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Biodegradation of UV Treated LDPE by *Penicillium* Isolated from Dumpsite Soil

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Abstract: ***Synthetic polymers are widely used both in food or commercial industry. Plastic one of its products is known to be expanding with changing life styles and increasing population. Plastics are polymeric material that has the capability of being moulded or shaped, usually by the application of heat and pressure. However, they have an undesirable influence on the environment and cause problems with waste deposition and utilization. In the present study, biodegradation of low-density polyethylene (LDPE) have been performed using Penicillium. The fungal strain adhered to the LDPE strip was used for biodegradation. Fungal growth was observed on the surface of the polyethylene when cultured on potato dextrose agar medium. Degradation was carried out using UV treated polyethylene, Degradation is monitored by observing weight loss, the Fourier transform infra-red (FT-IR), X-ray diffraction (XRD) Scanning electron microscopy (SEM) which showed the hyphal penetration and degradation on the surface of polyethylene. Atomic force microscope (AFM) analysis showed increased surface roughness after the treatment with fungal isolate. A network of hyphae formation was observed on the surface of polyethylene strips. These results show that the Penicilliumis potential in biological degradation of polyethylene.***

Keywords: Biodegradation, Polyethylene, *Penicillium*, SEM, FTIR, AFM, XRD

1. Introduction

Developments in science and technology, especially over the last two decades, have increased the amount of synthetic polymers produced world-wide each year. Each year approximately 200million tonnes of synthetic polymers are produced. (Leza & Lewandowiez 2010). During the past decade polyethylene materials have gained widespread use in various fields and have become indispensables. (Gajendrian *et al*., 2016). Plastics are polymers of carbon along with hydrogen, nitrogen, sulphur and other and inorganic components are made from fossil fuel. Plastics are non-biodegradable, strong, durable, moisture resistant, light weight. (Kumari *et al.,* 2013, Sura 2019). Chemical engineering involves new technologies employing highly novel materials whose unusual response, at the molecular levels endows them with unique properties. In recent years, polymers have gained an increased presence in the chemical industry with their highly defined properties and uses. Natural and synthetic polymers find a vital and pervasive use in day to day life. (McCrum *et al*., 1997, Aishwarya & Harshini 2018) due to their versatile traits (Paul*et al*., 1997, Aishwarya& Harshni 2018). Polymers are the molecule whose structure contains multiple repeating units which results into a compound of high relative molecular mass and associated properties. (http: //goldbook. Iupac. org/Mo3667. html).

As the world's population continues to grow the amount of plastic wastes produced by human beings (Morsy *et al*., 2017). Polyethylene/ Polythene is a polymer of ethylene gas (CH_2=CH_2) is commonly used in our day to day life like grocery bags, shampoo bottles, bullet proof vests etc. Several kinds of polythene are known with most having the chemical formula (C_2H_2) $_nH_2$ (Alka *et al*., 2015). LDPE, mainly used to make plastic carry bags and food packaging materials, is the most abundant petroleum-polymer on earth, and represents up to 64% of single use plastics that are discarded within a short period after use, resulting in massive and rapid accumulation in the environment. (Zahra *et al*., 2020, Ragaert et al., 2017, Harshavardhan et al., 2013). Low-density polythene is a major cause of environmental pollution due to high tensile strength, lightness, resistance to water and microbial attack. (Muhonja *et al*., 2018).

Polythene/ Polyethylene are one of the synthetic polymers of high hydrophobic level and high molecular weight. In natural form it is not biodegradable. Thus their use in the production of disposal or packaging materials causes dangerous environmental problems. (Potts, 1978, Mahalakshmi 2012).

The first synthetic plastic-Bakelite was produced in 1907, marking the beginning of the global plastic industry. However, rapid growth in global plastic production was not realized until the 1950s. Over the next 65 years, annual production of plastics increased nearly200-fold to 381 million tonnes in 2015. (Hannah *et al*., 2018).

The increase of global plastic producing measured in tonnes per year; from 1950 to 2015. In 1950 the world produced only two million tonnes per year since then annual production has increased nearly 200-fold, reaching 381 million tonnes in 2015. Plastics are man-made hazardous long chain synthetic polymers. Their low cost, excellent moisture barrier properties, bio-inertness and light weight make them excellent packaging materials. (Das & Kumar 2015, Ronan *et al*., 2017)

Biodegradation is processes which include microorganisms like bacteria and fungi as sources of carbon and energy for their growth which can degrade the polythene and therefore the process of biodegradation is an upcoming trend in the field of degradation (Albertsson, Gu. *et al.*, 2000).

