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"LDPE-biodegradation using microbial consortium by the incorporation of Cobalt Ferrite Nanoparticle as the enhancer for biodegradation"

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Abstract:- *Plastics are the synthetic materials created out of the fossil fuel resources. As a result they remain in the environment for a very long time without any degradation because of microbial attack resistance. It has given rise to serious threats to the environment due to its poor waste disposal. Plastics have various drawbacks like inducing lower water absorption of soil, reduction in the fertility of soil. Therefore, the degradation of Low Density Polyethylene (LDPE) by mixed microbial consortium with Nanoparticles seems to be a good choice.Nanoparticles studies have shown their ability to degrade the synthetic polymer with the help of microbes. Cobalt Ferrite Nanoparticle with size ranging 15-40 nm were synthesized and characterized through XRD, FT-IR spectra, simultaneous TG-DTG-DTA, vibrational sample magnetometry (VSM) and transmission electron microscopy (TEM).Nanoparticles influence exponential and stationary phase. It acts by reducing the duration of lag phase and increasing the duration of exponential and stationary phase. These nanoparticles influence the growth profiles of LDPE degrading microorganisms and enhance the rate of Biodegradation. This review has its emphasis on LDPE degradation aspects of mixed consortium associated with Nanoparticle act as enhancers of biodegradation.*

Introduction

Polyethylene is a synthetic material created through fossil fuel resources.The annual global production of plastics in India is 4.3 million tones in year 2001-03 and that of the year 2006-07 was approximately 8 million tones **(Gnanavel *et al*.,2012).** Plastics are used in many applications due to their durability, processability and light weight **(Sivasankari *et al*.,2011)**.The present demand for plastics is very high because of their use in daily life. Various different types of polyethylenes used in packaging are (LDPE, MDPE, HDPE and LLDPE polystyrene (PS), polyurethane (PUR), poly(ethylene terephthalate) (PET), poly- propylene (PP), poly (butylene terephthalate) (PBT), nylons, polyvinyl chloride (PVC) **(Ghosh *et al*.,2013).**

They do not break down in the environment easily because they are resistant to microbial attack, due to their excessive molecular mass, unusual bonds, high number of aromatic rings or halogen substitutions. As a result they remain in the environment for a very long time without any degradation. It has given rise to the severe environmental pollution **(Dey *et al*.,2012).**These polyethylenes reduce the fertility of soil as well as reduce water absorbance power of soil **(*S.B.* Gupta *et al.,2012*).** On burning, it releases some toxic gases and other chemicals thereby polluting environment. This in turn leads to diseases that affect the lungs and skin **(Hadad *et al.*,2004).** Plastics are used in all sectors of economy such as packaging, health and medical, construction, food, clothing, shelter, transportation **(Tribedi & Sil *et al*.,2013).**

The reason of popularity of these plastic is long time durability and there light weight. As a rule, widely used plastics do not degrade naturally due to high molecular weight Aromatic rings **(J Arutchadvi *et al.*, 2004).**There are four mechanisms by which polyethylenes degrade in the environment photodegradation, thermooxidative degradation, hydrolytic degradation and biodegradation with the help of microorganisms**(I Kyrikou *et al.*, 2007).** Natural degradation of plastics begins with photodegradation, which leads to thermooxidative degradation. Ultraviolet light from the sun is responsible for natural degradation because it provides the activation energy required to initiate the incorporation of oxygen atoms into the polymer **(Hayden K *et al.*, 2013)**. This causes the long chain of polymer to break into small sized monomers or breakdown of higher molecular weight polymer into lower molecular weight monomers.

Nanoparticles are the new advancement in study of plastic degradation. We found, the nanoparticles influenced microbial growth profile and their incorporation was responsible for the enhancement of degradation. Any particle having a size range between 1-100 nm is called a nanoparticle. Some previous studies have shown that various types of nanoparticles enhance thermal, mechanical and physiochemical stability along with biodegradability **(Bhardwaj *et al.*, 2013).**

Cobalt ferrite nanoparticle were produced by co-precipitation, combustion, precipitaion method and the particle size between 15 to 24 nm, as determined by X-ray diffraction (XRD) , Raman, therm-ogravimetric differential thermal analysis and transmission electron microscope FT-IR spectra, simultaneous TG-DTG-DTA, vibrational sample magnetometry (VSM) and transmission electron microscopy (TEM) **(Houshiar *et al.*,2014).**These types of nanoparticles are very promising tools for several applications like biomedical aspects(drug delivery), ferrofluids, storage devices etc. According to (**Marjorie *et at.*, 2004)** Cobalt ferrite nanoparticle gives the closer view of interaction between the electric polarity of microbe.

