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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

BIOREMEDIATION OF PREPROCESSED PLASTIC WASTES THROUGH MICROBIAL CONSORTIUM

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ABSTRACT

The plastic pollution is one of the anthropogenic effects which causes harm to ecolife through toxic compounds. The main objective of this present work is to imply the effect of pre-treatment of plastic wastes with microbial consortium. This work includes collection of soil sample from plastic dumped sites, isolation, identification, screening and compatibility analysis of microbial consortium, pre-treatment of polyethylene sheets by physical, chemical and Microbial degradation. Polyethylene sheets were pre-treated by a UV radiation and acetone. The pre-treated polyethylene strips were further degraded by using microbial communities that is isolated from plastic dumped sites. Nine types of bacterial colonies and fourteen types of fungal colonies have been isolated by serial dilution. The polyethylene degraders were screened by using two types of screening methods such as well cut and petri plate methods. There are four types of microbes were screened namely *Bacillus* sp., *Aspergillus* sp. (A1), *Aspergillus* sp. (A2), and *Aspergillus* sp. (A3). Four isolates and consortium of these microbes were analyzed for their ability in degrading plastics. The weight loss of polyethylene strips were detected after 30 days of incubation. In this study, maximum degradation of polymer shown by consortium with a reduction of 12%, *Bacillus* sp., showed 10% reduction and *Aspergillus* sp. (A1) showed 10% reduction under laboratory conditions respectively. Consortium of *Bacillus sp.,* and *Aspergillus sp.,* did not show any antagonistic effect over other microbes on Muller Hinton agar plates. Hence, it was concluded that this consortium could be potentially applicable for the bioremediation of pre-processed plastic wastes in future with less cost effective.

Keywords: Plastic degradation, Physical and Chemical degradation, Microbial degradation, polyethylene degradation.

1. INTRODUCTION

Plastics are organic polymers of high molecular mass. The word plastic is derived from its property of plasticity [1]. Plastic pollution is caused by accumulation of plastic objects and particles (e.g. plastic bottles, bags and micro beads) in the earth's environment that adversely affects wildlife and humans. Plastics act as pollutants and release hazardous chemicals into soil, which categorized into micro, meso or macro debris, based on size. Every year the high amount of plastics produced find themselves in the ecosystem as industrial waste products [2]. About 30% of plastics are used worldwide for packaging of foods, pharmaceuticals, cosmetics, detergents and chemicals and this is at a high rate of 12% and still expanding [3]. Some of the plastics, such as polyethylene are used extensively in packaging and other industrial and agricultural applications. These plastics are characteristically inert and are resistant to microbial attack, leading to their accumulation in the environment. Use of microorganisms for degradation of polymer wastes gives more significant impact than physical and chemical disposal methods. In other words, it protects our environment and also cost effective [4].

Plastics are the most commonly used polymers for routine applications. Plastic wastes accumulating in environment are posing an ever increasing ecological threat [5]. Reduce, Re-use and Recycle concept of solid waste management is enhancing but this has not addressed the problem of polyethylene which remains scattered in the environment as recorded [6]. Biodegradation is defined as destruction of contaminant molecules by the action of enzymatic machinery of biological system. Biodegradation is the process by which the organic substances are broken down by living organisms. Microorganisms play a significant role in the biological decomposition of material [7]. The high molecular weight, 3-dimensional structure, hydrophobic nature and lack of functional groups in the LDPE,

interfere with the microbial attack.Biodegradation overcome the usage of physical and chemical degradation method that could be a gain for the microbes to act upon. The UV radiation (photo-oxidation), thermal and chemical oxidation of polyethylene prior is exposed to a biotic environment enhancing biodegradation [8]. This method was cheap and effective, so that it can be used widely for the pre-treatment of polyethylene [9]. Biodegradability of the polyethylene is mainly evaluated by weight loss. Polyethylene solid waste disposal problems pose various threats. So an attempt has been made to isolate the potent bacteria and fungi that degrade polyethylene from soil environment [4, 10].