2. Materials and Methods

1) Collection of soil sample:
Soil sample was collected from a local dumpsite of Raichur city, brought to the laboratory, preserved under laboratory conditions for further use.

2) Isolation and identification of fungi from soil:
Enrichment procedure was used for the isolation of fungus where polyethylene was used as sole source of carbon. Isolated fungus was identified based on its microscopic appearance using standard manuals (Ellis Ellis1976, Pitt 1979, Domsch *et al.*, 1980, Subramani 1983, Ellis & Ellis 1997; Gilman 2001; Nagamani *et al.*, 2006, Soumya *et al.*, 2014). Colonies were preserved at 4°C in agar slants of Malt and Yeast extract medium (Yamada-Onodera *et al.*, 2001; Sowmya *et al.*, 2014).

3) Screening of fungi for polyethylene degradation:

a) Degradation of polyethylene:-
The pre-weighed plastic strips of UV-treated polythene of 3cm diameter prepared from polyethylene bags were aseptically transferred to the conical flask containing 50ml of mineral salt medium. Surface sterilization was done by washing polyethylene strips for 2min in tap water, then washed and disinfected with a solution containing 7ml Tween-80, 10ml bleach and 983 ml sterile water, further the strips were then transferred aseptically into 70% (v/v) ethanol solution for 30 min then followed by drying them over night in the laminar chamber.

b) Confirmation of polyethylene Degradation:-
Polyethylene degradation was confirmed using scanning electronic microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-RD analysis, AFM.

c) Screening of fungi to degrade treated polyethylene:-
Polyethylene strips were UV-irradiated for 72 hours and the strips were inoculated to mineral salt medium.

4) Analytical methods to check biodegradation of polyethylene:-

a) Observation of colonies grown on medium by stereo-binocular:
Growth of colonies on the medium was observed under the stereo-binocular microscope.

b) Observation of strips using SEM:
The morphology of plastic strips was characterised by scanning electron microscopy (SEM) at University of Agricultural sciences, Raichur. For the structural morphology observation of the plastic strips, their surface was coated with a thin layer of gold.

c) Evaluation of chemical structure of the plastic strips:
Fourier transform infrared spectroscopy (FTIR) analysis was performed by using SHIMADZU at room temperature to investigate the changes in chemical structure of the plastic strips with *Aspergillus* sp. Each sample was recorded with different scans at a resolution of 4cm^{-1} in the scanning range from 4000 to 500 cm^{-1} at the University of Agricultural Sciences.

d) Surface morphology study of LDPE:
AFM analyses for the plastic strip were obtained with a scan speed of 1.0 Hz and resolution 40k.

e) X-ray diffraction (XRD) analysis:
The X-ray diffraction patterns of the strips were measured with an X-ray diffractometer which is operated fully automatically the scattered radiation was registered in the angular interval (2Ɵ) from 2° to 40°. A current of 30 mA and a voltage of 40 kV were used. All diffraction patterns were examined at room temperature and under continuous operating conditions.

3. Results and Discussion

1) Isolation and identification of fungi:
Penicillium was isolated from the dumpsite soil and identified based on its morphological characters. *Penicillium* was selected for the study because of its predominant presence in soil contaminated with waste polyethylene plastic bags.

Table 1: Weight loss of surface sterilized polyethylene in milligrams (mg)

S. No	Initial weight (mg)	Final weight (mg)	Weight loss (mg)	Weight loss (%)
1.	45	44.6	0.88	0.9%

Table 2: Weight loss of surface sterilized polyethylene in grams (gm)

