

Biodegradation of Polypropylene By *Pseudomonas Aeruginosa* Isolated From Wastewater Associated Soil; A Potential Method To Eliminate The Plastic Pollution To Save Ecosystem

Vijayalakshmi Selvakumar

Kangwon National University

Preethi Gopalsamy

Askoscen Probiotics (R&D)

Karnan Muthusamy

National Institute of Animal Science

Thenmozhi Manivel

Askoscen Probiotics (R&D)

Ramachandran Chelliah

Kangwon National University

Dinesh Kumar Sundarraj

Bharathidasan University

Sathammai Priya Narayanan

Affiliated to Bharathidasan University

Deog-Hwan Oh (✉ deoghwa@kangwon.ac.kr)

Kangwon National University <https://orcid.org/0000-0002-7472-0436>

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Abstract

The face mask introduction in SARS-CoV-2 pandemic situation, one of the precautionary measure to reduce the rate of transmission of infection from person to person. There are many type of of face masks enter into our global market with various size and designs. Among those, surgical mask belongs to secure an important place and this aimed to evaluate the degrading efficiency disposable face masks (single use face masks). The present study dealt with, biodegradation of face masks which is made of polypropylene with the help of bacterial strain *Pesudomonasaeruginosa* isolated from the plastic waste dumping sites in an around the Tiruchirappalli, India. The bacteriacould degrade PP mask via the formation of biofilm on a solid medium. To degrade the PP, the mask pieces were incubated with the culture of *P. aeruginosa* in three different solid and liquid medium (nutrient agar, Bushnell Haas agar and mineral salt medium) for 30 days at 37°C. The microbial degradation (up to 33% of weight reduction of PP films within 30 days) was proved by surface changes along with the variation in the intensity of functional groups as well as carbonyl index variations using Field-Emission Scanning Electron Microscopy (FE-SEM) and Fourier Transform Infra-Red Spectroscopy (FTIR) analysis. These results suggested that *P.aeruginosa* strain can prove to be a suitable candidate for polypropylene mask biodegradation without causing any impairment to our health or environment.

Introduction

The ongoing quick universal spread of SARS-CoV-2 pandemic situation has led to the critical efforts to diminish transmission, leading to significant and extensive socioeconomic disruption (Sohrabiet al, 2020). On or after 3rd March 2020, the confirmed COVID-19 cases are about 90,870. From that confirmed cases 80,304 were confined to China. The remaining 10,566 cases were belongs to other 72 countries including Japan, United Nations and Australia of these cases 166 were fatal (the Japan, Philippines, Italy, France, Iran, Australia, Korea, Thailand and the United Nations). It is important to note that these data's were depicted laboratory – confirmed results only (WHO, 2020). Near July 2020, over 10 million cases have been reported globally, as well as around 50,000 death cases were recorded with going on spread in most of the parts of our earth (COVID, 2020). The corona virus (COVID-19) pandemic has caused the development of emergency responses and progressive moves were aimed to deal with and overcoming COVID-19 virus while reduces the exposures and supporting healthy life of all people around the world. One of such precautionary measure designed to minimize the community spread of COVID-19 transmitted via droplet is the compulsory wearing of plastic- based personal protective equipment (PPE) by the healthcare workers and face masks for the public leaving their homes for important necessary reasons (Benson et al, 2021).

The use of face masks by public people can minimizes the community spread of COVID-19, but it also develops severe troubles for our ecosystem. The number of face masks used in 49 countries of Asia was estimated from the COVID-19 pandemic database on July 31, 2020. The result of estimated value of the daily face mask use in Asia is 2.2 billion (Sangkham, 2020). There are three types of face masks can use in current situation i) Respirator Mask (N95, FFP2), ii) Surgical or Medical mask and iii) Non-certified

disposable- mask (cloth mask)(Dharmaraj et al, 2021). Top and bottom layers of disposable (single use) face masks were made up of woven fabric and the middle layer were meltdown polypropylene polymers. It was estimated that the authors models were generated 6.88 billion (approximately 206,470 t) around the world each day, which are eventually sent to land filling or incinerated after use(Nzediegwu and Chang, 2020). Also, the used face masks can be inappropriately spotted everywhere from the city streets to local parks, due to its lightweight, the rain water and wind can move this mask freely into city streets or rivers and oceans, where it was fragmented into microplastics(Prata et al, 2020; Saberian et al, 2021). In addition, the face masks used as single purpose are made up of non- biodegradable and take more than hundreds of years to break down in the environment(Saberian et al, 2021; Dhawan et al, 2019). As a result, a multidisciplinary approach is needed immediately to fight against the COVID-19 pandemic as well as to reduce the environmental troubles associated with the disposal of used personal protective equipment's.

In order to overcome the negative environmental issues due to the pandemic situation many authors works to reduce the pandemic-generated waste management in an innovative way. One of the best and eco-friendly approaches is the use of microorganisms for waste management. The investigation of microorganisms towards polypropylene and polyethylene degradation is well studied by several authors. Biodegradation is one of the best ability of microorganisms to persuade abiotic degradation by physical, chemical or enzymatic action(Albertsson et al, 1987). For biodegradation process microorganisms were well suited for their ability to possess enzymes and the small size make them able to contact with the surface of the plastic(Devi et al, 2016). The microbial exo-enzymes can break the complex polymers to simpler one which was then easily pass through the semi-permeable outer membranes and then it can be used as carbon and energy sources of the microbes(Gu, 2003). A number of bacterial and fungal genera were reported by several researchers for microplastic degradation process including *Pseudomonas* sp.(Gupta and Devi, 2020; Vague et al, 2019; Muniret al, 2018; Roberts et al, 2020). The ability of microbes to mediate plastic degradation directly there are several gut bacterial species were isolated from worms and verified. *In vitro* depolymerization of PE can be done by the bacterial strains namely *Enterobacterasburiae* YT1 and *Bacillus* sp. YP1 isolated from the waxworms(Yang et al, 2014). *Paeruginosa* isolated from the guts of superworms can have extraordinary biodegradative ability on four types of plastics (PE, PP, PPS and PP). On the other hand the efficiency of biodegradation varied from one plastic to another, the fastest biodegradation occurs on PE(Lee et al, 2020). The present study was aimed to evaluate the biodegradation ability of *Paeruginosa* on surgical face mask with eco-friendly manner and to save our environment from pandemic- generated wastes.

Materials Methods

Chemicals and Reagents

The soil sample was collected from the plastic waste dumping sites around the Tiruchirappalli district, Tamil Nadu, India. Then the sample was carefully transported for our laboratory in order to isolate the plastic degrading *Pseudomonas* sp. The used face masks were not allowed to use in our laboratory test due to the laboratory rules and regulations during this covid-19 pandemic situation. Therefore, clean

surgical face masks were used in the experiments (ear loops were removed from the masks). The chemicals used in our study were purchased from Hi-media laboratory, India.

Isolation of bacteria from soil sample

Serially diluted soil sample was inoculated onto the previously prepared sterile nutrient agar medium (NAM) and incubated at 37°C for 24-48 hours in order to isolating bacteria from the sample. Individual colonies obtained after incubation were used for the preparation of pure culture. The pure colonies were subjected to Gram's staining for primary identification. Then the isolated bacterium was maintained on Nutrient agar at 4°C in our laboratory for performing the biodegradation experiment in our present study.

