Kochi Chapter

Indian Geotechnical Conference
IGC 2022
15th – 17th December, 2022, Kochi

Biodegradation of Disposable Mask in Municipal Solid Waste Management Soil through Bioaugmentation

Sangeetha S[1], Hari Krishna P[2] , T Naveen Kumar[3]

[1]Assistant Professor, VNR Vignana Jyothi Institute of Engineering and Technology, Telangana -500 090
[2] Professor, Department of Civil Engineering, NIT, Warangal-506004
[3]M.Tech student, VNR Vignana Jyothi Institute of Engineering and Technology, Telangana-500090

e-mail: mailsangeethasundar@gmail.com

Abstract: Soil is the ultimate destination of any waste generated by the mankind. Disposable facial masks are quite common constituent of municipal wastes under new-normal and post COVID-19 situation at the solid waste management sites. Most of the commonly used disposable masks are made out of polypropylene which is one of the long lasting non-degradable polymers. Physical disintegration may happen due to burial in soil and photo oxidation upon exposure to sunlight that results in the formation of micro plastics. Studies in the past have proved the ability of micro-organisms especially bacterial species to degrade polypropylene under controlled laboratory condition. The micro-organisms utilise these polymeric products as sole source of carbon in the absence of other carbon source in their environment. *Bacillus Circulans* is the one among such species to degrade polypropylene. But most of the previous research workswere limited to controlled laboratory conditions. In the present work, the disposed polypropylene masks are physically fragmented and mixed with the soil collected from Greater Warangal Municipal Corporation Solid Waste Management (GWMC SWM) site, Rampur Village, HanumaKonda, Telangana, India. In this study efforts are made to study the role of soil, purpose of minimal salt media supplemented, effect of bioaugmentation using *Bacillus Circulans* in the degradation of these masks. FTIR analyses were performed for different treatment combinations at regular intervals. The transmittance at the prominent wave numbers (peak value) was found to increase during degradation process which indicated the reduction of bonds and hydrocarbons. Also, Carbon dioxide evolution test as suggested by ASTM D5988 for aerobic degradation of plastic wastes was also conducted to estimate the amount of degradation effected by the bioaugmentation technique.

Keywords: *Polypropylene, Disposable facial mask, Biodegradation, MSW soil, Bacillus Circulans, Bioaugmentation.*

1. Introduction

The process of degradation of plastic waste is not even comparable with the pace by which they are being littered in the soil. The amount of waste dumped in GWMC SWM park is keeping on increasing since its inception. In order to control the volume of persistent waste, they were burnt uncontrollably and the extent of smoke can be witnessed in the Satellite imagery portrayed in Fig 1. Pertaining to existing literatures

it has been witnessed that, soil characteristics that supports the growth of a particular microorganism and degradation mechanism of plastic waste in natural soil condition is not explored completely. The potentiality of microbes to destroy plastic waste was identified [1][11]. Bioaugmentation is the phenomena of addition of microorganism to the soil environment so as to enhance the process of bioremediation. The enhancement of soil environment with additional nutrients to support microbial degradation is termed as biostimulation [5][7]. The survival of identified micro-organism in soil under the landfill condition has to be identified. *Bacillus Circulans* (BC) is one of the species unveiled by the researchers to degrade polypropylene under laboratory condition [6]. Enormous quantity of disposable facial masks in post COVID -19 situation are dumped into the landfill site. The biodegradability of facial masks that are mostly made out of polypropylene in soil-like fraction available at the landfill has to be investigated. In the present study, biodegradability of facial mask introduced into the soil-like fraction collected from GWMC SWM site is effected through bioaugmentation with *Bacillus Circulans* and bio-stimulation with carbonless minimal salt media.

