

Biodegradation of disposable face mask by *Tenebrio molitor* larvae (Mealworm) and its metagenomic characterization

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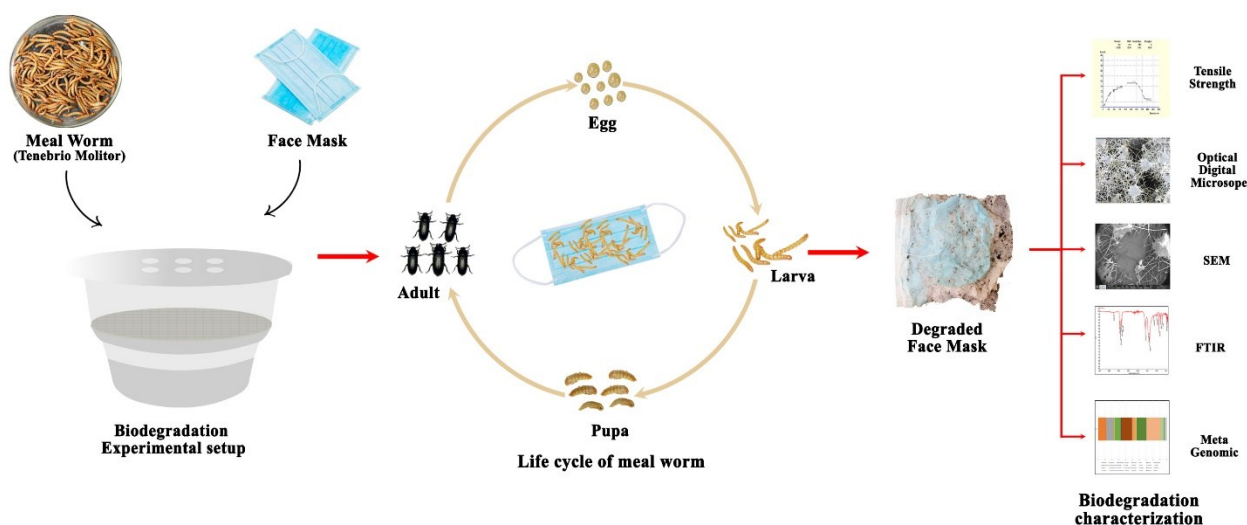
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Graphical Abstract



Abstract

In recent years, the surgical face mask is mandatory for human health safeguards due to the worldwide pandemic disease COVID-19. The improper disposal of medical face masks polluted the environment. *Tenebrio molitor* (mealworm) larvae were shown to have the capacity to chew and consume polypropylene medical face masks. Mealworm survival rates were determined for 30 days under three different feeding conditions such as (i) medical mask as a sole diet, (ii) fed bran as a sole diet, and (iii) mealworms are starved, the survival rates results are $89.25 \pm 4.5\%$, $95.35 \pm 1.5\%$ and $58.2 \pm 3.5\%$ respectively. While the biodegradation of the medical face mask by mealworms, it consumed 47.5% of the total mask and lost tensile strength by about 80% which showed that

biodegradation of the medical mask. The biodegradation of the mask was confirmed by instrumentation analysis, which included digital microscopy, Fourier transform infrared spectroscopy, and scanning electron microscope microscopy. The results revealed that the qualitative mask had lost its original characteristics, such as damaged pore size, many scratches, and functional groups that had changed, and that mealworms could degrade the medical face masks. In addition, mealworm gut metagenomic analysis was also performed for the microbial diversity, ten genera were found as the most abundant following *Delftia*, *Pseudomonas*, *Flavobacterium*, *Chryseobacterium*, *Spiroplasma*, *Stenotrophomonas*, *Bacillus*, *Rhodococcus*, *Brevundimo* and *Acinetobacter*.

Keywords: Biodegradation; Next-generation sequencing; Medical face mask; Tensile strength; *Tenebrio molitor*

1. Introduction

Coronavirus disease (COVID-19) is caused by a new coronavirus that causes severe acute respiratory infection (SARS-Cov-2) which is the pandemic measure. Coronaviruses are single-strand RNA viruses that infect a wide range of humans and also animals (Shereen et al., 2020). Across the globe, people have been affected by COVID-19, which began in China in December 2019 and has generated global concerns (Huang et al., 2020). The World Health Organization (WHO) declared it a global health emergency on January 30th, 2020 (Sohrabi et al., 2020).

COVID-19 has accelerated the global generation of healthcare (medical) waste. Because of inadequate management systems, developing nations are the most affected by this harmful and toxic medical waste (Dihan et al., 2023). In particular, plastic pollution has been made worse by the COVID-19 epidemic (Wang et al., 2023). To avoid stockpiling and polluting communities with potentially infectious medical waste (MW), as well as to assure sustainability in the present and post-COVID-19 eras, a safe and efficient medical waste management (MWM) system must be developed and implemented (Tushar et al., 2023).

During the pandemic WHO suggests that wearing a medical face mask (SFM) in public places is mandatory to prevent the virus easy spreading, which resulted in a vast use of SFM. In 2020, over 129 billion SFM were predicted to be used globally every month (Prata et al., 2020) and 3.4 billion of those were discarded every day (Benson et al., 2021). In this regard, because of the limited disposal capacity and the overwhelming problem of medical waste, waste researchers and policymakers must act quickly. (Cao et al., 2023; Purnomo et al., 2021).

These face masks are composed of liquid-resistant plastic-based materials, and their half-life is greater in soil and water environment (Chellamani et al., 2013; Dharmaraj et al., 2021). The disposable SFM is made up of various polymers such as polypropylene, polyester, polystyrene, polycarbonate, polyurethane, or polyethylene (Aragaw 2022; Potluri & Needham., 2005). As per WHO and every national healthcare body instructed the people, SFM should not be used for more than one day and discarding them to medical waste (Sangkham 2020). The used SFMs are not properly discarded and managed, it was directly discarded into the environment these abandoned SFMs do affect the environment, specifically mistakenly eaten as food by animals, soil pollution, water pollution, and a new type of plastic pollution it resulting in eco-toxicological effects on the environment. (Patrício Silva et al., 2021; Knicker & Velasco-Molina 2022).