Thus the current study is involved in plastic waste management to protect the environmental system from hazardous pollution. The aim of this study is to isolate and identification of potential bacteria and fungi that have the capacity to degrade plastic pollutant.

2. MATERIAL AND METHODS

2.1. Sample collection

Soil samples were collected from Plastic dumped site at Vellalore, Coimbatore, Tamil Nadu (10°57'32.3"N76° 59'59.7"E). The dump yard was chosen because the microbial communities would have acclimatized to the plastic wastes dumped there and would therefore be a rich source of probable microbes that could degrade the pre-treated plastic material. Polyethylene bags were collected from local market and Plastic powder was collected from Arunachal industries located in Pannimadai, Coimbatore, Tamil Nadu (11°04'43.8"N76° 54'45.9"E).

2.2. Isolation of polyethylene degrading microorganisms

One gram of soil sample was transferred into a conical flask containing 99ml of sterile distilled water. This content was shaken and serially diluted. To isolate microorganisms associated with materials, (polyethylene bag) pour plate method was adopted using nutrient agar for bacteria and rose bengal agar for fungi. The plates were then incubated at 30°C for 2-7 days. The developed colonies were isolated and sub cultured repeatedly to get pure cultures and it was preserved in slant at 4°C [11].

2.3. Screening of polyethylene degrading microbes

In this study, we selected two types of screening techniques to screen the effective microbes for degradation of polyethylene strips.

2.3.1. Clear Zone Method

Polyethylene powder was added into the mineral salt medium at a final concentration of 0.1% (w/v) and the mixture was sonicated for 1 hour at 120 rpm in shaker. After sonication, the medium was sterilized at 121°C and pressure of 15 lbs/inch² for 20 minutes [11]. The medium was sterilized and poured into sterile plates before cooling and it was allowed to solidify. Wells were cut using well cutter and 20µl culture of isolated organisms was added to the well [10] and then incubated at 25-30°C for 2-4 weeks. The organisms producing zone of clearance around their colonies would selected for degradation studies [11].

2.3.2. Petri Plate Method

Isolated bacterial colonies were streaked on minimal salt agar. Polyethylene bag was cut into pieces of 2×2 cm and placed on the minimal salt agar plates. After incubation microbes, which effectively grew on the top of the polyethylene strips were chosen for further degradation analysis [5].

2.4. Identification of bacteria and fungi

The identification of bacteria was performed on the basis of macroscopic and microscopic examination and bio-chemical test according to Bergey's manual of determinative bacteriology. The fungal culture was identified by lacto phenol cotton blue staining technique [11].

2.5. Pre-treatment of Polyethylene sheets

2.5.1. Physical Degradation

The Polyethylene sheets were taken and cut into small pieces of 2×2cm. The sheets were exposed to UV light at 254 nm in a Double UV chamber. The exposed polyethylene sheets were weighed and then were subjected to chemical treatment, which were then used as substrate for growth of the isolated bacteria and fungi to analyze their ability to break poly ethylene [12].

2.5.2. Chemical Degradation

UV light exposed polyethylene sheet was treated with acetone which was placed in a glass saucer with 100 ml of acetone and it was kept for a week [13]. After a week, the pretreated polyethylene sheets were subjected to microbial degradation.

2.6. Microbial Degradation

2.6.1. Compatibility analysis of microbial consortium

The compatibility analysis was done with the screened microbes. The Muller Hinton Agar plates were swabbed with one of the selected microbe. Four wells were cut

and 10 µl of the culture supernatant (72 hrs old culture) of the other organisms were added to the well. The test was repeated by changing the swabbed organism with the selected cultures used in this study and the culture supernatants of other cultures which were not swabbed. The organisms were considered to be incompatible with other when the zone of clearance was observed around the well [14].

2.6.2. Growth pattern of isolated microorganisms

Optical density (OD) measurements of the microbial growth are one of the most common techniques used in microbiology. Initial and final optical density of cultures was measured by UV light at 660nm. In Mineral salt medium, the microorganism was inoculated and the initial value of culture was measured and final optical density of culture was measured after incubation. This measurement was taken for quantitative analysis of the plastic degrading bacteria and fungi. These microbes utilize the plastic powder as carbon source and growing well which was measured by optical density method [15].

