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Biodegradation of LDPE with Mixed Consortium in presence of Super Paramagnetic Iron Oxide Nanoparticle (SPION) as the Enhancer for Biodegradation by Accelerating Growth Profiling of Microbes

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Abstract

Low density polyethylene (plastics) is used in different sectors of applications like building materials, packaging, consumer products and much more. Due to wide range of application, plastics are accumulating in the environment and harm the flora and fauna. LDPE-biodegradation especially by making microbial consortium will be a good choice. Super paramagnetic iron oxide nanoparticle (SPION) has varied potential applications. Studies with SPION nanoparticles have shown their ability in enhancement of polymer degradation due to accelerating growth profiling of microbes. Super paramagnetic iron oxide nanoparticles (SPION) with size ranging 10.6 nm were used and study the degradation potential of microbes. There are three consortium were used and the effect of SPION nanoparticle different on each consortium. Mixed consortium of isolated bacterial and fungal strains shows the higher degradation percentage of LDPE. Degraded products are recovered and tested by FT-IR spectrophotometer. The FT-IR spectra of LDPE have chemical bond shifting that conform degradation take place. SPION improved the exponential phase durability. SPION influence the growth profiles of LDPE degrading microorganisms and consortium to augment the biodegradation rate. The research article primarily focuses on the biodegradation where SPION acted as enhancers of biodegradation. The significance of microbial-nanoparticle interactions which can dramatically influences LDPE-biodegradation.

Keywords: Low Density Polyethylene, Super Paramagnetic Iron Oxide, Soil Microbes, Consortium and FT-IR.

1. Introduction

Plastic (LDPE) are an integral part of our day to day life and are being used in, building materials, packaging and for many other purposes (Gnanavel *et al.*, 2012). Plastic (LDPE) has gained remarkable indispensable character in all fields of activities (Fig 1). The worldwide utilization of polyethylene is explored at a rate of 12% annum and approximately 140 million tons of synthetic polymers are produced worldwide each year. One kind of the plastic waste that hard to degrade is polyethylene (LDPE).

Polyethylene (LDPE) is a linear hydrocarbon polymers consisting of long chains of the ethylene monomers (Tokiwa *et al.*, 2009).

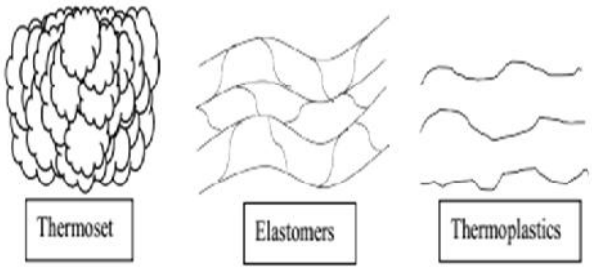


Figure 1: Classification of Petroleum Plastics (Griffey, 2014)

Plastic (LDPE) growing problems in water and land pollution, the need of degradability and taking space for landfills have led to concern about plastics allied with huge accumulation in the environment. It leads to long-term environmental, economic and waste management problems. It is now widely accepted that polyethylene degradation occurs by Oxo degradation mechanism which is a two-step process. The first step is oxidative degradation, which is normally abiotic by means of sun light, heat and the second step is biodegradation in which the oxidation products are degraded by micro-organisms (Chiellini *et al.*, 2006).

The synthetic polymers like LDPE, HDPE are absorbs solar ultraviolet radiation and undergoes photolytic, photo oxidative, photo- chemical and thermo oxidative reactions that result in the abiotic degradation of these materials (Kyrikou *et al.*, 2011). The degradation of polymers depends on the enzymes produced by the microbes to convert the polymers to oligomers and then to monomers. These monomer products are further absorbed by the microbial cells as carbon source where they are metabolized (Vasile, 2000).

There are some nanoparticles that enhance growth cycle, mechanical and physiochemical stability along with biodegradability. Nanoparticle cobalt-ferrite have reported to enhance the growth of *Escherichia coli* and *Corynebacterium xerosis* (Flores *et al.*, 2004). Nanometric silicon particles have also reported that it accelerate the growth profiles of bacteria (Pérez *et al.*, 2002). There are varied inorganic nanoparticles, including silica, silica/iron oxide, and gold have been shown to exhibit no negative influence on the growth and activity of *E. coli* (Williams *et al.*, 2006).