Fig. 1. Satellite Image of GWMC SWM Park (Source: Google Earth)

2. Methodology

2.1 Materials:

Bacillus circulans (NCIM Accession No:2107) vials were obtained from NCIM, Pune. Liquid broth and solid media with agar suggested by NCIM as mentioned in Table 1 was prepared and autoclaved. The autoclaved solid media was poured into petri dish and solidified. The lyophilized bacterial content was initially transferred to 10ml of liquid broth. The inoculating loop was sterilised and dipped into the inoculated broth and streaked on the solid media prepared on the petri dish. Bacterial colonies were observed after 48 hours in the perti dish. Liquid nutrient broths were prepared in two conical flasks of 250 ml each. A single colony was isolated and introduced into the

liquid broth. The Optical Density (OD) of the inoculated media was observed periodically at 600 nm. OD was observed as 0.4 absorbance after 48 hours of inoculation which represented that the bacterial population was at the end of the exponential phase and ready for harvesting through centrifuging [2]

Table 1. Culture medium for *Bacillus Circulans*

Ingredients	Quantity
Peptone	5.0 g
Meat extract	3.0 g
Agar	15.0 g (for solid media)
Distilled water	1000 ml
pH	7

The top soil from the GWMC SWM site was collected and basic characteristics were determined as per Indian standards. Commercially available disposable facial masks were shredded into pieces in a blender and artificially added to the soil from dumping yard.

2.2 Experimentation

Seven different treatment batches were prepared in 250 ml conical flasks as mentioned in Table 2. The soil sample collected from the dumping yard were steam sterilized using autoclaved at 120˚C and 1 kg/cm^2. In the treatment batches numbered 1,2,5 and6, 100 g of autoclaved soil was taken and 1 g (1% by weight) of shredded disposable facial masks made out of polypropylene was added. 1 g of shredded mask pieces wereadded in rest of the treatment batches to maintain uniformity. For the treatment batches 2,3,4 and 6, 50 ml of inoculated nutrient media mentioned in the previous section was taken separately. The nutrient broth was centrifuged and the bacterial pellets were obtained at the bottom of the centrifuge tubes. The pellets were transformed to the conical flasks of respective batches.

Table 2. Treatment Batches

Combination no.	Content
1	Autoclaved Soil + PP+DW
2	Autoclaved Soil+ PP + BC+DW
3	PP + BC+DW
4	PP + BC+MS
5	Autoclaved Soil+ PP + MS
6	Autoclaved Soil+ PP + MS + BC
7	PP + MS

Note: PP - Shredded disposable Poly Propylene masks , *BC- Bacillus Circulans*, MS - Minimal salt media, DW- Distilled water

The Minimal Salt Media (MS) as given in Table 3 was prepared and 100 ml of MSM was supplemented in the treatment combinations numbered as 4, 5, 6 and 7. 100ml of Distilled water was added to the batches 1,2 and 3. The prepared batches in the conical flasks were kept inside the incubator at 32˚C throughout the study. FTIR analysis was performed after 30,60 and 90 days of treatment for all the seven prepared batches and studied. The conical flasks with prepared treatment batches weighed and the moisture content losses were restored with the addition of water

Table 3. Composition of Minimal Salt Media (MS for Bacillus sp.) [8]

Chemicals	**Quantity per litre**
Potassium nitrate (KNO_3)	3.3 g
Di potassium phosphate (K_2HPO_4)	2.2 g
Mono potassium phosphate (KH_2PO_4)	0.14 g
Sodium chloride (NaCl)	0.1 g
Magnesium sulphate ($MgSo_4$)	0.6 g
Calcium chloride ($CaCl_2$)	0.4 g
Ferrous Sulphate ($FeSo_4$)	0.2 g
Stock solution *	0.5 ml
Stock solution*	**Qty in 1 litre**
1g of Ethylenediaminetetraacetic acid (EDTA)	1 g
Copper Sulphate Pentahydrate, $CuSO_4.5H_2O$	1 g
Manganese sulphate tetrahydrate ($MnSO_4.4H_2O$)	1.78 g
Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$)	2.32 g
1g of Ethylenediaminetetraacetic acid (EDTA)	1 g