S. No.	Initial weight (gm)	Final weight (gm)	Weight loss (gm)	Weight loss (%)
1.	0.045	0.0446	88	88%

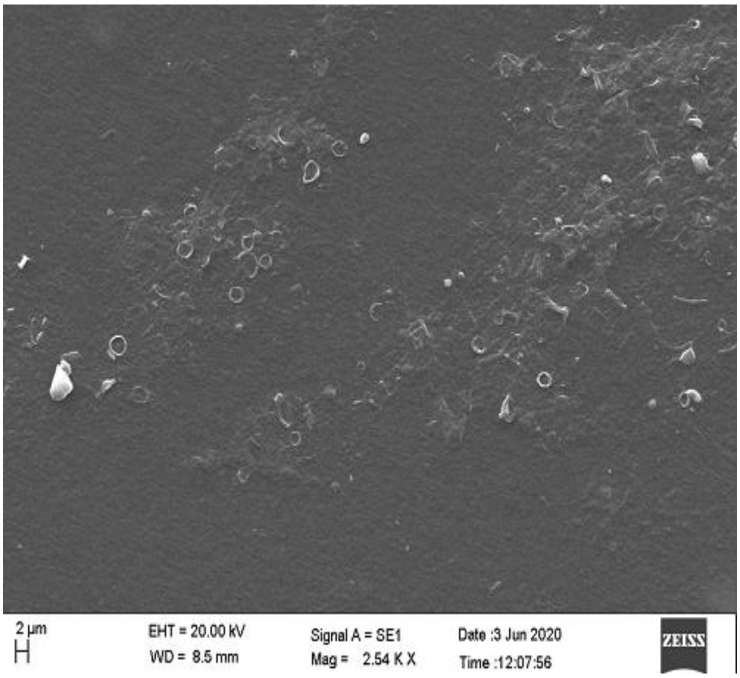
2) Screening fungi for polyethylene degradation:
*Penicillium*was able to grow on PDA medium containing polyethylene as sole carbon source. This showed its capacity to utilize polyethylene as carbon source and to degrade polyethylene.

3) Degradation of UV-treated polyethylene:
Penicillium was able to degrade UV treated Polyethylene more efficiently than surface sterilized or untreated or unsterilized polyethylene strips. The weight loss of UV treated polyethylene was 55.4%. Degradation was more here because of pre-treatment using UV light. UV light is known as initiator of polyethylene oxidation and enhances the fungal degradation. UV treatment causes pro-oxidant and photo-oxidant to produce free radicals on the long chain, causing the material to lose some of its physical properties to become oxidised and more accessible to microbial biodegradation. The fungal attachment was found on the surface of the plastic and it indicates possible utilization of plastic as carbon source.

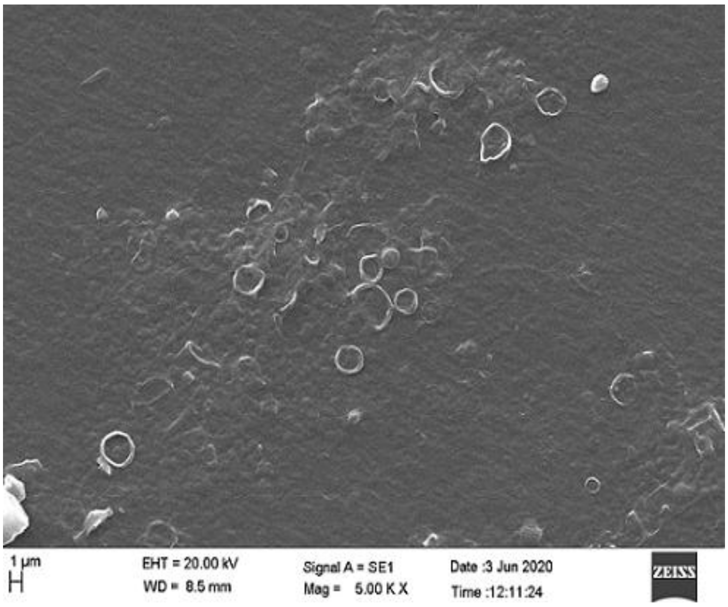
Figure 1: Degradation of UV-treated polythene strips