Among a variety of ferrite based Nano-forms, recently SPION have broad applications in several fields including magnetic fluids, magnetic drug delivery, microwave devices and high-density information storage. These SPION exhibit magnetic properties that might to interact with the electric polarity of the bacteria and influence its growth (Flores et al., 2004).

The microbial consortium was documented to degrade synthetic polymers like epoxy and epoxy silicone blends. The participating strains have also been used in combination with other microbes to degrade HDPE (Satlewal *et al.*, 2008), non-poronized and poronized LDPE (Soni *et al.*, 2009). The present investigation deals with the influence of SPION particles on the LDPE biodegradation efficiency with mixed consortium.

2. Materials and Methods

2.1. Soil Sampling

Soil samples were collected from a plastic dumping zone of Bhanpura Bhopal aseptically from a depth of 5 cm - 15 cm. The samples were sieved (mesh size < 2 mm), sorted to remove stones, plant debris.

2.2. Nanoparticle: Super paramagnetic iron oxide (SPION) nanoparticle were procured from Nano Green Technology Gwalior.

2.3. Media for culturing:

Luria-Bertani (LB) broth and agar are the most widely used media for the growth of bacteria. Potato dextrose broth (PDB) was used for suspension culture and Potato dextrose agar (PDA) was used for platting and striking. Both PDB and PDA were procured from Sigma –Aldrich (for fungal strains). Nutrient agar and broth are used for mixed consortium.

2.4. Isolation of microbes:

Soil was collected from plastics dumping zone. Serial dilution of soil was done by dissolving soil in water and then taking 1ml of the solution and making up volume to 10 ml and the same process was repeated 9 times. Then the microbes left in solution was platted on solid basal media. Different colonies was picked and platted on other petridish to form isolated colonies which was further be used for degradation of LDPE (Zahra *et al.*, 2010)

2.5. Growth profiling of individual strains without nanoparticles through Spectrophotometer

Overnight cultures of all individual strains were transferred into fresh media and OD was observed after every 2 hours at 540 nm for fungal, 560 nm for mixed and 600 nm for bacteria and a graph was plotted for OD against time to determine the growth curve of strains (Kapri *et al.*, 2010).

2.6. Determination of optimum tolerance for nanoparticles

Five different concentrations of nanoparticles (0.1%, 0.25%, 0.50%, 0.75%, and 1%) were made from stock. Microbial cultures were inoculated in these different concentrations and allowed to grow for overnight. Then these overnight cultures were grown on agar plates each for one nanoparticle for six hours to perform spot assay. Spot assay results were evaluated to see what concentration of nanoparticle is toxic to which microbial strains (Sah *et al.*, 2010).

2.7. Growth profiling of individual strains with nanoparticles

Overnight cultures of all individual microbial strains were transferred into fresh media containing nanoparticles and OD was observed after every two hours at 600nm (bacterial), 560 nm (mixed) and 540 nm (fungal) and a graph was plotted for OD against time to determine the growth curve of microbial strains (Kapri *et al*., 2010).

2.8. Formation of microbial consortium:

Different microbial species were collected from plastics dumping zone. Strains were grown on nutrient basal media contents and maintained at optimum conditions. Cfu/ml for each strain was measured and microbial consortia was formed accordingly.

2.9. Growth profiling of microbial consortium without nanoparticles

100 ml of nutrient media was added to a 250ml flask. 300 µl of active log phase consortia was added to it. Samples was incubated at optimum conditions. OD would be taken at 600nm (bacterial), 560 nm (mixed) and 540 nm (fungal) at interval of 2 hours for each consortium (Kapri *et al*., 2010).

2.10. Comparative growth profiling in the presence of nanoparticles

100 ml of nutrient basal media was added to a 250ml flask. 300 µl of active log phase consortia was added to it. Working solution of nanoparticles was added to it. Samples was incubated at optimum conditions. OD was taken at 600 nm (bacterial), 540 nm (fungal) and 560 (mixed) at interval of 2 hours for each consortium (Kapri *et al*., 2010).

2.11. *In -vitro* LDPE biodegradation assay using nanoparticles

100 ml of media was added to 250-ml flask containing LDPE film (1 cm, 4 mg). The flasks was inoculated with 300 µl of active consortium containing nanoparticles. The assay was performed with respective positive (minimal broth + consortia) and negative (minimal broth + LDPE) controls with and without selected 10.6 nm SPION. The flasks was incubated at optimum temperature 37^{o}C for bacterial consortium, 32^{o}C for mixed consortium and 25^{o}C for fungal consortium with continuous shaking (120 rpm). The assays was monitored for microbial growth by measuring the OD at 600 nm for bacterial, 540 for fungal and 560 for mixed consortium (Kapri *et al*., 2010).

