In vitro and *in situ* studies on microbial detoxification of plastic wastes

S. Bharathi, Deepthi Varier, C. Lakshmi, R.A. Sujan

Department of Microbiology, The Oxford College of Science, Bangalore, Karnataka, 560102, India, Email: vpscience@theoxford.edu

ABSTRACT

Plastic waste accumulation in the environment is causing an ever increasing ecological threat as it causes serious damage, both during its production and disposal. In the present study, potent microbial strains with the ability to degrade plastics (viz., HDPE, LDPE, PE and PVC) were isolated using enrichment culture technique. The samples were collected from Mavalipura dumpsite, Bangalore that is rich in plastic waste. At the end of the study, 6 fungal species were identified and isolated on MSV medium supplemented with emulsified plastic. The microbial species associated with the PE materials was identified as *Aspergillus flavus*, PVC materials were identified as *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus oryzae*, *Cladosporium*; LDPE material was identified as *Aspergillus flavus*; HDPE materials were identified as *Aspergillus niger*, *Aspergillus flavus* and *Pencillium*. The biodegradation of the respective plastics was analyzed after 3 months of incubation by liquid culture method and 80.6% weight loss for PE, 81% weight loss for PVC, 80.4% weight loss for LDPE and 82% weight loss for HDPE was observed. Optimization methods were employed to monitor different parameters like pH, temperature and concentration of plastics for the increased biodegradation of the respective plastics.

Keywords: *Aspergillus*, biodegradation, enrichment culture, plastics

INTRODUCTION

Plastics are synthetic substances that can be classified based on the chemical process that is used in their synthesis. Though plastic brings a lot of convenience to people's life, at the same time it also causes long term harms [1]. Plastics are advantageous as they are strong, durable and light-weighted [2]. Originally plastics were mimicking and replacing natural products (lacquer, shellac, tusks, and horns) but today they are largely synthetic materials made from crude oil which is a nonrenewable resource [3]. With time, the stability and durability of plastics have been improved continuously, and hence these groups of materials are considered as a synonym for materials that are resistant to many environmental influences [4]. They are durable and degrade very slowly; the chemical bonds that make plastic so durable make it equally resistant to natural processes of degradation. Due to their durability and visibility in litter, plastics have attracted public and media attention more than any component of solid waste stream [5].

Environmental pollution by synthetic polymers, such as waste plastics and water soluble synthetic polymers in waste water has been recognized as a major problem [6]. Synthetic plastics like polyester polyurethane, polyethylene with starch blend, can biodegrade, although most commodity plastics used now are either non-biodegradable or take decades to degrade. This growing concern about degradable polymers has raised and promoted research activity worldwide to

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either modify current products to promote degradability or to develop new alternatives that are degradable by any or all of the following mechanisms: biodegradation, photo degradation, thermal degradation and environmental erosion [7].

The plastic waste stream emerges from domestic, industrial and municipal refuse. The plastic waste is usually disposed off through incineration, land filling and recycling [8]. Biodegradation of plastic waste using microbial strains could offer a solution to this problem due to their diverse metabolic capability, adaptability to different environments and possibility of isolation using semi synthetic minimal media. Environmental factors not only influence the polymer to be degraded, they also have a crucial influence on the microbial population and on the activity of the polymer to be degraded. Parameters such as Humidity, Temperature, pH, Salinity, the presence or absence of oxygen and the supply of different nutrients have important effects on the microbial degradation of polymers [9], so these conditions must be considered when the biodegradability of plastics is tested. The plastics used in the study were obtained from the Mavalipura dumpsite, 38 km towards North of Bangalore. Among the samples collected, PE, PVC, LDPE and HDPE were selected for the degradation study.

MATERIALS AND METHODS

Collection of microbial source samples

Samples from Mavalipura dumpsite were collected to be used as a microbial source for the isolation of the plastic degrading microorganisms. The plastic materials found in the sites, either openly visible or partially buried in the soil were initially identified and aseptically transferred to a clean container. Soil samples which are highly polluted with plastic waste were also collected from the same dumpsite. Plastic samples collected: PE, PVC, LDPE and HDPE.

Soil burial technique

Soil burial technique was used for obtaining samples of microbial population stimulated to plastic degradation [10]. The soil obtained from the dumpsite was sorted out and screened to remove large clumps, plant debris, etc. Soil was filled in equal amounts in five different jute bags. The different plastic sample obtained from the dumpsite were cut into small strips of 0.5 g each and buried in the soil at a depth of 5 cms. The bags were stored in laboratory at room temperature, frequently watered to avoid drying up of the soil and left for a period of 30 days. After one month, strips were removed and washings of the strips were used as inoculum for enrichment culture technique.