Biochemical identification of the bacterium

The isolated bacterial strain was identified based on the basic biochemical tests. The biochemical tests were conducted on 24 hours old cultures grown on Nutrient agar medium at 37°C. Indole test was performed in order to determine their tryptophan utilization ability according to the supplier protocol. Catalase analysis was conducted according to Kumari et al, 2013. Citrate metabolism was studied by inoculating bacterial colonies on Simmon's Citrate agar (According to supplier protocol). Oxidase test was conducted in order to check the bacteria that possess the enzyme cytochrome oxidase. Sugar fermentation test (Glucose, Lactose, and Sucrose) was studied by inoculating bacteria onto the TSI agar medium (According to supplier protocol). Motility and H₂S production test were conducted using SIM medium (According to supplier protocol). The MR-VP test was done to identify the isolate as facultative anaerobe by their sugar fermentation profiles. Also the bacterium was streaked onto the Cetrimide agar for further clarification of the bacterial identification processes.

Investigation of Biodegradation of Polypropylene Mask

Pretreatment of Polypropylene mask pieces

The metal strips and ear loops were removed from the masks. Then the masks were pretreated by the method previously described by Gupta and Devi (2020). For this process the masks were cut into (3×3 cm) pieces and soaked into the solution containing 7ml of Tween- 80, 10ml of bleach and 983ml of sterile water for 30-60 minutes with continuous stirring. Then the mask pieces were washed with distilled water at room temperature. Then the pieces were surface sterilized with 70% ethanol for 30 minutes and allowed to dry at 45°C. After drying the mask pieces were weighed using the weighing balance and initial weight of the pieces was noted.

Biodegradation Experiment

The bacterium *Paeruginosa* was aseptically inoculated by carpet culture method on to Nutrient agar, BHM agar and MSM agar plates. After inoculation the pretreated polypropylene mask pieces (0.1 g) were aseptically placed over the inoculated plates using sterile forceps and incubated at 30°C and 37°C separately for one month (30 days). The agar plates containing the same quantity of mask pieces without bacterial inoculation were maintained at the same temperature as negative control. Also the

pretreated mask pieces were incubated with *Paeruginosa* in NB, BHM broth and MSM broth (each media contain 0.1g of mask pieces) and the liquid media containing same quantity of mask pieces without culture maintained at the same duration as control.

Experimental set up

T1A- 0.1 g of pretreated mask pieces+ *Paeruginosa* (in NA plates)

T1B- 0.1 g of pretreated mask pieces+ *Paeruginosa* (in Nutrient Broth)

C1A- 0.1 g of pretreated mask pieces in NA plates (without inoculums)

C1B- 0.1 g of pretreated mask pieces in nutrient broth (without inoculums)

T2A- 0.1 g of pretreated mask pieces+ *Paeruginosa* in Bushnell Haas agar plates (BHM agar)

T2B- 0.1 g of pretreated mask pieces+ *Paeruginosa* (in BHM Broth)

C2A- 0.1 g of pretreated mask pieces in BHM agar plates (without inoculums)

C2B- 0.1 g of pretreated mask pieces in BHM broth (without inoculums)

T3A- 0.1 g of pretreated mask pieces+ *Paeruginosa* in Mineral Salt agar plates(MSM agar)

T3B- 0.1 g of pretreated mask pieces+ *Paeruginosa* (in MSM Broth)

C3A- 0.1 g of pretreated mask pieces in MSM agar plates (without inoculums)

C3B- 0.1 g of pretreated mask pieces in MSM broth (without inoculums)

Monitoring the planktonic growth of *P.aeruginosa* strain

The growth of the *Paeruginosa* in the liquid media such as NB, BHM and MSM owing to the presence of PP film and the characteristics of the biofilm produced on the surface of the polymer throughout the period of 30 days (1 month) with 15 days interval was scrutinized as explained below. Before analyzing the biofilm, polymer samples were taken away from the media by means of sterile forceps and cautiously rinsed with sterilized distilled water in order to confiscate the slackly adhered bacteria. Then the biofilm was removed from the surface of the polymer by subjecting the sample to a mild water bath sonication in 1 ml of 0.85% saline solution, for a total of 4 min at 1 min intervals. Thus obtained saline solution was serially diluted up to 10^{-7} and the aliquots were spreaded on Nutrient agar and the number colonies were calculated as CFU/ml (Arkatkaret *al*, 2010).

Viability testing of Surface attached bacteria

The viability of bacterial strains attached on the surface of the PP films was tested as per the method described by Andes *et al*(2004). PP films were removed from the media at 10 days interval and washed with sterile distilled water then it was subjected to mild bath sonication with 0.85% saline solution. The

obtained solution was serially diluted and plated on NA and incubated at 35°C for 48 h. Number of viable bacteria species were obtained as CFU/ml.

Assessment of cell surface hydrophobicity of *P.aeruginosa*

The hydrophobicity of the bacterial cell surface was determined using the BATH test by little modifications (Harshvardhan and Jha, 2013) was explained as follows: the bacteria were cultured in NB medium until the growth of bacteria reach the mid-log stage. Then it was centrifuged and washed twice with phosphate urea buffer (PUM containing 17g K₂HPO₄, 7.26g KH₂PO₄, 1.8g Urea and 0.2g MgSO₄.7H₂O per liter). After washing the cells were resuspended in PUM buffer to an OD at 400nm (OD₄₀₀) value of 1.0-1.2. Aliquots of 1.2 ml of above obtained suspension were added to a series of test tubes containing increased volumes of xylene (ranges from 0-0.2ml) and shake well for 10 min then allowed to stand for 2 min in order to facilitate phase separation. The OD_{400nm} of the lower aqueous phase was then measured and noted as OD₁. The cell surface hydrophobicity was stated as the percentage of the xylene –bound cells described as the fraction of the cells expelled from the aqueous phase. Plain PUM buffer served as the blank. Therefore, the percentage of adhering cells are represented using the following formula: Cell Surface Hydrophobicity (%) = ((OD₀-OD₁₀)/ OD₀) × 100 (OD₀ is the initial OD of the aqueous phase).

Determination of Dry weight of the recovered Mask pieces

The residual mask pieces were recovered from the culture plates after the completion of one month incubation. The bacterial biomass adhering on the polypropylene mask surface was washed with the sodium dodecyl sulfate (SDS) solution (2%v/v) for 2 hours. After the pieces were rinsed with distilled water in order to remove any impurities on the surface and dried overnight at 45°C. Then the degraded mask pieces were weighed using weighing balance and the percentage of bio degradation (weight loss) was calculated using the following formula

$$Biodegradation (\%) = \frac{Initialweight - Finalweight}{Initialweight} \times 100$$

Surfaceanalysis of mask pieces

Scanning electron microscopy

The mask pieces treated with the bacterium *P.aeruginosa* for 30 days were removed from the medium and subjected to FE-SEM for the observing biofilm formation and surface erosion. Bacterial morphology of biofilm on PP mask surface was observed. Before the observation, the treated mask pieces were washed with 0.01M phosphate buffer solution for 2 min in order to remove the excess medium adheres with the bacterial colonies. For observing the surface alteration of treated mask pieces, the pieces were washed with 2% SDS along with warm water for 10-20 min to facilitate the complete removal of bacterial biomass. After the process was completed, the mask pieces were fixed in 4% glutaraldehyde at 4°C for 2 hours and dehydrated with 50% ethanol for 30 min. Then the recovered mask pieces were incubated

overnight in 70% ethanol at room temperature. Then the pieces were dried, mounted and sputter coated with gold for 40s and scanned through FE-SEM.