2.6.3. Microbial degradation of pre-treated polyethylene sheets

The pre-treated polyethylene sheets were aseptically transferred into the conical flask containing 100ml of mineral salt medium and then inoculated with isolated and identified polyethylene degrading microorganisms' individually in separate flasks and in another flask microbial Consortium of both bacteria and fungi was inoculated. Polyethylene sheet in the microbe free medium acted as control. This setup was kept in a shaker at 30°C, 150 rpm for one month period. The plastic strips were taken away from shaker after one month and were washed thoroughly using distilled water and diluted ethanol. Sheet was allowed to dry and then weighted to check the final weight of plastic sheets [11].

2.7. Weight loss measurement

After the degradation, the plastic sheets were then allowed to float on 2% SDS solution for 4 hours at 50°C to remove the residual adherent cells. It was then disinfected with ethanol for 5 min which was dried in hot air oven at 60°C for overnight. These films were weighted by digital balance. The degraded plastic sheets were compared with control. The percentage degradation of polyethylene sheets was calculated by following equation [12]:

Percentage Degradation (%) = {(Initial weight –Final weight)/ Initial weight} x 100

3. RESULTS

3.1. Isolation of polyethylene degrading microorganisms

The Serial dilution plates of bacteria and fungi were showed in Fig. 1 & 2 respectively. Based on the different colony morphology, several microorganisms were isolated from dilution plates. From this, 9 bacterial colonies and 14 fungal colonies were selected and sub cultured.