2.3 Carbon dioxide evolution test

FTIR, SEM and other advanced analysis could qualitative ascertain the process of biodegradation [11]. The ultimate end product of aerobic degradation of hydrocarbon ends up in carbon dioxide and water. This principle is used to estimate the amount of degradation of plastic polymers. Carbon dioxide evolution test was performed for three treatment combinations as per ASTM D5988 to quantify the amount of degradation effected by the microorganism in the dumping yard soil and in the autoclaved dumping yard soil with some modifications [4]. The treatment combinations maintained for Carbon dioxide evolution study were listed in Table 4. The treatment combinations were kept at the bottom of the desiccator. In each of the

treatment batch 300 g of respective soil was taken and 3 g of shredded disposable mask fragments were added. 300 ml of water and bacterial pellets centrifuged from 150 ml of inoculated media were added to the first and the second treatment combination. The third combination was kept without any bacterial addition and maintained as a control. In the upper portion of the desiccator, 100 ml of 0.5 N KOH was kept in a glass beaker. The KOH solution reacted with carbon dioxide evolved from the treatment batch. The unreacted KOH was titrated against 0.25 N HCL at regular intervals. The weight of the desiccator with prepared soil was initially measured and maintained constant throughout the study by the addition of water.

Table 4. Carbon dioxide evolution study treatment combinations

Combination Label	Content
A	Dump yard Soil + PP+ water +BC
B	Dump yard Soil + PP+ water
C	Autoclaved Soil+PP + BC+ water

Note: PP - Shredded disposable Poly Propylene masks, *BC- Bacillus Circulans*,

3. Results and Discussions

Preliminary tests were conducted on soil collected from dumping yard and the test results are mentioned in Table 5. As the soil is a mixture of degrading heterogeneous garbage, the field dry density was only 0.89 t/m^3 and specific gravity was around 2.2.

Table 5. Basic properties of soil-like material collected from GWMC SWM Park.

Properties	Codal Provision	Results/Values
Field dry density	IS: 2720 (Part 29): 1975	0.89 t/m^3
Water content	IS: 2720 (Part 2): 1973	40.6%
Specific gravity	IS:2720 (Part 3):1980	2.27
Grain size distribution Sieve and Hydrometer Analysis	IS: 2720 (Part 4): 1985	Gravel– 0 % Sand– 73 % Silt– 25.2% Clay– 1.8 %
Soil classification	IS: 1498 (1970)	Silty Sand (SM)
Maximum Dry Density Optimum moisture Content	IS: 2720 (Part 7): 1980	1.864 t/m^3 12.3%
pH	IS: 2720 (Part 26): 1987	8.7 at 29.3˚C
Electrical Conductivity	IS: 14767: 1973	0.731 mS at 29.3˚C
Salinity	Probe method	0.622 ppt at 29.3˚C

3.1 FTIR results

Fourier Transform Infrared spectroscopy is proved to be an efficient tool for identification and interpretation of different bonds present in hydrocarbon compounds [3][9][10]. FTIR analyses were conducted for the random samples collected from the treatment batches after 30, 60 and 90 days of treatment. FTIR spectra of each treatment batch observed at regular time intervals were overlapped to witness the progress of degradation. The overlapped graphs are given from Fig. 2 to 8. Presence of peak in the FTIR spectra at 1630-1670 cm^{-1} corresponds to alkenes. Broadband at 3300-3600 cm^{-1} refers to O-H group or presence of H_2O. Presence of peak between 1720-1740 cm^{-1} indicates C=O of aldehyde. Biodegradation of PP is expected to produce oxidised products such as alcohol, aldehyde and ketone. Increase of transmittance indicates reduction in concentration of the bond. Disposable facial masks were made out of poly propylene that contains alkene bonds. The presence of sharp peak at 1641 cm^{-1} in the FTIR spectra of treatment batches indicates polypropylene.