Hence, SFM needs to decompose/degrade through the non-hazardous process and eco-friendly methods for the environment. There are several methods involved in plastic degradation such as Photo-oxidative, Thermal, Ozone, Mechanochemical, Catalytic, and Biodegradation (Zeenet et al., 2021). *T. molitor* is essential to the ecosystem's functioning and has the potential to expand into other scientific domains . The treatment techniques now used in several companies take a lot of energy and area, and they frequently have negative environmental effects. Consequently Even at the national and worldwide level, the biodegradation of plastic waste employing different kinds of insects is still in its infancy. However, it offers a sustainable option, keeps plastic waste separate from other natural resources, and protects the environment. In addition, recently researchers have developed new technologies for plastic degradation and their management. Among the technologies, one of the superior methods is insects chewing and ingesting plastics under biodegradation and the plastics were degraded more rapidly (Lou et al., 2021; Przemieniecki et al. 2022). The use of insects in the biodegradation of polymer-based material is one of the most recent and prominent methods for pollutant removal (Yang et al., 2020). Several insects are involved in the biodegradation, they are wax worms (*Achroia grisella*), super worms (*Zophobas atratus*), mealworms (*Tenebrio Molitor*), Indian meal moth (*Plodia interpunctella*), the bigger wax moth (*Galleria mellonella*), snail, etc., have been demonstrated in plastic polymer biodegradation (Ali et al., 2021). Especially, the insect of *Tenebrio molitor* can consume polypropylene, polyethylene, polyvinyl chloride, polylactic acid, and polystyrene for metabolic development (Peng et al., 2023; Peng et al., 2022; Peng et al., 2021; Peng et al., 2020 Yang et al., 2021; Liu et al., 2022; Lou et al., 2021;). Mealworms are omnivorous, it secretes emulsifying factors that contributions the gut microorganisms and then involved the plastics (polystyrene and polyethylene) biodegradation and converting natural polymers into smaller bits that enhance enzymatic reactions (Brandon et al., 2021). Another insect of greater wax moth can ingest and diet on plastic material, During the biodegradation of plastics by

worms, plastics act as a sole carbon source, it enhanced protein synthesis to support larval growth in worms. The gut microbes associated with diet, the microbial diversity of worm gut *Citrobacter* sp. And *Kosakonia* sp. and greater wax moth larvae gut microbes *Bacillus* and *Serratia* were considerably connected to together polystyrene and polyethylene degradation, and the depolymerization pathway of polystyrene and polyethylene (Brandon et al., 2018; Ali et al.,2022).

In this study, the biodegradation of SFM by insects was investigated; Mealworms (*Tenebrio molitor*) were used and explore the biodegradation of SFM under optimal conditions. At various intervals, the analysis of SFM changes has been assessed, including mechanical, chemical, and morphological before and after biodegradation. Worm survival rates and SFM consumption rates are investigated for mealworms. Additionally, mealworm gut metagenomic analysis and microbial diversity were discovered. This study found and provide the scientific community with a new approach to managing the disposal of face masks, leading to overall better medical waste management.

2. Materials and Methods

2.1 Medical face mask

The disposable SFM purchased from commercially available medical/pharmacy shops was used for this experiment, the SFM is covered by bundle warp sheets by the manufacturer. The purchased masks are directly transported into the laboratory and stored in aseptic conditions. The SFM photograph shown in Fig. 1A, dimensions of SFM 17.5 cm x 9.5 cm (length and breadth) with 2.7 g of weight, it was made up of three layers, a skin-friendly non-woven fabric makes up the inner layer, a melt-brown non-woven fabric serves as the middle layer's filter for small particles and germs, and a hydrophobic non-woven fabric serves as the outside layer's filter for big particles. The SFM is dominantly made up of polypropylene polymers (i.e. known as plastic). Before starting experiments, SFM was sterilized under ultraviolet (UV) radiation in laminar air flow for 30 minutes.

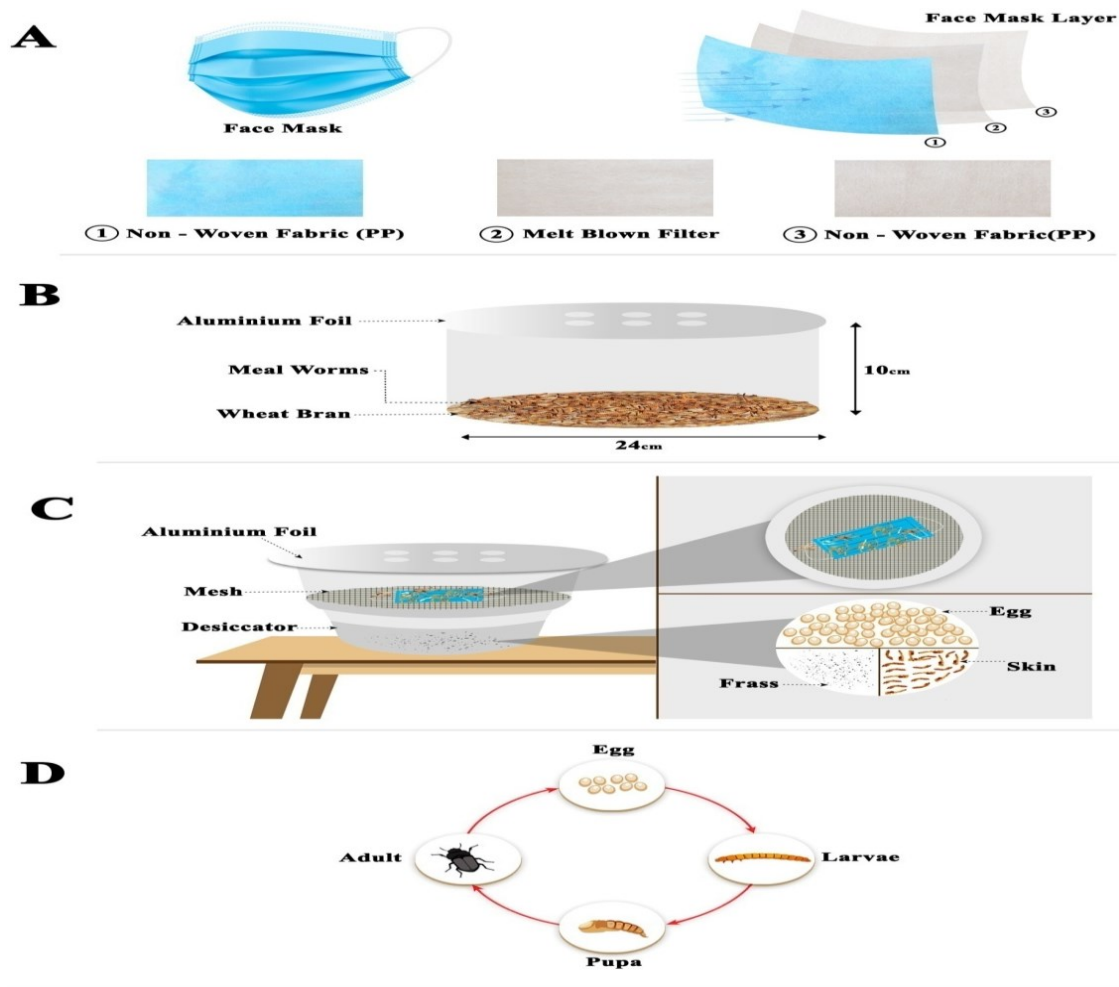


Fig.1. (A) Layers of surgical facemask, (B) Meal worms rearing setup, (C) Experimental Setup for biodegradation, (D) Mealworm life cycle