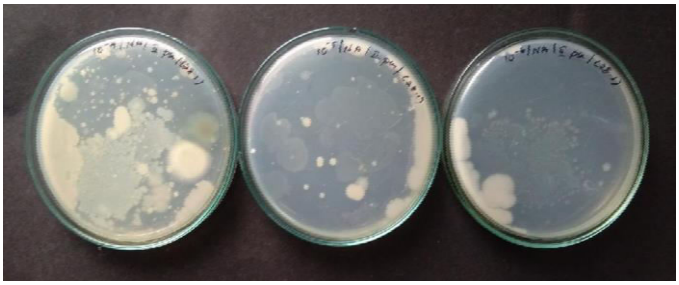


Fig. 1: Serial diluted plates of Bacteria

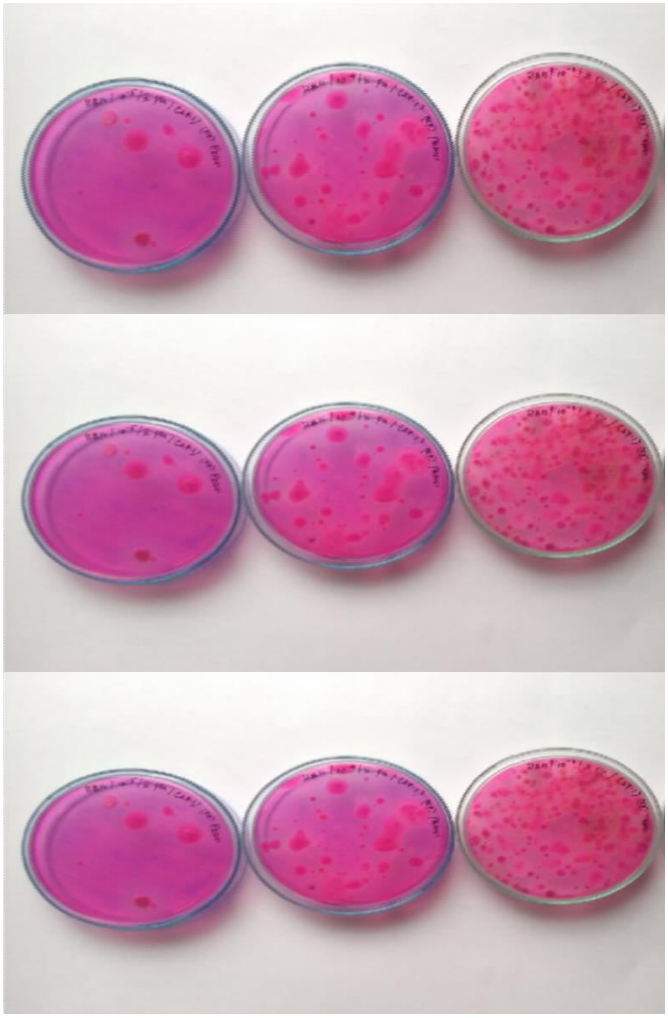


Fig.2: Serial diluted plates of Fungi

3.2. Screening of polyethylene degrading Microorganisms

The clear zone and petri plate screening techniques, showed four potential microorganisms which utilize polyethylene as a carbon source. These four microbes were found to have a greater ability to utilize carbon source for their growth compared to other isolated microbes from serial dilution. Thus screening methods provide an efficient four polyethylene degraders, which was used for further polyethylene degradation studies.

3.2.1. Clear Zone Method

Fig. 3 shows the polyethylene degraders of bacteria and fungi. Clear zone screening method provided four polyethylene degraders having higher degradation ability. One bacterial colony and three fungal colonies were isolated from screening plate which used plastic powder as a sole source of carbon in minimal salt agar medium. The other isolates did not show any significant growth on minimal salt agar plates because of notutilizing the carbon source. Hence, clear zone producing four microorganisms were selected as polyethylene (PE) degraders.

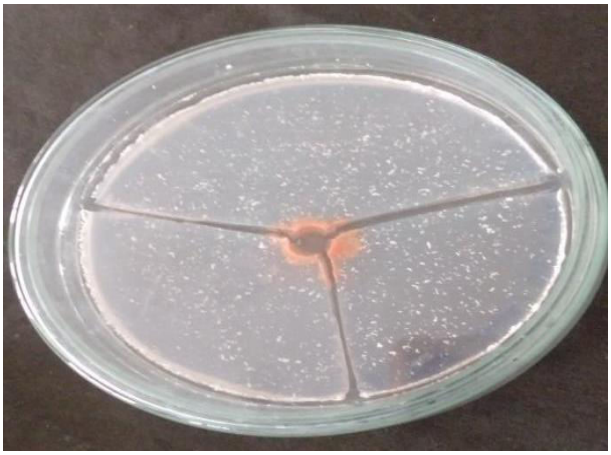


Fig. 3: Screening of polyethylene degrading organism by Clear Zone Method

3.2.2. Petri Plate Method

Fig. 4 shows the growth of microbes on minimal salt agar medium containing PE strips. Those microbes have higher degradation capacity. Four isolates were selected as PE degraders out of other isolated microbes for the reason of growth on PE strips. Other isolated microorganisms didn't show the growth on PE strips. Hence, these four microbes were selected only for further degradation studies. Thus the obtained four isolates are similar to screened microbes of clear zone method.

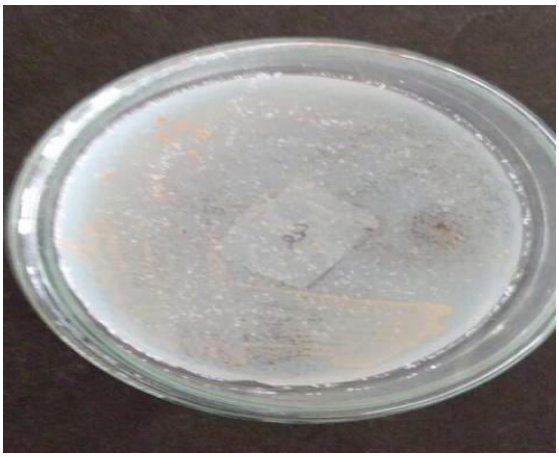


Fig. 4: Screening of polyethylene degrading microorganism by Petri plate method

3.3. Identification of polyethylene degrading bacteria

The screened and isolated organisms were characterized according to Bergey's manual of determinative microbiology.