Biodegradation by Micro-organisms:

It has already been reported in pureculture studies with various microorganisms such as *Streptomyces* sp (Bailey *et al*.,1991),*Phanerochaetesp***(Ali *et al*.,2009),***Pseudomonas, Xanthomonas, Flavobacterium, Micrococci, Streptococcus, Staphylococcus, Bacillus* **(Gupta *et al., 2012*)** *Penicillium Alcaligenes, Fusarium, Amycolatopsis sp., Comamonas acidovorans1, Alternaria, Spicaria* spp., *Aspergillus*,**(Ibrahim *et al*., 2011**) *Aureobasidium, Poecilomyces* **(Borghei *et al*., 2010)** after chemical degradationwas initiated and with their correspondingextracellular enzymes. Studies have also reported the usefulness of *Microbacteriumsp*. Strain MK3, *Pseudomonas putida* strain MK4, Bacterium Te68R strain PN12, *P. aeruginosa* strain PS1, P. putida strain PW1, and *P. aeruginosa* strain C1(**Sah *et al*., 2010)***Brevibacillus borstelensis* **(Hadad *et al*., 2009)** in successful degradation of plastic films. It has been reported that *Lysinibacillus xylanilyticus* was able to degrade 29.5% of plastic film and *Aspergillus niger* 15.8%. Experiments have also been conducted using *Microbacterium* sp. strain MK3, *Pseudomonas putida* strain MK4, *Bacterium* Te68R strain PN12, *Pseudomonas aeruginosa* strain PS1, *Pseudomonas putida*strain PW1,*Pseudomonas aeruginosa* strain C1, *Acanthopleurobacter pedis* strain SPA1, *Bacillus cereus* strain SPA2, *P. otitidis* strain SPT1, *Bacillus aerius* strain SPT2, *Acanthopleurobacterpedis* strain SPT3, *Bacillus cereus* strain SPK1which were proven to degrade plastic. *Phanerochaetechyrosporium***(Ali *et al*., 2009)** Geomyces pannorum was found to be the predominant fungi consisting 22–100 % of the polyester degrading fungi (Barratt *et al*., 2006). white rot fungus *Basidiomycetes* , *Ascomycetes, Deuteromycetesetc* etc. these fungus produces lignin degrading laccase enzyme that can degrade the plastic**(white rot fungai ref**). *Aspergillus niger, Asp.flavous, Asp. Fumigates,Asp. Oryze* Etc. all are common fungi use in degradation of plastic**.(Pandey *et al*., 2015*)(* Sivan *et al*,.2011).**

Synthesis of Cobalt ferrite Nanoparticle:

Cobalt ferrite nano-particles were synthesized by co-precipitation method followed by annealing treatment. The nanoparticle size, microstructure and composition were confirmed by X-ray diffraction, Raman, thermo gravimetric-differential thermal analysis and transmission electron microscope (TEM), FT-IR spectra, simultaneous TG-DTG-DTA. The sample were prepared by controlling the concentration of mixed solution of Co2+ and Fe3+ ions**.** Nanoparticle prepared by adding the mixed solutions of Co2+ and Fe3+ ions in to the NaOH solutions were observed by small saturation magnetization and enhanced coercivity. It was related to the formation of outer layer with poor crystallization on the surface of the cobalt ferrite nano crystal **(Zhang *et al*.,2010).**

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Characterization of Cobalt ferrite Nanoparticle:

Cobalt ferrite nanoparticle was summarized by the following reaction, which involve the precursor precipitation and its ulterior conversion into the anhydrous ferrite through a dissolution recrystallization process.

Co2+ + 2Fe3+ + NaOH _ CoFe2(OH)8 _ CoFe2O4 + 4H2O.

Sizes of the cobalt ferrite were produced by the co- precipitation method in the range of 15 and 40 nm, as determined by HRTEM. The average size estimated from the XRD. The values of room-temperature coercivity and magnetization are in order of 55-60 emu/g and 600-700 Oe, respectively **(Flores M *et al*.,2004).**

Particles size determined by scherrer formula and average size of particles calcined at 600oC were found to be 15nm, 17.5nm, 21± 3nm with size controlling by NaOH salt solution. A larger size particle as the growth rate begins to exceed the nucleation rate by annealing 15nm particles at 800, 900, 850 and 1000oC respectively for 10 hours, particle sizes of 24, 26, 32 and 38± 3nm were obtained.