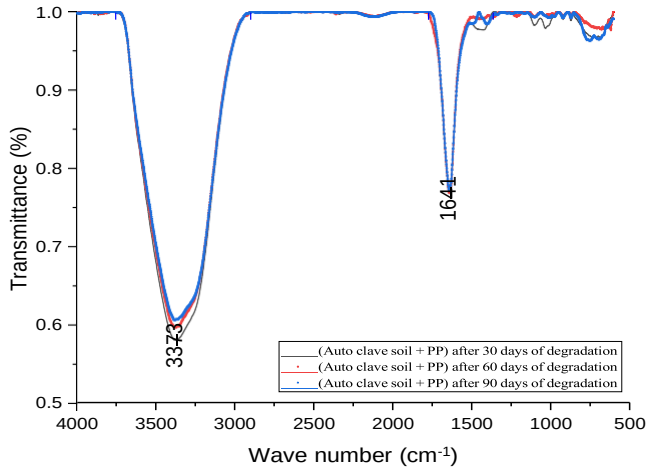

Fig. 2.(Autoclaved soil + PP) after 30,60 and 90 days of degradation

From Fig. 2, it has been observed that there is no variation in the peak at 1641 cm^{-1} for the batch that has only autoclaved soil loaded with shredded polypropylene pieces. Therate of degradation of treatment batch bioaugmented with *Bacillus Circulans* can be witnessed as given in Fig. 3. After 90 days of treatment, there is a reduction and shifting of peak at 1644 cm^{-1} is observed along with changes in the minor peaks around 1400-1200 cm^{-1} region. Bioaugmentation of autoclaved soil with BC has reduced the concentration of polypropylene. The breakage of polypropylene has resulted formation, followed by reduction of new bonds in the adjacent regions.

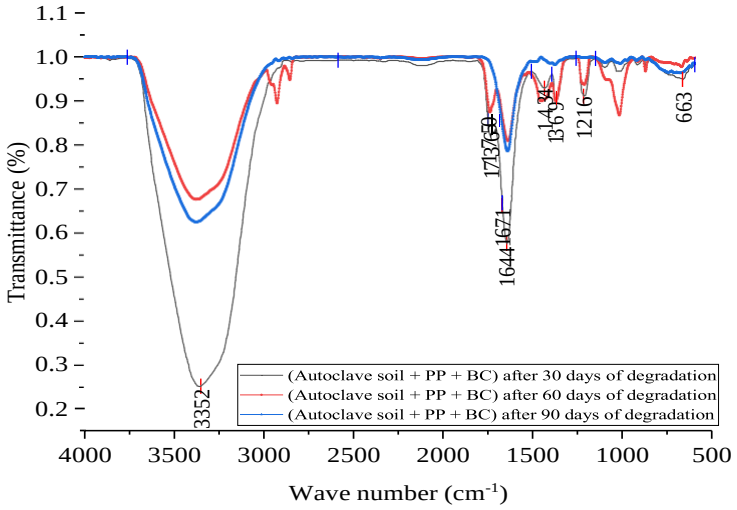

Fig. 3. (Autoclaved soil + PP +BC) after 30,60 and 90 days of degradation

When the shredded polymeric fragments are only exposed to bacterial species, there are no much variations in transmittance of alkene bonds as observed in Fig. 4 due to the unavailability of soil that essentials provides the macro and micro nutrients. The provision of essential salts through minimal salt media to the polymeric pieces and bacterial species did not create significant changes in the peaks observed at 1641 cm^{-1} of Fig. 5. This indicates the important of soil substrate in the process of degradation.