Fourier Transform Infra- Red Spectroscopy (FTIR analysis)

The structural and functional group alteration in the polypropylene mask pieces were analyzed by FTIR spectra in the frequency range of 4000-400 cm^{-1} were used at a 1 cm^{-1} resolution. The relative absorbance intensities of the keto carbonyl bond, ester carbonyl bond, terminal double bond (vinyl) and the internal double bond with methyl bond were evaluated using the following formula: Keto carbonyl bond Index (KCBI) = 1715/1465; Ester Carbonyl bond index (ECBI) = 1740/1465; Vinyl Bond Index (VBI) = 1650/1465 and the Internal Double Bond Index (IDBI) = 1908/1465. The degree of biodegradation was measured using the Carbonyl index because its value depends on the degradation. The percentage of crystallinity of the polypropylene mask film was measured according to the method described by Zerbi et al (1989) (Zerbi et al, 1989).

Results And Discussion

Isolation and Identification of *Pseudomonas* sp.

Typical colonies of bacterial isolates were sub-cultured on nutrient agar and incubated at 37°C for 24 h. Isolated identification was performed according to the morphological, staining reaction, cultural and various biochemical characteristics by following the Bergey's Manual of Systematic Bacteriology. Based on presumptive identification of bacteria by Gram's staining protocol *Pseudomonas* sp. was identified as gram negative rod. Depending upon the different biochemical characterization the isolated bacteria was identified as *P. aeruginosa* strain (Fig. 1). It was further confirmed by streaking the strain onto the Cetrimide agar which is selective media for isolating *Paeruginosa*. The isolates were showed green pigmented, circular and opaque colony morphology after incubation. Similar to our study, the another author reported that the isolated bacteria were also grown in milk agar with cetrimide for the preliminary detection of *Pseudomonas* spp. (Szita et al, 1998). Based on the results it was reasonable, to identify the isolated bacterium as *P. aeruginosa*. This identified strain was used for the biodegradation of pre-treated polypropylene mask pieces. The bacterium *Paeruginosa* isolated from Sisdol land fill site and Sanothimi household garbage site soil in Nepal has similar biochemical properties (Badahit et al, 2018). It has been reported that *P. aeruginosa* ISJ14 showed tremendous efficiency to degrade low density polyethylene (LDPE) in BHM (Gupta and Devi, 2020). In contrast, the present study reports for the first time that *Paeruginosa* strain is capable of biodegradation of surgical face mask made up of hardy PP like substance.

Cell surface hydrophobicity of bacteria

The capability of bacteria to use any substrate depends upon its development on and adherence to that substrate. The adhesion ability of bacteria to either hydrophilic or hydrophobic surfaces is addressed by a number of physical factors, together with the forces which help the bacterium to adhere to solid

substrates, properties of that substrate and the nature of bacteria. For the most part, a hydrophobic bacterium favors a hydrophobic surface for adhesion, though the inverse is substantial for bacterium with hydrophilic properties (Gupta and Devi, 2020). In the current investigation, the hydrophobicity of mid-log phase cells of *Paeruginosa* at 0.2ml concentration of xylene, we showed a significance increase in hydrophobicity (30.39%) (Table 1 and Fig. 2). These results are in agreement with Gupta and Devi (2020), who observed that bacterial cells in log phase are more hydrophobic in nature. Previous study also documented similar findings, where, maximum increase in hydrophobicity i.e, the isolates *Kocuriapalustris*M16 and *Bacillus subtilis* H1584 showed approximately 24% turbidity reduction at 0.25 µl and a maximum reduction of turbidity 32% at 150µl concentration of hydrocarbon like hexadecane (Harshvardhan and Jha, 2013). Another recent research also reported that the hydrophobicity of *L.monocytogenes* strain CICC 21332 showed lowest hydrophobicity (12.5%) and the strain FSIS 57034 displayed highest percentage of CSH (74.81%) at 1ml of xylene concentration (Fun et al, 2020).

Table-1. Cell surface Hydrophobicity of *Paeruginosa*

S. No	Conc. Of Xylene	OD value	%Hydrophobicity
01	0	1.02	0
02	0.05	0.89	12.74
03	0.10	0.82	19.61
04	0.15	0.76	25.49
05	0.20	0.71	30.39

Growth of *Paeruginosa* planktonic cells and surface attached cells on PP films

The growth pattern of bacteria attached on the surface of PP film was examined in planktonic cells and viable count of surface attached bacterial species (Arkatkar et al, 2010; Andes et al, 2004). The data illustrated in Figures 3 and 4 show a surface attachment pattern of bacteria on the PP film. The bacterial cell growth was characterized by a precipitous raise in planktonic cells after 15 and 30 days of incubation and is also revealed by a raise in the surface-attached bacterial mass. After 15-20 days of incubation the growth of *Paeruginosa* was able to reach a steady, almost 10^7 CFU/ml in all the liquid media used. In all the three media, biofilm formation patterns exhibited analogous to that of the growth of planktonic cells. The results recommend that *Paeruginosa* cells exhibit better colonization, formation of biofilm and fractional biodegradation of PP film in all the three media. These observations not only specify high affinity of *Paeruginosa* cells for the PP film but also increase the possibilities of *Paeruginosa* cultures to form biofilms by hydrophobic interactions at low levels of carbon availability. In considering of concurrence model, the course of action of biofilm formation by microbes is began when the planktonic growth of cells achieves high density and help for the attachment of bacterial cells to a surface through

cell signaling, likewise bringing about the development of microcolonies that will eventually frame the mature biofilms (Costerton et al, 1999). This biofilm population is diverse and comparatively steady intended for an extensive period of time (Bodtker et al, 2008). Conversely, this circumstance is not inevitably familiar to the entire biofilm forming bacterial species. In our investigation, PP films provided as a substrate for the attachment and biofilms formation as well as a source of carbon for *Paeruginosa* strain. The extended incubation of *Paeruginosa* with PP films resulted in a solid biofilm on PP surface which may lead to the fractional deduction of this polymer. Likewise, the formation and maintenance of active biofilm throughout the 30 days of incubation possibly will perhaps by the utilization of low molecular mass materials in the polymer. Similar findings were recorded in earlier studies such as biodegradation of untreated films of polyethylene by *P.putita* IRN22, *Micrococcus luteus* IRN20, *Acinetobacter pittii* IRN19 (Montaveret et al, 2019) and other bacterial genera including *Delftia*, *Stenotrophomonas* and *Comamonas* (Peixoto et al, 2017) and *Galleria melonella* isolated from the gut of the wax worm also have been established the capabilities of PE degradation (Cassone et al, 2020).