3.3.1. Microscopic examination

Gram staining of screened bacteria was shown in Fig.5, which is Gram positive, rod shaped, and is motile bacteria. This bacterium is further characterized by macroscopic examination of biochemical tests.

Table 1: Microscopic examination

Microscopic observation	Result
Color	Purple
Shape	Rod
Motility	Motile

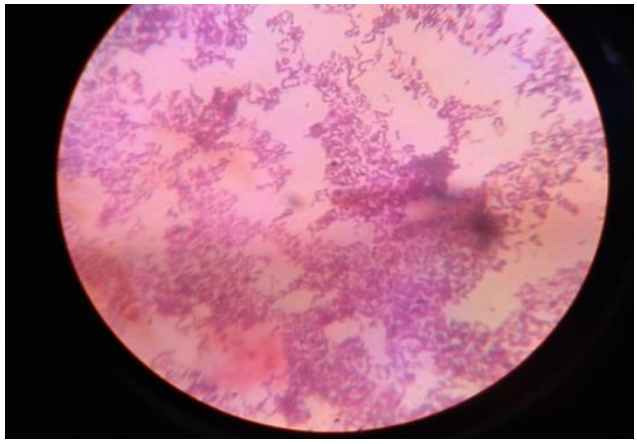


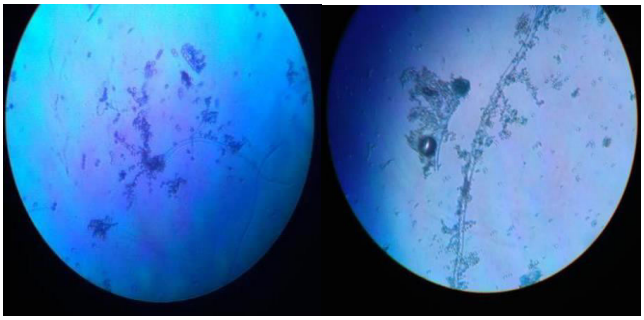
Fig.5: Gram positive rod shaped bacteria

3.3.2. Macroscopic examination

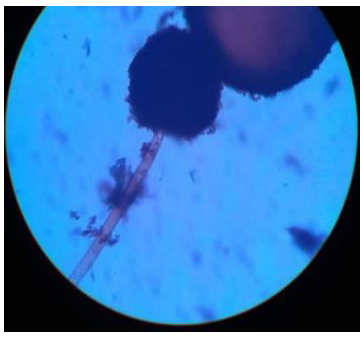
Table 2 shows that the biochemical characteristic of isolated bacteria. The isolated and screened bacterium belonged to the genus *Bacillus*.

Table 2: Biochemical characterization of isolated bacteria

Biochemical test	Result
Indole test	Negative
Methyl Red test	Negative
Voges-proskaeur test	Positive
Citrate test	Positive
Catalase test	Positive
Urease test	Negative
Nitrate Reduction test	Positive
Gelatin Hydrolysis test	Positive
Gas production	No Gas produced
Lactose	Negative
Sucrose	Positive
Mannitol	Positive
Glucose	Positive
Probable organisms is ***Bacillus* sp.,**	



(a) **(b)**



(c)

(a) Aspergillus sp., (A1) (b) Aspergillus sp., (A2) (c) Aspergillus sp., (A3)

Fig. 6: Microscopic examination of Fungi

3.4. Identification of polyethylene degrading fungi

3.4.1. LPCB Staining

Among the 14 isolates of fungi, three isolates were reported as potential PE degrading fungi. The selected efficient fungal degraders stained with LPCB shows small conidiophores under microscope (Fig. 6) which belongs to the genus *Aspergillus sp.,*

3.5. Pre-treatment of polyethylene strips

3.5.1. Physical Degradation

The polyethylene sheets were pre-treated with physical method of UV radiation. The UV radiation causes photo-oxidative degradation. The polyethylene strips absorbs UV radiation, UV energy excites as photons. It creates some free radicals which causes photochemical effect within the polymer structure. The UV rays were penetrated into the polymer then broke the polymer chains and reduced the molecular weight.

