A REVIEW: LDPE-BIODEGRADATION USING MICROBIAL CONSORTIUM BY THE INCORPORATION OF SUPER PARAMAGNETIC IRON OXIDE NANOPARTICLE (SPION) AS THE ENHANCER FOR BIODEGRADATION

Krishna Murari Patel[1], Dr. Archana Tiwari[2], Dr.Mahavir Yadav[3]

1. *Student of school of biotechnology (RGPV Bhopal) India*
2. *Professor of school of biotechnology (RGPV Bhopal) India*
3. *Associate Professor of school of biotechnology (RGPV) India*

Abstract: *Plastics have become a most important part of modern life and are used in different sectors of applications like packaging, building materials, consumer products and much more. The worldwide utilization of polyethylene is expanding at a rate of 12% annum and approximately 140 million tons of synthetic polymers are produced worldwide each year.Biodegradation especially by making microbial consortium will be a good choice for plastic degradation. Nanoparticles have varied potential applications. Studies with nanoparticles have shown their ability in enhancement of polymer degradation. Superparamagnetic iron oxide nanoparticles (SPION) with size ranging 10.6-37.8 nm were synthesized and characterized through XRD, FT-IR spectra, simultaneous TG-DTG-DTA, vibrational sample magnetometry (VSM) and transmission electron microscopy (TEM). SPION accelerating the microbial growth, these nanoparticles also improved the exponential phase durability. SPION influence the growth profiles of LDPE degrading microorganisms to augment the biodegradation rate. The review primarily focuses on the biodegradation where SPION acted as enhancers of biodegradation.The significance of microbial-nanoparticleinteractions which can dramatically influences key metabolic processes like biodegradation.*

Keywords- *SPION, XRD, FT-IR spectra, TG-DTG-DTA, VSM, TEM, biodegradation, enhancer of biodegradation*

I. INTRODUCTION

Plastics are an integral part of our day to day life and are being used in packaging, building materials and for many other purposes [1]. Plastic has gained remarkable indispensable character in all fields of activities (Fig 1). It has helped to substitute and save limited natural resources. The worldwide utilization of polyethylene is expanding 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. Polyethylene is a linear hydrocarbon polymers consisting of long chains of the ethylene monomers [2].

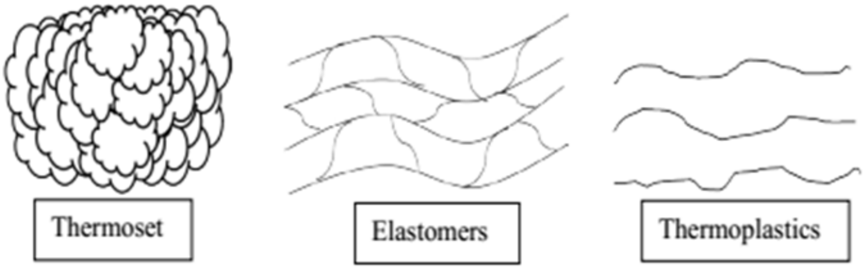

Figure 1: Classification of Petroleum – Plastics [3]

According to the United Nations Environmental Programme an estimated 22 to 43 percent of the plastic used worldwide is disposed offin landfills, where its resources are wasted thus the material takes up valuable space. 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-stage process. The first stage is oxidative degradation, which is normally abiotic and the second stage is biodegradation in which the oxidation products are degraded by micro-organisms [4].

A wide variety of synthetic polymers absorbs solar ultraviolet radiation and undergoes photolytic, photo oxidative, photo- chemical and thermo oxidative reactions that result in the degradation of these materials[5]. The ability to degrade polymers depends on the enzymes produced by the microbes to convert the polymers to oligomers and then to monomers. These water soluble enzymatically cleaved products are further absorbed by the microbial cells as carbon source where they are metabolized [6]. Polyethylene is remains in the environment for a long period of time as it lacks functional groups in polythene required for the microbial degradation [7].

