaOH solution for 24 h at room temperature in order to remove proteins and alkali soluble polysaccharides, as well as to soften and swell the fungal biomass. After NaOH extraction samples were washed with tap water and mechanically ground by shredder Blendtec 725 (USA) at 360W for 30 seconds. Obtained fungal hyphae were dried at room temperature and kept in dry state until used.

Figure 1. Kraft fibre sheets

Figure 2. Fruiting body of basidiomycete *Ganoderma applanatum*

Producing

In order to obtain material with target grammage, certain mass of both composite components (Kraft fibres and fungal hyphae pulp) was put in glass baker and soaked in 1 L distilled water for 8 h, then disintegrated in 75000 revolutions in the Disintegrator (Frank PTI, Austria). Material sheets (Fig. 3) with diameter ~20 cm were produced according to ISO 5269-2 with a Rapid Köthen paper machine (PTI, Austria) (Fig. 4).

Figure 3. Fungal hyphae and cellulosic fibre composite material KF FF 50/50

Figure 4. Rapid Köthen paper machine

Characterisation methods

The microstructure of fibre and composite material samples was examined by a light microscope Leica DMLB at magnification of 200×. The images were captured using a video camera Leica DFC490 using calibrated image analysis software (Image-Pro plus 6.3, Media Cybernetics, Inc.).

For scanning electron microscopy (SEM) images, samples (surface; cross sections of cut and torn samples) were coated using gold plazma using K550X sputter coater (Emitech, UK), and examined with Vega TC (Tescan, Czech Republic) with software 2.9.9.21.

Thickness of material was measured according to ISO 534 with a micrometer F16502 (Frank-PTI, Austria).

Samples for mechanical testing were prepared with Strip Cutter and tested according to ISO 1924-1 and 2758, using Tensile Tester Vertical F81838 and Burst Tester for Paper (both: Frank-PTI, Austria).

Air permeability of material was measured according to the three different standards, taking into account its potential applicability in personal protective equipment. Thus, air permeability was measured according to ISO 5636-3 (Air Permeability Tester, Lorentzen & Wettre, Sweden), to ISO 9237:2001 and EN 14683+AC:2020 (Air Permeability Tester M021S, SDL Atlas, USA.).

FTIR spectrum values were recorded using Nicolet iS50 spectrometer (Thermo Fisher Scientific, USA) at a resolution of 4 cm^{-1}, 32 scans per sample.

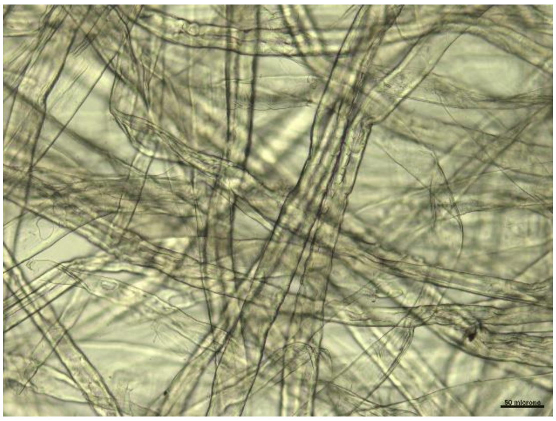
Total content of carbon, hydrogen and nitrogen was determined with Elementar Analysensysteme GmbH, Vario MACRO CHNS (Germany).

A general test for determining the potential of biodegradability of the samples was carried out using OxiTop system. The main indicator of this test is BOD (biochemical oxygen demand - the amount of oxygen required for microbial metabolism). Biochemical oxygen demand was measured in mg per 1 L of the substrate. The principle of the operation is a pressure drop in the closed system due to absorption of the released CO_2 in NaOH. 100 g of the substrate (dw 78%) was placed in a bottle. The compost (producer SIA “Zeltābele”, N≤0,4%, P_2O_5≤0,15%, K_2O≤0,06%, pH 6-7, organic ≤16%) was used as a substrate. Fresh NaOH granules were added for each incubation set. Manometric measurement of the changes in the vacuum was performed automatically every day. The incubation was performed at 20°C without agitation. Data collected during the incubation were sent to the controller through the infrared interface and then to the computer using AchatOC software.