2.2 Rearing of insects

The active *Tenebrio Molitor* larvae were purchased from local fish farm vendors and cultivated to feed fish. The worm's average length ranges from 2.5 to 3.5 cm. *Tenebrio Molitor* was reared in a circular glass container with a diameter of 24 cm and a depth of 10 cm that was covered with aluminium foil with air circulation holes, the graphical picture was shown in Fig. 1B. Before beginning the process, the circular glass container was three times rinsed-washed with 70% ethanol and distilled water after the drying process glass container was sterilized under UV radiation in laminar air flow for 30 minutes. These larvae were transported into the laboratory placed in an aseptic room under controlled conditions and fed wheat bran for three days then allowed to empty their stomach for one day before using them for experimentation (Bulak et al., 2021).

2.3 Experimental setup

The biodegradation experiments were conducted within a glass desiccator in the laboratory shown in Fig. 1C. The desiccator was divided into three parts such as top, middle, and bottom; the top of the desiccator was covered with aluminium foil with appropriate aeration, 1 millimetre of metal

mesh sheet was fixed at the centre of the desiccator, which to hold SFM and mealworms. The excreta, skin peels, and eggs of the larvae were collected at the bottom of the desiccator. The mealworm life cycle Fig. 1D is divided into four stages: egg, larvae, pupa, and beetle. Darkling Beetles reproduce eggs, which hatch into tiny mealworms, grow into pupae, and then the pupa matures into a Darkling Beetle. The whole life cycle of mealworms was observed while being fed the medical mask. This arrangement must be away from ants because they may harm the worms. I confront the Obstacle.

2.4 Biodegradation of SF and Survival Rate of mealworms

There were 900 mealworms utilized in this experiment, which is divided into three groups; (i) 300 mealworms were fed simply bran, (ii) 300 mealworms were fed sterile medical face masks, (iii) 300 mealworms are unfed. All the experiments were conducted in aseptic conditions. During the experiments every five days analysis of mealworm survival rate, and visual morphological examination. At the end of the experiment, it was calculated the percentage of live mealworms divided by the total number of live mealworms at the beginning of the experiment was used to compute survival rates (Brandon et al., 2021).

2.5 Characterization of medical face mask

2.5.1. Percentage of mask consumption rate

The SFM weight loss was determined using a 0.001g accuracy digital balance Gibertini E42s electronic balance. Mask samples were taken every 5 days during the biodegradation process to monitor weight loss. The effectiveness of the biodegradation process was determined based on the change in weight (%) of the SFM (Bulak et al., 2021).

2.5.2. Mechanical strength

Mechanical strength/tensile strength was done on the medical mask with a Hounsfield Universal testing machine. The SFM sample was cut into 50 mm x 25 mm pieces before being placed in the loading device. The test was carried out at a speed of 5 mm/min. tensile stress variations were observed on the 0th, 15th, and 30th days. The tests were carried out in an air-conditioned environment at a temperature of 20 °C (Suresh et al., 2011). The value for this experiment is the mean of the three samples obtained.

2.5.3 FESEM analysis

The surface morphological changes of the medical mask layers were examined using a field-emission scanning electron microscope (FESEM, Apreo S, Thermo Scientific, USA) with the following parameters: Work distance 7.18-10.6 mm, magnification 100x—500X, spot size 8, and an accelerating voltage of 15 kV, aperture size: 300m-500m, Signal SE detector with scanning mode and noise reduction for pixels. The samples were rinsed with ethanol and air-dried to remove the

majority of the dust without harming the surface. The SFM pieces were approximately 5 mm × 5 mm in size. The specimens were mounted to aluminium stubs using carbon tape (Saliu et al., 2021). The structural alterations of each layer of the mask were examined and compared to different biodegradation intervals.

2.5.4 Optical microscopy

Analysis with a digital microscope aid in determining the differences in the surface morphology of medical masks caused by biodegradation. The digital microscope & FESEM was used for morphological analysis of the medical mask at room temperature (Make:dinolite, Taiwan, ModelAM4515T) with a magnification capacity of 220x. The experimental materials were collected during the intervals of the 0th, 15th, and 30th days of the experiment, and the following parameters were employed in this analysis: The magnification is 58.6x, the working distance is 0.5 mm, and the pixel size is 1280 x 1024 (Moraisa et al., 2021).

2.5.5 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR, Perkin Elmer 2400 CHN Elemental Analyser) (including make country) was used to assess how the chemical structures of each layer of 3-ply SFM before and after biodegradation. The spectral scanning frequency range of 4000–450 cm⁻¹ and a resolution of 4 cm⁻¹ were set to identify the chemical structure (Aragaw 2020) of the mask for different intervals of degradation (0th, 15th & 30th days).

2.6 Microbial community analysis

2.6.1. Genomic DNA extraction

After 30 days of consuming a medical mask, ten mealworms were chosen from the group for a gut microbiota study. All larvae were immersed for five minutes in 75% ethyl alcohol and rinsed twice in sterile saline water. A commercially available kit (Qiagen, Zymo Research, Thermo Fisher) was used to extract the genomic DNA from the mealworm gut, and producers followed according to the manufacturer's recommendations (Brandon et al., 2021). This process was repeated three times. After the isolation of DNA, the Nano Drop spectrophotometer was used to check the purity and quantity of DNA. Finally, measure the quantity of double-stranded DNA in a fluorometer (Elumalai et al., 2020).

2.6.2. Generation of amplicons

The gut microbiome of this worm was studied using the Illumina MiSeq technology with 16s Gena variable regions (V3 & V4) using a pair of bacterial primers: forward primer 16sF (5'-AGAGTTTGATGMTGGCTCAG3') and reverse primer 16sR (5'TTACCGCGGCMGCSGGCAC3') (Luo et al., 2021). The PCR mixes and thermal cycling conditions were carried out as described by

the previous publication (Elumalai et al., 2020) and 1% agarose gel was used to test the DNA's integrity after the amplified 16S PCR product had been purified.