2.12. Recovery of degraded products

The biodegraded samples obtained after the assay were analysed by FT-IR, and different peaks relative to CH_2 deformation, CH_2 bending (symmetrical), CH_2 bending (asymmetrical), CH_2 stretching (asymmetrical and symmetrical), CH_2 rocking, CHstretching, and C-O bond were compared by taking pure LDPE as a reference. Bending, stretching, and rocking vibrations have been depicted by δ, ν, and ρ, with asymmetrical and symmetrical absorptions represented by subscripts “as” and “s,” respectively (Kapri *et al*., 2010).

2.13. Fourier Transform Infra-Red spectroscopy

After the consortium had attained stationary growth phase, the degraded compound was recovered from the broth and subsequently washed with ethanol and then analysed by FTIR-ATR and using pure LDPE film as the control (Kapri *et al*., 2010).

In infrared spectroscopy, IR radiation was passed through a sample. Some of the infrared radiation was absorbed by the sample and some of it is passed through (transmitted). The spectrum of sample represents the molecular absorption and transmission, creating a molecular fingerprint of the sample.

3. Results and Discussion

3.1. Growth kinetic studied for selection of similar microbial strains:-

Total sixteen (16) bacterial strains were isolated. In these isolated bacteria only four strains were showing similar growth pattern and further used for toxicity assay.

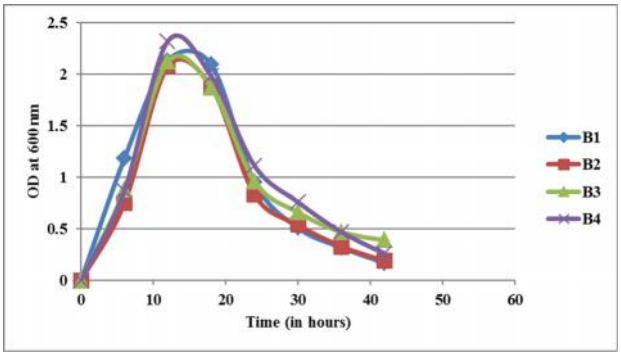


Figure 2: Growth kinetic study of isolated bacterial strains.

Total thirteen (13) fungal strains were isolated by serial dilution method. In these thirteen fungal strains four strains were selected on basis of growth pattern.

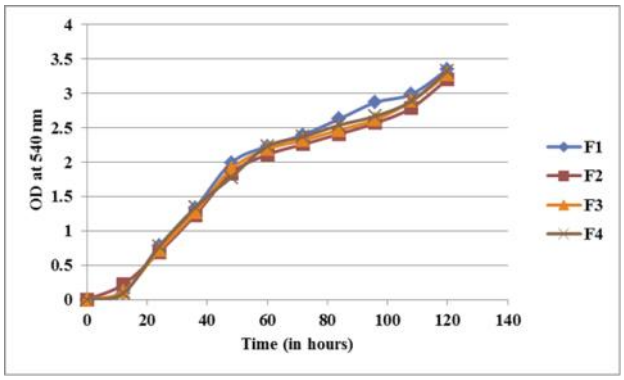


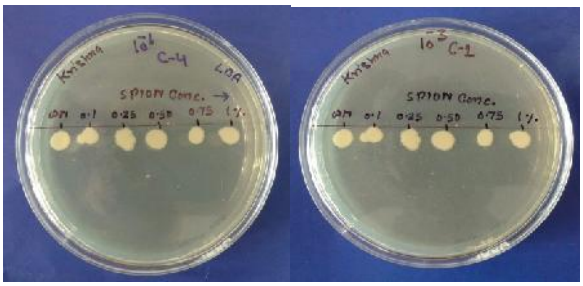
Figure 3: Comparative growth profiling of isolated fungal strains.

The isolated fungal strains F1, F2, F3 and F4 shows similar growth pattern, hence these four strains were used for further experiment.