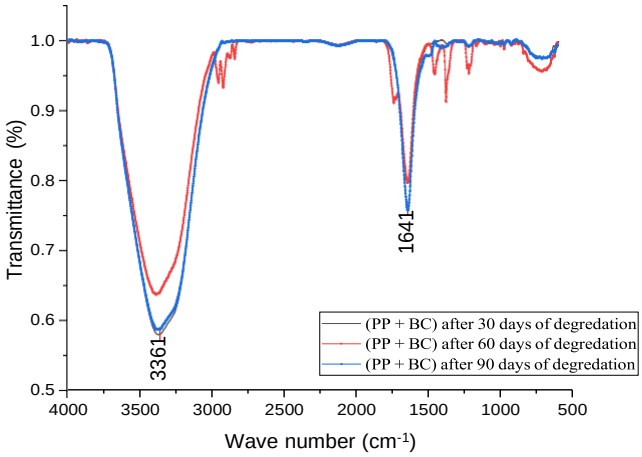

Fig. 4. (PP +BC) after 30, 60 and 90 days of degradation

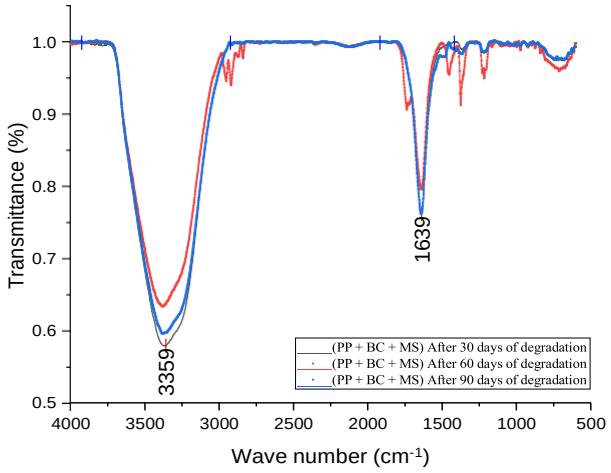

Fig. 5. (PP +BC + MS) after 30, 60 and 90 days of degradation (Incubator)

There is a reduction in alkene peak when autoclaved soil is bio-stimulated with minimal salt media as depicted in Fig. 6. Bioaugmentation by BC along with bio-stimulation in autoclaved soil also shown increase of transmittance at 1641 cm^{-1} which can be observed in Fig. 7.

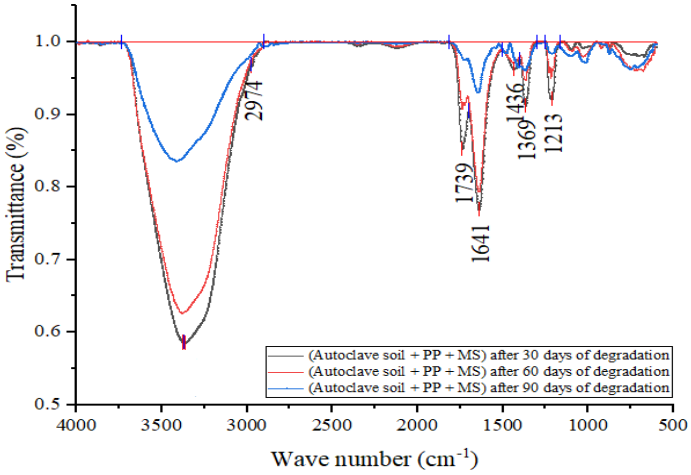

Fig. 6. (Autoclaved soil + PP + MS) after 30, 60 and 90 days of degradation

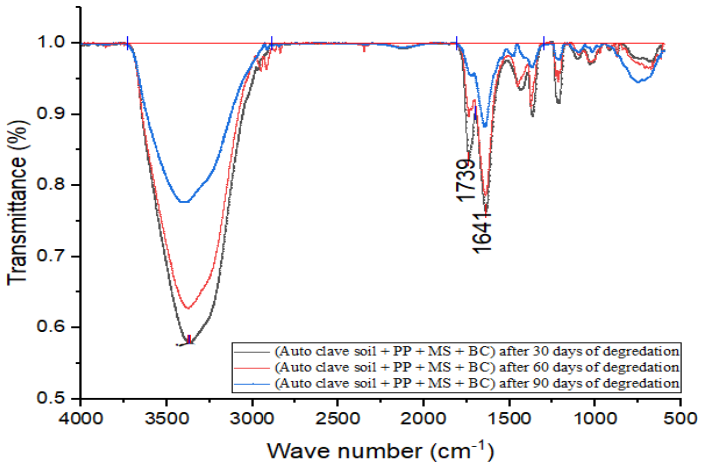

Fig. 7. (Autoclaved soil + PP + MS + BC) after 30, 60 and 90 days of degradation

In the Fig. 8., it has been observed that the minimal salt media remains inert with the Shredded mask pieces as there is no change in the peak observed at 1641 cm^{-1}.