2.6.3. Library clean-up and sequencing

Ampure beads were used to filter out unnecessary primers from each sample's amplicons before performing an additional eight cycles of PCR with barcoded Illumina adapters to construct the sequencing libraries. Following purification with Ampure beads, the libraries were tested using the Qubit ds DNA High Sensitivity test kit. For the sequencing, an Illumina Miseq with a 2x300PE v3&v4 sequencing kit was used. The SILVA operational taxonomic units (OTUs) database was used to classify worm gut microbial diversity using a ribosomal database project (RDP) classifier. Fig. 9 illustrates the complete information of the worms' gut, including total consensus, reads, chimeric sequences, pre-processed reads, OTUs, and the distribution of the class, phylum, order, genus, & family based on OTUs and reads (Prakash et al., 2021; Elumalai et al., 2020).

2.6.4. GEN Bank submission accession number

After the sequencing raw data was obtained, those raw data were submitted to the NCBI's sequence read archive (SRA) portal. The NCBI was assigned an accession number ID SUB11591708.

3. Results and Discussion

3.1 Mealworms Survival Rate and Mask consumption efficiency

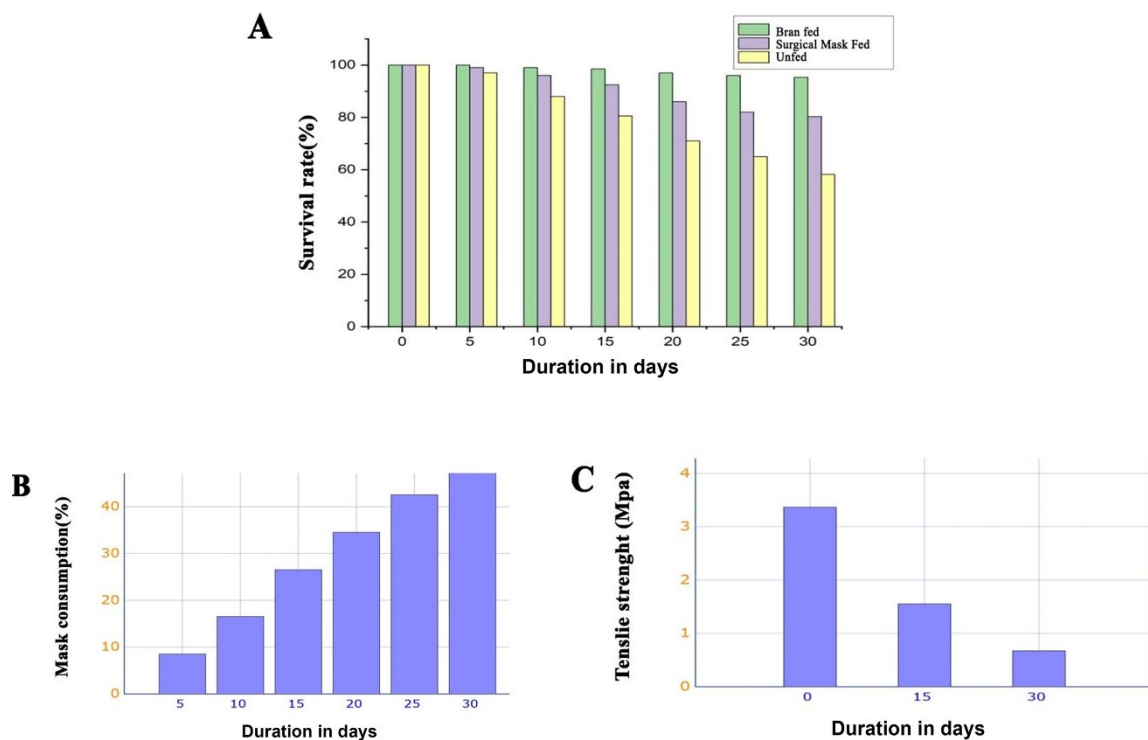


Fig.2. (A) Percentage of consumption of surgical facemasks throughout the experiment, (B)Percentage of Meal Survival rate throughout the experiment, (C) Tensile strength losses throughout the experiment

At the end of incubation, three groups of experimental survival rates of mealworms were identified; a)fed on bran, b)fed on the medical mask, and c)not fed followed by $95.35 \pm 1.5\%$, $89.25 \pm 4.5\%$, and $58.2 \pm 3.5\%$ respectively. The survival rate graph shown in Fig. 2A, the mealworm survival rate was significantly higher than the mealworms not fed and not significantly lower than mealworms fed on bran supplement. From an initial weight of 2.7g SFM and after a degradation weight was 1.28g, feedstock consumption increased progressively by the mealworm, it reached about 47.5% of SFM consumption efficiency at the end of the biodegradation experimental system shown in Fig. 2B. The results of survival and consumption rate confirmed that mealworms completed their entire life cycle (larvae, pupae, beetle & egg) and all metabolic activities after consuming the SFM chewed and penetrated during the experimental period. A recent study reported that the SR of meal worms consuming solely PP was reported to be $88.7 \pm 0.7\%$, which is not significantly different from the present study results (Yang et al., 2021). Worm mortality can be reduced by changing the diet and giving resting time.

3.2 Mechanical Strength of Mask

The tensile strength of the SFM before and after biodegradation was shown in Fig.2C. The results demonstrated that the SFM mechanical strength was drastically reduced because of worms consuming the SFM textures. The initial tensile strength of the SFM was 3.361 MPa. After the 15th day of degradation, it dropped to 1.156 MPa (65.6%) and 0.67 MPa (80%) after the 30th day. The decrease in tensile strength was evidence of the SFM biodegradation. According to the observation of these authors, (Obasi et al., 2013), Polypropylene films were subjected to soil burial for 90 days of degradation, and tensile strength losses were 40% which indicates that this study's efficiency was greater. Soleimani et al., 2020 reported that low-density polyethylene film subjected to biodegradation by terrestrial Actinobacteria species showed a maximum of 62% of tensile strength loss at end of 60 days of incubation. The tensile strength of SFM had been lost by the mealworms, it indicated that the present study was degraded in a short period and with a high tensile loss. As a result, mealworms are more capable of reducing the strength of polypropylene medical face masks.

3.3 Surface morphological studies

The surface deterioration occurred on SFM by mealworms through biting and chewing, SFM layers such as outer, middle, and inner morphologically changed during and after the biodegradation periods shown in Fig. 3. In the beginning, the fibre material appeared with a smooth surface and

interconnected with other fibres by a sponge-like structure at a consistent distance, which was promoted to holding fibres with sustained elastic strength Fig. 3A, B, C. After 15 days of biodegradation, there was a flattened biting surface noted at SFM fibre edges, which also occurred at all the layers and significant cracks and pores have appeared showed in Fig. 3D, E, F. Similar occurrences have noticed two times increased damages after 30 days of biodegradation shown in Fig. 3G, H, I. In Fig 3. showed control, it possesses a smooth surface with no pits, cracks, or damage. This analysis confirmed that SFM consumed by mealworms then leads to the downfall of fibres, the microbes in the worm gut were able to utilize the mask fibres for their growth and reproduction. In a recent study, medical masks were exposed to the soil for six months and findings revealed that the degradation of the masks did not visually change their appearance, indicating low degradation (Knicker & Velasco 2022). According to Saeed et al., 2022 the polymer's surface area adhesion of fungi and bacteria by the development of biofilms caused minor erosion, pits, and fractures. For better visualization, an optical digital microscope was used to evaluate the changes in SFM morphology shown in Fig. 4A, B, C, three layers of the SFM before biodegradation representing continuous bonding, after 15 days of degradation shown in Fig. 4D, E, F and evident breakdown of bonds and pits were identified in layers of the mask. The surface biting and penetration increased at 30 days shown in Fig. 4G, H, I. After a mealworm attack, the surface of the mask was physically weakened and easily disintegrated.