3.5.2. Chemical Degradation

Physically pre-treated polyethylene sheets were treated with chemical method using Acetone. The results in the formation of aldehydes and acids can negatively influence the fluid viscosity and lubrication and cause corrosion. Polyethylene sheets react within few days with acetone. After few days hard surface of polyethylene sheets was changed to soft, smooth and fluffy.

3.6. Microbial Degradation

3.6.1. Compatibility analysis of Microbial Consortium

The efficient screened microbes of one bacterium and three fungal isolates were subjected to compatibility analysis. There was no zone of clearance found around the well on Muller Hinton Agar plates. This shows that the consortium of *Bacillus* sp and *Aspergillus* sp., do not exhibit any antagonistic effect over the other microbes in the consortium. From the results it was concluded that this consortium has potential for significant degradation of polyethylene.

3.6.2. Growth pattern of Microorganisms on Synthetic Medium

The initial and final growth rate of plastic degrading microorganisms of bacteria and fungi was measured by using optical density method which is shown in table 3. *Bacillus* sp., *Aspergillus* sp., (A1) and microbial consortium were efficient enough in growing in the minimal media supplemented with plastic as the sole carbon source compared than other *Aspergillus* sp., (A2&A3).

Table 3: Growth rate of Isolated Microorganisms

Bacteria/ Fungi	Name of the Micro Organisms	Initial OD Value (660nm)	Final OD Value (660nm)
Bacteria	*Bacillus* sp.,	0.04	0.12
Fungi	*Aspergillus* sp., (A1)	0.04	0.12
Fungi	*Aspergillus* sp., (A2)	0.06	0.09
Fungi	*Aspergilus* sp., (A3)	0.03	0.06
Both bacteria and fungi	Microbial Consortium	0.02	0.14

3.6.3. Microbial degradation of Pre-treated polyethylene strips under laboratory conditions

After one month, the microbes show its potential to polymer degradation ability. *Bacillus* sp., *Aspergillus* sp., and microbial consortium were utilizing the polymer as a carbon source, which leads to loss of plastic weight up to 10 %. The finding of this study is similar to Muhonja et al, 2018 [16]. The common soil fungi like *Aspergillus, Mucor, Penicillium, Fusaarium* etc has the ability to degrade low density polyethylene up to 36% [17]. The present study has explored that the isolated genus have the greater capacity to degrade polythene films.

3.7. Weight Loss Measurement

After 30 days of treatment by isolated polyethylene degraders, the weight loss of the pre-treated polyethylene sheets was noted and percentage of degradation was calculated. Fig. 7 shows the degraded polyethylene sheets. Fig. 8 graphically represents the percentage weight reduction of polyethylene sheets. Weight loss of polyethylene sheets with different kinds of degraders did not show the same efficiency. *Bacillus* sp, A1, A2, A3 and microbial consortium showed 10%, 10%, 5%, 3% and 12% weight reduction respectively whereas highest weight loss was recorded in PE treated with consortium of microbes.