There are varied nanoparticles that enhance growth cycle, mechanical and physiochemical stability along with biodegradability. The cobalt-ferrite nanoparticles have reported to enhance the growth of *Escherichia coli* and *Corynebacteriumxerosis*[8].Nanometric silicon particles have also reported that it alter the growth profiles of bacteria [9]. Several 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*[10].

Among a variety of ferrite based nanostructures, recently SPION have broad applications in several fields including permanent magnets, magnetic fluids, magnetic drug delivery, microwave devices and high-density information storage. These nanoparticles exhibit magnetic properties that might to interact with the electric polarity of the bacteria and influence its growth [8].

The consortium was documented to degrade synthetic polymers like epoxy and epoxy silicone blends. Moreover, the participating strains have also been used in combination with other microbes to degrade HDPE [11], non-poronized and poronized LDPE [7]. The present study deals with the influence of SPION particles on the LDPE biodegradation efficiency with microbial consortium.

II. MICROORGANISMS FOR BIODEGRADATION

Recently, the biodegradation of plastic waste and the use of microorganisms to degrade the polymers have gained notable importance because of the inefficiency of the chemical and physical disposal methods used for the pollutants, as they causes many environmental hitches. Microorganisms play a substantial role in the biological decomposition of material, fungus also have capability to degrade polyethylene [12].

List of microorganisms reported for degradation of polyethylene materials:

Fungus	**Bacteria**
Aspergillusniger 1[13]	*Bacillus amyloliquefaciens*[14]
Penicilliumsimplicissimum[15]	*Rhodococcus rubber*[16]

III. SYNTHESIS OF SPION

There are three size variants of SPION viz. 10.6, 20.0 and 37.8 were synthesized by controlling the concentration of Fe(II) and Fe(III) chloride solutions, followed by the co-precipitation with ammonia. The size of SPION was deduced from Debyee-Scherrer equationthrough XRD measurement [17]. These were used for growth profiling studies of LDPE-degrading microbial consortium.

Based on the screening study, three potential strains, *Microbacterium sp*. strain MK3, *Pseudomonas putida*strain MK4 and *Bacterium Te68R* strain PN12 (accession numbers DQ318884, DQ318885 and DQ423487 respectively), were selected for consortium development and LDPE biodegradation, as standardized earlier [11].

IV. CHARACTERIZATION OF 10.6 NM SPION

X-ray diffraction (XRD) pattern of samples were used and recorded at 25°C over a Rigaku-Geigerflexdiffractometer using Cu-Kα radiation ($\lambda = 0.154056$). The XRD peaks were observed at 2θ corresponding hkl values were calculated. The unit cell volume and particle size of corresponding SPION were calculated using the Debye—Scherrer equation. The magnetic properties of EFNCs were studied by using a vibrating-sample magnetometer VSM, EG&G, Princeton Applied Research Vibrating Sample Magnetometer, model 155 at room temperature. FT-IR spectrum were recorded on Perkin Elmer FT-IR Spectrophotometer in KBr. Simultaneous thermogravimetric-derivative thermogravimetry-differential thermal analysis (TG-DTG-DTA) was performed over Perkin Elmer (Pyris Diamond) thermal analyzer under nitrogen atmosphere (200 ml/ min) from 28 °C to 500 °C at 5 °C/min. Further, the structural morphology of SPION was determined at 80 kV TEM using JEOL (model JEM 1011) transmission electron microscope[18].