Enrichment and isolation of plastic degrading microbial strains

The plastic samples were used as inoculants for enrichment culture. Each plastic sample of 0.5g was added to 100ml of Mineral Salt Vitamin (MSV) [11] media containing (g/L):NH_4NO_3, KH_2PO_4, K_2HPO_4, $MgSO_4.7H_20$, NaCl, $FeSO_4.7H_20$, $ZnSO_4.7H_20$, $MnSO_4.4H_20$, Biotin and Vitamin B12. Powdered form of plastic sample for PVC & HDPE was prepared as one set whereas PE and LDPE were cut into small strips and added in another set of conical flasks. The flasks were incubated at 37°C in a shaker incubator for 2 months.

Emulsification of plastics

0.1 g of LDPE and PE sheets were taken for emulsification. The pre weighed plastics were immersed in 0.5ml of xylene and boiled for 15mins. The plastic residue so obtained was immersed

in 1ml ethanol and kept at 37°C overnight for evaporation of ethanol. The samples were then heated in a water bath for 30 minutes to remove the residual solvent and obtain dry plastic powder. PVC and HDPE in a powdered form were used for degradation study.

Isolation of plastic degrading microbial strains

Three types of Semi Synthetic Media (SSM) were prepared for isolation of plastic degrading microbial strains. Media 1: 0.1ml of inoculums from enrichment culture were spread plated on Mineral Salt Vitamin Media with 1g of respective plastics. Media 2: 0.1ml of inoculums from enrichment culture were spread plated on Mineral Salt Vitamin Media with 1g of respective plastics and 0.1% of yeast. Media 3: 0.1ml of inoculums from enrichment culture were spread plated on Mineral Salt Vitamin Media with 1g of respective plastics and 0.1% of dextrose. The plates were incubated at 37°C for 24-48 hours. Control for all the three Selective Synthetic Medium (SSM) was also prepared with the same composition of reagents and the plates were incubated at 37°C for 24-48 hours.

Identification of Fungi

Based on the colony morphology and characteristics of the organisms grown in the plate after incubation period, staining technique of Lacto phenol blue stain was performed and fungal species were identified.

Determination of weight loss

The different plastic samples [PE/PVC/LDPE/HDPE] were cut into small strips of 0.5g each and aseptically transferred to the conical flask containing 200ml of Mineral Salt Vitamin broth medium. Different flasks were maintained for each plastic sample and all the flasks were incubated at 37°C in a shaker incubator for 2 months. After 2 months of shaking, the plastic strips were collected, washed thoroughly using distilled water, shade dried and weighed for final weight. From the data collected weight loss of the plastics was calculated.

RESULTS AND DISCUSSION

Fungal isolates were identified as *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium*, *Aspergillus flavus*, *Aspergillus oryzae* and *Cladosporium*. Optimization methods were employed to monitor different parameters like pH (pH 6 and pH 8), temperature (28°C and 39°C) and concentration of plastic (0.05 % and 0.5 %) for the biodegradation of the respective plastics (Figure 1 and 2). The study deals with the isolation, identification, degradative ability and optimization parameters of plastic degrading microorganisms from the Mavalipura dumpsite. The microorganisms (fungi) with the ability to degrade respective plastics (HDPE, LDPE, PE, and PVC) were isolated on the Mineral Salt Vitamin medium containing plastics (Figure 3). The degradative ability of the microorganisms was observed after 3 months of incubation. Optimization methods were employed to monitor different parameters like pH of range 6 and 8, temperature of range 28°C and 39°C and concentration of plastic 0.05 % and 0.5 %. In the optimization of PE plastic, *A. flavus* was found to be more stable and was able to degrade the plastic effectively at a concentration of 0.5 % at pH 8 at 39°C when compared to 0.05 % plastic concentration. In the optimization of PVC plastic, *A. niger* was found to be more stable and was able to degrade the plastic effectively in the plastic concentration of 0.05 % at pH 8 at 28°C when compared to 0.5 % plastic concentration.