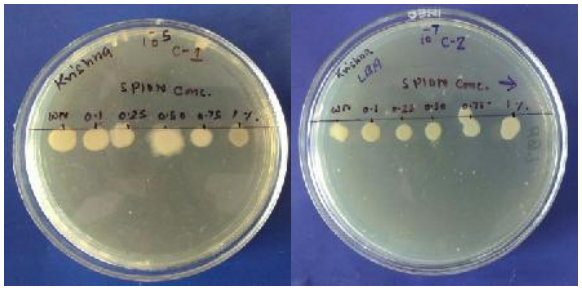
3.2. Toxicity assay of microbial strains with SPION nanoparticle at different concentration to check the tolerance level:

All the bacterial and fungal strains were tested for toxicity with the SPION nanoparticle at the five different concentrations (0.1%, 0.25%, 0.5%, 0.75%, and 1%) since optimum tolerance level of microorganism with nanoparticles is not known.

Bacterial strains:



Strain no. B1 **Strain no. B2**



Strain no. B3 **Strain no. B4**

Figure 4: Strain no. B1: Maximum growth shows at the point of 0.50 % concentration of SPION.Strain no. B2: Maximum growth shows at the point of 0.25 % concentration of SPION.Strain no. B3: Maximum growth shows at the point of 0.50 % concentration of SPION.Strain no. B4: Maximum growth shows at the point of 0.75 % concentration of SPION.

3.3. Growth kinetics of selected bacterial strains with and without nanoparticles:

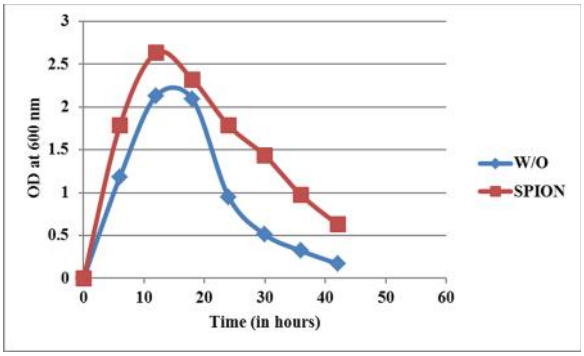


Figure 5: Growth curve of bacterial strain B1 with and without nanoparticles.

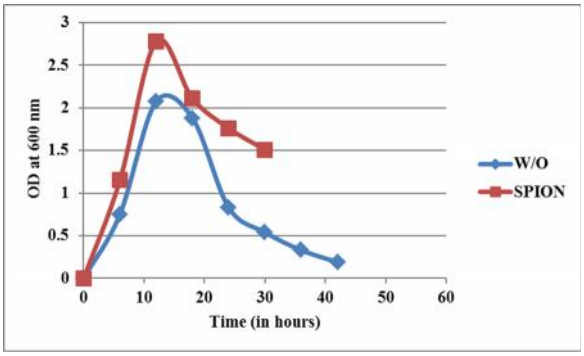


Figure 6: Growth curve of bacterial strain B2 with and without nanoparticles.

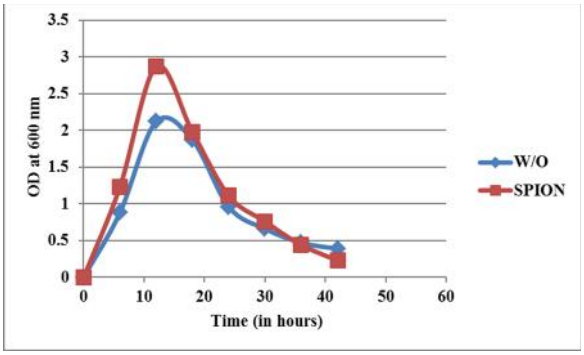


Figure 7: Growth curve of bacterial strain B3 with and without nanoparticles.