Properties of composite material

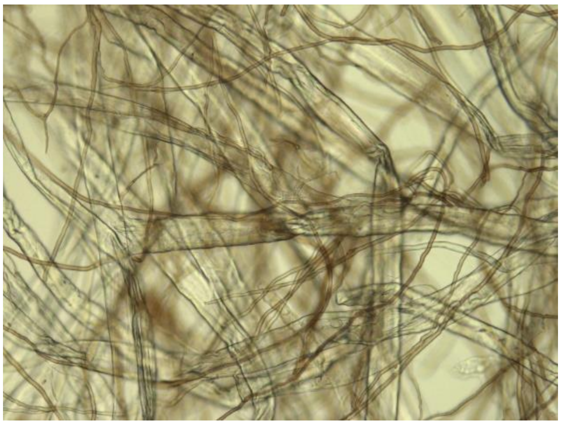
Light microscopy (LM)

Microscopic structure of the composite material KF FF 50/50 is shown in Figure 5 The kraft fibres (a) and fungal fibres (b) are mechanically bound together making a net of the composite material (c).



a

b



c

Figure 5. KF fibres 30 µm in diameter (a), fungal fibres (hyphae) 3-6 µm in diameter (b) and composite material KF FF 50/50 (c); LM 200x

SEM images (Fig.6) reveal the structure of the composite material KF FF 50/50 in details. Images confirm the observations of LM. Entangled fibres are shown, they are mechanically bounded together, forming non-oriented, layered network. Kraft fibres and fungal fibres have diameters 20-50 µm and 3-6 µm respectively. Rather loose network with pores is shown in cross section of composite.

Mechanical properties (Table 1) were appropriate for integrating the material in personal protective equipment (face mask/respirator) without damage caused by handling or processing. There are no specific requirements or standards regarding filtrating materials for face masks/respirators.