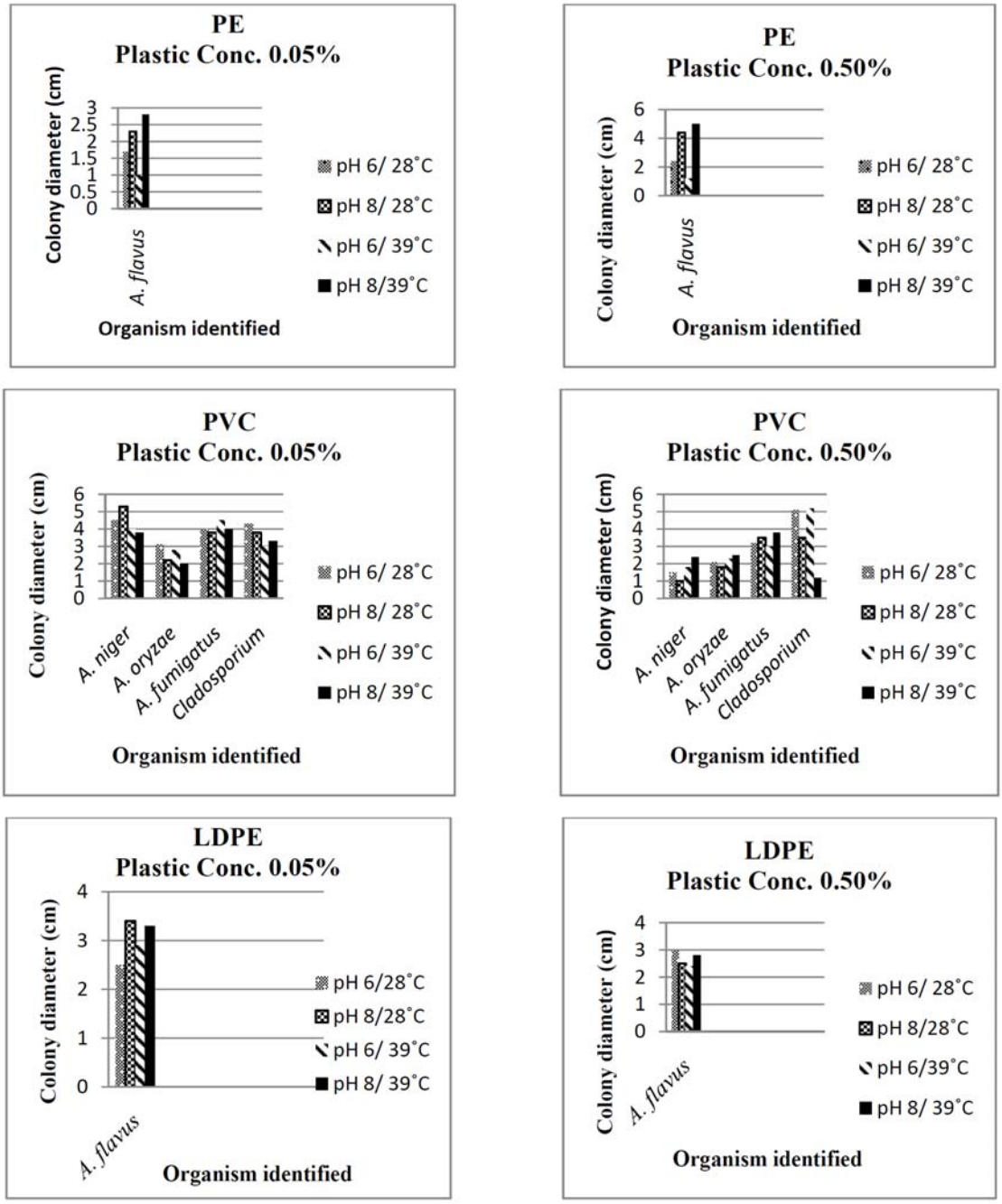

Figure 1. Optimization of plastic concentration, pH and temperature (PE, PVC, LDPE).