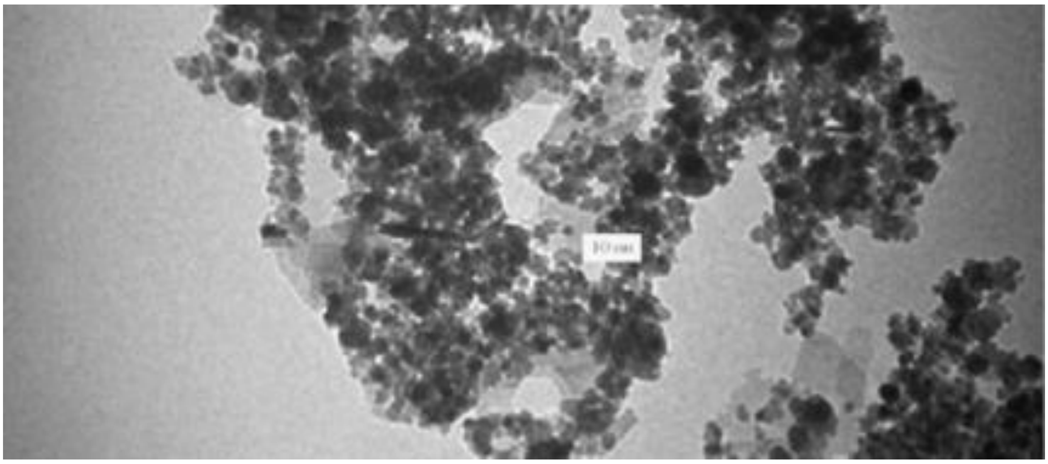

Figure2: SPION size 10.6 nm (TEM)[18].

V. GROWTH STATICS WITH SPION

The characteristic increase in the consortial growth of microorganism due to the addition of SPION was further supported by CFU/ml counts, which were significantly higher in their presence as compared to the control during active mid-logarithmic phases (Table 1). Among the three size variants, 10.6 nm particles accelerated the microbial growth maximally and nearly doubled the number of microbial cells per ml, as observed earlier in optical density measurements. Further, generation or doubling time (g) of the consortium was significantly reduced by 30 min in the presence of SPION unlikely to control. Based on the above studies, 10.6 nm SPION were selected for degradation experiments [18].

Table 1: Effect of sonicated SPION size variants on the colony forming units (CFU) and generation-time of consortium [18].

Time-interval (h)	Colony forming units/ml ($\times 10^6$) in different ferrite particle sizes			
	Control	10.6 nm	20.0 nm	37.8 nm
0	–	–	–	–
24	96 ± 3	168 ± 3	266 ± 2	142 ± 5
48	123 ± 2	230 ± 2	123 ± 5	176 ± 2
72	115 ± 4	256 ± 3	151 ± 1	182 ± 7
96	62 ± 4	222 ± 4	116 ± 4	136 ± 3
120	65 ± 5	115 ± 2	76 ± 2	124 ± 3
Generation time, g (min)	120	90	105	105

VI. BIODEGRADATION

The biological environment includes the biological agents such as bacteria, fungi, algae and their enzymes responsible for the deterioration of polymeric substances. They consume a substance as a carbon and energy source (Fig 3).

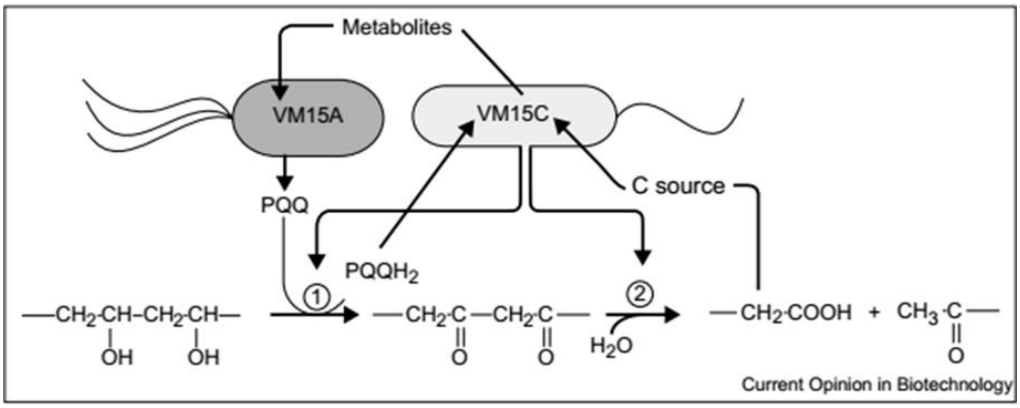

Figure3: Symbiotic PVA utilization by bacteria. Two strains, mixture of the two strains is able to grow on PVA, however, in a symbiotic relationship. Strain VM15A releases the growth factor PQQ, which enables the growth of VM15C on PVA. 1- PQQ-dependent PVA dehydrogenase; 2, oxidized PVA hydrolase [19].