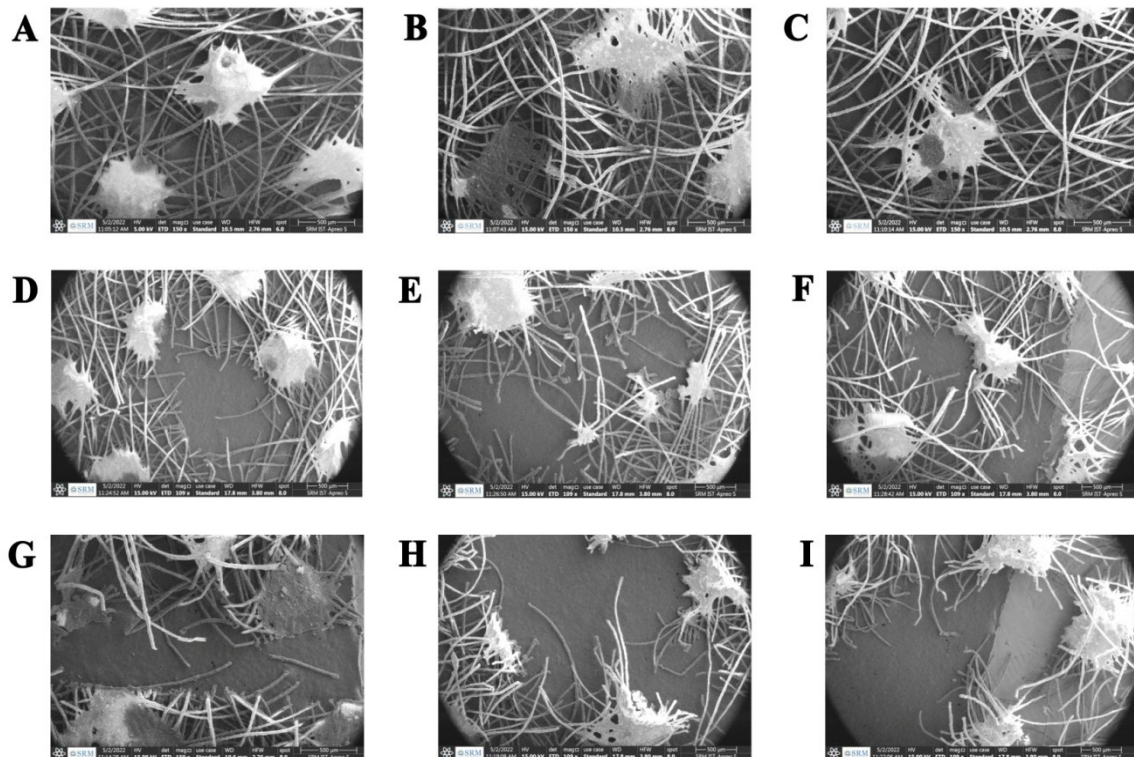


Fig.3. SEM micrographs showing the changes of surface morphology of mask by mealworms, (A, B, C) three layers of the mask before degradation, (D, E, F) three layers of masks at 15th day of degradation, (G, H, I) three layers of a mask at 30th day of degradation.

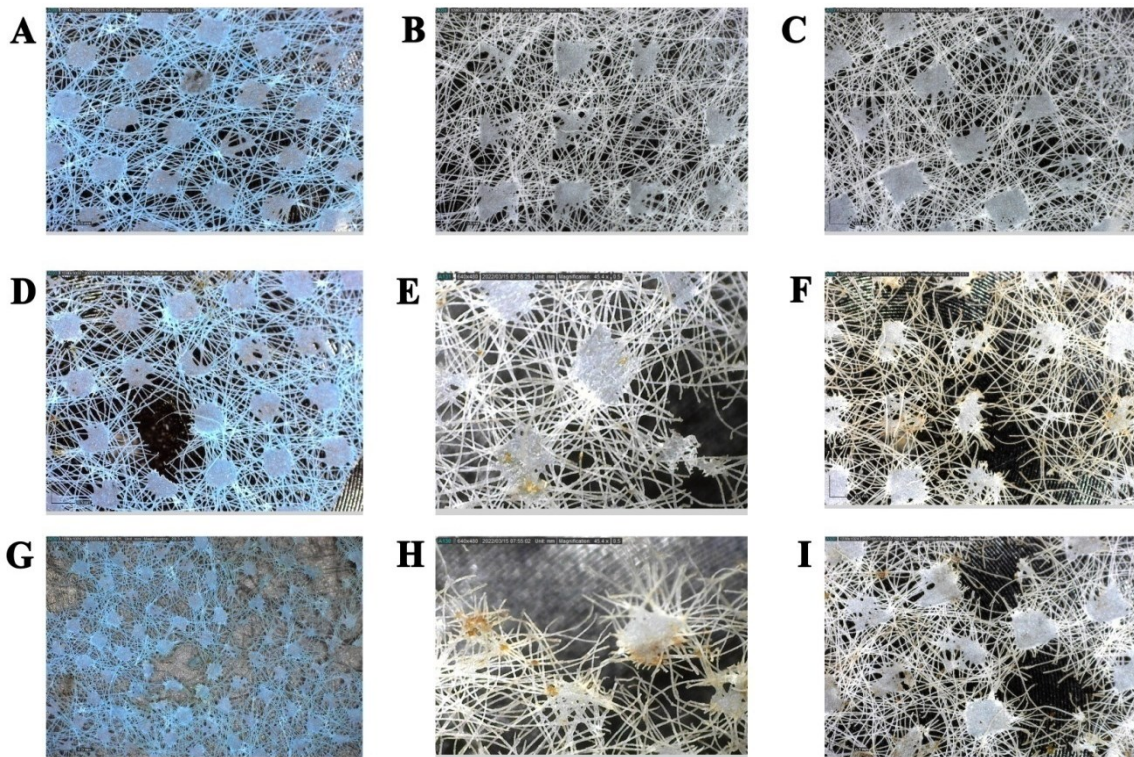


Fig.4. Digital microscope images showing the changes of surface morphology of mask by mealworms, (A, B, C) three layers of mask before degradation, (D, E, F) three layers of masks at 15th day of degradation, (G, H, I) three layers of a mask at 30th day of degradation.