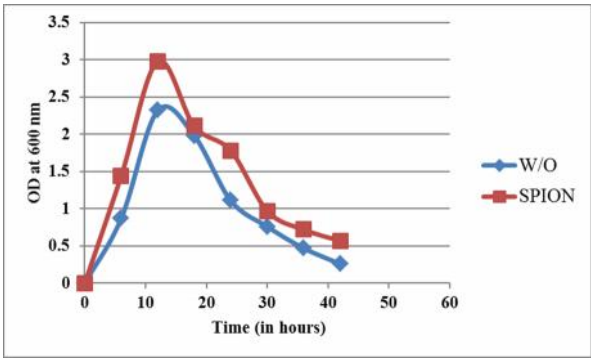
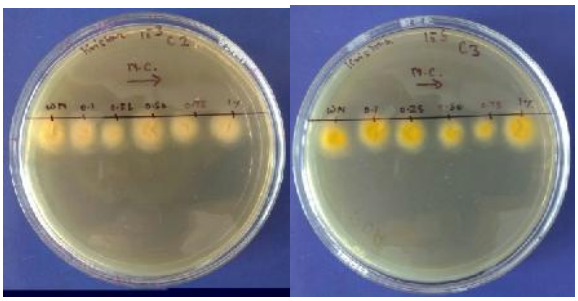
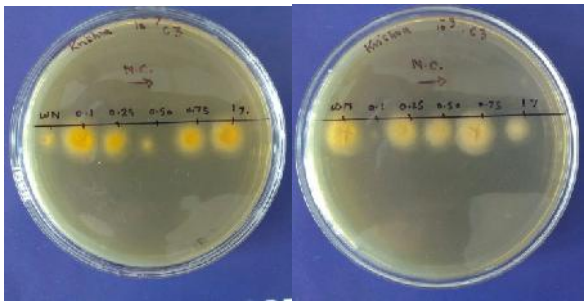


Figure 8: Growth curve of bacterial strain B4 with and

3.4. Fungal Isolated Strains:-



Strain no. F1Strain no. F2



Strain no. F3Strain no. F4

Figure 9: Strain no. F1: Maximum growth shows at the point of 0.50 % concentration of SPION. Strain no. F2: Maximum growth shows at the point of 1% concentration of SPION. Strain no. F3: Maximum growth shows at the point of 0.1 % concentration of SPION. Strain no. F4: Maximum growth shows at the point of 0.75 % concentration of SPION.

3.5. Growth kinetics of selected fungal strains with and without nanoparticles:

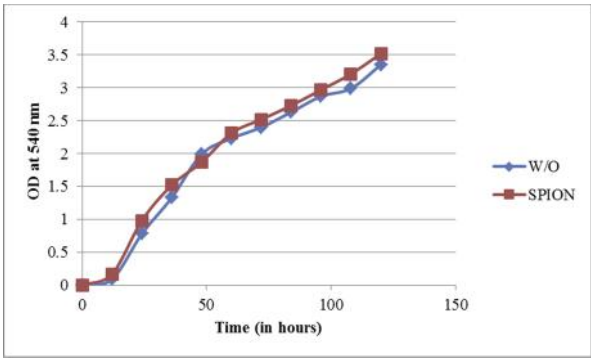


Figure 10: Growth curve of fungal strain F1 with and without nanoparticles.

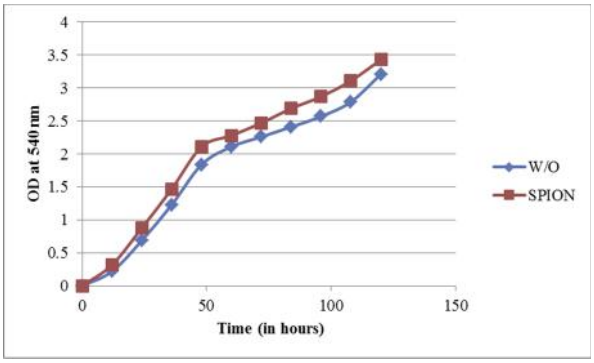


Figure 11: Growth curve of fungal strain F2 with and without nanoparticles.

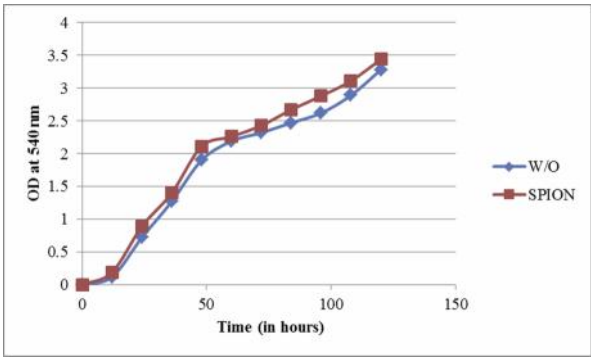


Figure 12: Growth curve of fungal strain F3 with and without nanoparticles.