VII. MODE OF BIODEGRADATION

The extracellular enzymes secreted by microorganisms depolymerize the cell wall components and polymers. These, extracellular and intracellular depolymerases are actively involved in biological degradation of polymers. During degradation, exoenzymes from microorganisms break down complex polymers to short chains or smaller molecules, e.g., oligomers, dimers, and monomers (Fig5)that are smaller enough (water soluble) to pass the semipermeable outer membranes and then to be utilized as carbon and energy sources [20].

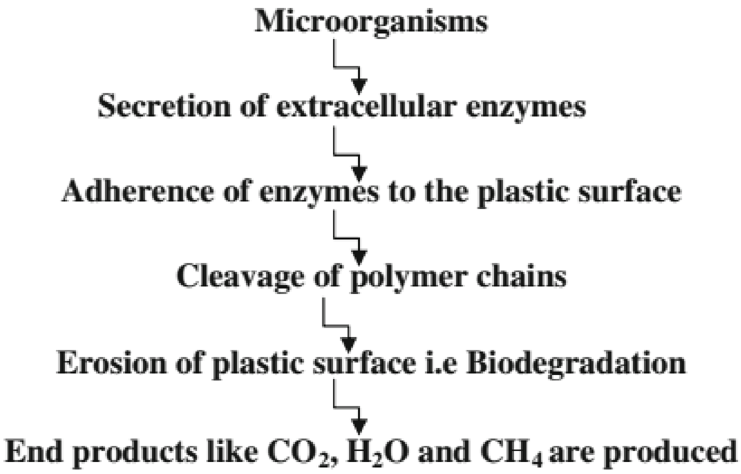

Figure4: Mechanism of enzymatic biodegradation of LDPE[21].

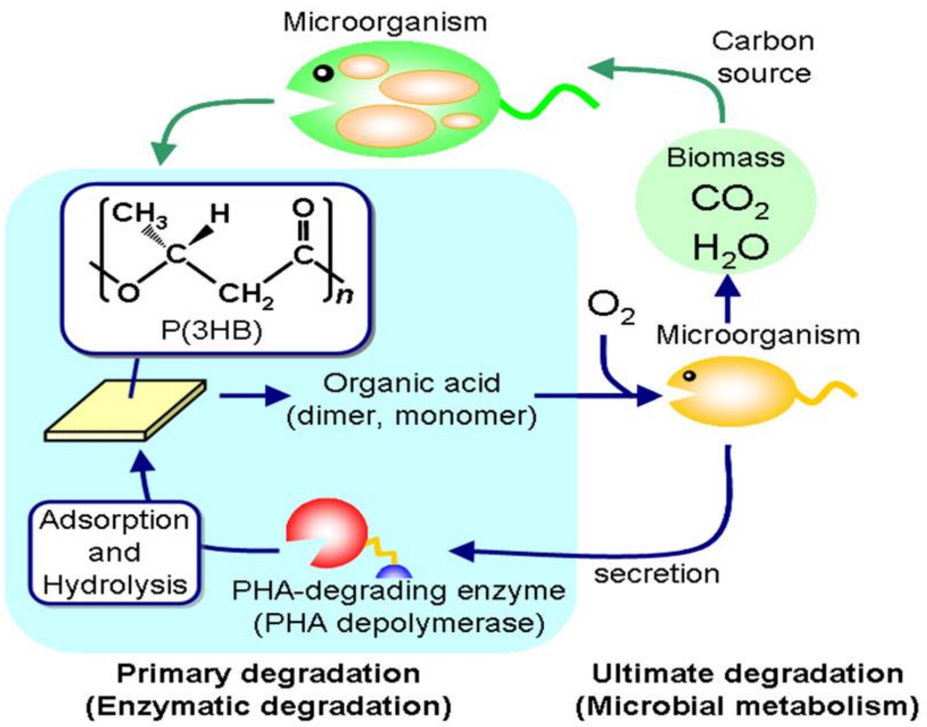

Figure5: Biosynthesis and biodegradation process of PHA in a natural environment [22].