3.4 FTIR spectra analysis

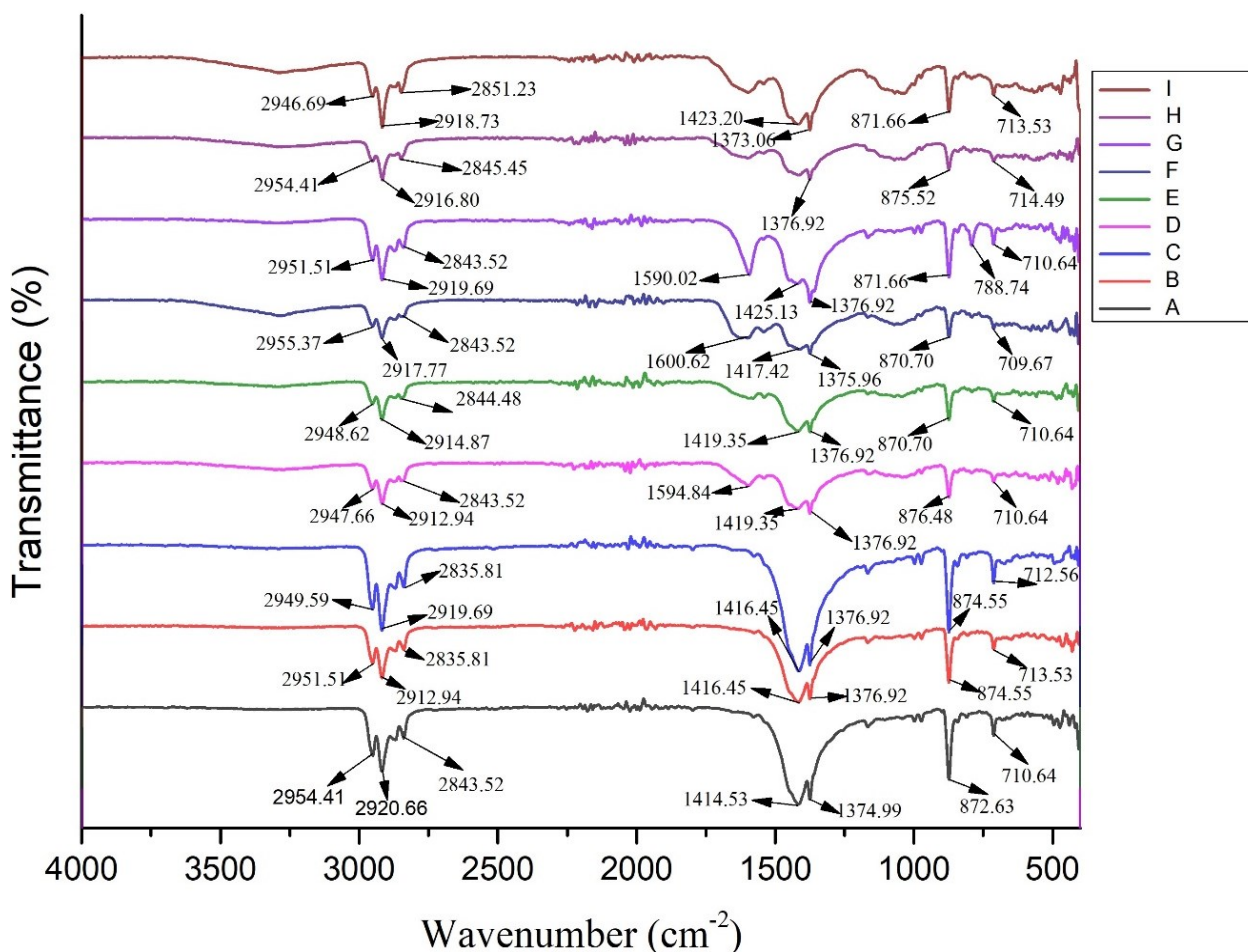
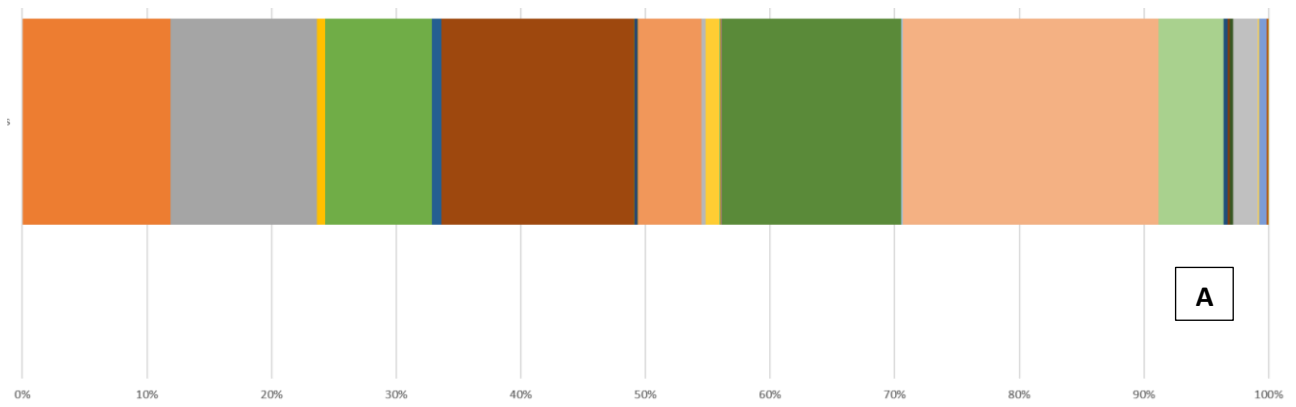


Fig.5 FTIR spectrum of surgical facemask, (A, B, C) change of chemical composition before degradation, (D, E, F) Change of chemical composition at 15th day of degradation, (G, H, I) Change of chemical composition at 30th day of degradation.