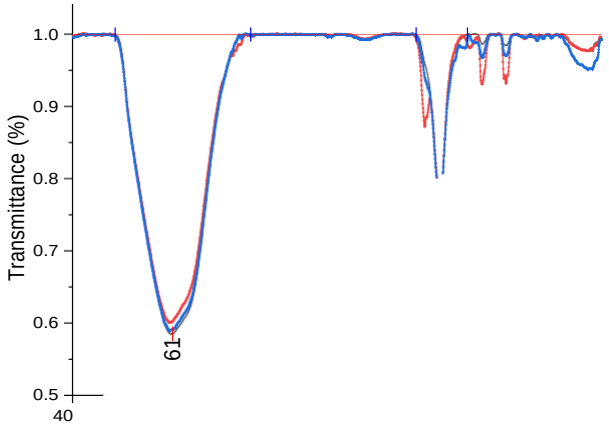

Fig. 8. (PP + MS) after 30, 60 and 90 days of degradation

In order to evaluate the performance of different treatment combinations, variations of transmittance at 1641cm^{-1} that corresponds to alkene group after 30 and 90 days of treatment are compared in Fig. 9. The following observations are made.

- Batches that contain soil has only shown increase of transmittance, which is represented by the reduction in intensity of bonds.
- Treatment batch that has only autoclaved soil and PP has not undergone any reduction of peak due to absence of microbial activity
- Bioaugmented, Bio-stimulated and combined bioaugmented & bio-stimulated

batch has undergone reduction in peak

- The highest amount of increase in transmittance is reported in case of bio-augmented sample (Autoclaved soil +PP+BC)
-

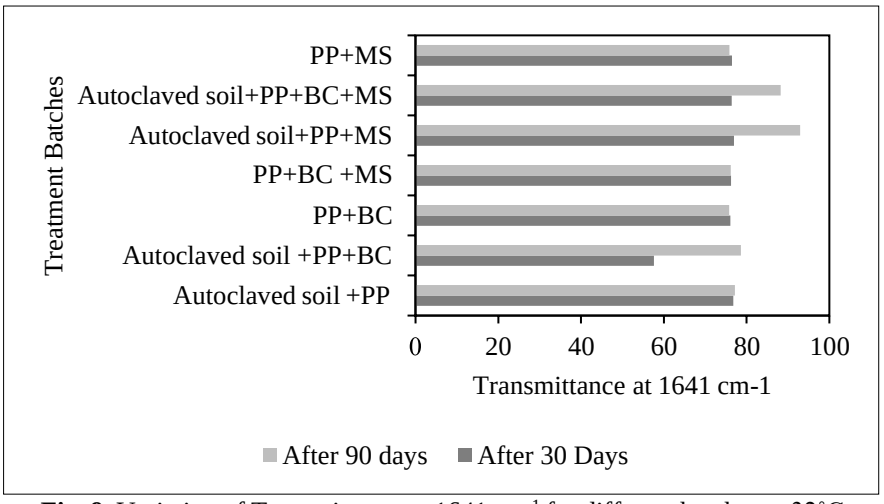

Fig. 9. Variation of Transmittance at 1641 cm^{-1} for different batches at 32˚C

3.2 Carbon dioxide evolution test results:

The amount of carbon dioxide evolved as a result of degradation is measured for a period of 6 months as per ASTM D5988 and the results are given in Table 6. The cumulative amount of carbon dioxide evolved during degradation is calculated from Titration test results. Theoretical amount of carbon dioxide can be calculated from the molecular formula of the propylene monomer with an assumption that the mask is predominantly made of out poly propylene. It has been witnessed that degradation can be affected by indigenous microbes present in the dumping yard soil as well.