4) **Confirmation of polyethylene degradation:**

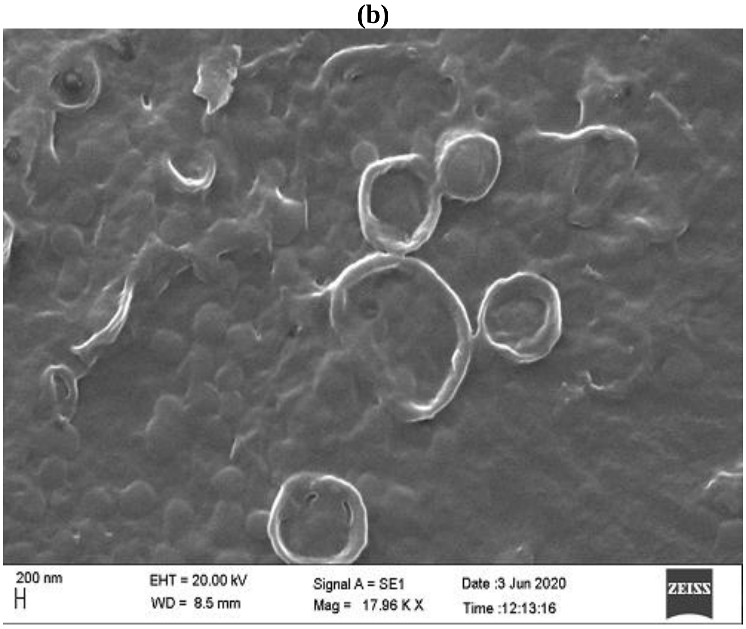
Observation of strips using SEM:
UV treated polyethylene showed morphological changes when observed through SEM. Formation of holes, disruption of polyethylene structure confirmed degradation of polyethylene structure confirmed degradation capacity of *Penicillium*.



(a)


1 µm
EHT = 20.00 kV
WD = 8.5 mm
Signal A = SE1
Mag = 5.00 K X
Date :3 Jun 2020
Time :12:11:24
ZEISS

(b)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Signal A = SE1
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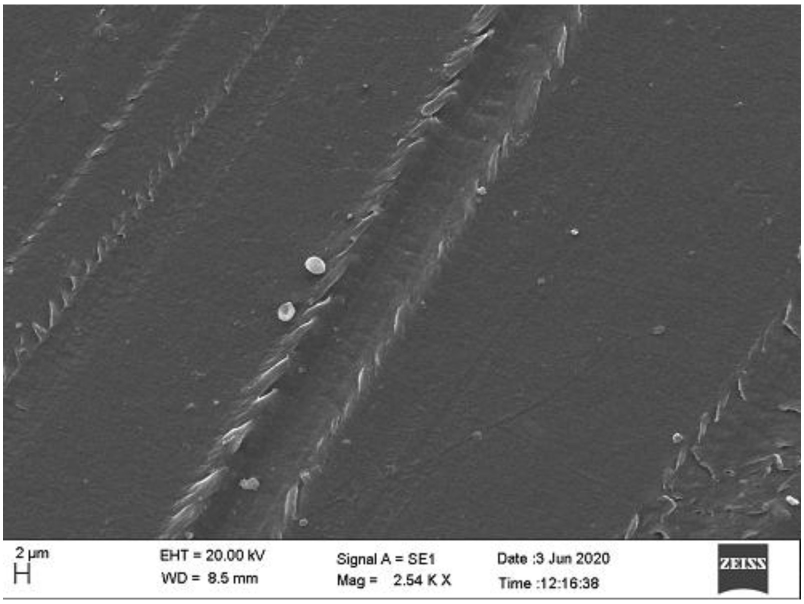
Time :12:13:16
ZEISS

(c)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WD = 8.5 mm
Signal A = SE1
Mag = 2.54 K X
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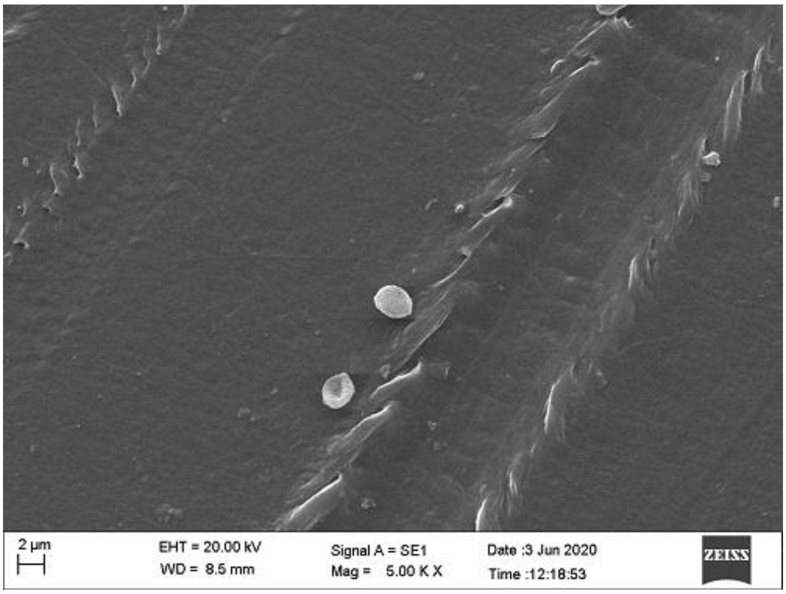
Time :12:16:38
ZEISS