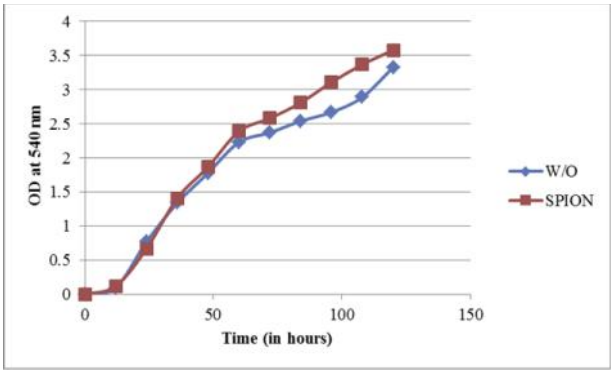


Figure 13: Growth curve of fungal strain F4 with and without nanoparticles.

3.6. Toxicity assay of bacterial consortium with SPION nanoparticles:

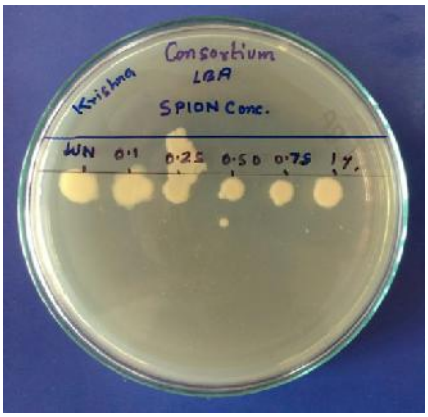
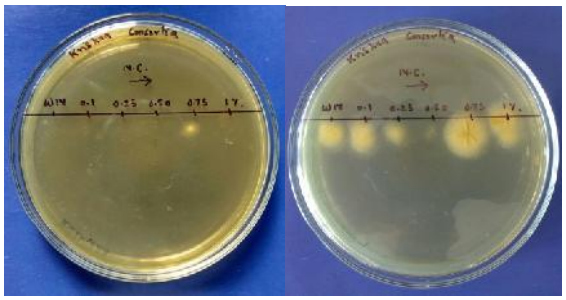


Figure 14: Toxicity assay of bacterial consortium at different concentration of SPION nanoparticles.Maximum growth shows at the point of 0.25 % concentration of SPION.

3.7. Toxicity assay of fungal consortium (FC) with SPION nanoparticles:



After 8 hours **After 36 hours**

Figure 15: Toxicity assay of fungal consortium at different concentration of SPION nanoparticles. Maximum growth shows at the point of 0.75 % concentration of SPION.

3.8. Toxicity assay of mixed consortium (Sridhar & Kumar) with SPION nanoparticles:

Figure 16: Toxicity assay of fungal consortium at different concentration of SPION nanoparticles. Maximum growth shows at the point of 0.50 % concentration of SPION.

3.9. Growth profiling of bacterial consortium with and without nanoparticles:

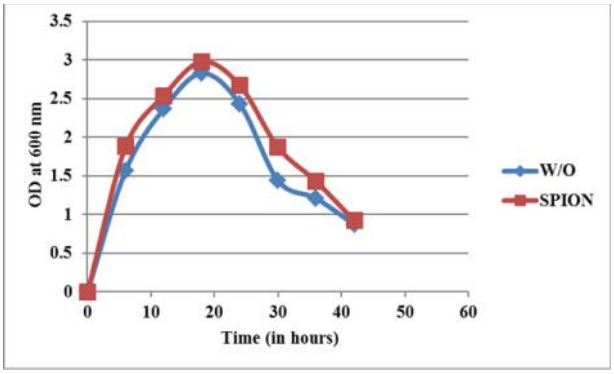


Figure 17: Growth curve of bacterial consortium (BC) with and without nanoparticles.

3.10. Growth profiling of fungal consortium with and without nanoparticles:

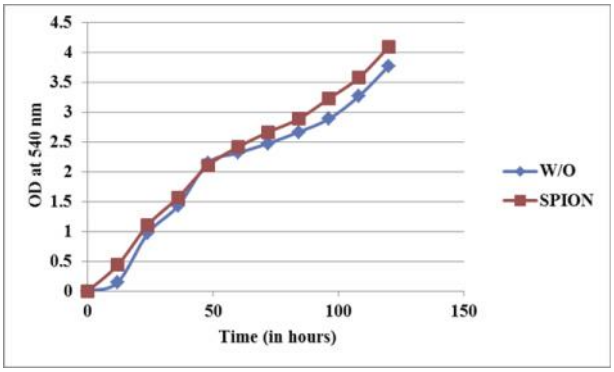


Figure 18: Growth curve of fungal consortium (FC) with and without nanoparticles.