Table-2. Viability of *Paeruginosa* on different day interval

S.No	Days	Cfu/ml in NB(10 ⁻⁷ dilution)	Cfu/ml in BHM (10 ⁻⁷ dilution)	Cfu/ml in MSM (10 ⁻⁷ dilution)
1	0	1.07×10 ⁵	0.8×10 ⁵	0.92×10 ⁵
2	10	2.01×10 ⁶	1.9×10 ⁶	1.92×10 ⁶
3	20	2.02×10 ⁷	1.95×10 ⁷	1.99×10 ⁷
4	30	1.97×10 ⁷	1.98×10 ⁷	1.98×10 ⁷

Table-3. Monitoring the planktonic growth of *Paeruginosa* (log cfu/ml)

Media	0 DAY	15TH DAY	30TH DAY
NB	0.9×10 ⁵	2.0×10 ⁵	2.08×10 ⁵
BHM	1.1×10 ⁷	1.9×10 ⁷	2.07×10 ⁷
MSM	1.2×10 ⁷	2.0×10 ⁷	2.12×10 ⁷

Dry Weight Determination of recovered Polypropylene Mask pieces

The residual polypropylene mask strips were recovered from the media after one month (30 days) of incubation. The adhered media and bacterial biomass were washed with appropriate solutions and allowed to air dry. The air dried films were weighed and the final weight loss for *Paeruginosa* in respective

culture media (NA, NB, BHM agar, BHM broth, MSM agar and MSM broth) has been provided in table 4 and Fig. 5a and 5b. The growth kinetics of the *Paeruginosa* strain in media demonstrated its colonization on PP mask pieces surface consequently, reduction in weight was observed as a result of utilization of pp mask film as nutrient source. Our study describes the potency of the *Paeruginosa* strain was very high because it shows 26 and 33 % of weight reduction in case of PP films placed in NB and NA, 28 and 31% weight reduction observed in BHM agar and BHM broth and 24 and 23% weight reduction was noted in MSM agar and MSM broth after one month duration (30 days). Similar findings were reported by several other researchers on LDPE surface (Kapriet al, 2010; Shah et al, 2015; Sahet al, 2010). Whereas, the LDPE film treated by *Paeruginosa* strain showed 20% reduction after 120 days of treatment also has been reported (Kyawet al, 2012). Several other researchers also reported the same findings on LDPE surface (Kapriet al, 2010; Shah et al, 2015; Sahet al, 2010). Whereas, the LDPE film treated by *Paeruginosa* strain showed 20% reduction after 120 days of treatment also has been reported (Kyawet al, 2012). However, the recent study provide strong evidence for PP microplastic degradation by *Rhodococcus* sp. strain 36 with 6.4% degradation and *Bacillus* sp. strain 27 with 4% also depicted the good degradation capacity of bacterial strains (Autaet al, 2018).

Table-4. Weight reduction of PP mask pieces after 30 days treatment with *Paeruginosa* on different media sources

Media sources	Initial weight (g)		Final weight(g)		% Weight reduction	
	Control	Treated	Control	Treated	Control	Treated
Nutrient agar	0.1	0.1	0.1	0.074	0.0	26
Nutrient broth	0.1	0.1	0.1	0.067	0.0	33
BHM agar	0.1	0.1	0.1	0.072	0.0	28
BHM broth	0.1	0.1	0.1	0.069	0.0	31
MSM agar	0.1	0.1	0.1	0.076	0.0	24
MSM broth	0.1	0.1	0.1	0.077	0.0	23

Surface Changes Analysis

The surface morphology changes on the PP mask pieces before and after biotic exposure was investigated with the help of Field- Emission Scanning Electron Microscope (FE-SEM). The Fig. 6a, 6b and 6c showed that the surface changes recorded during FE-SEM analysis both the PP mask films treated with *Paeruginosa* strain (T1A, T1B, T2A, T2B, T3A and T3B) and untreated negative control (C1A, C1B, C2A, C2B, C3A and C3B) after 30 days of treatment. It was examined that signs of surface deterioration appeared on the PP mask films treated with *Paeruginosa* strain after 30 days of incubation. On the other hand, the control film (un-treated with *Paeruginosa* strain) keep hold of a smooth surface under same incubation condition was noted. Similar findings were reported in surface morphology of the LDPE films treated with

Pseudomonas spp. by SEM after 40, 80 and 120 days of incubation (Kyawet al, 2012). The another study also reported that the *Paeruginosa* ISJ14 used to treat LDPE film showed maximum deterioration after 60 days of treatment when observed under the FE-SEM (Gupta and Devi, 2020). A cross reference to the earlier research studies on LDPE biodegradation, many authors have reported the similar morphological changes on LDPE degradation by *Aspergillus* spp. (Zahra et al, 2010). *A.clavatus* JASK1 (Gajendiranetal, 2016).

Structural Analysis using FTIR

Structural changes in biologically treated PP mask films were further analyzed with the help of FTIR. This investigation of the degraded PP films had shown the stretching of numerous functional groups after incubation with the *Paeruginosa* strain. This was established by the alterations in the peaks of the FTIR spectra amidst the control and test samples in all the media used. Table 5a, 5b and 5c recapitulate the sort of functional group implicated in stretching by the role of *Paeruginosa*, the wave number and IR band position on the PP films. A considerable reduction in the Carbonyl Index was observed on the samples incubated with *Paeruginosa* strain for 30 days. The drop in CI with respect to the negative control was showed in Fig. 7a-7l. In our study, we observed maximum reduction in CI on PP mask films incubated with *Paeruginosa* strain for 30 days. Spectrophotometric variations of PP mask films and the value of CI which determines the maximum degradation when compared to un-treated negative control. Thus, our results suggested that the *Paeruginosa* strain has great ability to degrade the PP mask films. The LDPE film incubated with *Paeruginosa* PA01 showed maximum reduction in CI was reported by (Kyawet al, 2012; Skariyachanetal, 2015). In the present study, FTIR analysis affords a close view of N-H stretching of aldehydes group at 3190.18 cm^{-1} . The C-C absorption peaks were shifted as evident at 1255.18 , 1302.34 , 1794.87 , 2427.49 and 2617.26 cm^{-1} . A similar observation was reported by several authors (Howard and Hilliard, 1999). Our results were supported by various previous research studies noticed the formation of functional groups and disappearance of these groups in the LDPE degradation using the strain *Bacillus amyloliquefaciens* (Das et al, 2015). In relation to our study, (Gajendiran et al, 2016) have also noticed visible modifications in the synthetic polymers undergo biodegradation, before and after exposure to microbes by FTIR analysis. The conformational changes on PP mask film were supported by the changes in the peak values of almost all functional groups (Fig .7a-7l).

Table-5a. Comparison of IR band position in the PP films after incubation with *Paeruginosa* in NB and NA(both control and test)

S.No	Incubation period	IR band position in BHM broth control-test (cm ⁻¹)	IR band position in BHM Agar control-test (cm ⁻¹)	Functional group involved
1	30 days	572.18 - 460.23	1002.24 – 808.93	C-X stretching
2		2950.10 – 2838.92	2918.97 – 2917.61	C-H stretching
3		2916.61 – 2722.13	2947.73 – 2838.92	C-H stretching
4		3762.08 - 3189.68	3761.52 - 3189.68	O-H stretching

Table-5b. Comparison of IR band position in the PPfilms after incubation with *P.aeruginosa* in BHM broth and BHM agar (both control and test)

S.No	Incubation period	IR band position in BHM broth control-test (cm ⁻¹)	IR band position in BHM Agar control-test (cm ⁻¹)	Functional group involved
1	30 days	572.18 - 460.23	1002.24 – 808.93	C-X stretching
2		2950.10 – 2838.92	2918.97 – 2917.61	C-H stretching
3		2916.61 – 2722.13	2947.73 – 2838.92	C-H stretching
4		3762.08 - 3189.68	3761.52 - 3189.68	O-H stretching