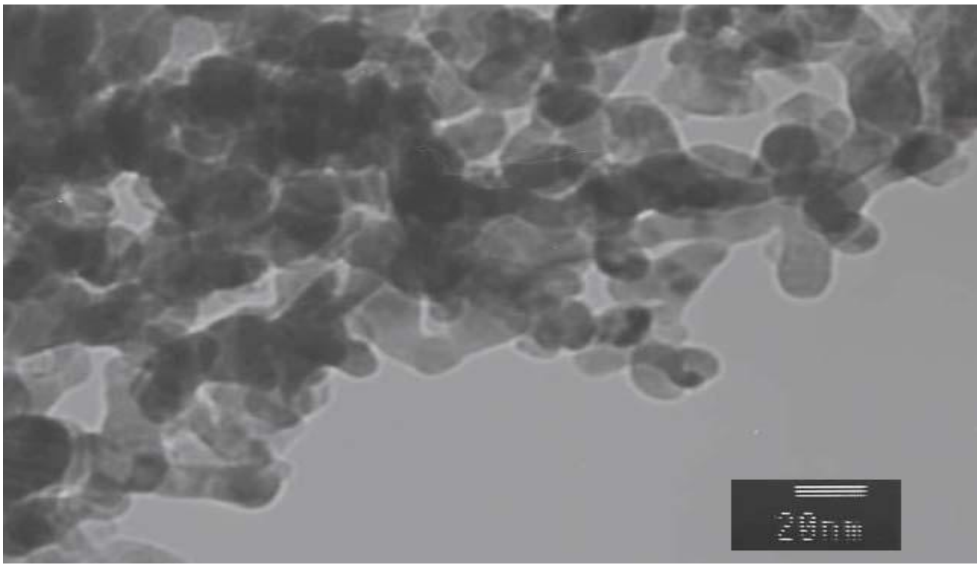

Figure1: Cobalt ferrite Nanoparticle size 20 nm (TEM) (K.Maaz *et al.*,2014)

Growth statics of Cobalt ferrite nanoparticle and nanopartice – cell interaction:

Nanoparticles are feasible for many microorganisms like bacterial strains and some of fungal strains. Present investigation is centred on the growth curve study of E.coli and C.xerosis in presence of cobalt ferrite nanoparticle, where as cobalt ferrite has been reported to increase the growth of Escherichia coli and Corynebacterium xerosis. The standard growth curves were prepared using 5.0 ml of culture strain in 130 ml of Nutrient Broth and 20ml of nanoparticle suspension. The mixture is kept at 37°C with homogeneous shaking. The growth was measured by Spectrophotometer named Genesis 5 UV-VIS Single Beam at 640nm in the visible range of 30-min for 20hr.