3.11. Growth profiling of mixed consortium with and without nanoparticles:

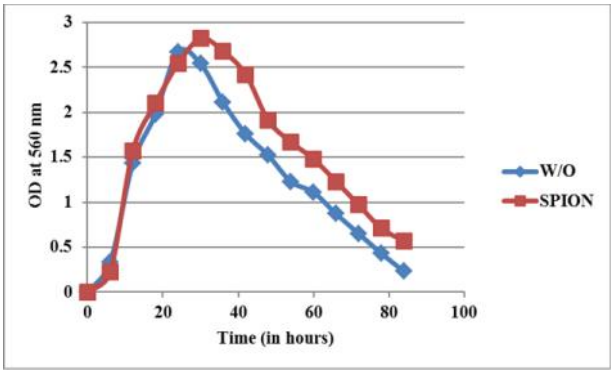


Figure 19: Growth curve of mixed consortium (MC) with and without nanoparticles.

3.12. Weight Loss % by bacterial strains:

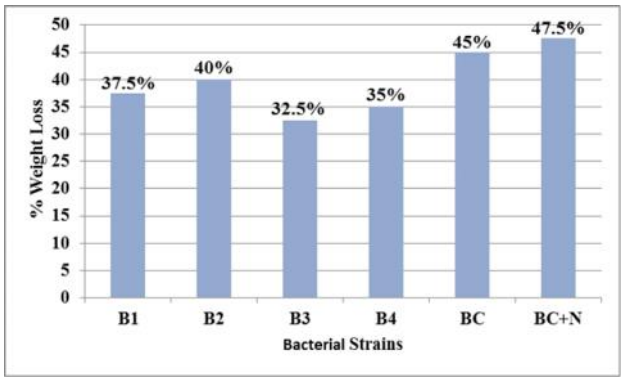


Figure 20: Polymer degradation percent achieved by respective bacterium.

3.13. Weight Loss % by fungal strains:

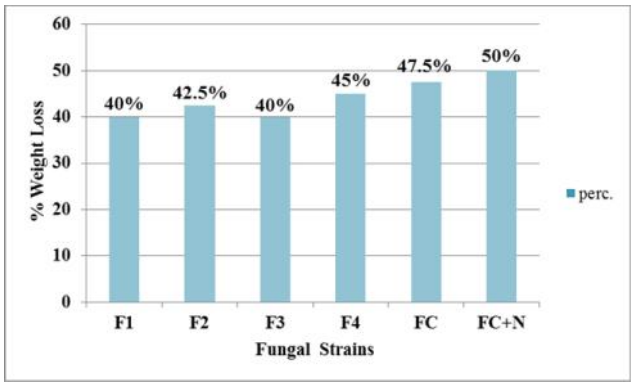


Figure 21: Polymer degradation percent achieved by respective fungal strains.

3.14. Comparative study of Weight Loss % by microbial consortium:

Figure 22: Polymer degradation percentage achieved by respective microbial consortium.

3.15. FT-IR spectra of LDPE film before and after degradation in consortium with nanoparticle

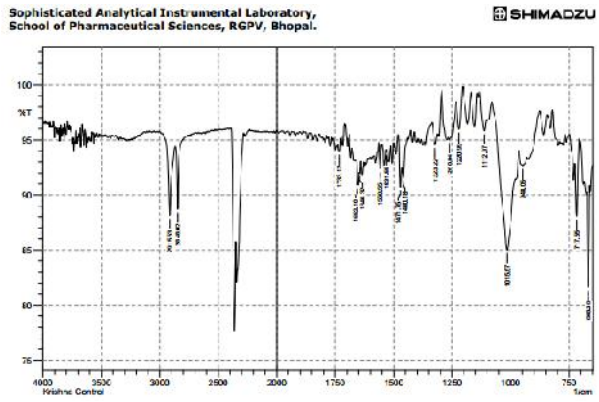


Figure 23: Just to check the degradation process control was taken as reference which was kept without any consortium and nanoparticle. Figure shows pure LDPE film illustrated FT-IR absorptions corresponding to ρ CH_2 (668.36), δ CH_2 (1471.75), >CH_2 deformation (1558.55), υ_s CH_2 (2848.02), υ_{as} CH_2 (2915.53), respectively, carbonyl absorption bands can be observed at 1733.12, methylene band at 1460.18.