Table-5c. Comparison of IR band position in the PPfilms after incubation with *P.aeruginosa* in MSM broth and MSM agar (both control and test)

S.No	Incubation period	IR band position in MSM broth control-test (cm ⁻¹)	IR band position in MSM Agar control-test (cm ⁻¹)	Functional group involved
1	30 days	562.12 – 458.98	460.00 – 459.35	C-X stretching
2		2839.02 – 2722.15	2839.57 – 2838.65	C-H stretching
3		2916.76 – 2838.83	2918.00 – 2917.14	C-H stretching
4		3760.91 - 3346.85	3351.15 - 3190.32	O-H stretching

Conclusion

In sum, this work offered a versatile biological process to evaluate the degradation of disposable face mask used in this SARS-CoV-2 pandemic situation. The *in vitro* biodegradation of *P.aeruginosa* strain in three different solid and liquid medium reflects the eco friendly approach. We observed a tremendous biodegradation efficiency of our isolates towards the PP based disposable face mask whose molecular weight was as high as 228,000. The isolate has a capability to forming biofilm on PP surface and utilized it as a sole nutrient source for growth was reflected by weight reduction of PP mask films in comparison with un-treated control films (up to 33% of weight reduction within 30 days). Based on the results of FE-

SEM as well as FTIR analysis, the *Paeruginosa* strain is a best suited candidate for PP degradation without an UV treatment. However, further studies on enzyme based metabolic passages of *Paeruginosa* strain are also recommended to better understanding of its tremendous role in the process of biodegradation.

Declarations

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Author contribution: **Vijayalakshmi Selvakumar**: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing; **Preethi Gopalsamy**: Methodology, Data curation, Writing - original draft, Writing - review & editing; **Kaman Muthusamy**: formal analysis; **Thenmozhi Manivel**: writing and formal analysis; **Ramachandran Chelliah**: writing—review and editing; **Dinesh Kumar Sundarraj**: Software, Data curation, Writing - review & editing; **Sathammai Priya Narayanan**: formal analysis and **Deog-Hwan Oh**: Conceptualization, Methodology, Supervision, Project administration, writing- review&editing.

Declarations of interests

None

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Figures

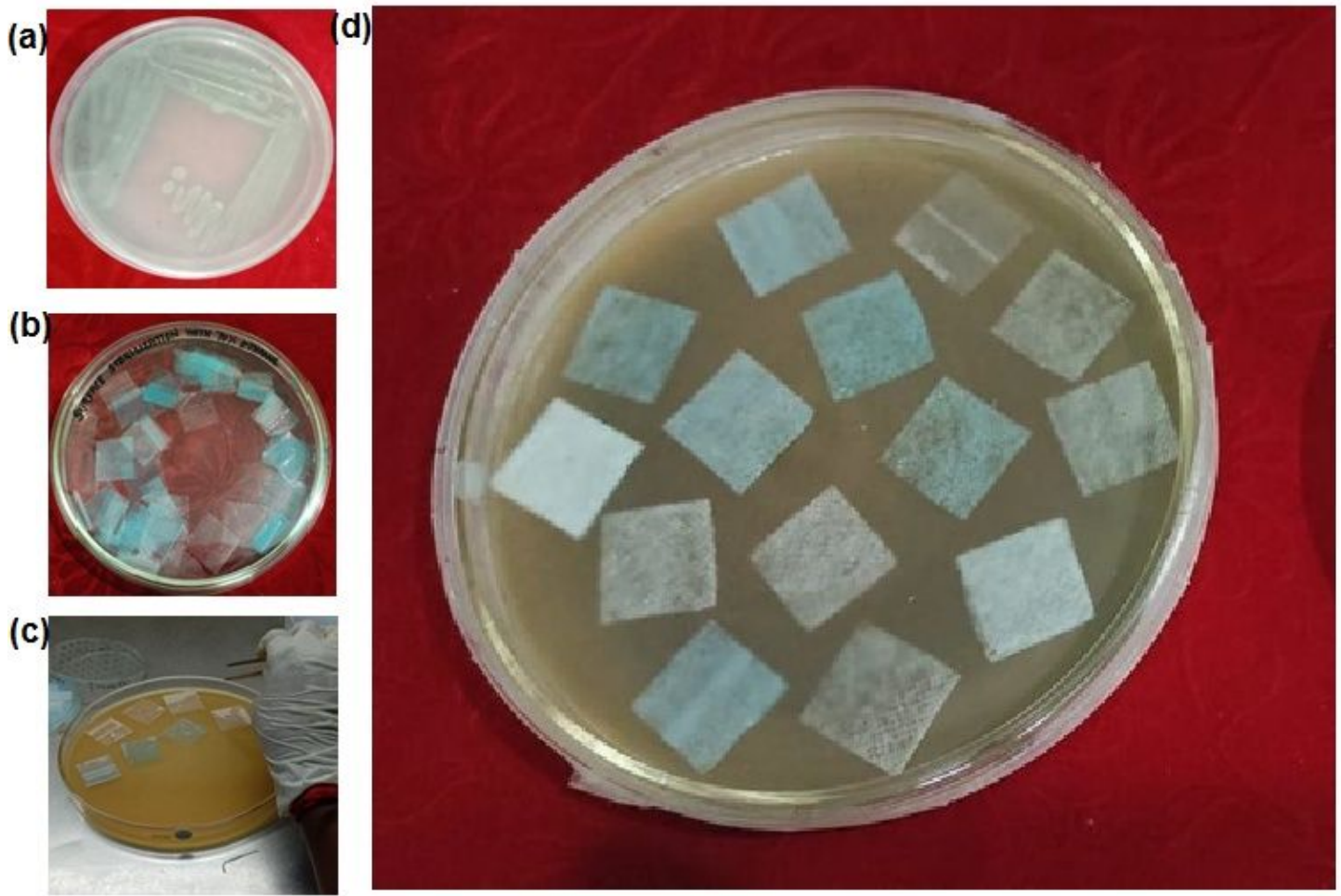


Figure 1

A-Pure culture of *Pseudomonas aeruginosa* strain; B- Pre-treated PP mask pieces; C- PP mask placed on to the Culture inoculated Nutrient Agar plate; D- PP mask pieces on Nutrient agar after 30 days.