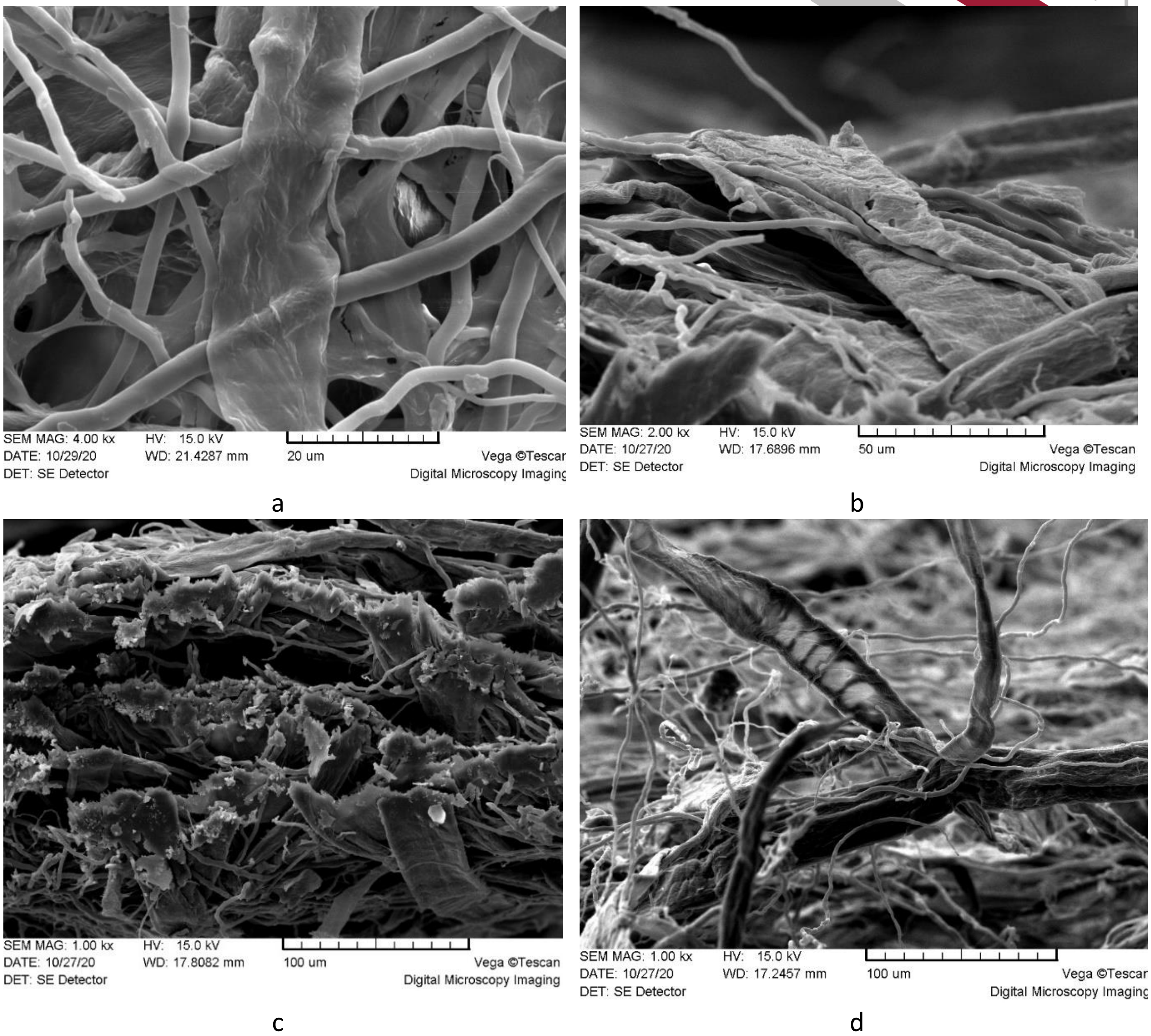


Figure 6. SEM microscopy of fungal hyphae and cellulosic fibre composite material membrane: a,b surface structure; c – cross section, cut; cross section, torn.

Table 1. Mechanical properties of KF FF 50/50

Property	Value
Tensile index, dry	13.86 Nm/g
Tensile index, wet	1.3 Nm/g
Burst index	1.0 kPa m^2/g
Tensile energy absorption	17.7 J/m
Breaking length	1.4 km
Stretch	2.04 %

Air permeability properties (Table 2) reveal the appropriateness of composite to be used as filtrating material - it is breathable, however slightly does not reach the number set in the standard EN 14683 for face masks (60 Pa/cm^2) and in the next stage of development it is necessary to overcome detected shortcoming. It is expected to reach the needed number simply decreasing the grammage, because significant correlation was detected between grammage and air permeability of sample.

Table 2. Air permeability properties of KF FF 50/50

Standard	**Value**
ISO 5636-3	23990 ml/min
EN 9237	65.5 mm/s
EN 14683	90.8 Pa/cm^2

Chemical properties

FTIR spectra of composite KF FF 50/50 show (Fig.7) all the typical Kraft fibre and fungus fibre peaks and bands, no other peaks have appeared, therefore it is clear that there are only mechanical bonding and H bonds among fibres in composite material KF FF 50/50.

For spectra of fungus fibres, the broad band at 3400 cm^{-1} is attributed to the -NH_2 and -OH groups stretching vibration and intermolecular hydrogen bonding. The bands at 2929 cm^{-1} and 2880 cm^{-1} represent the C–H a symmetric stretching, and have been reported as representative bands for chitin. Bands at 1640 cm^{-1} (C=O stretching) and 1550 cm^{-1} (N–H bending) are related to the amide I, amide II respectively. Region 1160–980 cm^{-1} is typical for saccharides of fungus biomass, C-C and C-O stretching vibrations in glycosidic bonds and pyranoid rings have been detected.

In the spectra of Kraft fibres typical bands of cellulose are shown. The band observed at ~3400 cm^{-1} is attributed to O-H vibration, mainly caused by the hydrogen bonds in the cellulose, peak at ~2900 cm^{-1} is associated with C–H stretching vibrations, and the peak at ~1640 cm^{-1} is related to the absorbed water and sharp peaks at 1055 cm^{-1} and 896cm^{-1} are associated with the C–O stretching and C1–H deformation vibrations of cellulose.