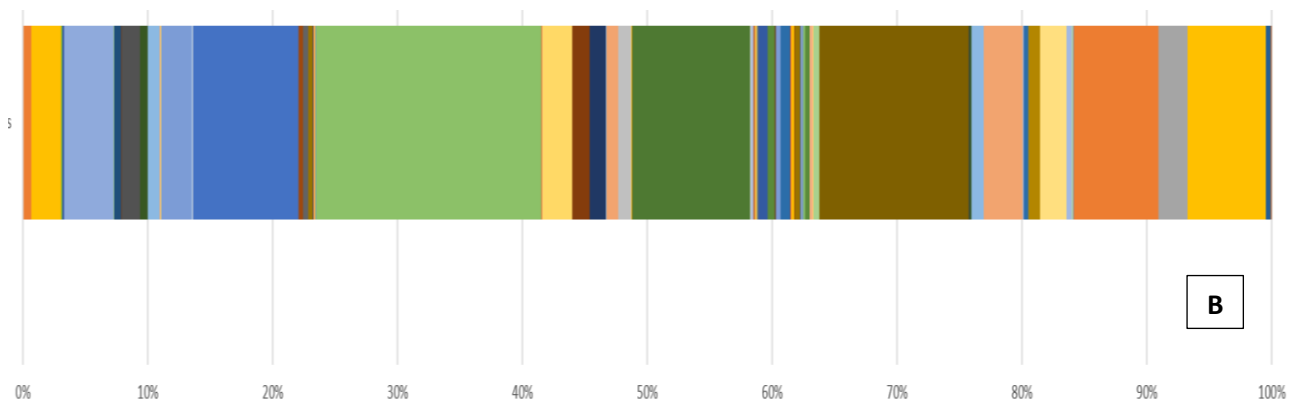
The FTIR results demonstrated that functional group changes in SFM before and after biodegradation shown in Fig. 5. The peaks changes occurred at different intensities such as 2946 cm^{-1} , 2919 cm^{-1} , 1415 cm^{-1} , 1373 cm^{-1} , 871 cm^{-1} , and 709 cm^{-1} . The functional groups befallen follows in -CH₃ stretching asymmetric and symmetric, -CH₃ bending (in-plane) asymmetric and symmetric, CCH bending symmetric, -CH₃ rocking, and CH₂ rocking. From the 0th day all peaks are normal after 15 and 30 days of incubation of mealworm with SFM intensity of peak highly reduced in each layer of the SFM; interestingly, at the end of 30th-day peak intensity decreased greater than 15 days. The enlargement of peaks at 2500-3500 cm^{-1} is connected with the hydrogen bond of hydroxyl groups and/or carboxylic acid groups, proposing an alteration from hydrophobic to more hydrophilic surface possessions (Yang et al., 2018). The overall peak changes confirmed that SFM is evidence of degradation by mealworms. The FTIR confirmed that the shortening of peaks was caused by mask polymer breakdown.

3.6 Worm gut microbes



A

- | | | | | | | | |
|-----------------------|---------------------------|-----------------------|-------------------------|--------------------|--------------------|--------------------|----------------------|
| ■ Acidobacteriia | ■ Actinobacteria | ■ Alphaproteobacteria | ■ Anaerolineae | ■ Ardentecatenia | ■ Bacilli | ■ Bacteroidia | ■ Betaproteobacteria |
| ■ Candidatus Babeliae | ■ Candidatus Brocadiaceae | ■ Chitinophagia | ■ Chlamydiia | ■ Chloroflexia | ■ Clostridia | ■ Coriobacteriia | ■ Cytophagia |
| ■ Deinococci | ■ Deltaproteobacteria | ■ Elusimicrobia | ■ Epsilonproteobacteria | ■ Erysipelotrichia | ■ Fibrobacteria | ■ Fimbriimonadia | ■ Flavobacteriia |
| ■ Fusobacteriia | ■ Gammaproteobacteria | ■ Gemmatimonadetes | ■ Ignavibacteria | ■ Lentisphaeria | ■ Mollicutes | ■ Negativicutes | ■ Nitrospira |
| ■ Oligoflexia | ■ Opitutae | ■ Phycisphaerae | ■ Planctomycetia | ■ Rubrobacteria | ■ Saprospira | ■ Sphingobacteriia | ■ Spirochaetia |
| ■ Tepidiformia | ■ Thermolephilia | ■ Thermomicrobia | ■ Tissierellia | ■ Verrucomicrobiae | ■ Vicinamibacteria | | |



B

- | | | | | | |
|-----------------------|----------------------------------|-----------------------------|-------------------------------|-------------------------|-------------------------|
| ■ Acetobacter | ■ Achromobacter | ■ Acidaminococcus | ■ Acinetobacter | ■ Actinomadura | ■ Actinomyces |
| ■ Actinoplanes | ■ Aeromicrobium | ■ Aeromonas | ■ Aggregatibacter | ■ Akkermansia | ■ Alcaligenes |
| ■ Alcanivorax | ■ Alistipes | ■ Altererythrobacter | ■ Alteromonas | ■ Anaerococcus | ■ Anaerohalospaera |
| ■ Anaeromyxobacter | ■ Anaerostipes | ■ Anoxybacillus | ■ Arachidococcus | ■ Arcobacter | ■ Asticcacaulis |
| ■ Atopobium | ■ Azoarcus | ■ Azospira | ■ Azospirillum | ■ Bacillus | ■ Bacteroides |
| ■ Bartonella | ■ Bdellovibrio | ■ Bifidobacterium | ■ Blastochloris | ■ Blattabacterium | ■ Blautia |
| ■ Bosea | ■ Brevefilum | ■ Brevibacillus | ■ Brevibacterium | ■ Brevundimonas | ■ Bythopirellula |
| ■ Campylobacter | ■ Candidatus Atelocyanobacterium | ■ Candidatus Babela | ■ Candidatus Cloacimonas | ■ Candidatus Hodgkinia | ■ Candidatus Koribacter |
| ■ Candidatus Kuenenia | ■ Candidatus Promineofilum | ■ Candidatus Protochlamydia | ■ Candidatus Puniceispirillum | ■ Candidatus Solibacter | ■ Capnocytophaga |
| ■ Castellaniella | ■ Cellvibrio | ■ Chitinophaga | ■ Chloroflexus | ■ Chryseobacterium | ■ Chryseolinea |
| ■ Citrobacter | ■ Clostridioides | ■ Clostridium | ■ Collinsella | ■ Comamonas | ■ Conexibacter |
| ■ Coralococcus | ■ Corynebacterium | ■ Cupriavidus | ■ Curtobacterium | ■ Deinococcus | ■ Delftia |
| ■ Dermabacter | ■ Desulfallas | ■ Desulfobacter | ■ Desulfocapsa | ■ Desulfoglaeba | ■ Desulfomicrobium |
| ■ Desulfosarcina | ■ Desulfotomaculum | ■ Desulfovibrio | ■ Devosia | ■ Dialister | ■ Dietzia |
| ■ Dolosigranulum | ■ Dyadobacter | ■ Eggerthella | ■ Elusimicrobium | ■ Enterococcus | ■ Erythrobacter |
| ■ Escherichia | ■ Exiguobacterium | ■ Faecalibacterium | ■ Faecalitalea | ■ Ferrimonas | ■ Ferrovibrio |