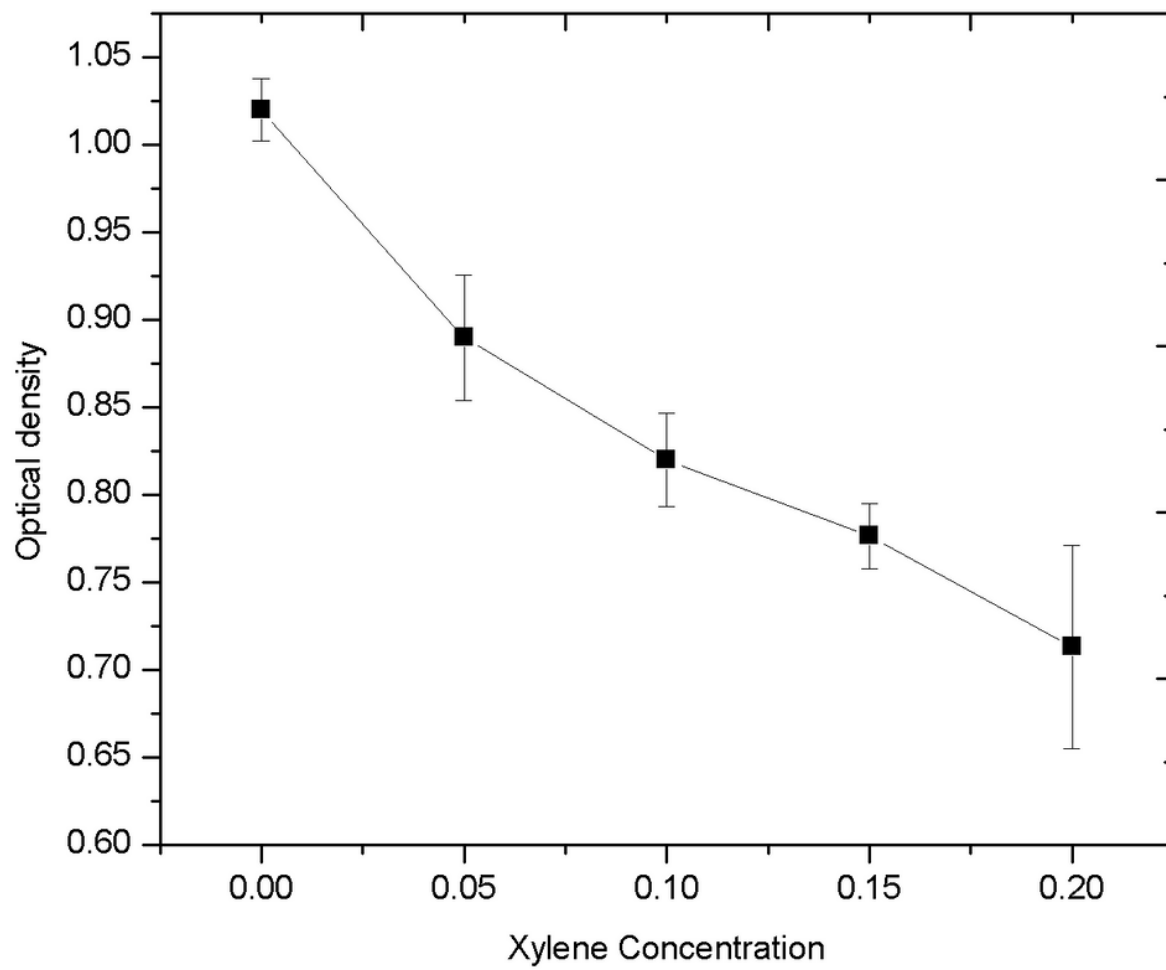


Figure 2

Cell surface Hydrophobicity of *Paeruginosa*

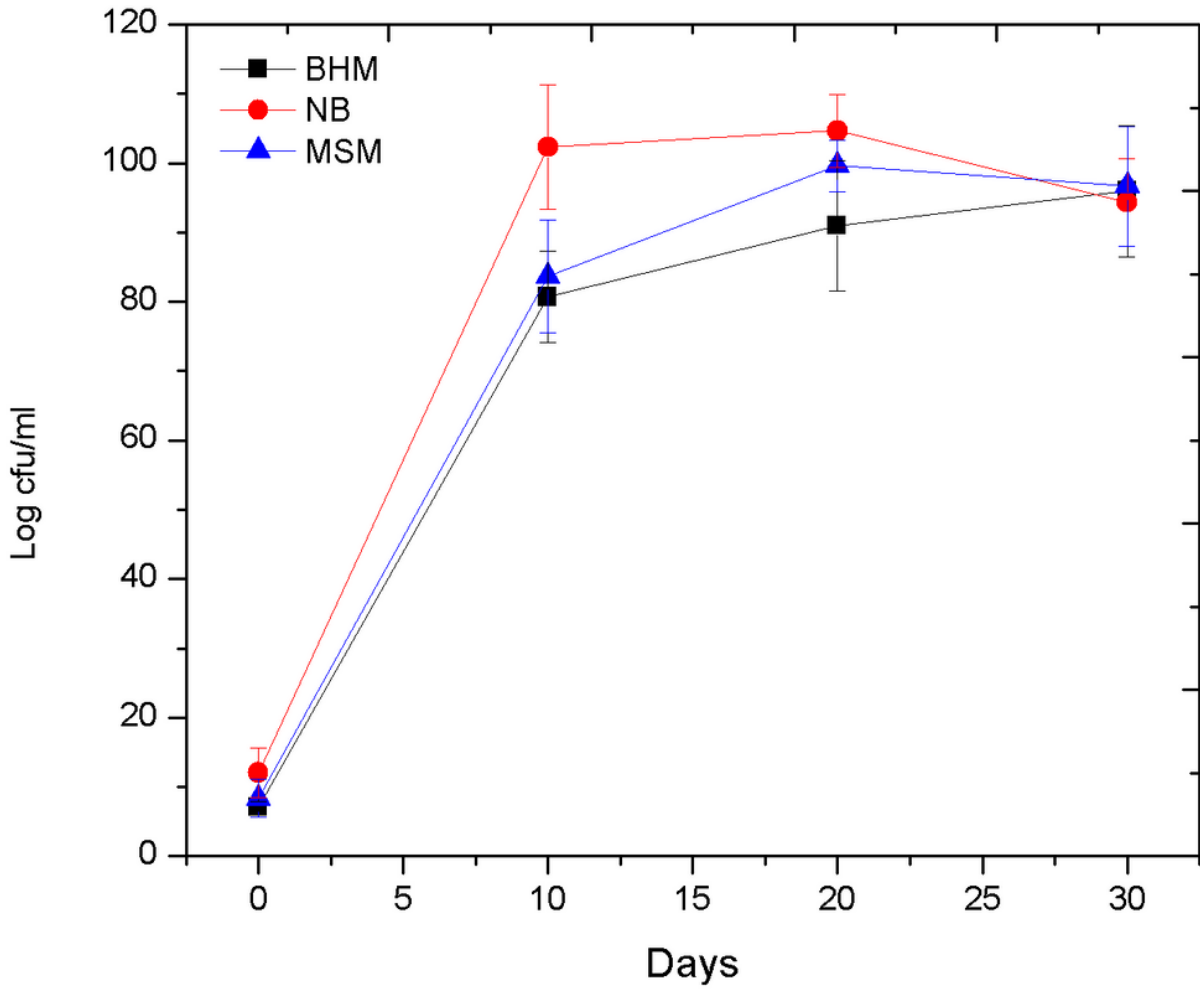


Figure 3

Viability of surface attached *Paeruginosa*

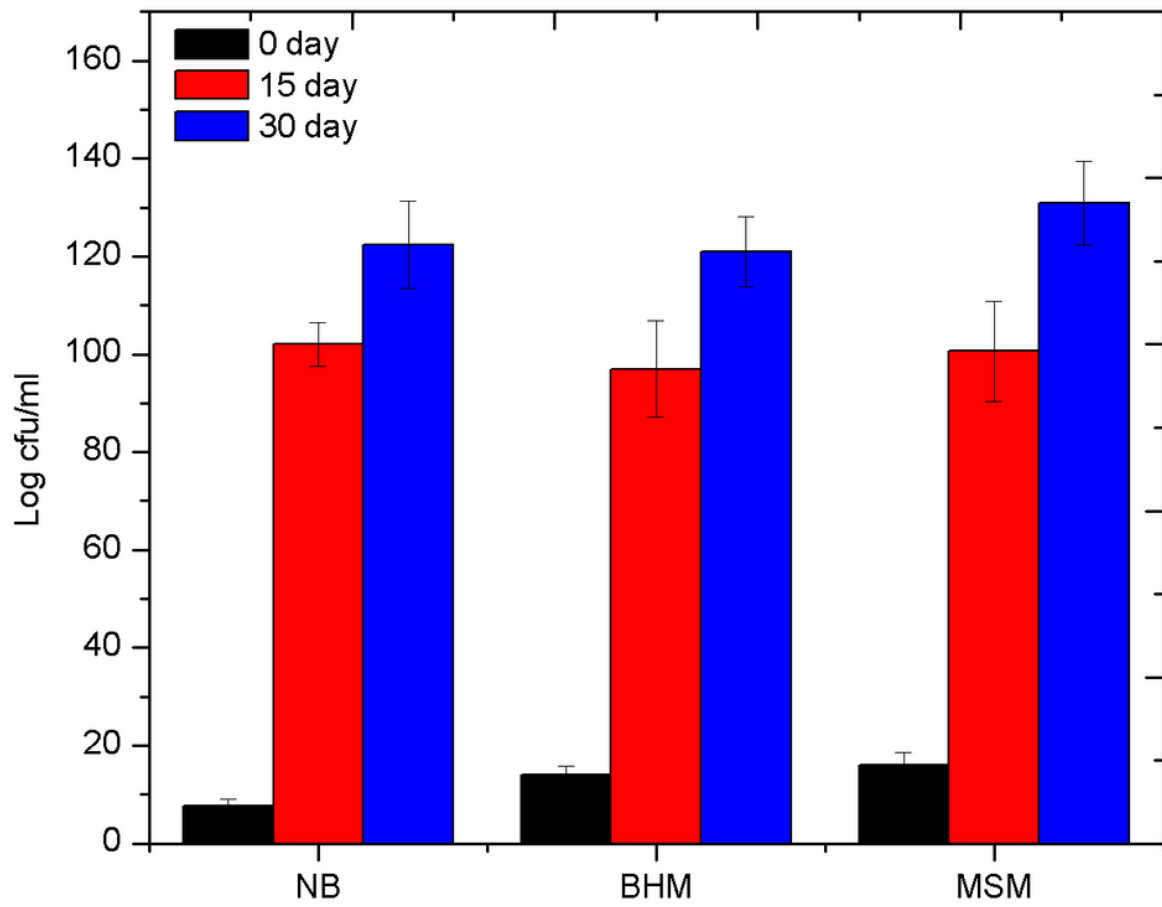