(d)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WD = 8.5 mm
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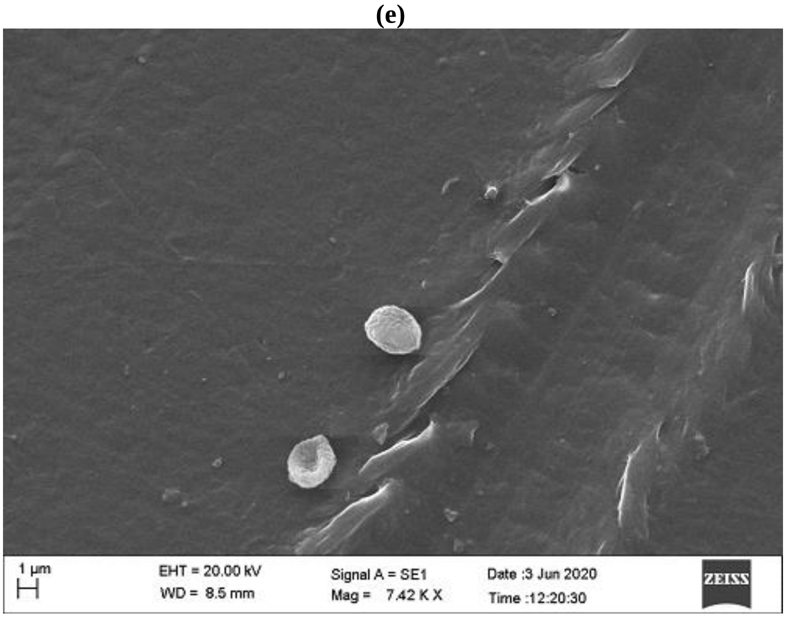
ZEISS

(e)


1 µm
EHT = 20.00 kV
WD = 8.5 mm
Signal A = SE1
Mag = 7.42 K X
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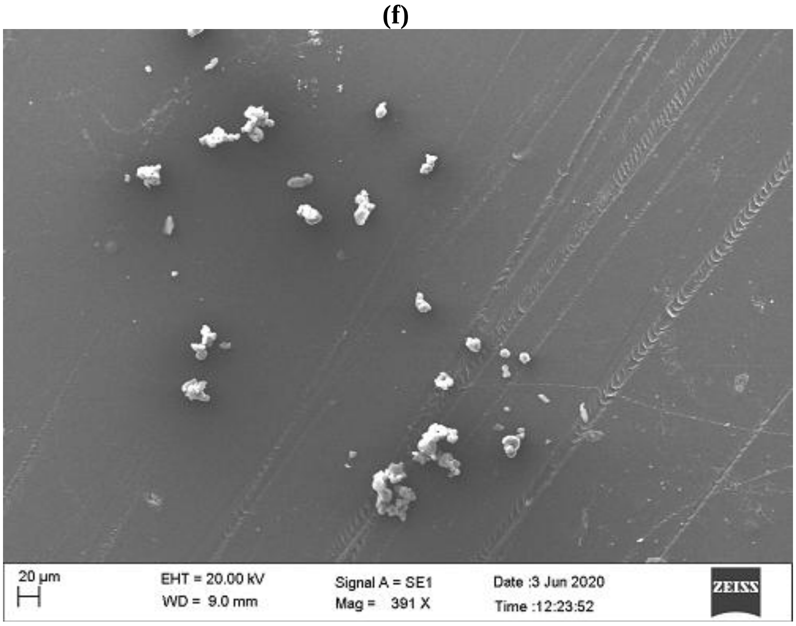
Time :12:20:30
ZEISS