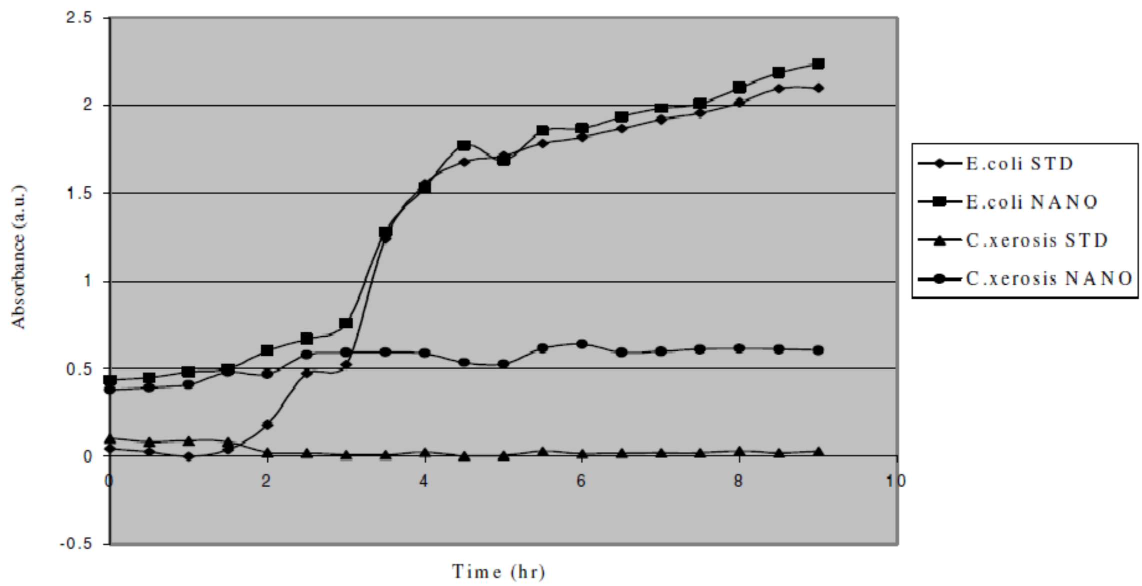

Figure2: Bacteria growth curve with Cobalt ferrite Nanoparticle (Flores M *et al.*,2004).

The growth curves of the bacteria E. *coli and* C. *xerosis* in a medium containing Cobalt Ferrite Nanoparticles. In case of E. *coli* bacteria, the standard curves and the one with nanoparticle behave the same way after the third hour possibly because of the presence of a critical population of bacteria **(Flores M *et al.*,2004).**

Nanoparticle-cell interaction enhances the mechanical, thermo chemical and physicochemical stability along with growth of organism. Cobalt Ferrite allows a closer view of the interaction between the electric polarity of the bacteria and the external magnetic field. Bacterial cell-interaction of the electric polarity with a magnetic property of the Cobalt Ferrite involved the observed phenomena. Changes in the concentration of Cobalt Ferrite was done to determine the interaction of the electric field produced by the particles with the polarity of the bacteria in the growth curve this changes are the responsible of this effect. Another possible cause of this behaviour is attributing in to the creation of cofactors produced in the medium in presence of the Nanoparticles. These cofactors are not yet determined. Cobalt ferrite nanoparticle provides larger surface area for microorganism to adhere and initiate degradation. Plastic being hydrophobic in nature does not allow microbes to attach on its surface. Initially nanoparticle attaches with plastic surface and provides hydrophilic surface for microorganism to adhere and grow properly. Specially at cellular level Cobalt ferrite nanoparticle behave in relation to microorganisms since the interaction between the particles and the membrane is non-specific rather than specific between the nanoparticle and a particular component of the membrane such as a surface expressed proteins. Enzymes secreted by microbes play important role in degradation **(Sangale *et al.*,2012)**. Biodegradation is determined by weight loss of the plastic sample, morphological changes by Scanning Electron Microscopy, spectroscopy analysis by Fourier transform infrared spectroscopy (**H.Negi1 *et al.*,2012**).

Biodegradation of plastics:

In the degradation of plastics by the help of micro-organisms, the degradation rate is slower **(Sangale *et al.*,2012).**Micro-organisms secrete a variety of enzymes to degrade the plastics. There are two types of enzymes involved in the process of degradation, intracellular and extracellular depolymerases. Exoenzymes first break the complex polymer down in to short chains or monomers. These are small enough to permeate through the cell membrane to be utilized as carbon and energy source sothis process is called as depolymerization **(Dey *et al*.,2012)**.After the degradation of plastics they release the end product as water, methane or carbon dioxide after that this process is called mineralization.

Mode of Action (Dussud & Ghiglione *et al.*, 2014):

There are several steps that occur in the plastic biodegradation process (Figure 1) and could be identified by specific terminology.

Bio-deterioration:

Deterioration leads to superficial degradation that modifies physical, chemical and mechanical properties of plastics. In most of the cases biotic parameters contribute to weaken the polymeric structure of plastic. In some other cases it is responsible for the initiation of biodegradation process **(Shah *et al.*,2008).** Bio-deterioration defines the action of microorganisms; these microbes are grown on the surface and inside of plastic and form microbial biofilm. The development of biofilm is dependent on the environmental condition, structure and composition of plastic. (**Helbling *et al.*, 2006; Ipekoglu *et al.*, 2007).**

Chemical and physical deterioration by microbial biofilm:

Physical deterioration:

The microbial biofilm secretes extra cellular polymeric substances (EPS) that adhere to plastic surface. The extra cellular polymeric substance then enters the pores. This results in Microbial growth inside the plastic which in turn increases the pore size and promotes crack formation **(Bonhomme *et al.*, 2003).**

Chemical deterioration:

Microorganisms form biofilm on thesurface of plastic as well as inside the plastic. They release acids such asnitrous acid (e.g. *Nitrosomonas* spp.), nitric acid (e.g. *Nitrobacter* spp.) or sulphuric acid (e.g. *Thiobacillus* spp.) and some organic acids such as oxalic acid, citric acid, fumaric acid, gluconic acid, glutaric acid, glyoxalic acid by chemolithotrophic bacteria. The modified pH inside the pores results in a progressive degradation and change inthe microstructure of plastic.