3.16. FT-IR spectra of LDPE with bacterial consortium (BC) and SPION:

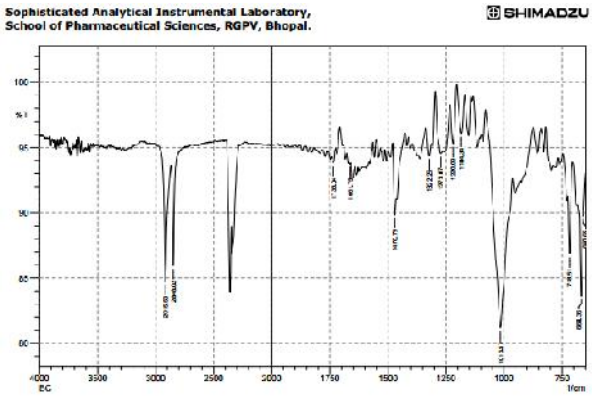


Figure 24: Results of degradation by showing the peaks to ρCH_2 (668.36), δCH_2 (1471.75), υ_s CH_2 (2848.02), υ_{as} CH_2 (2915.53), respectively, with methylene band at 1470.79 in addition with the control. Various other additional peaks are also there at 660.65, 1184.34, 1220.03, 1273.07, and 1322.26.

3.17. FT-IR spectra of LDPE with fungal consortium and SPION:

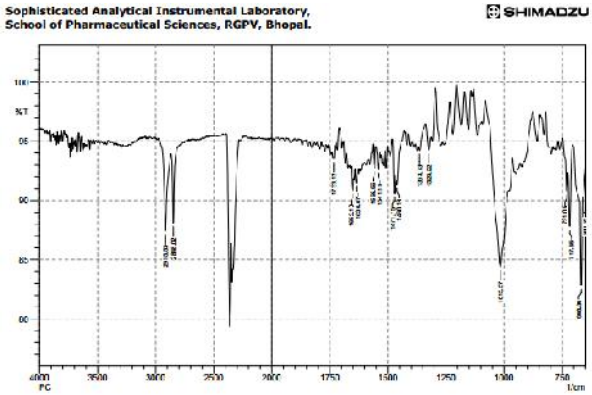


Figure 25: Results of degradation by showing the peaks to ρCH_2 (668.36), δCH_2 (1471.75), υ_s CH_2 (2848.02), υ_{as} CH_2 (2915.53), respectively, with methylene band at 1460.18 in addition with the control. Various other additional peaks are also there at 717.55, 731.05, 1015.57, 1323.22, 1363.73, 1541.19, 1558.55, 1636.67, and 1652.10.

3.18. FT-IR spectra of LDPE with mixed consortium and SPION:

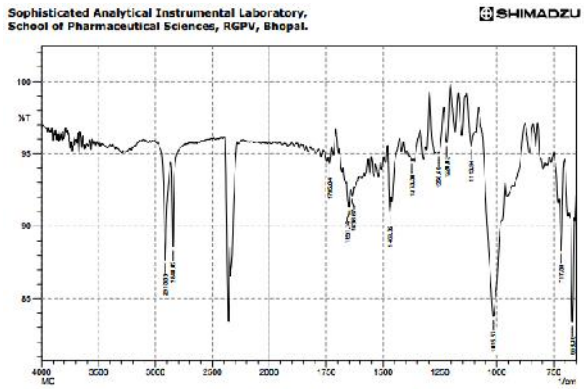


Figure 26: Results of degradation by showing the peaks to ρCH_2 (668.36), δCH_2 (1471.75), υ_s CH_2 (2848.02), υ_{as} CH_2 (2915.53), respectively, with methylene band at 1469.82 in addition with the control. Various other additional peaks are also there at 1113.94, 1256.68, 1373.38, and 1651.14.

4. Conclusion

Super paramagnetic iron oxide (SPION) has been able to enhance rate of degradation of LDPE with the help of microbial consortium. The study reveals that a more stable suspension of SPION brings about an increase in the growth of polymer-degrading microbes which increases its LDPE biodegradation efficiency. Weight loss percentage of LDPE filmconfirms that SPION nanoparticle with mixed consortium is showing maximum degradation in 50 days i.e.; 55% compare to other test samples. It would be therefore facilitate the efficacy of plastic biodegradation and prove to be an important step in devising waste management strategies.

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