(f)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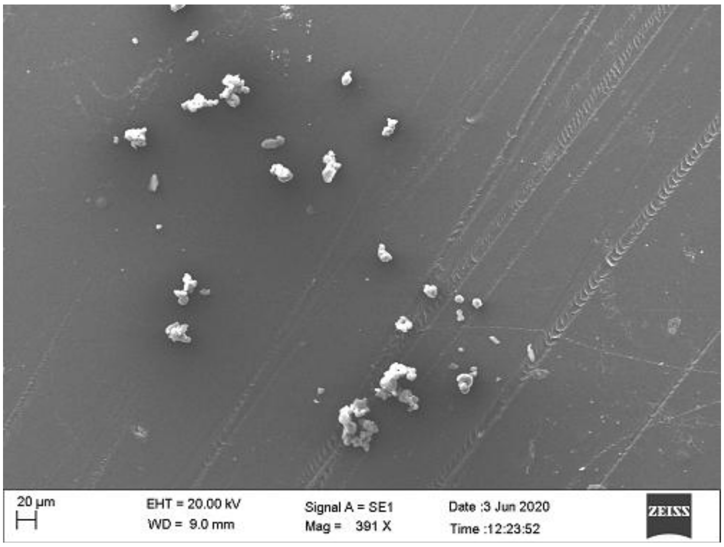
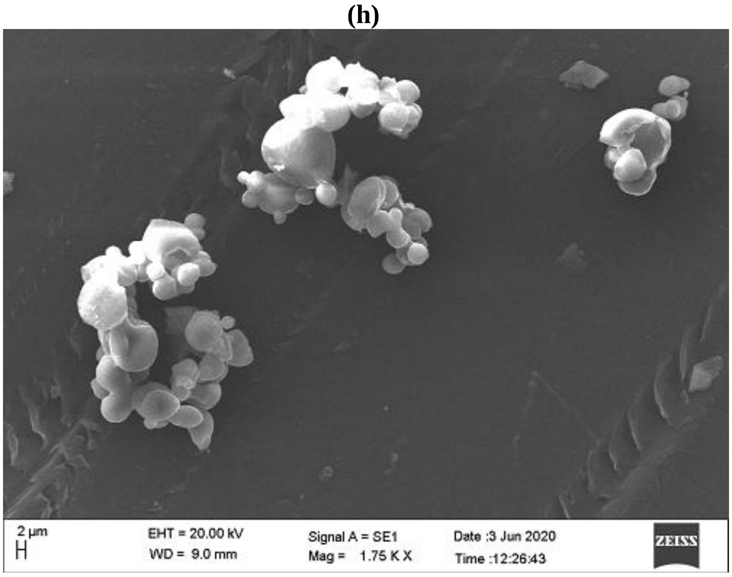
WD = 9.0 mm
Signal A = SE1
Mag = 391 X
Date :3 Jun 2020
Time :12:23:52
ZEISS

(g)



(h)



(i)

Figure 2: SEM images of UV-treated polyethylene

Observation of strips using FTIR:

In FTIR spectrum of UV-treated polyethylene showed formation of carboxylic acids, aldehydes, alcohols, ethers, esters, aromatics and phenolic groups indicating their degradation by *Penicillium*.

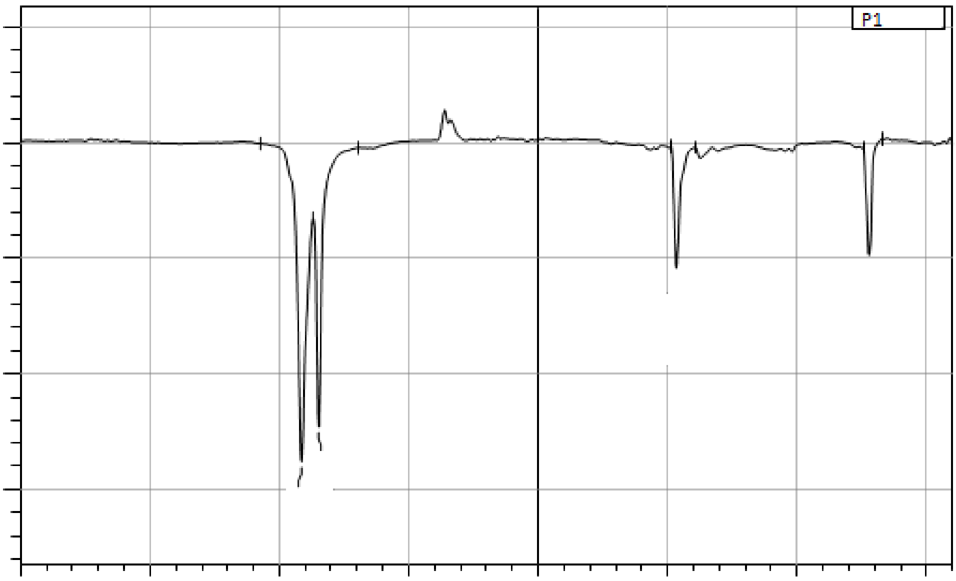
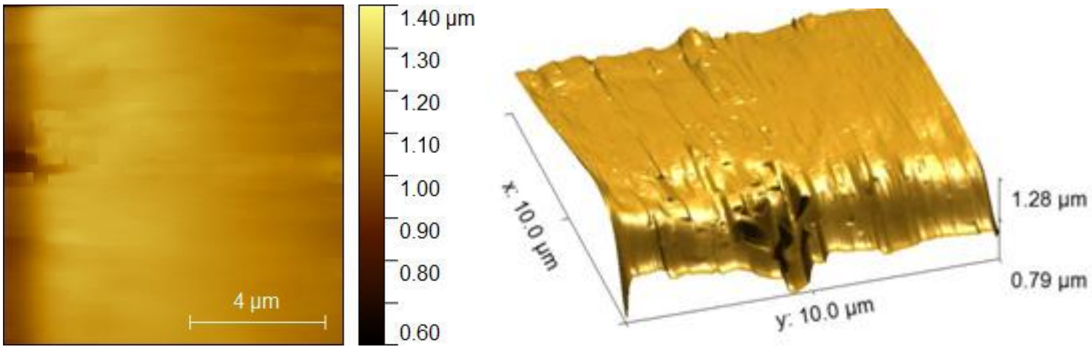