Figure 4

Planktonic growth of *P.aeruginosa* in different culture media used

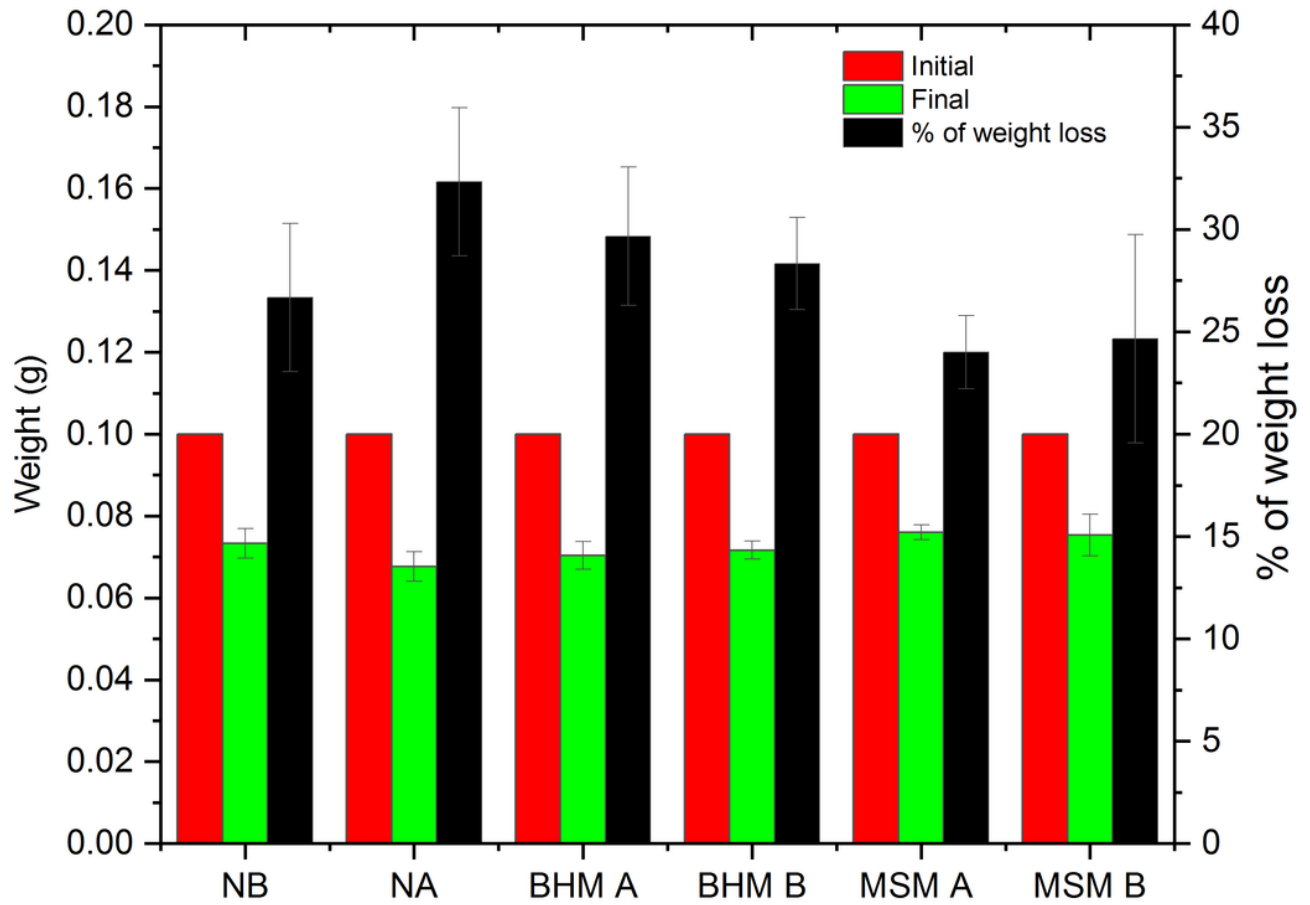


Figure 5

Dry weight of PP films after 30 days of incubation (control and treated)