Table 6. Carbon dioxide evolution test results

Combination Label	Total m moles of CO_2	Weight of CO_2 in mg	% of Degradation
A	18.175	799.7	25.47
B	20.825	916.3	29.18
C	18.675	821.7	25.46

4. Conclusions

The study on biodegradation through different treatment batches indicated that even if bacterial population and micro nutrients are supplemented, soil plays a crucial role in

the process of degradation of polymeric product. The treatment batch that contains soil has only shown degradation. The treatment batches that are bioaugmented with *bacillus sp.*, biostimulated with minimal salt media, and the combination of both has shown effective bioremediation of shredded polypropylene waste. But the studies are conducted in autoclaved soil. The phenomena of mass culturing of bacteria and introducing it into the heterogeneous landfill soil which has its own microbial population may not be feasible in all cases. Hence, in order to enhance the process of biodegradation of polymers in the real field condition, it is recommended to study the nutrient level, water content of the participating soil and bio-stimulate the soil media accordingly.

5. References

[1] Bahl, Shashi, Jigmat Dolma, Jashan Jyot Singh, and Shankar Sehgal. 2020. "Biodegradation of Plastics: A State-of-the-Art Review." *Materials Today: Proceedings*, 4–7. https://doi.org/10.1016/j.matpr.2020.06.096.

[2] Hall, B. G., Acar, H., Nandipati, A., & Barlow, M. (2014). Growth rates made easy. *Molecular Biology and Evolution*, *31*(1), 232–238. https://doi.org/10.1093/molbev/mst187

[3] Coates, J. (2006). Interpretation of Infrared Spectra, A Practical Approach. In Encyclopedia of Analytical Chemistry (eds R.A. Meyers and M.L. McKelvy). https://doi.org/10.1002/9780470027318.a5606

[4] D5988-03, ASTM. 2012. "Standard Test Method for Determining Aerobic Biodegradation in Soil of Plastic Materials or Residual Plastic Materials After Composting." *ASTM International* 08: 1–6.

[5] Katukojwala, Sravya, S. Sangeetha, and P. Hari Krishna. 2021. "Effectiveness of Bioremediation Technique on Oil Contaminated Marine Soil." In *Lecture Notes in Civil Engineering*, 159:73–86. Springer Science; Business Media Deutschland GmbH. https://doi.org/10.1007/978-981-16-2260-1_7.

[6] Khanna, N. Deepika, Inderjeet Kaur, T. C. Bhalla, and Neena Gautam. 2010. "Effect of Biodegradation on Thermal and Crystalline Behavior of Polypropylene-Gelatin Based Copolymers." *Journal of Applied Polymer Science*, June, n/a–. https://doi.org/10.1002/app.32434.

[7] Margesin, R., and F. Schinner. 2001. "Bioremediation (Natural Attenuation and Biostimulation) of Diesel-Oil-Contaminated Soil in an Alpine Glacier Skiing Area." *Applied and Environmental Microbiology* 67 (July): 3127–33. https://doi.org/10.1128/AEM.67.7.3127-3133.2001.

[8] Mukherjee, S., Das, P., Sivapathasekaran, C. and Sen, R. (2009), Antimicrobial biosurfactants from marine Bacillus circulans: extracellular synthesis and purification. Letters in Applied Microbiology, 48: 281-288. https://doi.org/10.1111/j.1472-765X.2008.02485.x

[9] Raghavan, D., and A. E. Torma. 1992. "DSC and FTIR Characterization of Biodegradation of Polyethylene." *Polymer Engineering & Science* 32: 438–42. https://doi.org/10.1002/pen.760320609.

[10] Rouillon, C., P. O. Bussiere, E. Desnoux, S. Collin, C. Vial, S. Therias, and J. L. Gardette. 2016. "Is Carbonyl Index a Quantitative Probe to Monitor

Polypropylene Photodegradation?" *Polymer Degradation and Stability* 128: 200–208. https://doi.org/10.1016/j.polymdegradstab.2015.12.011.

[11] Shah, Aamer Ali, Fariha Hasan, Abdul Hameed, and Safia Ahmed. 2008. "Biological Degradation of Plastics: A Comprehensive Review." *Biotechnology Advances* 26: 246–65. https://doi.org/10.1016/j.biotechadv.2007.12.005