VIII. SPION NANOPARTICLE USE AS ENHANCER FOR BIODEGRADATION

In biodegradation, nanoparticles are used as nanoclays by forming the nano-composites that have ultra large surface area to volume ratio, also known as clay nano-composites.These large surface areas provide space to microorganism where they attached in large cell count and produces enzyme. The enzymes play important role in the biodegradation. There are varied size of SPION nanoparticles that enhance mechanical, thermal, and physiochemical stability along with biodegradability. Using nanoparticles as fillers help in enhancing the material properties along with biodegradation. They help in achieving the activation energy faster for a process than the neat polymer. This effect can be caused by the catalytic role of the SPION. Super paramagnetic iron oxide nanoparticles (SPION) of particle size ranging 10.6-37.8 nm were synthesized and characterized by XRD, FT-IR spectra, simultaneous TG-DTG-DTA, vibrational sample magnetometry (VSM) and transmission electron microscopy (TEM). Super paramagnetic iron oxide nanoparticles (SPION) have been studied in the similar way as that of NBT and Fullerene 60 nanoparticles. Nanoparticle has been synthesized through co-precipitation method with ammonia using ferrous chloride and ferric chloride solutions. Super paramagnetic iron oxide nanoparticles improve the exponential phase durability by 36 h thus accelerating the bacterial growth. Further, shifting in lag-phase and the additives effect of sonication was also documented on growth profiling.The SPION of particle size 10.6 nm were found to significantly enhancing the biodegradation of efficiency of microbial consortium as revealed by λ-max shifts, Fourier transform infrared spectroscopy (FT-IR) and simultaneous thermogravimetric-differential thermogravimetry-differential thermal analysis (TG-DTG-DTA) [18].

IX. MECHANISM OF NANOPARTICLES

- **a)** Attractive forces are generated between positively charged nanoparticles and negatively charged bacterial cells (fig 5, 1a).
- **b)** Once in contact with microbial membranes, eventually allowing ingress of nanoparticles into the periplasm or cytoplasm (fig 5, 2e) and effect microbial environment [23]