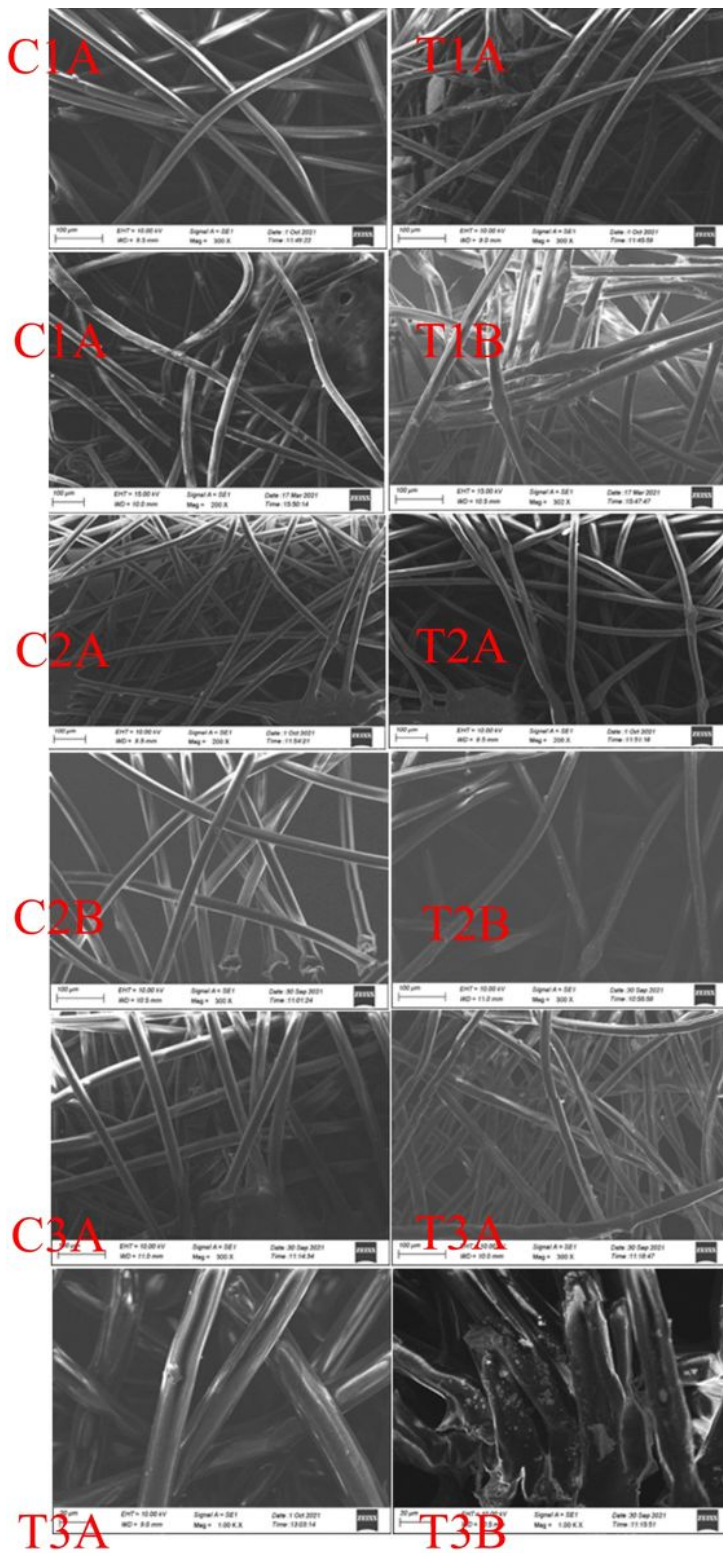


Figure 6

SEM images of PP films placed in Nutrient medium, BHM, and MSM; C1A, C1B- control; T1A and T1B- treated with *P. aeruginosain* NA and NB media respectively. C2A, C2B- control; T2A and T2B-treated with *P. aeruginosa* in BHM agar and broth respectively. C3A, C3B- control; T3A and T3B- treated with *P.aeruginosain* MSM agar and broth respectively

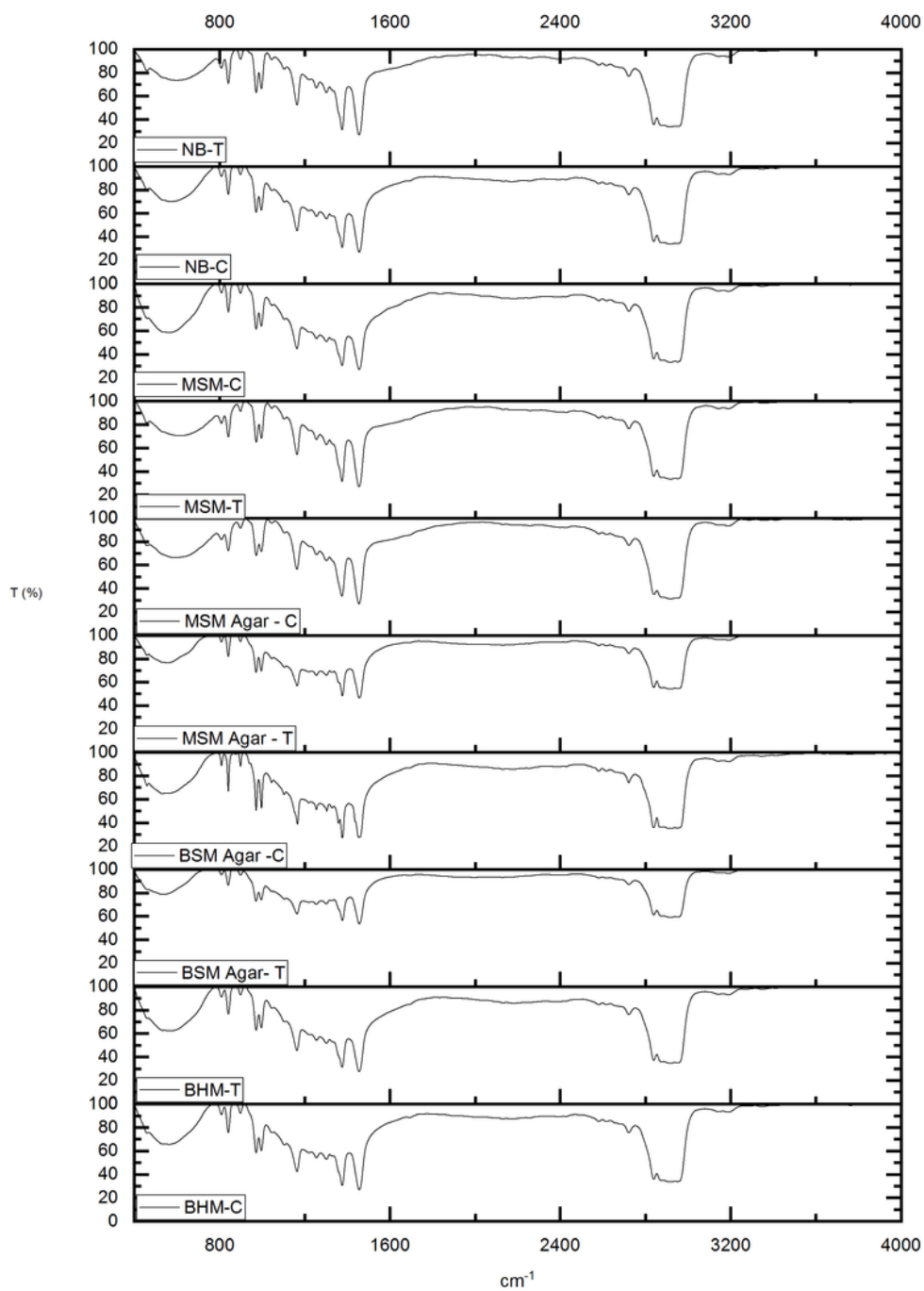


Figure 7

FTIR spectra of PP films