Figure 3: FTIR spectrum of UV-treated polyethylene

Observation of strips using AFM:
The surface morphology of plastic strip was observed by AFM micrographs of plastic strip with UV treated fungus incubation with *Penicillium* showed hyphal growth on the surface of plastic and degradation of the polyethylene around the fungal cells in the biofilm, causing the formation of grooves in the plastic after the incubation.



(a) (b)

Figure 4: (a) AFMof blank polyethylene strip**, (b**) AFM UV-treated polyethylene strip

X-ray diffraction (XRD) analysis:
The X-ray diffraction patterns of the strips were measured with an X-ray diffractometer.

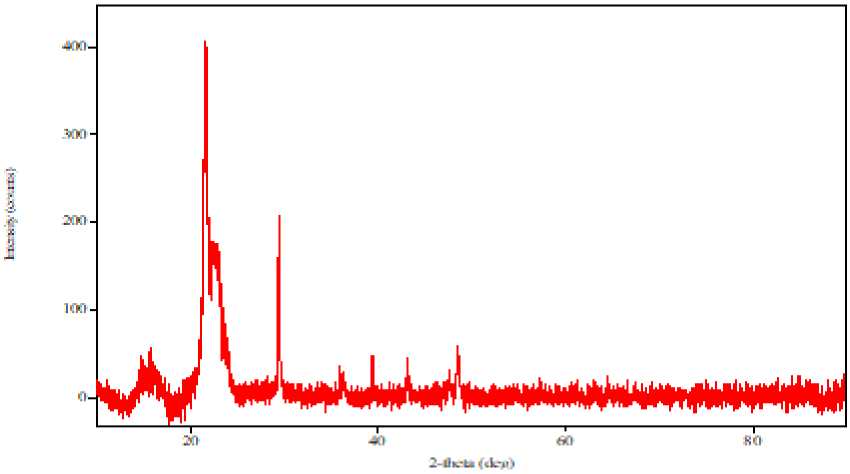


Figure 5: XRD spectra of UV-treated polyethylene strips after incubation in medium

4. Conclusion

Penicillium was isolated from local dumpsites of Raichur, the fungus identified based on both macroscopic and microscopic observations. This fungus was grown on medium containing polyethylene and agar. After the growth of fungus on polyethylene containing medium, it was screened for degradation of UV treated polyethylene. *A niger* was able to degrade UV treated (55.5%). *Penicillium* was able to degrade more effectively with UV light. The research article deals with efficient degradation of LDPE by the isolate *Penicillium*for the duration of four months' exposure. To the best of our knowledge, there are research articles supporting polyethylene degradation by *Penicillium*. Though there are many works on LDPE degradation there is no detailed analysis of AFM and XRD. In future studies, various analysis can be performed along with the molecular mechanism for degradation.

5. Acknowledgement

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