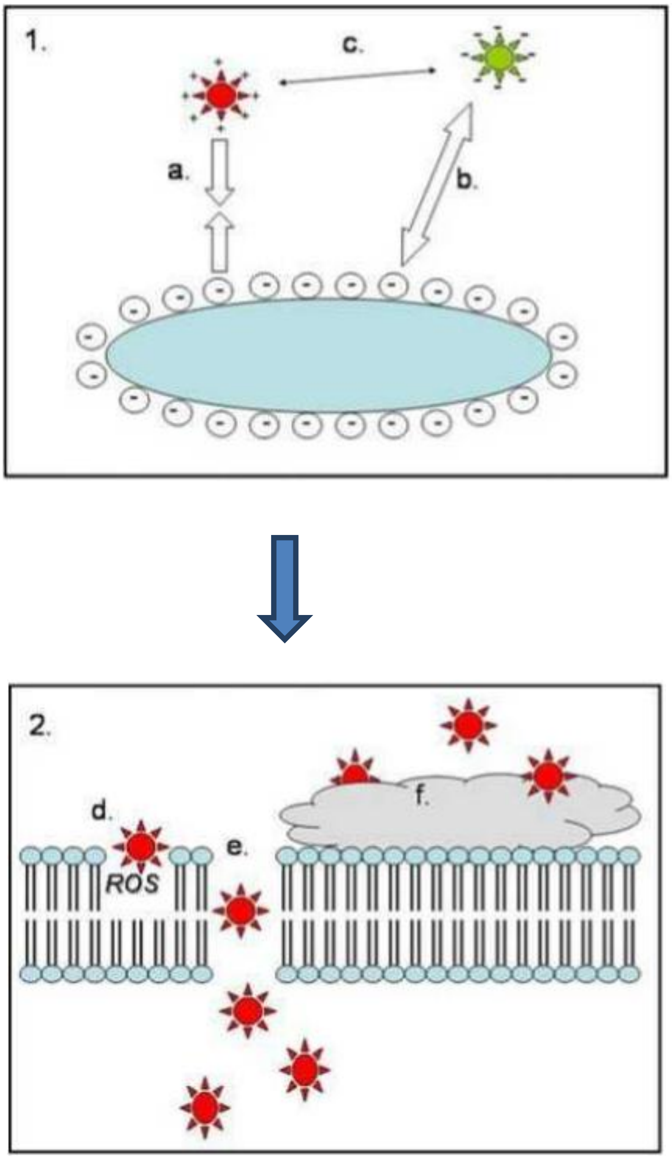

Figure 5: Mechanism of nanoparticles with microbial cells [23]

X. NANOPARTICLE-CELL INTERACTION STUDIES USING TEM

After characterizing and mechanism of nanoparticles, experiments were conducted to observing the relationship between the iron oxide (SPION) composite nanoparticles and *E. coli* in LB media. Cell-nanoparticle interactions were observed using a Zeiss EM10 CA transmission electron microscope at the University of Maryland Biological Ultrastructure Facility. *E. coli* samples were withdrawn at points during late exponential phase. After collection the sample, they were centrifuged and suspended at room temperature in 0.12 M Millonig's phosphate buffer at pH 7.3 and later with 2% glutaraldehyde. The cell pellets were then washed with buffer, and then secondary fixed with 1% OsO4. At this point, they were washed with distilled water and then postfixed with 2% uranyl acetate, rinsed in buffer and double distilled water, dehydrated in a series of ethanol and propylene oxide immersions, and embedded in Spurr's resin. A diamond knife was used to section the embedded microbial cells. The sections were post-stained with 2.5% aqueous uranyl acetate and 0.2% aqueous lead citrate. The micrographs of *E. coli*, in the presence of iron oxide (SPION) composite nanoparticles, indicate that the cells are able to maintain survival growth, showing no overt signs of toxicity[10]. Thenano-composites have large surface area where microbes attached and secrete enzymes which are responsible for degradation[24]. TEM appears to be an association of the nanoparticles with the cell membrane of microbes, as shown in Figures 6a and 6b for *E. coli* and composite iron oxidenanoparticles (SPION). Nanoparticles-cells interactions enhance mechanical, thermal, and physiochemical stability along with biodegradability. The cfu/ ml of the consortium were used that are more or less constant throughout the control assay (lacking nanoparticles) with a maximum value of 120 x 10^6 after 72 h. However, in the presence of SPION, the counts were significantly increased within 24 h of incubation (156 x 10^6cfu/ml) reaching a maximum value of 192 x 10^6cfu/ml after 48 h. The generation time was also found to be considerably decreased by 30 minutes in the presence of SPION nanoparticles.[10]. SPION nanoparticles increase degradation of polythene via providing large surface area for attachment of microorganism.

Generally plastics are hydrophobic in nature hence, microbes were not able to attach there surface. Nanoparticle firstly attached with plastics and then provide hydrophilic surface were microorganism adhere and grow properly. SPION behave in relation to microorganisms, particularly at the cellular level. The interaction between the particles and the membrane is non-specific rather than specific between the nanoparticles and a particular component of the membrane such as a surface expressed protein.Microbes play important role[25] in degradation via enzymes [26]. Biodegradation determined by weight loss of the plastics sample, morphological changes by Scanning Electron Microscopy, spectroscopy analysis by Fourier transform infrared spectroscopy [27]. Oxo-biodegradable polyethylene also shows morphological changes and determine by using FT-IR[28].