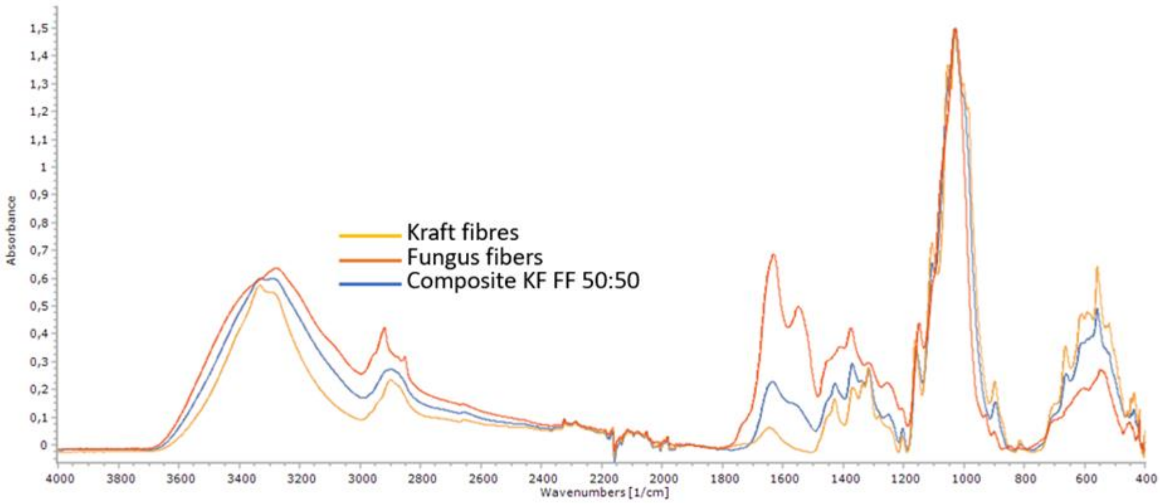


Figure 7. FTIR spectra of raw materials and composite material.

Presence of N containing components is confirmed by elemental compound analysis (Table 3).

Table 3. Total content of nitrogen, carbon, hydrogen and C/N ratio of KF FF 50/50 sample and raw materials.

	N, %	C, %	H, %	C/N ratio
KF FF 50/50	0.66	40.92	5.81	44.88
KF	0.22	42.50	5.90	189.21
FF	1.75	38.93	5.75	2.27

Biodegradability

Potential biodegradability was assessed by the evaluation of the biochemical oxygen demand (BOD) of substrate, in which composite samples were held to imitate the composting medium. Results of composite KF FF 50/50 show (Fig. 8) 496 mg/L of BOD, while control compost (without composite sample) has only 220 mg/L of BOD. Obtained numbers confirm the biodegradability of composite.

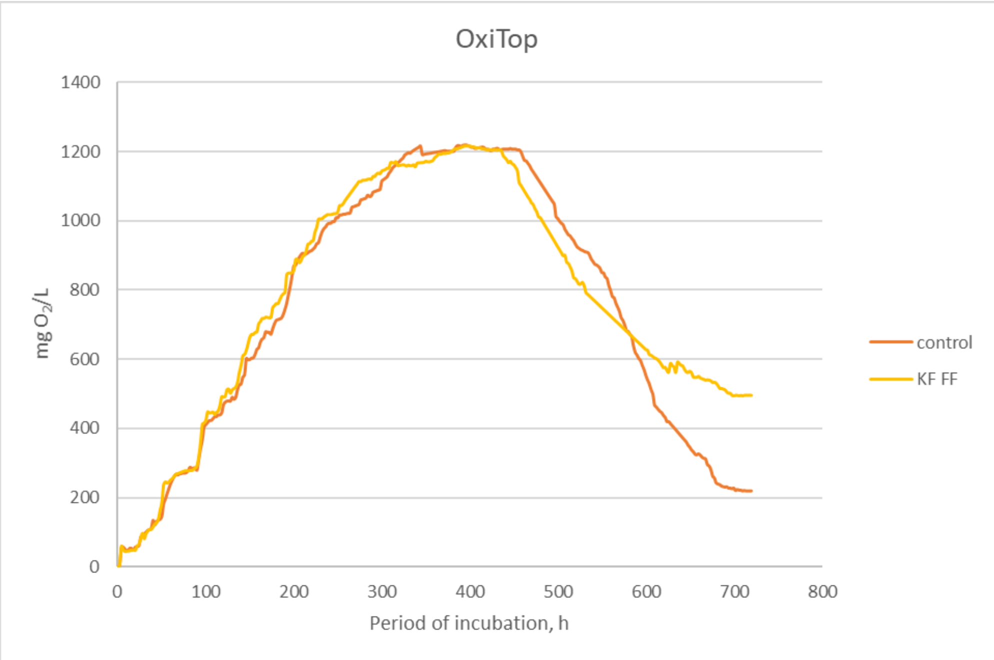


Figure 8. Biochemical oxygen demand during the incubation of KF FF 50/50 in the substrate

Table 4. Total content of nitrogen, carbon, hydrogen and C/N ratio of KF FF 50/50 sample and raw materials after composting.

	N, %	C, %	H, %	C/N ratio	Δ C/N,%
KF FF 50/50	1.50	43.19	5.67	28.78	53.9
KF	0.24	41.91	5.81	174.83	7.7
FF	2.68	45.49	5.49	16.96	23.8

The C/N ratio represents index of the maturity for organic substances as it significantly affects the microbiological growth. Concentration of C and N in composted samples after 30 days of experiment is shown in Table 4. Considerable differences among the tested samples were found. C/N ratio for the samples varied in the range of 17÷175. The decrease of C/N ratio by 54% of composite material KF FF 50/50 can be attributed to the more efficient conversion of organic matter in comparison with the raw materials.