Bio-fragmentation:

UV radiation, thermal, chemical and biological are some other ways of fragmentation of plastic into oligomers and monomers **(Sharma, *et al.*, 2008).**Microorganisms secrete extracellular enzymes (**exoenzymes)** that refer to the catalytic actions that cleave polymeric plastics into oligomers, dimers or monomers. It can be responsible for imbalance of electric charge and perform lysis.

Assimilation:

After Bio-fragmentation these monomers are transported in cytoplasm by specific carriers **(**
(Swapnil K. & Kale,*et al.,2015)*.Plastic monomers are oxidized through catabolic pathways to produce energy and integration of the atoms inside the microbial cell.

Mineralization:

The complete degradation of molecules results in the excretion of completely oxidized metabolites in form of carbon dioxide, nitrogen, water and some other substance.(CO_2, N_2, CH_4, H_2O).

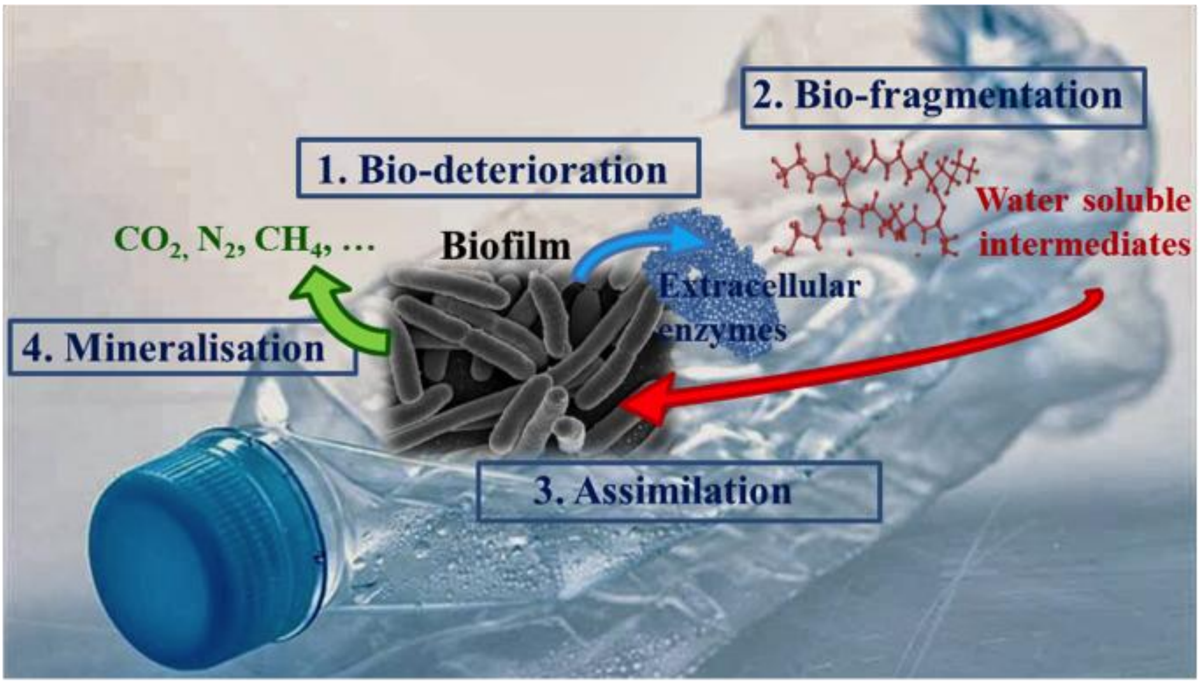

Figure 2: Mechanism of plastic biodegradation under aerobic condition **(ref 11 a).**