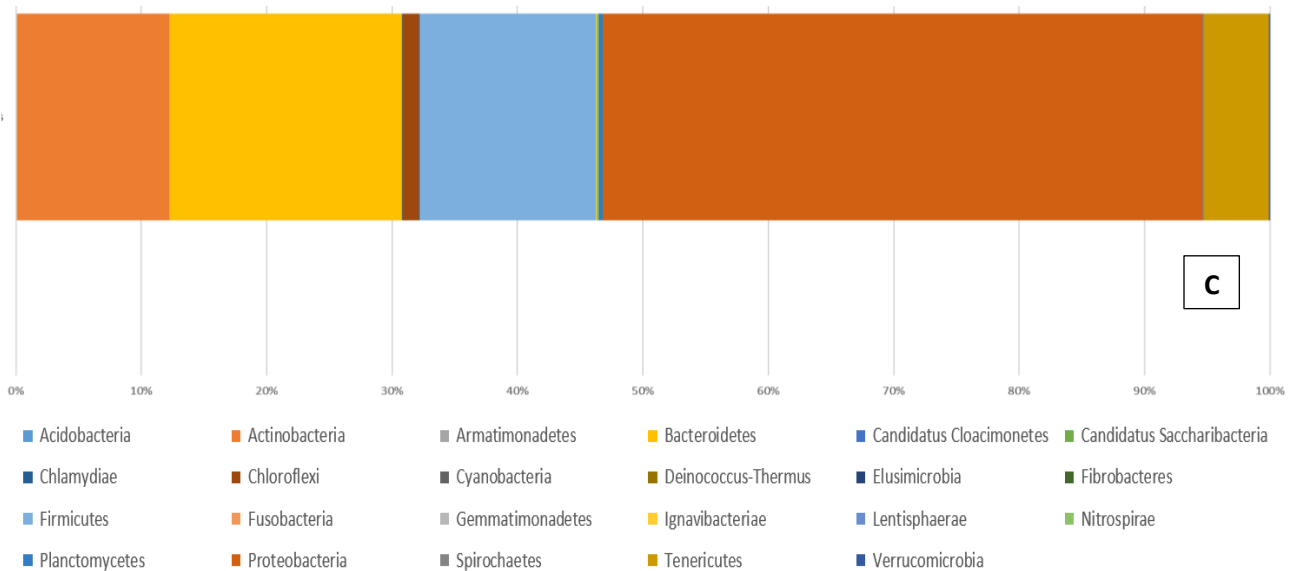


Fig.6. The bar graph depicting the composition of the microbial community revealed in the mask-eaten mealworm gut. Taxonomic levels of metagenomes: (A) Class, (B) Phylum, (C) Genus

The bacterial diversity of mealworm (*Tenebrio Molitor*) by Illumina MiSeq 16rRNA amplicon yielded 10,7227 reads and a relative abundance of bacterial communities in the worm gut of *Tenebrio Molitor* is categorized into 23 different phyla, 46 classes and 247 genera. The results of metagenomic demonstrate that the top 10 are classified. The most abundant bacterial OTUs class (%) in worm gut sample is as follows (Fig. 6(A)), *Gammaproteobacteria* (21), *Betaproteobacteria* (15), *Flavobacteriia* (14), *Actinobacteria* (12), *Alphaproteobacteria* (12), *Bacilli* (9), and *Mollicutes* (5). Similarly, the results of the genus-level analysis for the worms' gut were as indicated in Fig. 6(B). At the genus level, gut bacteria belong to *Delftia*, *Pseudomonas*, *Flavobacterium*, *Chryseobacterium*, *Spiroplasma*, *Stenotrophomonas*, *Bacillus*, *Rhodococcus*, *Brevundimo*, and *Acinetobacter*. Further, the Phylum level analysis was down which was displayed in Fig. 6(C). At the Phylum level gut bacteria predominantly belong to *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Tenericutes*. *Actinobacteria* have significant ecological roles in the environment, such as complicated polymer degradation, chemical recycling, and the production of bioactive molecules (Mawang et al., 2021). Recently, *Acinetobacter* from the *Galleria mellonella* gut genus appeared to be taking part in the biodegradation of polyethylene (Cassone et al., 2020).

At the order level, a diverse group of bacterial order found as *Burkholderiales*, *Flavobacteriales*, *Pseudomonadales*, *Rhizobiales*, *Micrococcales*, *Bacillales*, and *Clostridiales*. Most abundant families were *Flavobacteriaceae*, *Comamonadaceae*, *Pseudomonadaceae*, *Microbacteriaceae*, *Spiroplasmataceae*, and *Xanthomonadaceae* as dominant families in the gut. Biosurfactants are produced by *Pseudomonas* and *Bacillus*, which form a biofilm on the polypropylene surface, which has a greater carbohydrate and protein content. *Pseudomonas* and

Bacillus exploited polypropylene as a carbon source. It has capable of converting polypropylene to a hydrophilic state, which indicates deterioration (Arkatkar et al., 2010). *Rhodococcus sp.* were able to use PP microplastic for growth, as evidenced by a decrease in polymer mass (Auta et al., 2017). Under aerobic circumstances, *Delftia tsuruhatensis* degrades terephthalate (Shigematsu et al., 2003), while *Delftia sp.* AN3 degrades aniline (Zhang et al., 2008). In this study, the majority of gut bacteria are capable of degrading the polymers (Miri et al., 2022, Ghatge et al., 2020; Ali et al., 2021) those results revealed the abundance and diversity of the bacterial communities that help in the biodegradation process of medical face masks. Pilot scale and frass study will determine in future study.

4. Conclusion

This study investigated the biodegradation of surgical facemask by mealworms, which laid the foundations for the construction of efficient plastic-biodegrading reactor with mealworm. According to the results of this approach, mealworms can chew, burrow and consume the SFM material and gut bacterial communities can degrade the polymers. Mealworms consumed 47.5% of the surgical face mask over the course of 30 days, and by that time, the mask had lost 80% of its tensile strength, which indicating that the mask had biodegraded. Using digital microscopy, Fourier transform infrared spectroscopy, and scanning electron microscope imaging, as well as instrumentation analysis, the biodegradation of the mask was verified. The masks lost their strength by the end of the degradation period due to the mealworms consuming them at an increasing rate, which demonstrates the effectiveness of degradation. SEM and digital microscope results show the breakage & cracks of SFM fiber due to mealworms. In the gut, several bacterial communities were found and the majority of those identified microorganisms had unique properties to use polymer-based carbon sources as sole energy sources.

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