A. oryzae was able to degrade the plastic effectively in the plastic concentration of 0.05 % at pH 6 at 28°C when compared to 0.5 % plastic concentration. *A. fumigatus* was observed to degrade the plastic effectively at the concentration of 0.05 % at pH 6 at 39°C when compared to 0.5 % plastic concentration. *Cladosporium* was able to degrade the plastic effectively in the plastic concentration

of 0.5 % at pH 6 at both the temperatures when compared to 0.05 % plastic concentration. In the case of LDPE, *A. flavus* was found to be more stable and was able to degrade the plastic effectively in the plastic concentration of 0.05 % at pH 8 at 28°C and 39°C when compared to 0.5 % plastic concentration. For HDPE, *Penicillium* was found to effectively degrade at a concentration of 0.05 % at pH 6 at 39°C when compared to 0.5 % plastic concentration. *A. flavus* was able to degrade the plastic effectively in the plastic concentration of 0.05 % at pH 6 at 28°C when compared to 0.5 % plastic concentration. *A. niger* showed effective degradation at a concentration of 0.05 % at pH 8 at 39°C when compared to 0.5 % plastic concentration. The percentage of weight loss due to degradation was found more in the High Density Poly Ethylene (HDPE) at 83%.

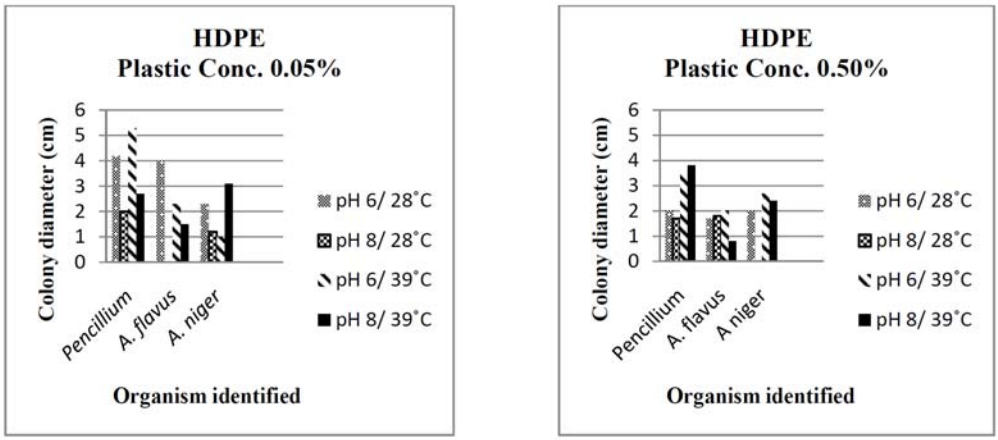

Figure 2. Optimization of plastic concentration, pH and temperature (HDPE).

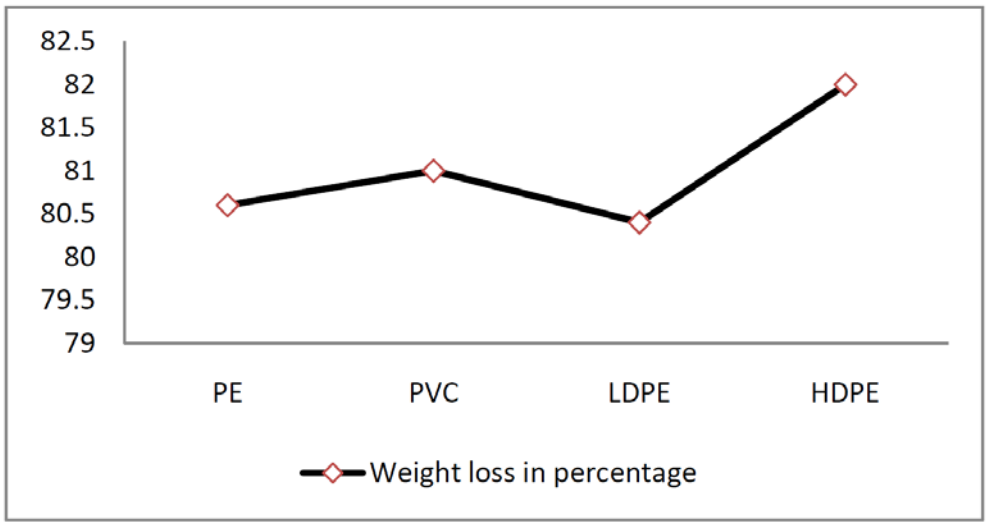

Figure 3. Degradation of plastic samples by microorganisms after 3 months.

Further research will focus on identification and isolation of enzyme or organic acids involved in plastic degradation by the isolated strain. Comprehensive Studies can be carried out to optimize the conditions for plastic degradation process, for example synergistic effect or effect of different co-substrates on the rate of degradation and molecular level characterization of the isolated organism which will pave way for identifying the strain that degrades the plastic material that are otherwise hazardous to environment.

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