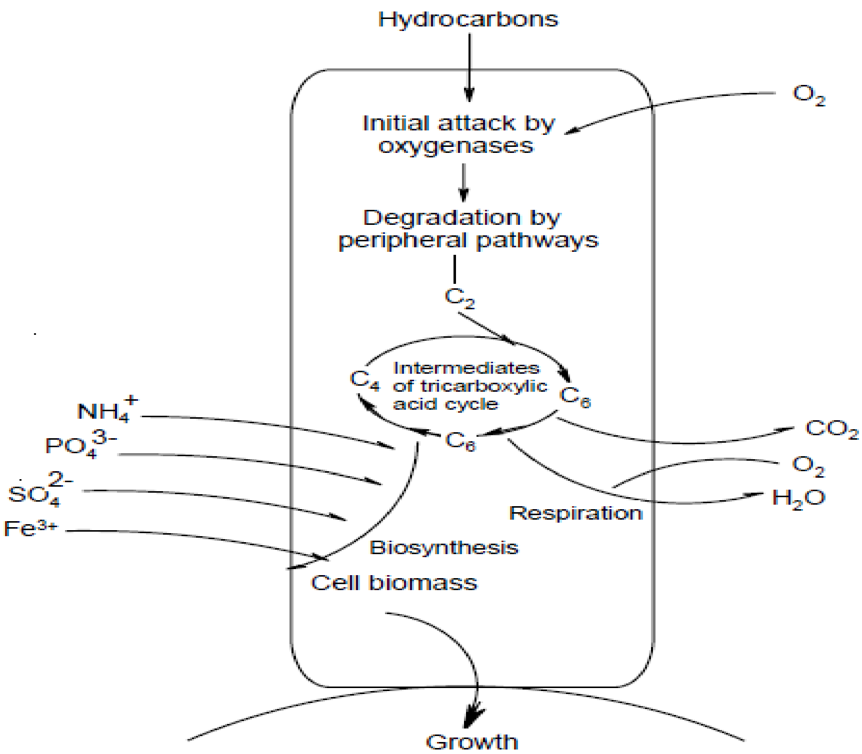

Figure3: Mechanism of enzymatic biodegradation of LDPE (Olajire AA *et al.,2014*)

Advantages of Cobalt ferrite Nanoparticle:

Nanoparticles have broad applications in several technological fields including permanent magnets, magnetic fluids, magnetic drug delivery, microwave devices, and high-density information storage so Magnetic ferrite is gaining more interest. Since Fe2O3 and Co-doped magnetite are already being used in recording media, renewed interest is being shown in cobalt ferrite (CoFe2O4). High density recording of Magnetic ferrite is possible because of as strong anisotropy and high coercivity at room temperature and moderate saturation magnetization, along with good mechanical hardness and chemical stability. However the production of cobalt ferrite particles with the desirable size and magnetic properties, are still a challenge **(Flores M *et al.*,2004).**. Cobalt ferrite (CoFe2O4) is a well-known hard magnetic material with high coercivity and moderate magnetization. These properties, along with their great physical and chemical stability, make CoFe2O4 nanoparticles suitable for magnetic recording applications such as audio and videotape and high-density digital recording disks etc. (**Maaz, *et al*.,2012).**

Conclusions

Polyethylene degradation is the biggest issue to deal with because it accumulates in environment, and it creates grave issues for the planet. There are many ways to degrade these plastics such as photodegradation, thermooxidative degradation, hydrolytic degradation and biodegradation. In this Review, we are focused on Biodegradation process. Biodegradation was firstly done with single microbial strain but that turned out to be occurring at a very slow rate. As light rise in degradation rate was observed when the same was done with consortium, but that was not enough because of higher rate of accumulation. Thus some researchers have found another approach to enhance the degradation rate byutilizing nanoparticles. They found that nanoparticles have the ability to enhance the biodegradation rate of plastics. Use of Nanoparticles is a new field to study biodegradation since it deals with growth profiling of microbes.

Cobalt ferrite nanoparticle has reported the enhance rate of degradation of LDPE with microorganisms. There is no work reported on the mixed consortium (bacterial and fungal) with cobalt ferrite nanoparticle

Therefore the new field has got potential to be explored with other nanoparticles like cobalt ferrite that influences the microbial growth profiling.

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36. C. dussud, and J.F ghiglione *et al.*, 2014 synthetic plastics are emerging environmental contaminants that have been found to accumulate within marine waters worldwide. in marine environments, microorganisms function as pioneering surface colonizers and drive critical ecosystem processes including primary production, biogeochemical cycling and the biodegradation of anthropogenic pollutants. this paper reviews the current knowledge on the biodegradation of synthetic plastics by microorganisms. the microbial biodegradation of plastic materials is a complex phenomenon that includes several steps that are described here.