Fig.7: Degraded plastic sheets

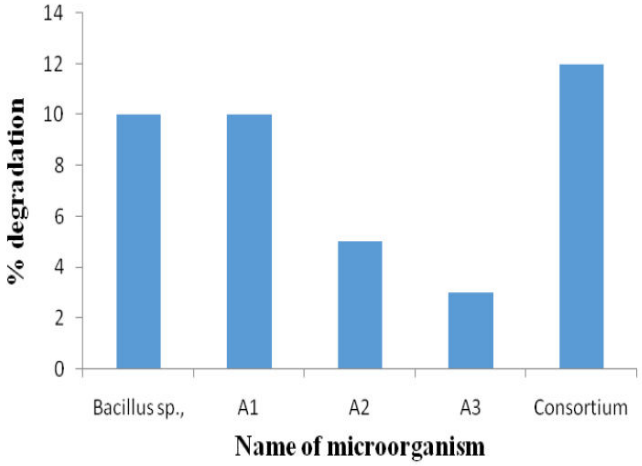


Fig. 8: Percentage degradation of Polyethylene sheets

4. DISCUSSION

The environmental pollution was mostly occurred by the accumulation of plastic wastes. So the degradation of plastic waste through the microorganisms is considered as an ecofriendly and cost effective method. In this study, plastic dumped soil samples were serially diluted and microbes were isolated from this for screening of polyethylene degraders. All the isolates may not have the ability to utilize plastic as carbon source and degrade PE. Therefore they were screened using well cut and petri plate method to check their ability of PE degradation. MSM contains all the other minerals, nutrients as required and polyethylene is used as carbon source by microbes. The isolates, which were able to utilize plastics as their carbon source showed growth on MSM agar plates around the wells in well cut method as well as growth on PE strips in petri plate method. As the organisms utilize the polyethylene, there is reduction in its weight. Thus reduction in weight of the polyethylene sheets indicates that it has been utilized by the organism. Not all the screened organisms showed higher efficiency in weight loss of polymer. This may be due to some factors responsible for microbial growth such as incubation period, concentration of inoculum, temperature and molecular

weight and size of polymer, etc., which can limit the biodegradability of microbes.

Pre-treatment of Polyethylene sheets using physical and chemical methods enhance the degradation process and it was subjected to microbial degradation with screened microbes. When PE exposed to UV radiation it causes structural changes on polymer as well as influence of acetone on plastic polymer results in breakdown the complex structure including their outward appearance. The result of this study shows that the selected microbes of both bacteria and fungi exhibit greater potential for LDPE biodegradation. *Bacillus* sp., was able to reduce the weight of the polyethylene by 10%. The fungi *Aspergillus* sp., (A1), *Aspergillus* sp., (A2) and *Aspergillus* sp., (A3) were able to remediate the polyethylene sheet upto 10%, 5% and 2.4% respectively. Microbial Consortium of bacteria and fungi showed more efficient weight loss than individual microbe treatment of PE and it gave 12% of weight reduction within a month. Weight reduction of polymer using microbial consortium was generally higher than bacteria and fungi.

Bacillus sp. and *Aspergillus* sp. was found to be efficient polyethylene degrader, it causes PE strips weight loss upto 10 % and the Microbial consortium was found to degrade effectively than the individual isolate of bacteria and it shows weight loss upto 12%. From this study, the microbial consortium of *Bacillus* sp., and *Aspergillus* sp., concludes the most effective bio-degrader. This finding of our study shows the similar result of Vatseldutt, 2018 [18]. The common soil fungi like *Aspergillus*, *Mucor*, *Penicillium*, *Fusaarium* etc have the ability to degrade plastic polymer upto 36% [17]. The present study has explored that the isolated and screened microbes are capable of degrading complex polymer films to smaller and simple units effectively.

5. CONCLUSION

In recent years, people have shown concern over the environmental issues. The problem of plastic pollution is now really a mess of the mankind. There is no part of the world untouched from its impact. Before making the commercial products globalize, manufacturers must have an idea about the protection of our earth. In the present study, physical and chemical treatment of PE films enhanced the degradation process. The isolated microbes were native to site of polyethylene disposal and it might show greater capacity of degradation in the natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media. This gives the suggestions that these microbes can used in both natural and artificial conditions for the purpose of degradation of polymers. Since, this study reveals that the microbial consortium enhances the bioremediation of preprocessed plastic wastes. In future, this microbial consortium can be used for bioremediation of plastic polymer wastes with less cost effective.

6. ACKNOWLEDGEMENT

The authors are grateful to the Management, Principal and Deans of Dr. N.G.P Arts and Science College (Autonomous) for their extended support**,** and the infrastructural facilities of DST-FIST Scheme, and DBT-Star Scheme. The authors also thank the Tamil Nadu State Council for Science and Technology (TNSCST) for their financial support (Communication number: Dr NGPASC 2020-21 BS027).

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