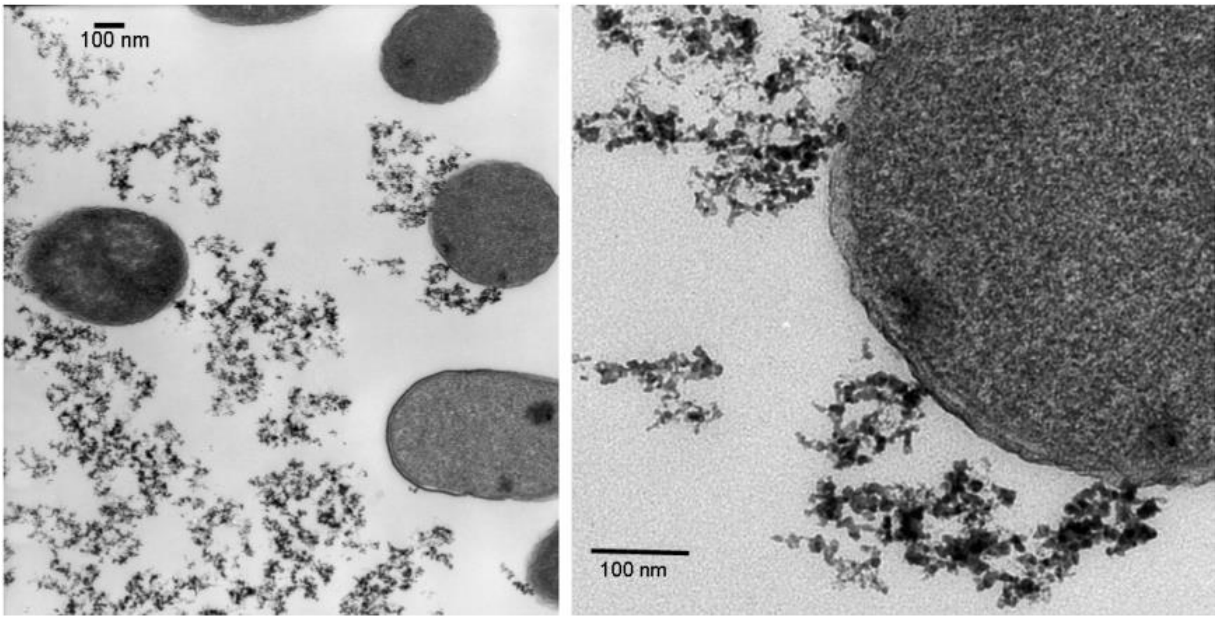

Figure 6: (a) Transmission electron microscope image of cross sections of E. coli grown with composite iron oxidenanoparticles. (b) A close up image showing the composite nanoparticles and a cell in contact with each other [10].

XI. ADVANTAGES OF SPION

Among a variety of ferrite based nanostructures, recently SPION nanoparticles have broad applications in several technological fields includingpermanent magnets, magnetic fluids, magnetic drug delivery, microwave devices and high-density information storage. These nanoparticles exhibit magnetic properties that might interact with the electric polarity of the bacteria and influence its growth [8].

In presence of SPION the metabolically active state of the consortium was not only attained much earlier, but was also prolonged by the action of SPION. The interaction of the electrical polarity possessed by this particle was cited as the probable reason.Formation of biofilm around SPION particles was also witnessed, which can dramatically increase the efficiency of cellular processes involved in polymer biodegradation.Iron is known to act as a catalyst in the production of reactive oxygen species inside living cellswhich might support the oxidative biodegradation mechanism[29].

XII. CONCLUSIONS

LDPE possesses excellent resistance against dilute and concentrated acids, alcohols, basesand accumulates day by day. Thus, to deal with this environmental problem, biodegradation with the help of micro-organisms appears to be the best choice. Initially use of single strain of microbes for the process of biodegradation. The percentage of degradation was low. Then the biodegradation of LDPE accelerated with microbial consortium. Theefficiency of degradation was increased slightly but not up to the mark. Hence scientists have found nanoparticles as enhancers of microbial degradation ability.

Super paramagnetic iron oxide (SPION) has been documented the enhance rate of degradation of LDPE with the help of microbial activity. The study reveals that a more stable suspension of SPION brings about an increase in the growth of polymer-degrading microbial consortium which increases its LDPE biodegradation efficiency. It would be therefore facilitate the efficacy of plastic biodegradation and prove to be an important step in devising waste management strategies. Till now no work has been done with mixed consortium (bacterial and fungal) along with SPION nanoparticle.

Therefore this can also be explored as rate of degradation with mixed consortium might be moreas per the available literature.

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